ELSEVIER

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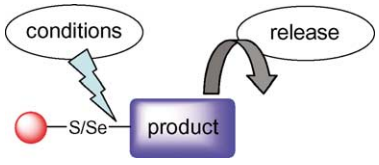
Contents

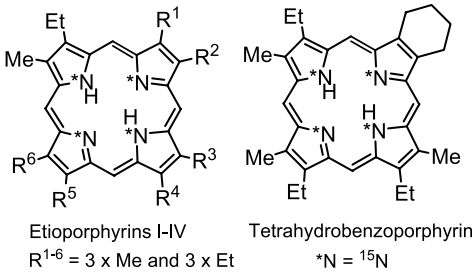
REPORT



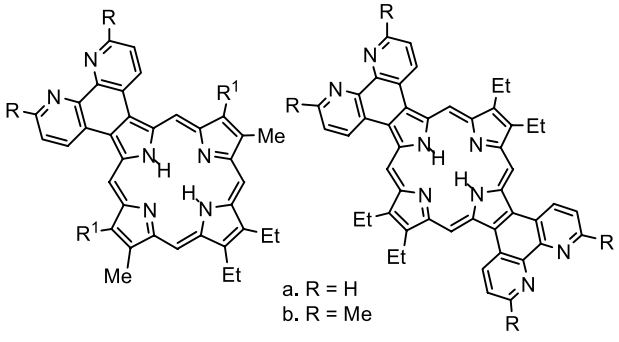
This review focuses on the use of sulfide, sulfoxide, sulfone, selenide and selenoxide linkers in solid-phase and fluorous-phase synthesis.

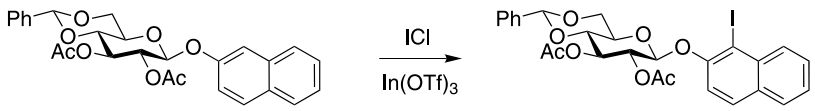
ARTICLES

Syntheses of per-nitrogen-15 porphyrins from labeled sodium nitrite and glycine are described. The pyrrolic precursors were prepared by the Knorr, Kleinspehn, and Barton-Zard condensations. per-^{15}N etioporphyrin-I was prepared via pyrromethene intermediates, while per-^{15}N etioporphyrins II–V and a related tetrahydrobenzoporphyrin were synthesized by the copper(II) mediated cyclization of a,c-biladiene intermediates.

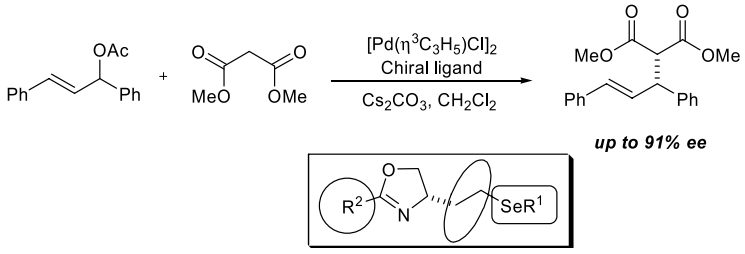


Syntheses of phenanthrolinoporphyrins have been developed using tripyyrane intermediate with a central phenantholinopyrrole component. Alternative procedures are also reported for the synthesis of diphenanthrolino-porphyrins and a triphenanthrolinoporphyrin.

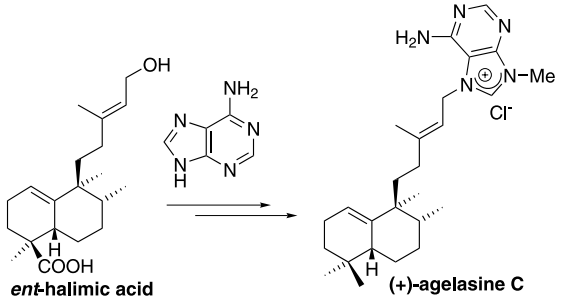


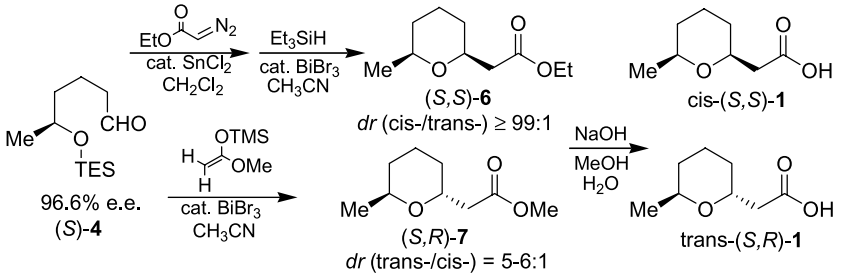


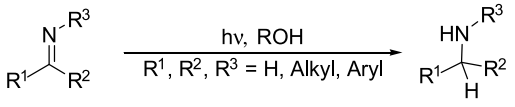
A new and mild method for aromatic iodination suitable for iodination of acid sensitive compounds is presented.

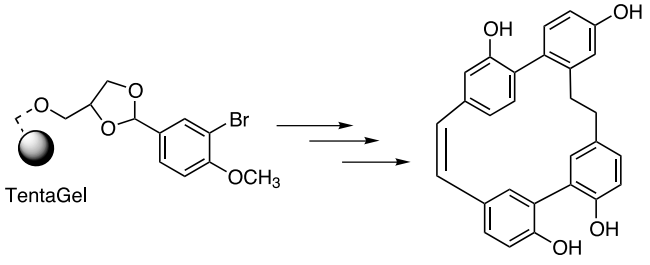


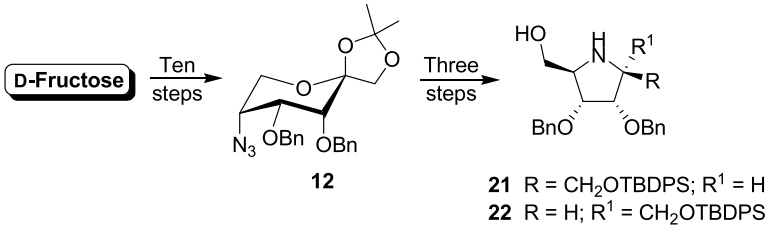
An efficient synthesis of (+)-agelasine C has been achieved from *ent*-halimic acid. The structures of (−)-agelasine C and *epi*-agelasine C has been revised.

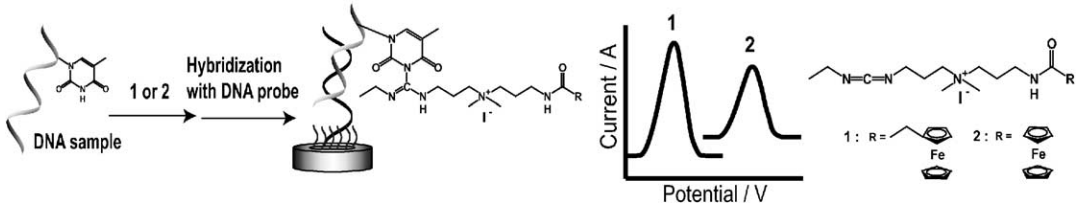




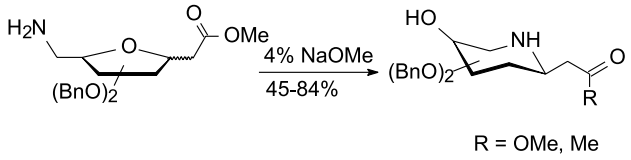


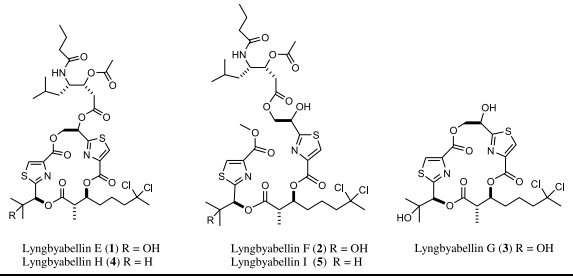


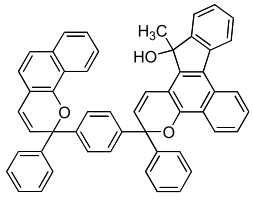


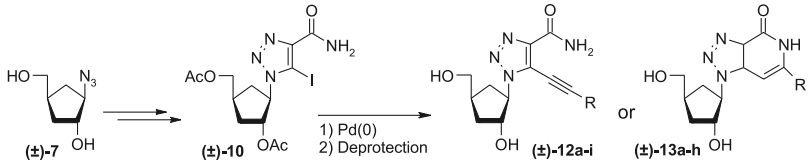


Ferrocenylcarbodiimides **1** and **2**, having different redox potentials, were used for convenient electrochemically active labeling reagents for nucleic acids. The amount of labeled DNAs could be quantitated by their current intensities at their individual potentials, allowing competitive detection of two different DNA samples.

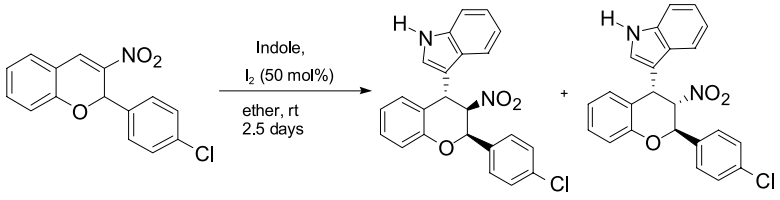


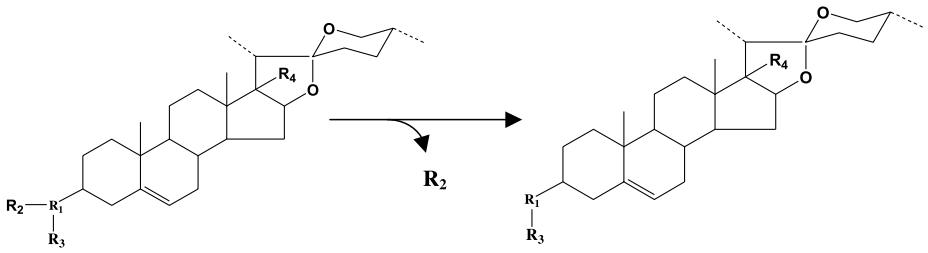






The synthesis of hitherto unknown 5-substituted-1,2,3-triazolo-3′-deoxycarbanucleosides (**7a**–**d**) is described, through a Pd(0)-catalyzed Sonogashira cross-coupling alkynylation. Their 8-aza-3-deazapurine analogues (**8a**–**c**) were also obtained.





R_1= Glc; **R_2= Rha (1→2);** R_3= Ara; Rha (1→4); R_4= H; OH

OTHER CONTENTS

*Corresponding author
ⓘ+ Supplementary data available via ScienceDirect

COVER

The cover graphic shows two examples of unusual porphyrin chromophores where the UV-visible absorption spectra are highly modified due to the presence of a fused heterocyclic subunit. The metallo-derivatives have strong absorptions above 600 nm that could lead to applications in photodynamic therapy. *Tetrahedron* **2005**, *61*, 11615–11627.
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Sulfide- and selenide-based linkers in phase tag-assisted synthesis

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Contents

Keywords: Solid phase; Linkers; Sulfur; Selenium.

Abbreviations: AIBN, 2,2′-azobis(2-methylpropionitrile); Alloc, allyloxycarbonyl; Boc, *t*-butoxycarbonyl; Cbz, benzyloxycarbonyl; CSA, 10-camphorsulfonic acid; DABCO, diazabicyclo[2.2.2]octane; DAST, diethylaminosulfur trifluoride; dba, dibenzylacetone; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; DCC, 1,3-dicyclohexyl carbodiimide; DEAD, diethyl azodicarboxylate; DIAD, diisopropyl azodicarboxylate; DIBALH, diisobutylaluminium hydride; DIC, 1,3-diisopropyl carbodiimide; DMA, *N*,*N*-dimethylacetamide; DMAP, 4-dimethylaminopyridine; DME, 1,2-dimethoxyethane; DMF, *N*,*N*-dimethylformamide; DMPU, 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidone; DMSO, dimethylsulfoxide; DMT, dimethoxytrityl; dppe, 1,2-bis(diphenylphosphino)ethane; dppf, 1,1-bis(diphenylphosphino)ferrocene; DTBP, 2,6-di-*t*-butyl-4-methylpyridine; EDCI, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; EE, ethoxyethyl; FDPP, pentafluorophenyl diphenylphosphinate; Fmoc, 9-fluorenylmethyoxycarbonyl; FSPE, fluorous solid-phase extraction; HATU, *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*,*N*-tetramethyluronium hexafluorophosphate; HFIP, 1,1,1,3,3,3-hexafluoropropan-2-ol; HMDS, 1,1,1,3,3,3-hexamethyldisilazane; HMPA, hexamethylphosphoramide; HOBt, 1-hydroxybenzotriazole; IBX, 2-Iodoxybenzoic acid; LDA, lithium diisopropylamide; *m*CPBA, 3-chloroperbenzoic acid; MMT, monomethoxytrityl; MOM, methoxymethyl; MS, molecular sieves; NBS, *N*-bromosuccinimide; NCS, *N*-chlorosuccinimide; NIS, *N*-iodosuccinimide; NMM, *N*-methylmorpholine; Pbf, 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl; PEG, poly(ethylene glycol); PMB, 4-methoxybenzyl; PPTS, pyridinium 4-toluenesulfonate; PyBOP, (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate; TBA, tetrabutylammonium; TBDMS, *t*-butyldimethylsilyl; TBDPS, *t*-butyldiphenylsilyl; TBS, *t*-butyldimethylsilyl; TBTU, *O*-benzotriazol-1-yl-*N*,*N*,*N*,*N*-tetramethyluronium hexafluorophosphate; TFA, trifluoroacetic acid; TFAA, trifluoroacetic anhydride; THF, tetrahydrofuran; THP, tetrahydropyranyl; TMEDA, *N*,*N*,*N*,*N*-tetramethylethylenediamine; TMS, trimethylsilyl; Tr, trityl; Ts, 4-toluenesulfonyl.

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1. Introduction

Phase tag-assisted synthesis is a well-established approach in modern organic chemistry. A phase tag can be simply thought of as a handle that allows a compound of interest to be 'lifted' out of a chemical mixture. Linkers are used to attach phase tags to starting materials before manipulation. The physical properties of the phase tag can be exploited to provide convenient and efficient means for the purification of intermediates in the synthetic sequence. Finally, cleavage of the linker releases products from the phase tag. An appropriate choice of linker is therefore crucial to the development of a successful phase tag-assisted synthesis. The design of powerful new linker systems represents a key activity in organic synthesis.

In recent years, the rich organic chemistry of sulfur- and selenium-containing compounds has found widespread application in the area of phase tag-assisted synthesis. Innovative linker strategies founded on 'traditional' sulfur and selenium organic chemistry have led to a wealth of powerful linker systems for the synthesis of myriad target classes. Many of these linker systems are traceless in nature, that is, cleavage of the linker leaves no residual functionality at the point of attachment to the phase tag, a feature vital to the synthesis of structurally unbiased libraries.[1–4] This review aims to highlight not only the power of sulfur and selenium chemistry, but also to illustrate how recourse to long-established chemistry can often prove the key in meeting challenges in emerging areas.

Several excellent reviews on linker chemistry have appeared and readers are referred to these earlier reviews for an introduction to the area.[5,6] This review will focus on the use of sulfide- and selenide-based linkers in both solid-phase and fluorous-phase synthesis. In particular, we will discuss linkers based on sulfide, sulfoxide, sulfone, selenide and selenoxide linkages. While Zaragoza has highlighted the importance of this emerging area,[7] a review of the subject has not previously appeared.

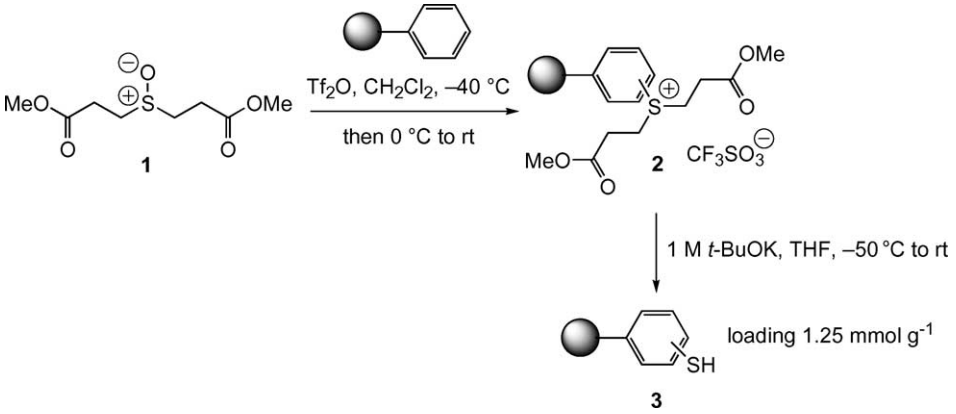
As many sulfur and selenium linkers utilise several different oxidation states to achieve the desired function, the material in this review is organised, where possible, according to the linker functional group initially present after immobilisation and formation of the link. Material is then sub-divided according to similarities in the methods of immobilisation, methods of cleavage, or application in the synthesis of related targets. Within each section and, where possible, reports have been ordered chronologically.

2. Sulfur-based linkers

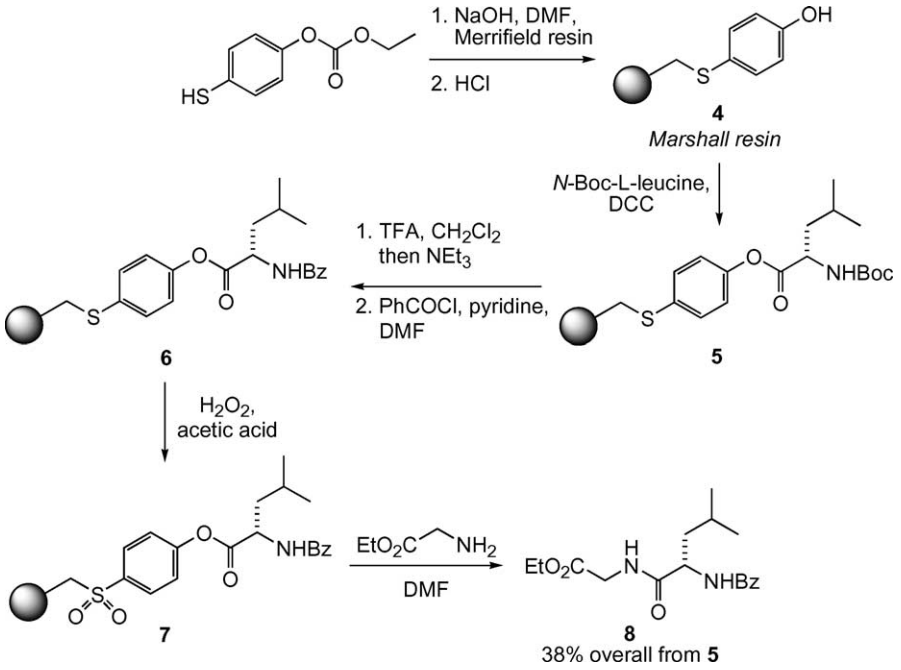
2.1. Sulfide linkers

A variety of methods have been developed for the introduction of sulfur-containing functional groups to simple resins. This is a common starting point for the construction of sulfur linkers. In an early study, Fréchet et al. described the lithiation of brominated polystyrene resin using *n*-BuLi in benzene followed by reaction with sulfur or dimethylsulfide to give thiol and methylsulfide resins, respectively.[8] Recently, Wagner, Mioskowski and co-workers have developed a mild and efficient two-step synthesis of a polystyrene thiol resin that avoids the harsh conditions commonly required for the solution-phase thiolation of aromatic compounds.[9] Polystyrene was treated with activated sulfoxide **1** to afford the sulfonium trifluoromethanesulfonate resin **2**. Smooth β-eliminative dealkylations using *t*-BuOK provided the polystyrene thiol resin **3** with a satisfactory loading (Scheme 1). Derivatisation of **3** with 4-nitrobenzyl bromide (NaOH, dioxane/H_2O, 60 °C) was used to assess the reactivity of the thiol resin.[9]

2.1.1. Early sulfide linkers. In an early example of the use



Scheme 1.



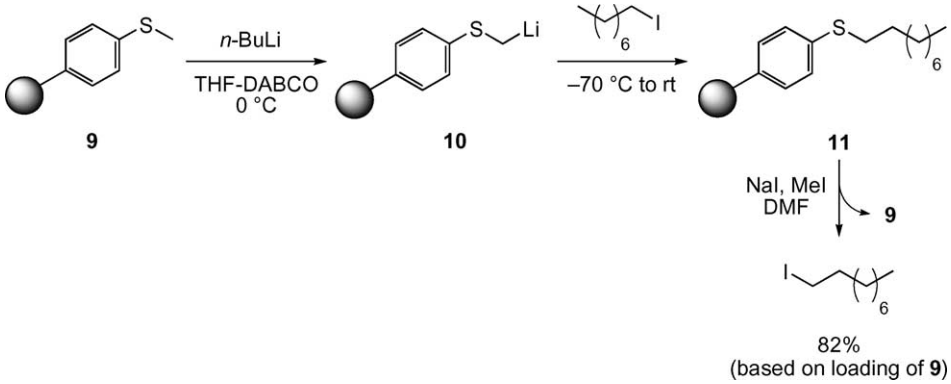
Scheme 2.

of a sulfur-based linker system, Marshall et al. reported a modification of Merrifield's solid-phase approach to peptide synthesis.[10] In Merrifield's original approach, harsh cleavage conditions were required to cleave the product peptide from the support. These conditions resulted in loss of N-terminal and side-chain protecting groups. Marshall's resin **4** is readily prepared from Merrifield resin and can be used to prepare peptides in the usual manner. Cleavage, however, is achieved by oxidation of sulfur in sulfides such as **6** (prepared from **5**), to give the corresponding sulfones **7**, thus activating the C-terminal ester towards nucleophilic attack (Scheme 2). Treatment of the activated resin **7** with an amino acid cleaves a product peptide such as **8** from the resin lengthened by one amino acid residue. Crucially, N-terminal and side-chain protecting groups are left intact when the technique is applied to the synthesis of more complex peptide products (Scheme 2).[10]

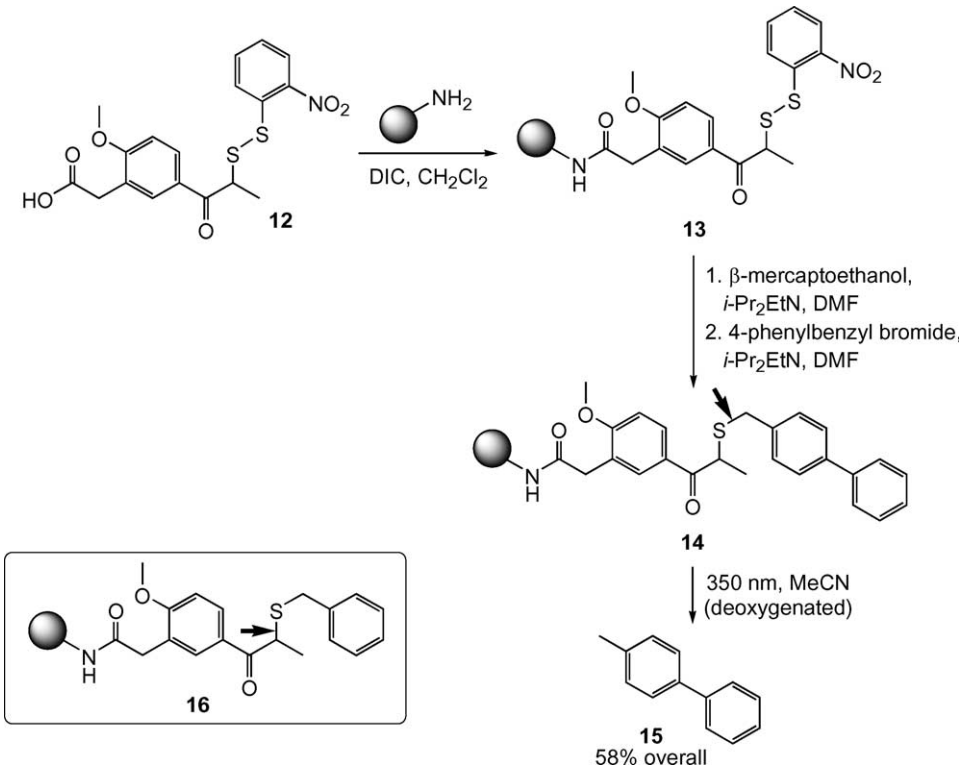
Crosby and co-workers used a polymer-supported phenylsulfanylmethyllithium reagent **10** in the homologation of alkyl iodides,[11] for example, reaction of **10** with 1-iodooctane gave sulfide resin **11**. Reaction with NaI and MeI regenerated methyl sulfide resin **9** and gave 1-iodononane in high yield (Scheme 3).[11]

The reaction of **10** with 1,4-diiodobutane was used to study the intraresin reactions of polymer-bound, ionic functional groups. Treatment of 1,4-diiodobutane with **10** gave a mixture of 1,5-diiodopentane and 1,6-diiodohexane resulting respectively from homologation at one or both of the terminal iodides. The ratio of these products is a direct indication of interaction between reactive sites on the polymer. It was found that 1,6-diiodohexane was often the major product. It is thought that the charged groups on the polymer lead to ionic clustering, thus generating regions of high functional group concentration in the polymer. This, in turn, leads to the extensive interactions between the functional groups seen in this study.[11]

Suchoeiki has developed a photocleavable sulfide linker for the cleavage of aliphatic molecules from solid support (Scheme 4).[12,13] The linking unit was constructed by immobilisation of disulfide **12** on amine resin. Treatment of **13** with β-mercaptoethanol gave a polymer-supported



Scheme 3.



Scheme 4.

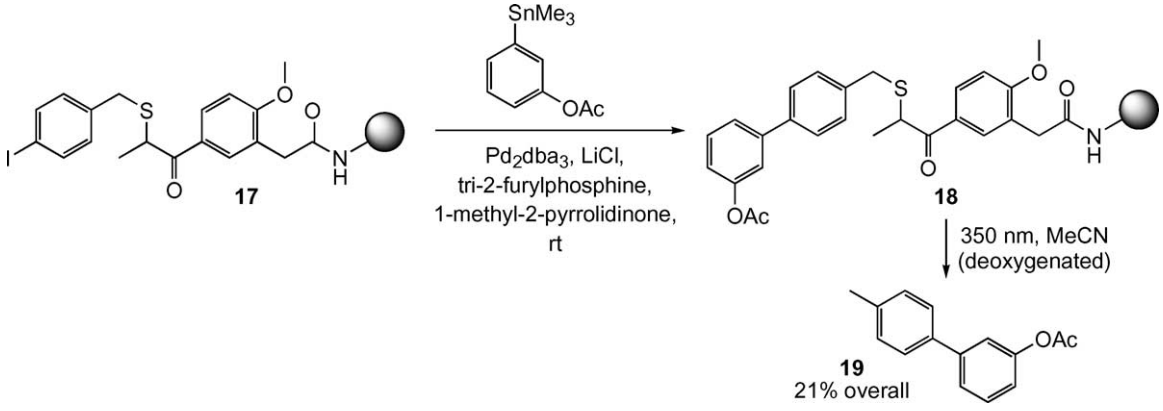
thiol that could be alkylated with benzylic halides to give sulfide-linked biaryl **14**. The benzylic carbon–sulfur bond was cleaved selectively under photochemical conditions to give **15** in 58% yield (Scheme 4).[12]

The cleavage reaction was shown to be sensitive to the nature of the benzylic system under study, for example, photolysis of sulfide resin **16** (Scheme 4), gave dibenzyl disulfide after cleavage of the carbon–sulfur bond α to the ketone carbonyl group.

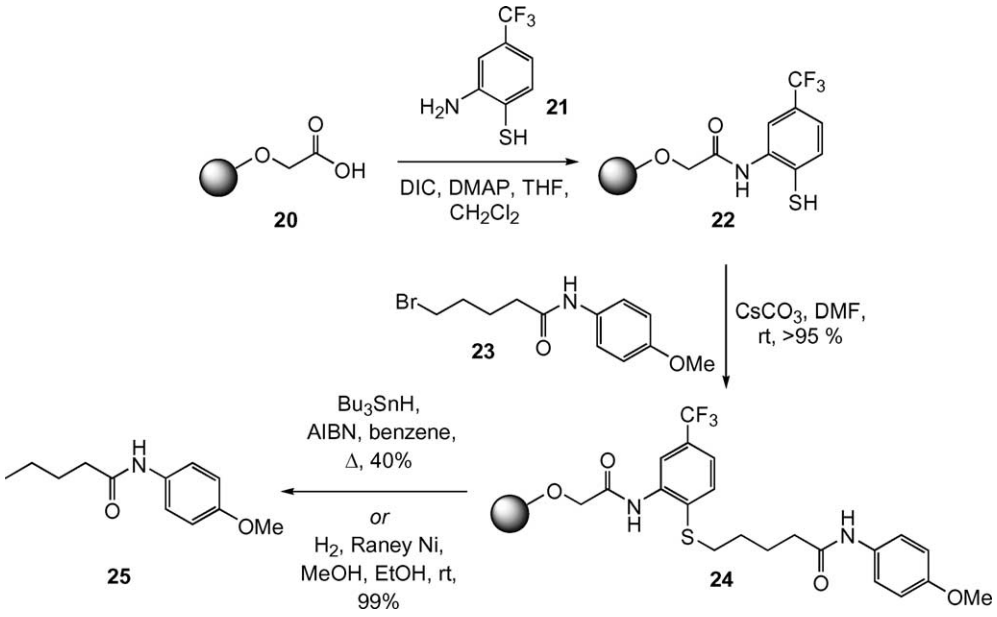
Sucholeiki and co-workers have utilised the linker in a solid-phase synthesis of biaryls using the Stille reaction.[13] Treatment of aryl iodide resin **17** with (3-acetoxyphenyl)-trimethyltin under palladium catalysis gave biaryl **18**. Irradiation of **18** triggered homolytic cleavage of the benzyl-sulfur bond and release of the biaryl product **19**, albeit in low yield (Scheme 5).[13]

Janda et al. played an important role in pioneering the use of sulfide linkers in solid-phase synthesis and developed several linkers for the traceless cleavage of aliphatic molecules from a soluble polymer support.[14] MeO-PEG acid-soluble polymer **20** was coupled with amino thiol **21** to form thiol resin **22**, that was then treated with various alkyl halides, such as **23**, to form a sulfide linkage. Treatment of sulfide **24** with Bu_3SnH and AIBN cleaved the carbon–sulfur bond, releasing the desired amide **25** in moderate yield. More efficient cleavage conditions involved the use of hydrogen and Raney nickel (Scheme 6). The use of soluble polymers allowed easy isolation and purification of the released product; precipitation of desulfurised MeO-PEG resin with ether and concentration of the filtrate gave the product in high purity.[14]

An improved sulfide linker system was developed by Janda and co-workers.[15] In this second-generation approach, the



Scheme 5.



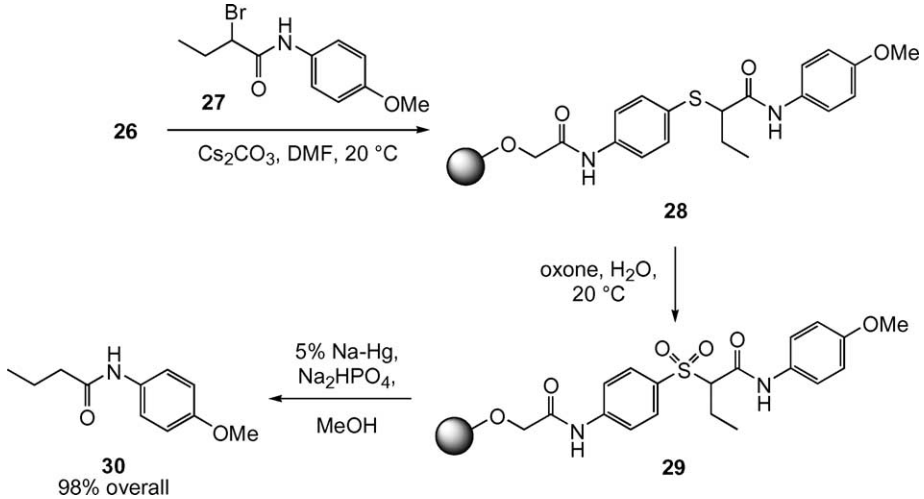
Scheme 6.

thiol precursor was prepared more efficiently by reduction of a disulfide using Cleland's reagent (dithiothreitol) (Scheme 7). Alkylation of thiol **26** using alkyl bromide **23** and cleavage using Raney nickel gave **25** (Scheme 6) in 99% yield.[15]

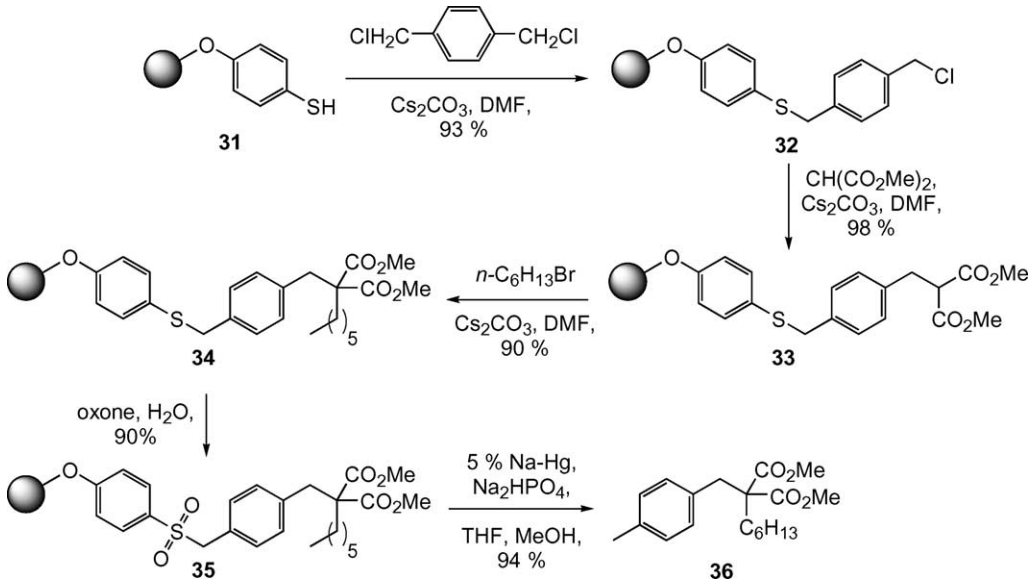
Scheme 7.

The strong reducing conditions required for the cleavage step limit the functional groups that can be present in the molecule being prepared. This problem was addressed by oxidation of the linking sulfur atom to the sulfone and the use of a more selective reducing agent. An Na–Hg reducing system was employed in an improved cleavage strategy (Scheme 8).[16] After attachment of the substrate **27** to the thiol resin **26**, the sulfide link in **28** was oxidised to the corresponding sulfone **29** using oxone. As oxone can oxidise sulfides to sulfones in the presence of alkenes and ketones, it is a more attractive reagent for this transformation than alternatives such as *m*CPBA. Unfortunately, the oxone reaction conditions are not compatible with all polymer supports. Reductive cleavage of the link gave product **30** in high yield (Scheme 8).[16,17]

The amide functionality in Janda's sulfide-linker systems renders them incompatible with reagents such as $LiAlH_4$ and strong base or acid. To increase the generality of these linkers, thiol **31** was synthesised and used to prepare a more robust, ether-based linker system. To demonstrate the utility of Janda's third-generation sulfide linker, a solid-phase synthesis of alkylated malonate derivatives was undertaken (Scheme 9).[18] Alkylation of thiol **31** with a dihaloalkane to form **32** was followed by addition of a malonate anion. The resulting polymer-supported malonate **33** was alkylated



Scheme 8.

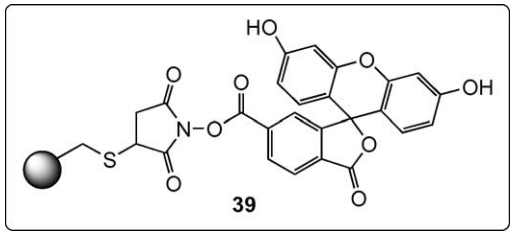


Scheme 9.

once more to furnish **34** before oxidation to the sulfone **35** and cleavage of the linker. Malonate derivative **36** was isolated in excellent yield (Scheme 9).[18] This linker system has also been applied in the synthesis of 3,5-pyrazolidinediones.[19]

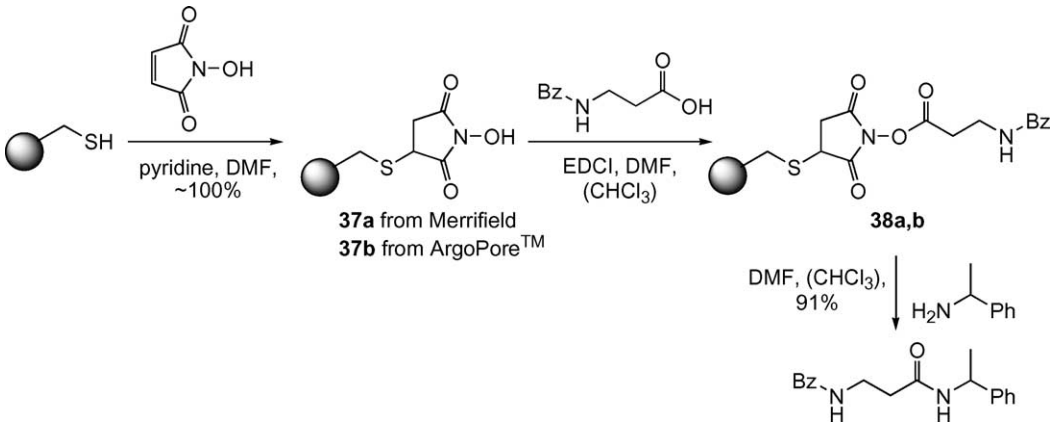
2.1.2. Sulfide linkers formed through Michael addition. Adamczyk et al. have reported the preparation of *N*-hydroxysuccinimide resins **37a**,**b** immobilised via a sulfide link to the support.[20] Michael addition of thiol resins, derived from Merrifield and ArgoPore™-Cl resins, to *N*-hydroxymaleimide provided efficient access to the supported reagents **37a**,**b**. Coupling with a range of carboxylic acids gave the active ester resins **38a**,**b** that underwent smooth reaction with primary and secondary amines to give the expected amides in high yield and purity (Scheme 10).[20]

Adamczyk and co-workers have utilised this approach to prepare solid-supported labelling reagents,[21] for example, active ester resin **39** derived from fluorescein was prepared and used in reactions with amines to prepare labelled amides.

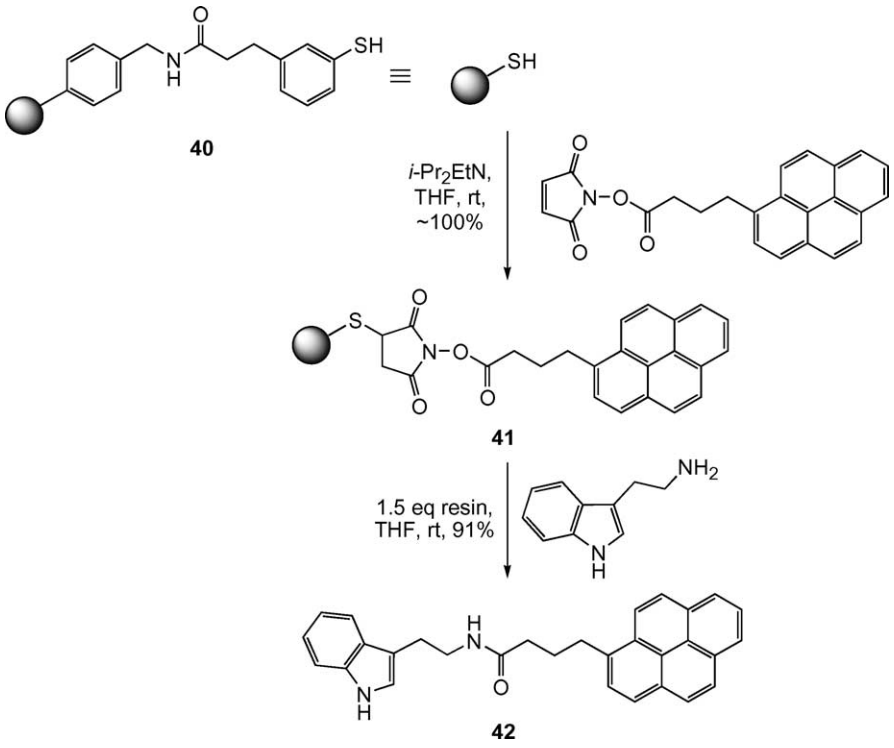


In an independent study, Sodeoka et al. described similiar polymer-bound *N*-hydroxysuccinimide esters that were prepared by Michael reaction of thiol resin **40** to various *N*-hydroxymaleimide esters,[22] for example, active ester resin **41** containing a pyrene fluorescent label was prepared. Treatment of resin **41** with tryptamine gave fluorescent-labelled amide **42** in excellent yield and high purity (Scheme 11).[22]

2.1.3. Sulfide linkers and Pummerer chemistry. Procter et al. have developed an approach to oxindoles that utilises a sulfide linker in the first Pummerer cyclisations on solid-phase.[23] Immobilised *N*-arylacetamides, such as **45**, were prepared by reaction of a benzyl thiol resin **44**[24] with

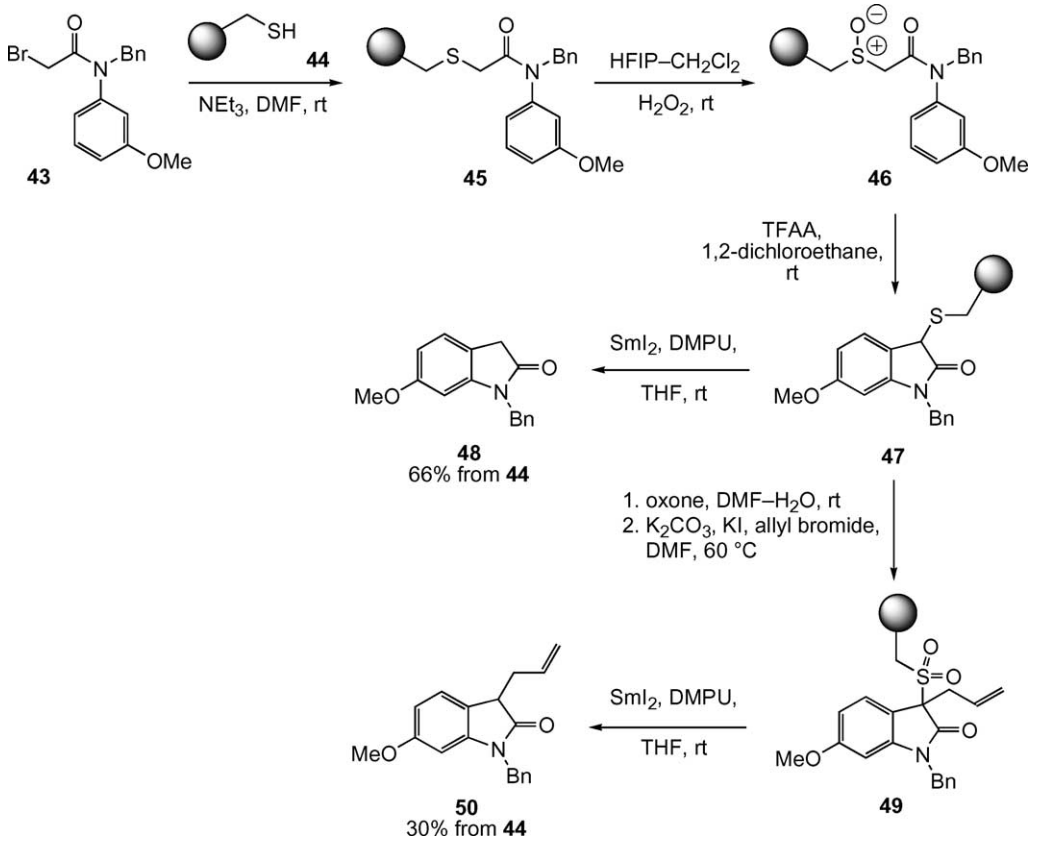


Scheme 10.

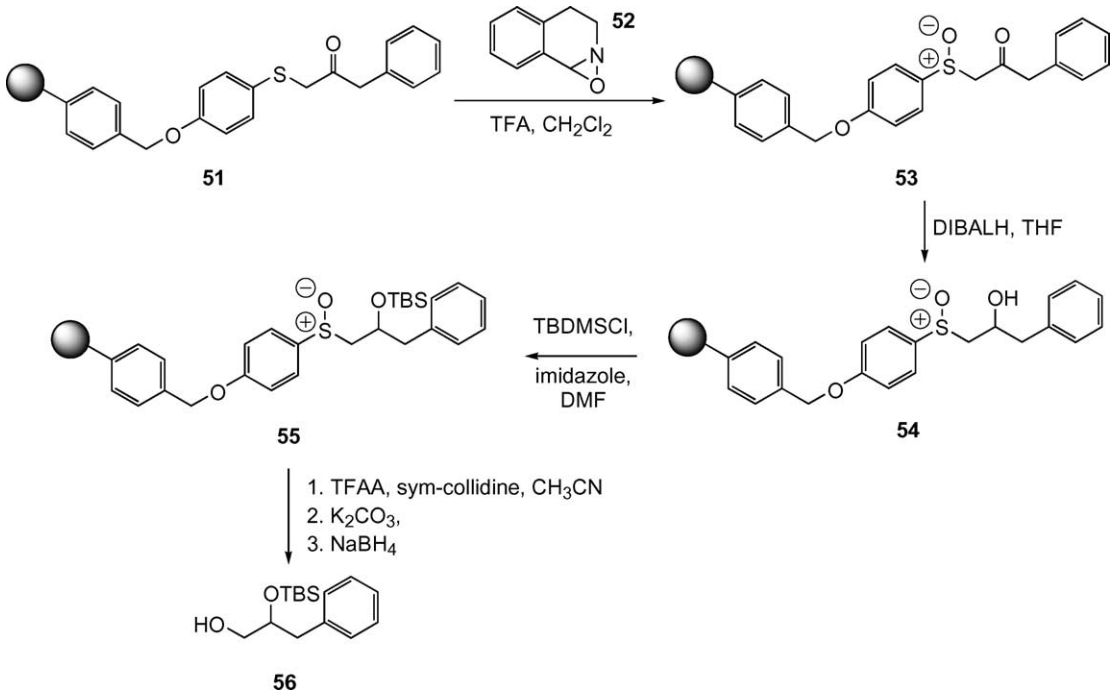


Scheme 11.

α-bromoacetamides **43**. Selective oxidation to the sulfoxide **46** and Pummerer cyclisation using TFAA gave oxindole **47** (Scheme 12). Cleavage of the sulfide linkage is based on the well-established reduction of α-heteroatom-substituted carbonyl compounds with the lanthanide, electron-transfer reagent, SmI_2. Thus, treatment of **47** with SmI_2 gave oxindole **48** in good overall yield (Scheme 12). As the sulfide linkage remains intact during the Pummerer



Scheme 12.

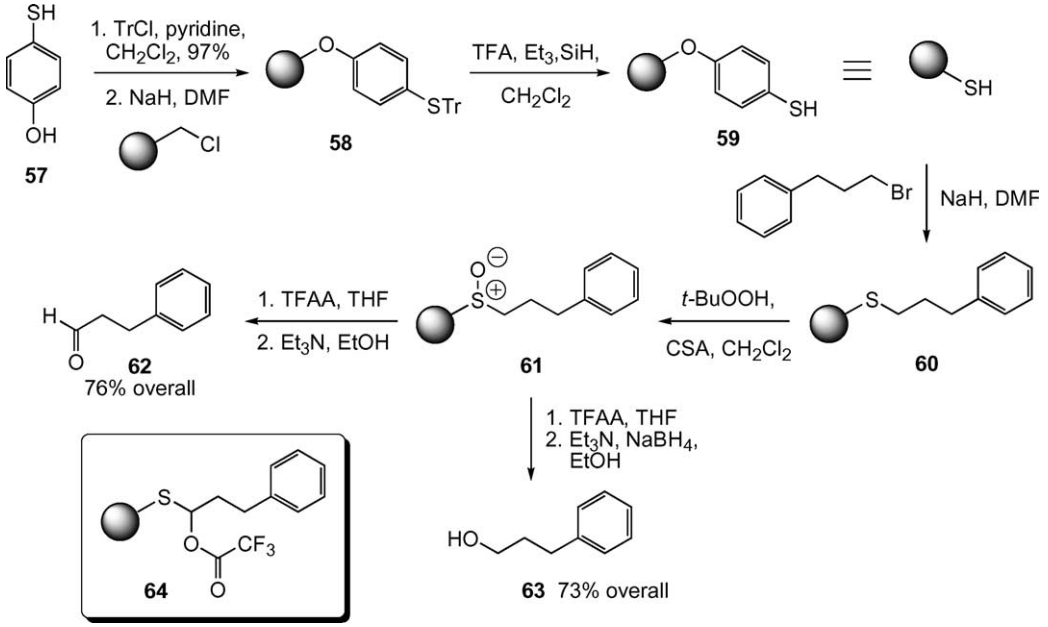


Scheme 13.

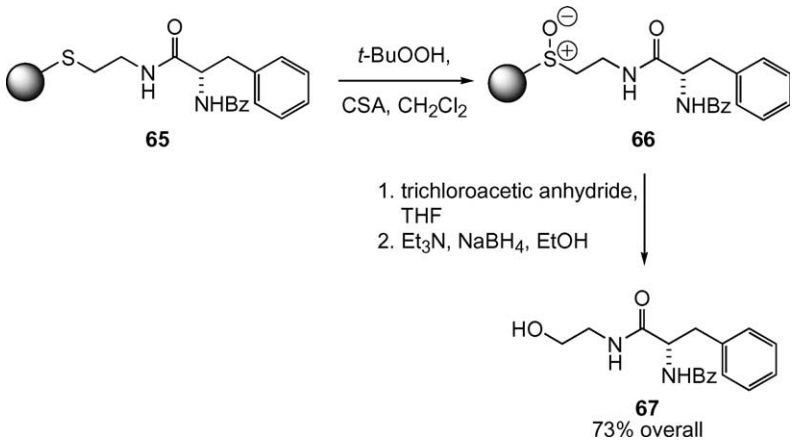
cyclisation, the sulfur atom can be used in further elaborations of the heterocyclic skeleton. Oxidation to sulfone facilitates alkylation to give **49** and SmI_2 cleavage provides **50** in 30% overall (Scheme 12).[23]

Solladié and co-workers have developed a sulfide linker for the synthesis of 1,2-diols employing a Pummerer rearrangement cleavage strategy (Scheme 13).[25] *p*-Hydroxyphenyl-α-ketosulfides were constructed in solution and attached to the Wang-based resin via the oxygen atom. Selective oxidation of the sulfide **51** to sulfoxide **53** was carried out using oxaziridine **52**. The sulfoxide link was then used to control the diastereoselectivity of a DIBALH reduction of the ketone group. Two diastereoisomers were obtained and a 95% de was reported, although the nature of the diastereoisomers was not reported. The resin-bound alcohol **54** was protected as silyl ether **55** prior to cleavage of monoprotected diol **56** by Pummerer rearrangement followed by hydrolysis and reduction (Scheme 13). Specific yields were not given although the cleavage was described as being almost quantitative.[25]

Li et al. have also reported a sulfide safety-catch linker that is activated by oxidation to the sulfoxide before cleavage using the Pummerer rearrangement.[26] The known, Merrifield-based thiol resin **59**,[27] prepared from **57** and **58**, was



Scheme 14.



Scheme 15.

treated with a range of electrophiles including alkyl halides, alcohols, tosylates, mesylates, and epoxides to give products immobilised through a sulfide linkage (Scheme 14), for example, thiol **59** was alkylated with 1-bromo-3-phenylpropane to give sulfide resin **60**. The sulfide linkage was then oxidised, employing *t*-BuOOH and 10-camphorsulfonic acid, to give polymer-bound sulfoxide **61**. Pummerer rearrangement, utilising TFAA, led to the rearranged intermediate trifluoroacetoxythioacetal **64** that was cleaved by treatment with Et_3N in ethanol, giving aldehyde **62** in good yield. An alternative cleavage strategy incorporating a one-pot Pummerer rearrangement–reduction sequence was also developed, to give alcohol **63** (Scheme 14).[26]

Amides were also prepared using this approach, but required modified cleavage conditions,[26] for example, immobilised amide **65** was cleaved by oxidation to the sulfoxide **66**, Pummerer rearrangement using trichloroacetic anhydride, followed by reduction to give alcohol **67** (Scheme 15). Trifluoroacetic anhydride was found to be unsuitable for the Pummerer rearrangement in these cases and led to by-products.[26]

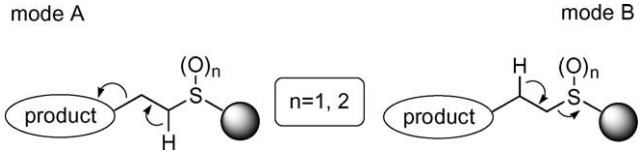


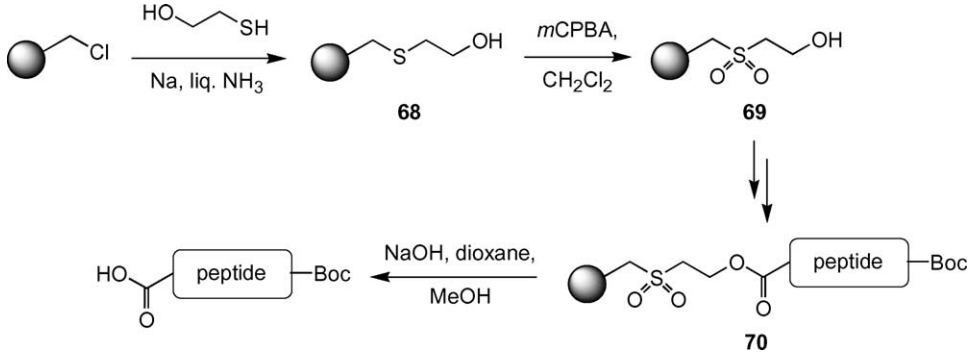
Figure 1.

2.1.4. Sulfide linkers cleaved by elimination. Oxidation of a linking sulfur atom to the corresponding sulfoxide or sulfone followed by elimination either of a group attached β to the linkage (mode A) or to the linking atom itself (mode B), is a common strategy for the release of products from sulfide linkers (Fig. 1).

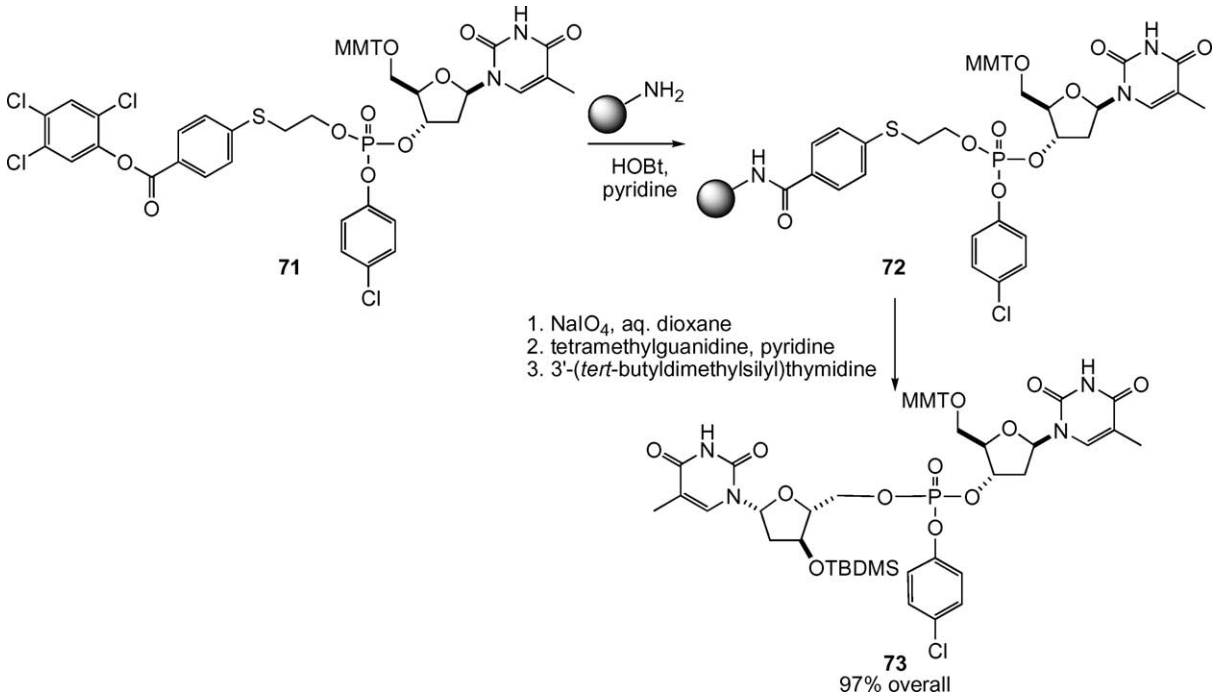
Cleavage mode A first found application in peptide synthesis. Tesser's group reported a sulfone linker prepared by modification of Merrifield resin with 2-hydroxyethanethiol and oxidation of the resulting sulfide **68** to the sulfone **69** using *m*CPBA.[28] Merrifield peptide synthesis can then be carried out after coupling of the C-terminal residue with **69** using DCC. The linker is cleaved by base-mediated β-elimination of intermediates such as **70** (Scheme 16).[28]

In a subsequent report, Tesser et al. compared the efficiency of a peptide synthesis using the sulfone linker **69** with the construction of the same target using Merrifield resin.[29] Although the yields of the final peptide were found to be similar using both linker systems, the cleavage step was faster and easier to carry out when the sulfone linkage was employed, thus reducing the risk of racemisation and product decomposition.[29]

Schwyzer and co-workers have used similar sulfur linkers for the synthesis of protected oligopeptides and oligonucleotides on solid-phase,[30] for example, the nucleotide active ester **71** was coupled to polydimethylacrylamide resin functionalised with ethylenediamine to afford **72** (Scheme 17). Upon oxidation to the sulfoxide, eliminative



Scheme 16.



Scheme 17.

cleavage released a protected nucleotide, which was condensed with 3-(*tert*-butyldimethylsilyl)thymidine to obtain the fully protected dinucleoside monophosphate **73** in 97% overall (Scheme 17).[30]

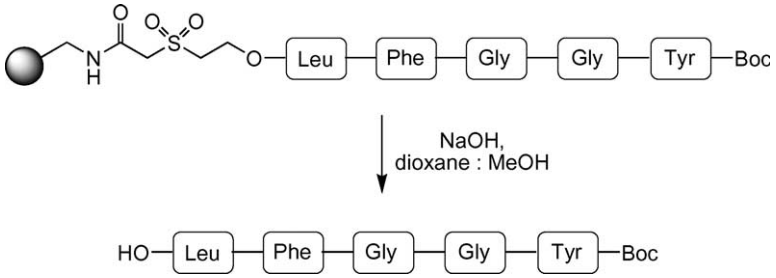
In a more recent study, Katti et al. have described a base-labile sulfone linker for the synthesis of protected peptide fragments.[31] The linker is compatible with both Boc and Fmoc chemistry (Scheme 18).

Canne and co-workers have used a similar base-labile linker in a purification strategy for solid-phase peptide synthesis.[32] After a Boc-solid-phase peptide synthesis, the products are often contaminated by peptides blocked at their *N*-terminus. Sulfone **74** has been used to treat mixtures of immobilised peptides from solid-phase synthesis. Only peptides having a free N-terminal amine can react and, thus, only the desired full-length peptides are tagged. The resultant amine **75** is then coupled with 4-oxopentanoic acid, giving **76** containing a ketone handle. Standard cleavage then gives a mixture of peptides, but the desired full-length peptides **77** can be easily sequestered using an oxime support and the unwanted, untagged peptide by-products can be washed away. Finally, the purified peptide product can be released by eliminative cleavage of the sulfone linkage in **78** (Scheme 19).[32]

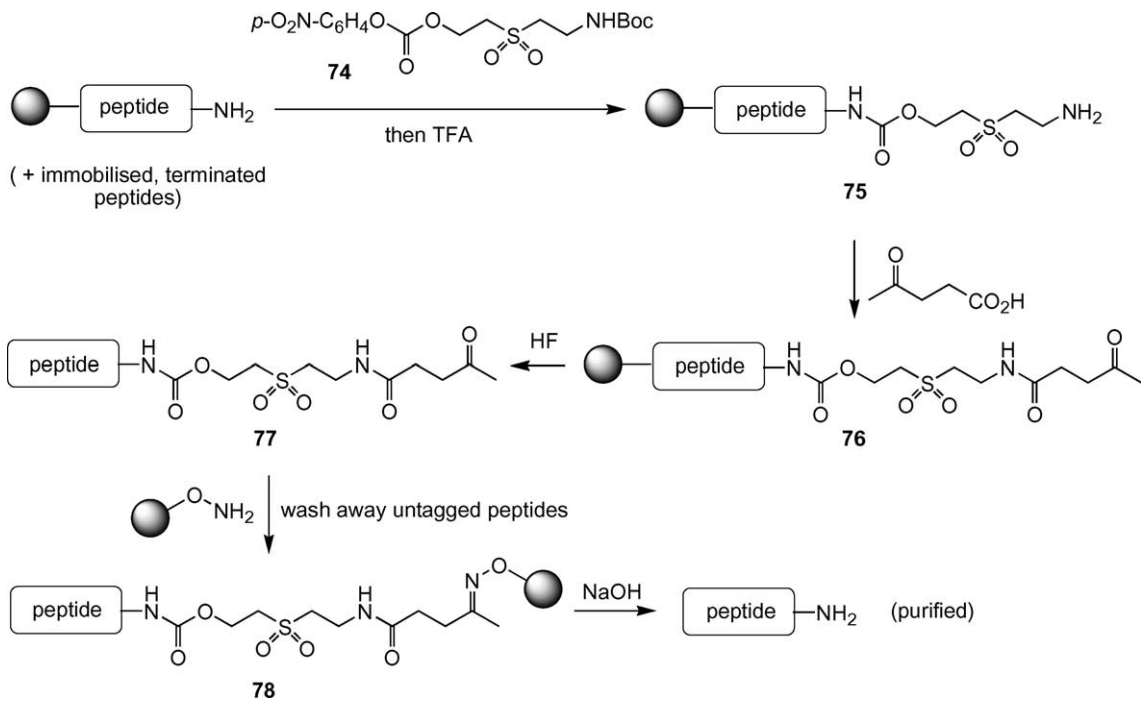
García-Echeverría has described the use of a similar sulfone-cleavage approach in the synthesis of arylsulfonamides.[33] Sulfide resin **79**, derived from 4-methyl-benzhydrylamine resin, was deprotected to give **80** and coupled first with 4-(chlorosulfonyl)phenylisocyanate, and then with three different amines to give immobilised sulfonamides, such as **81**. Oxidation of **81** to the sulfone and eliminative cleavage gave arylsulfonamide **82** in good yield (Scheme 20).[33]

Wade et al. have also utilised a sulfide safety-catch linker cleaved by oxidation to the sulfone and elimination.[34] Acid **83** was coupled to aminomethyl resin to give sulfide **84**. Reductive amination, *N*-acylation and *N*-alkylation gave quaternary ammonium salt **85**. Activation of the linker by oxidation to the corresponding sulfone **86** and base-induced elimination released amide **87** from the resin in good overall yield (Scheme 21).[34]

Barco et al. have utilised a sulfide linker cleaved by elimination mode B (Fig. 1) in a solid-phase route to



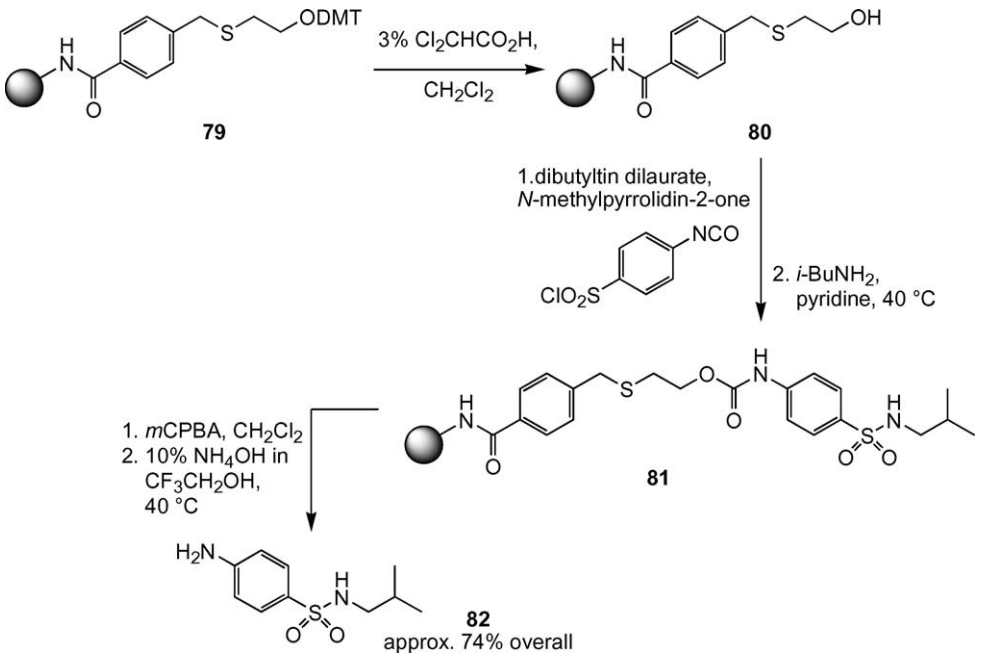
Scheme 18.



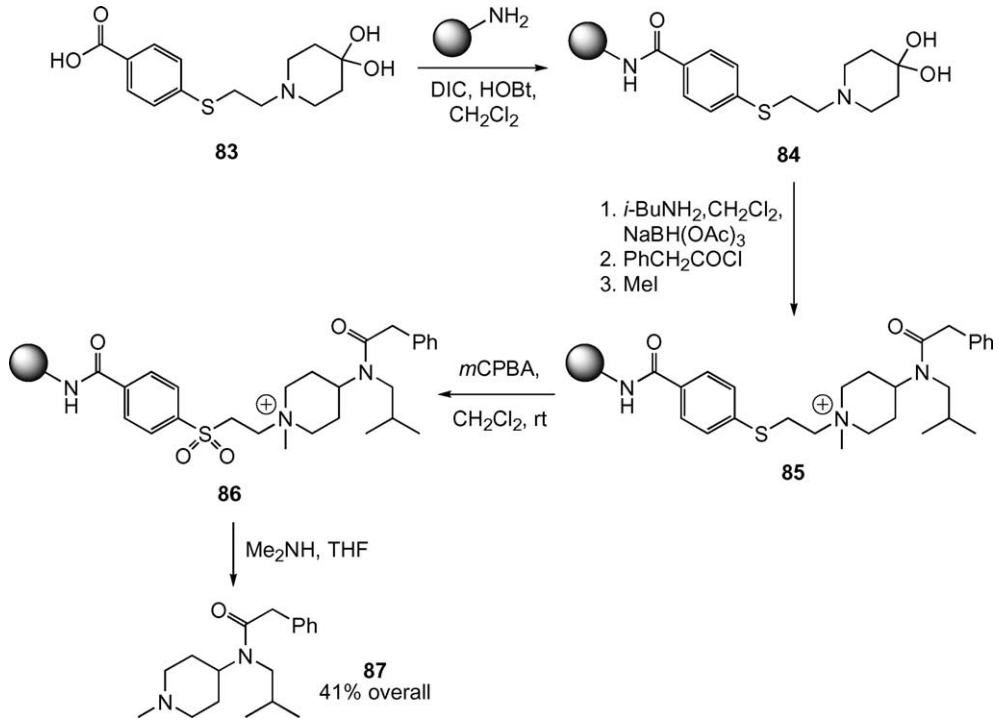
Scheme 19.

substituted piperidin-4-ones that proceeds via triphenylphosphoranylidene sulfone resin **90** (Scheme 22).[35] Addition of thiol resin to 3-buten-2-one and oxidation of the resulting sulfide link in **88** gave sulfone **89**. α-Bromination and treatment with triphenylphosphane and base then gave the key resin-bound ylide **90**. Wittig reaction with various aldehydes gave enones such as **91**. Subsequent treatment with benzylamine resulted in eliminative cleavage and cyclisation to give the substituted piperidin-4-one **92** (Scheme 22). Five different piperidin-4-ones were synthesised by varying the aldehyde used in the Wittig reaction.[35]

Biologically interesting dehydropeptides have been prepared by Yamada and co-workers using a solid-phase approach involving a sulfide linkage and a related cleavage strategy.[36] Immobilisation of cysteine via the sulfur atom gave resin **93**. Introduction of carbamate and ester protection and deprotection of nitrogen then gave amine resin **94** (Scheme 23). Coupling with protected phenylalanine under standard conditions gave the immobilised dipeptide **95**. Oxidation of the linking sulfur atom to the sulfone **96** and elimination using DBU gave dehydropeptide **97** in good yield and purity (Scheme 23).[36]



Scheme 20.

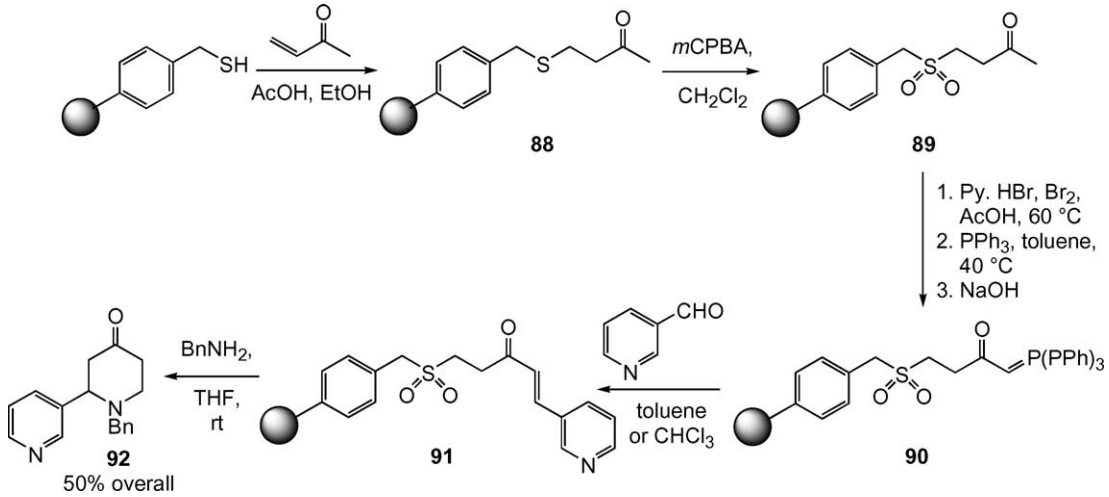


Scheme 21.

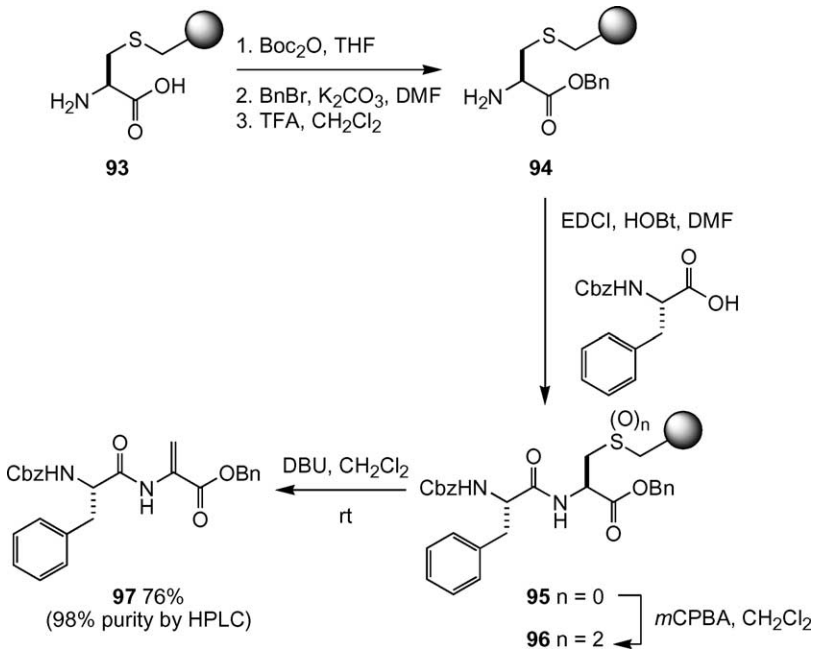
Bradley et al. have developed sulfoxide- and selenoxide-based thermally cleavable safety-catch linkers and have applied these in the solid-phase synthesis of indenones.[37] Indanone precursors **98a** and **98b**, with sulfur or selenium linking atoms α to the carbonyl group, were prepared in solution phase and attached to aminomethyl polystyrene resin via an amide coupling reaction (Scheme 24). At this stage, the linking sulfur or selenium atoms are unactivated and cleavage cannot be effected thermally. Selective oxidation of the sulfide linker to the corresonding sulfoxide **99a** was achieved using Bégué's hydrogen peroxide/1,1,1,3,3,3-hexafluoroisopropanol (HFIP) system.[38] These conditions allow a vast excess of hydrogen peroxide to be used without overoxidation to the sulfone, conditions ideal for use on solid-phase. HFIP is thought to act as an acid catalyst, enhancing the rate of the first oxidation to the sulfoxide and then co-ordinating to the sulfoxide, making it inert to any further oxidation by withdrawing electron density from sulfur.[38] Similar conditions were employed to prepare selenoxide **99b**.[37]

After oxidation to sulfoxide **99a** or selenoxide **99b**, thermal elimination occurred readily to release *exo* **101** and *endo* **100** indenone in greater than 95% HPLC purity and 45% overall. For the sulfur linkage, thermal elimination only occurred in activated systems. As expected, the selenium linker was found to be more efficient, with cleavage occurring at much lower temperature and with less activated substrates (Scheme 24).[37]

Finally, De Clercq and D'herde have utilised a sulfide linker in a solid-phase Julia-type olefination process



Scheme 22.

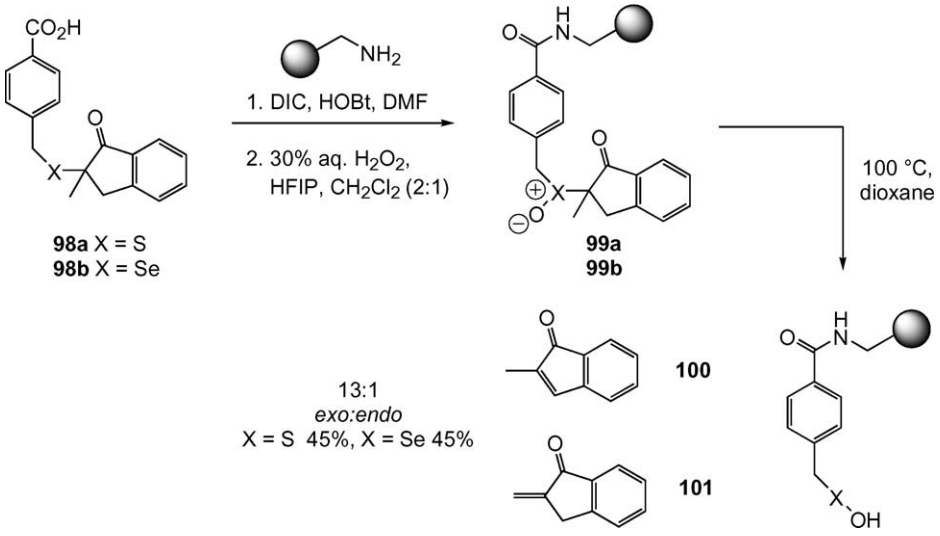


Scheme 23.

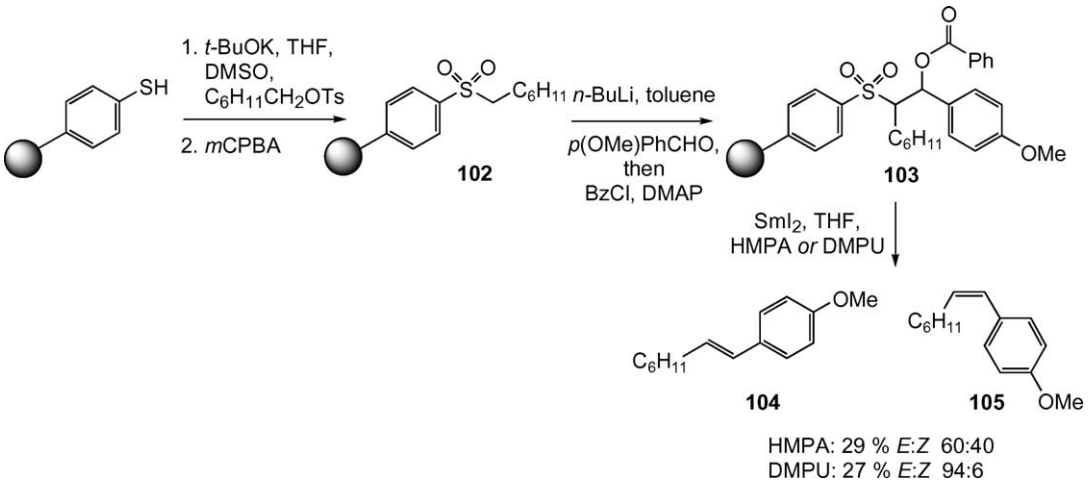
(Scheme 25).[39] Alkylation of aryl thiol resin followed by *m*CPBA oxidation gave supported sulfone **102**. Successive treatment of the resin with *n*-butyllithium and an aldehyde, followed by trapping of the resultant alkoxide with benzoyl chloride, gave resin-bound α-benzoyloxy sulfone **103**. Olefins **104** and **105** were released from the solid support upon reduction with an electron-transfer reagent and elimination of the sulfone linkage (Scheme 25).[39] Samarium diiodide proved to be the most suitable reagent for the process and was used with the promoters HMPA and DMPU. The stereoselectivity of the olefination was found to be strongly dependent upon the additive, DMPU giving rise to higher *E* selectivity than HMPA in the example shown (Scheme 25).[39]

2.1.5. Sulfide linkers cleaved by nucleophilic substitution. Obrecht et al. described the first use of a sulfide linker strategy in library synthesis based on the known nucleophilic displacement of 2-sulfonyl groups from pyrimidines.[40] Treatment of resin-bound thiouronium salt **106** with acetylenic ketones such as **107**, gave pyrimidine-4-carboxylic acids **108** after ester hydrolysis. After formation of amide **109**, activation of the sulfide linker by oxidation to the corresponding sulfone and nucleophilic cleavage with pyrrolidine gave **110** in good yield and in high purity (Scheme 26).[40]

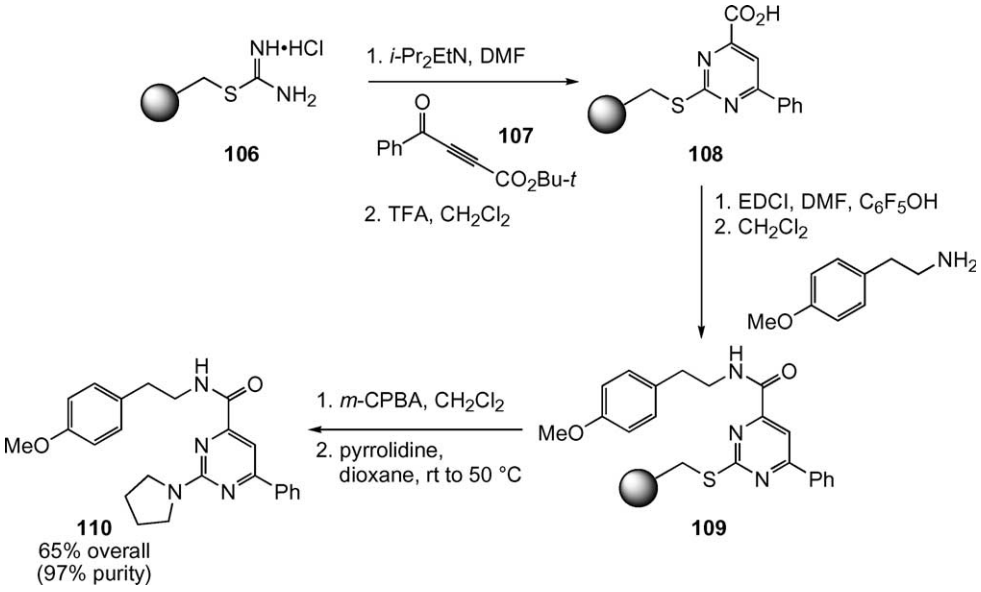
Suto and Gayo have also applied a similar sulfide linker in the synthesis of a pyrimidine library (Scheme 27).[41] Ethyl 2-chloro-4-trifluoromethylpyrimidine-5-carboxylate **111** was immobilised using a Tentagel thiol resin to give resin-bound pyrimidine **112**. The sulfide linker was stable to a variety of reaction conditions, remaining intact through ester hydrolysis, conversion of the resultant acid into the



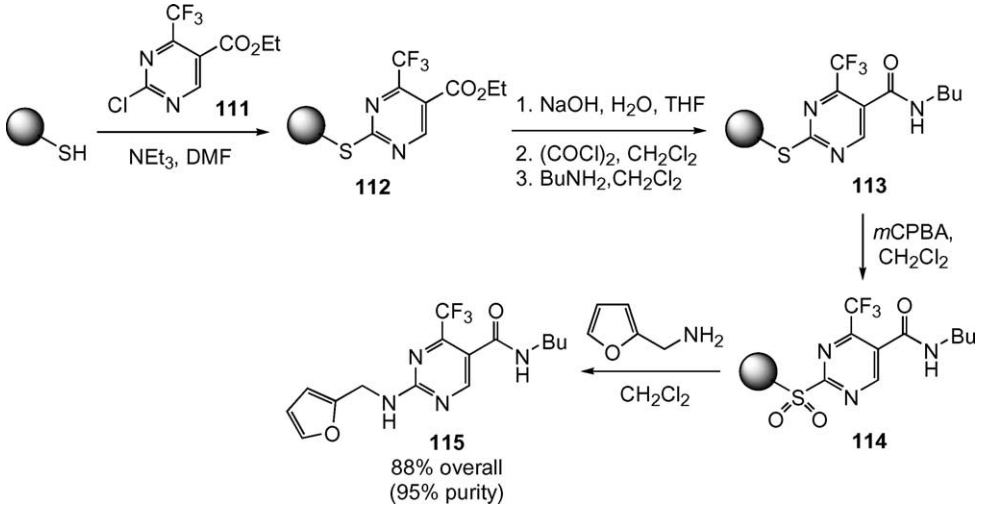
Scheme 24.



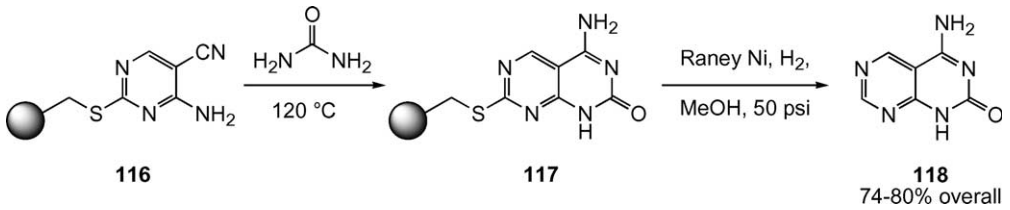
Scheme 25.



Scheme 26.



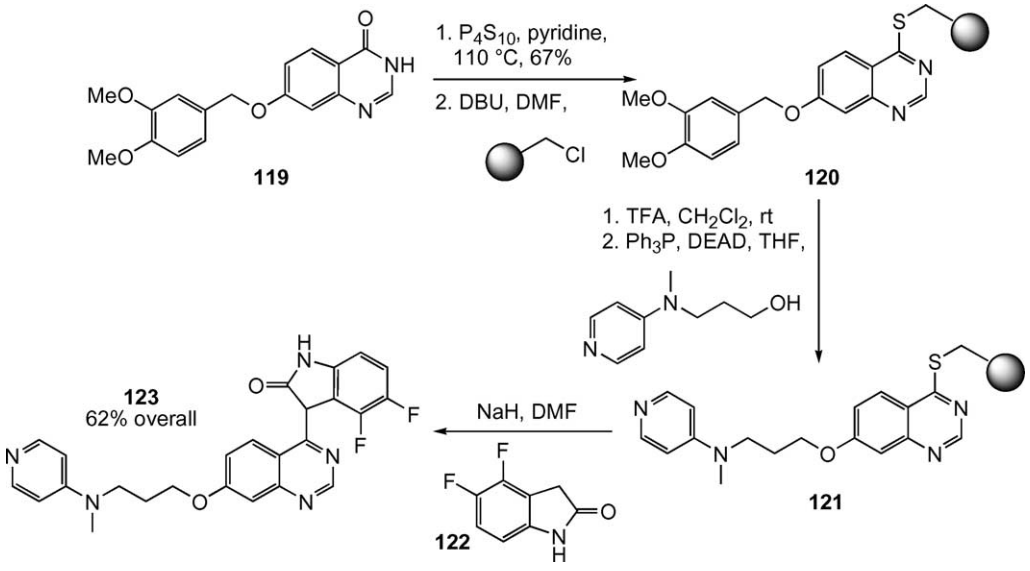
Scheme 27.



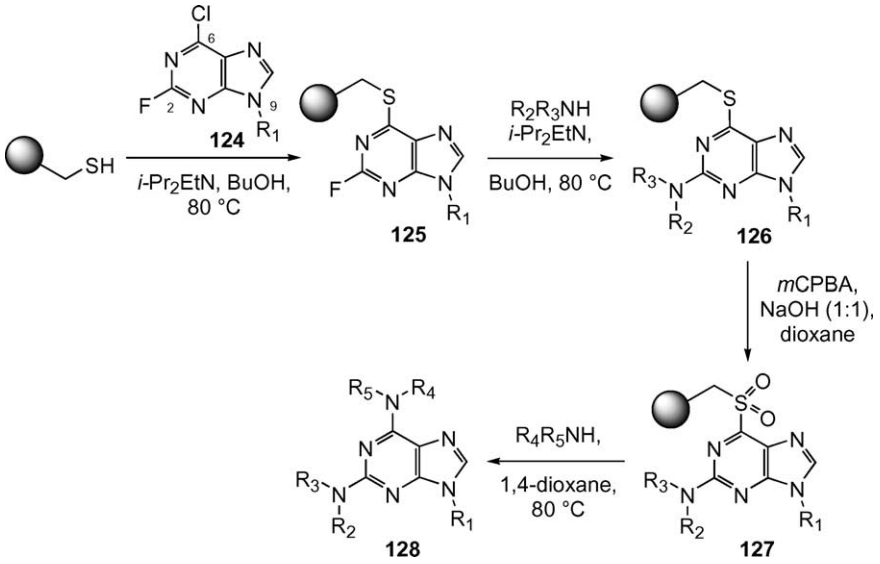
Scheme 28.

acid chloride and subsequent formation of amide **113**. The link was activated by oxidation with *m*CPBA to give sulfone **114**. Treatment with an amine such as furfurylamine resulted in nucleophilic cleavage of the highly functionalised pyrimidine **115** (Scheme 27).[41]

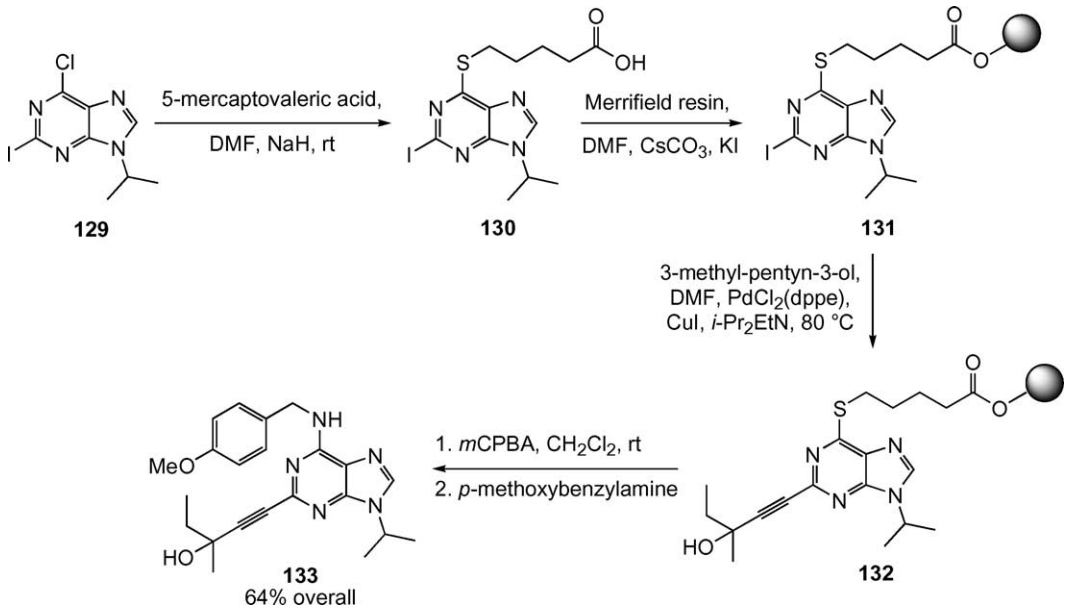
Chauhan et al. have also reported a route to pyrimidine derivatives, for example, **118**, using a sulfide linker **116**.[42] Cleavage in this case was achieved by reduction of substrates such as **117** at the sulfide oxidation state (Scheme 28).



Scheme 29.



Scheme 30.

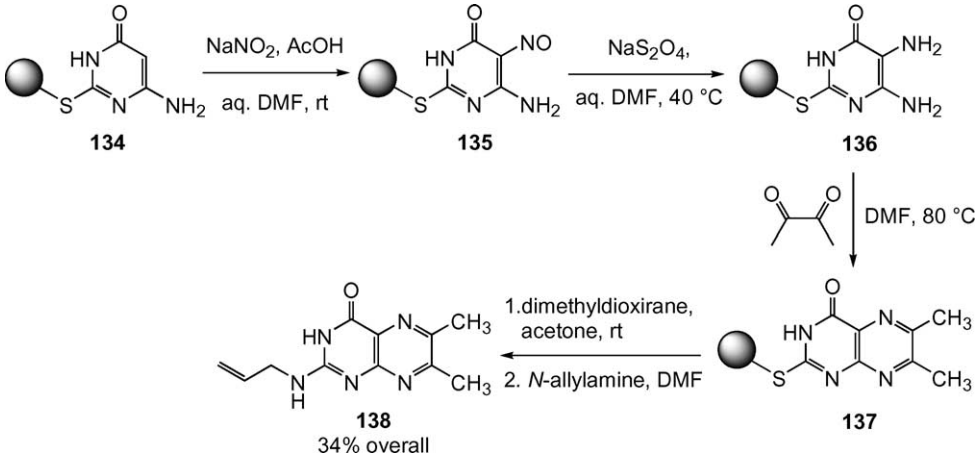


Scheme 31.

A sulfide linker cleaved by nucleophilic substitution has been utilised by Hennequin and Blanc in an interesting approach to oxindole-substituted quinazolines.[43] Quinazolinone **119** was converted into the corresponding thione before immobilisation using Merrifield resin to give **120** (Scheme 29). Deprotection and introduction of a range of side chains gave intermediates such as **121**. Cleavage of the sulfide link was achieved by displacement using the anion of oxindole **122**, releasing products such as **123** in good overall yield (Scheme 29).[43]

Schultz and co-workers have reported a combinatorial approach to the synthesis of 2,6,9-trisubstituted purines that employs a related sulfide linker strategy (Scheme 30).[44] N9-substituted purine **124** was attached to a polymer support at the C6 position using a sulfide link to the resin. Nucleophilic substitution at the C2 position of the purine occurred by treatment of resin **125** with an amine to give **126**. It was found that nucleophilic substitution at C2 in this way is not possible if the linker at C6 is amino-, rather than sulfur-based. Further derivatisation of the purine can be achieved by oxidation of the sulfide link to give sulfone **127**, thus activating the C6 position to nucleophilic substitution. Treatment of **127** with an amine results in nucleophilic cleavage of 2,6,9-substituted purines **128** (Scheme 30).[44] This route has been shown to tolerate the introduction of a broad range of substituents on the purine ring. A variety of sterically hindered primary and secondary amines can be added at the C2 position. Substitution at C6 can also be carried out with a wide range of primary and secondary amines and electron-rich anilines. A library of substituted purines was produced, with an average yield of 80 and $>85\%$ purity by HPLC.[44]

In an independent report, Legraverend et al. used a sulfide linker in a solid-phase approach to 2,6,9-trisubstituted purines that allows modification of the purine skeleton at all three positions.[45] Thiovaleric acid was attached to 6-chloro-2-iodopurine **129** to give a sulfide intermediate **130**, that was then anchored to Merrifield resin. The resin-bound sulfide



Scheme 32.

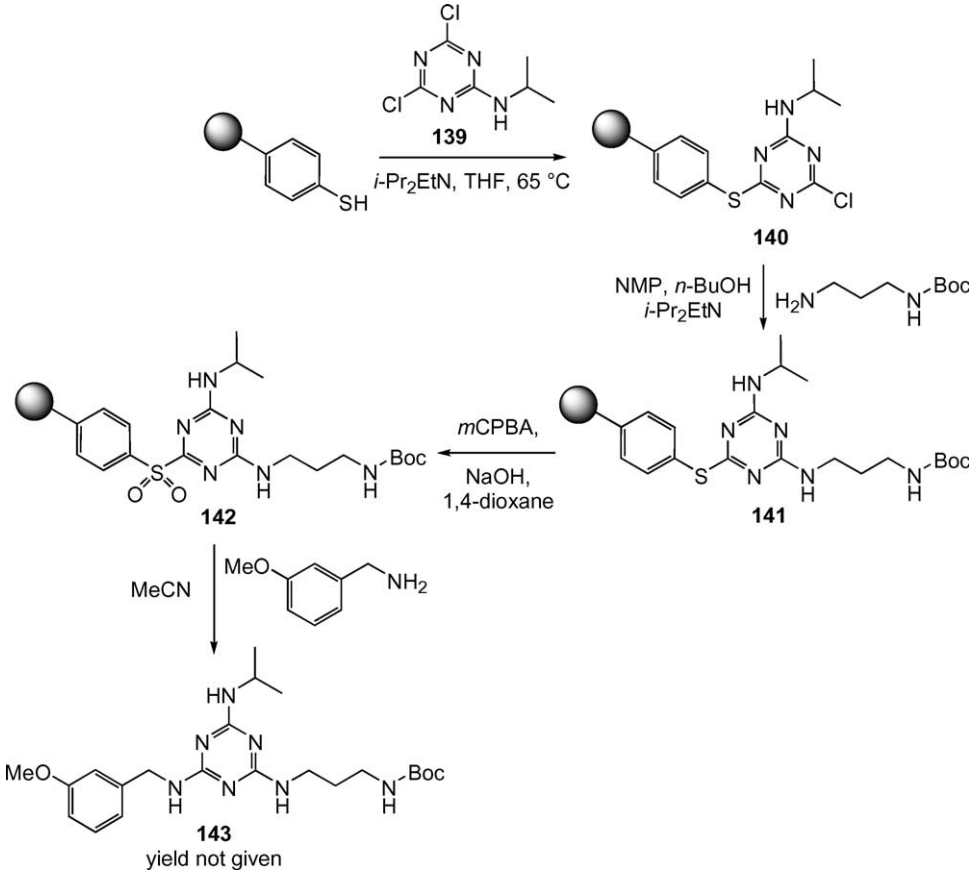
131 was coupled with 3-methyl-1-pentyn-3-ol using a stoichiometric Pd(dppe)Cl_2-CuI system to provide adduct **132**. Cleavage from the polymer support was then achieved by oxidation of the linking sulfur atom to the corresponding sulfone using *m*CPBA and nucleophilic cleavage using *p*-methoxybenzylamine. The trisubstituted purine derivative **133** was obtained in 64% overall (Scheme 31).[45]

Suckling and co-workers have applied a similar approach to the solid-phase synthesis of pteridines (Scheme 32).[46] Merrifield resin-bound 6-amino-2-sulfanylpyrimidin-4(3*H*)-one **134** underwent nitrosation to give **135** and reduction to furnish diaminopyrimidine **136**. Treatment with 2,3-butanedione gave resin-bound pteridine resin **137** and activation of the sulfide linker was achieved by oxidation with dimethyldioxirane. Nucleophilic cleavage with amines such as allylamine cleaved pteridines **138** from the support in 34% overall yield (Scheme 32). Nucleophiles such as azide and pyrrolidine were also used in the cleavage step.[46,47]

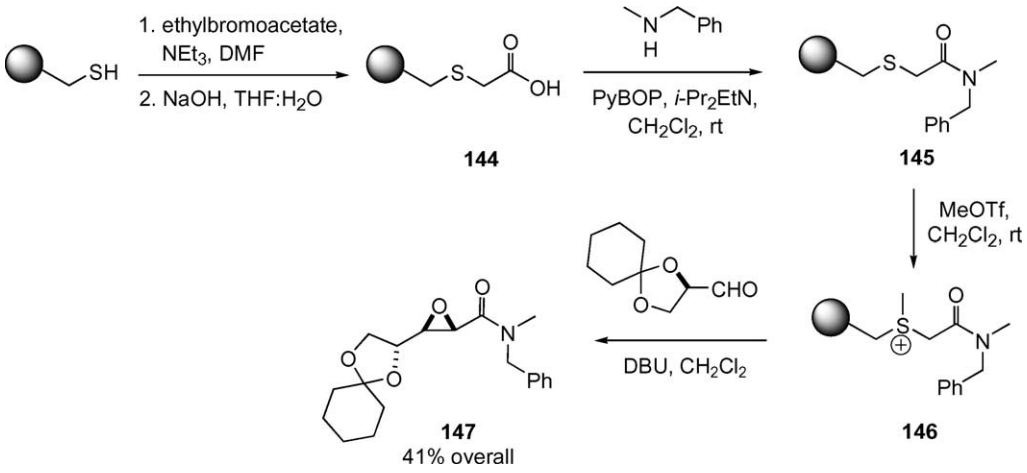
Finally, Chang and Khersonsky have developed a method for the orthogonal synthesis of trisubstituted triazines using a similar linker approach (Scheme 33).[48] Triazine **139** was immobilised using a thiophenol resin. A second substitution of **140** with an amine nucleophile gave disubstituted triazine **141**. Activation of the sulfide link by oxidation to sulfone **142** and nucleophilic cleavage from the support with an amine gave trisubstituted triazine **143** in high purity (Scheme 33).[48] A range of amines, anilines and alcohol nucleophiles were tested in the sulfone displacement step. Unactivated anilines were not successful in effecting cleavage, resulting in only a trace of triazine product. Phenol and sterically hindered amines such as dibenzylamine were also ineffective in the cleavage step. Unhindered primary and secondary amines gave the best results.[48]

2.1.6. Sulfide linkers cleaved via sulfonium ions. Gennari et al. have reported the solid-phase synthesis of epoxides and cyclopropanes using an elegant cyclative cleavage strategy employing supported sulfonium ylides.[49] A thiol resin prepared from an AgroGel-based polymer was used to synthesise supported amides such as **145** from **144** (Scheme 34). Activation of the linking sulfur atom by methylation gave sulfonium ion **146**. Treatment with base generated sulfur ylides and reaction with aldehydes led to the release of epoxides such as **147** from the solid support. An attractive feature of the cyclative cleavage strategy is that only the desired cyclised product is released into solution. By-products or unreacted components remain attached to the polymer, and the products are therefore obtained in high purity (Scheme 34).[49]

This methodology has also been applied in a cyclative-cleavage strategy for the synthesis of macrocyclic lactones

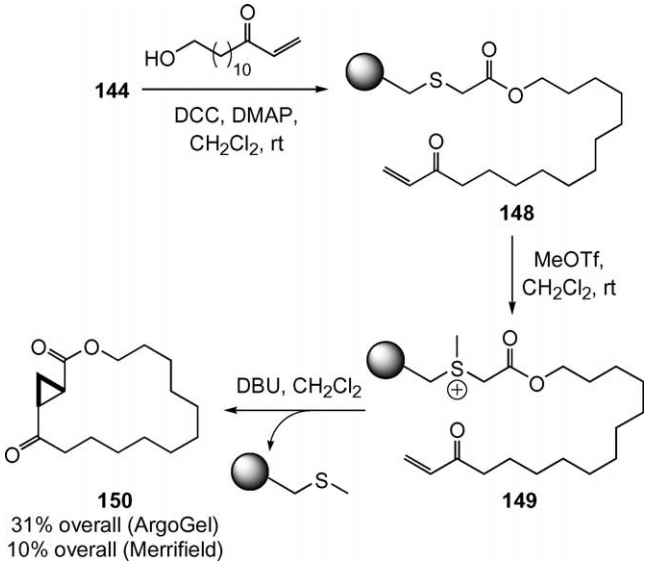


Scheme 33.



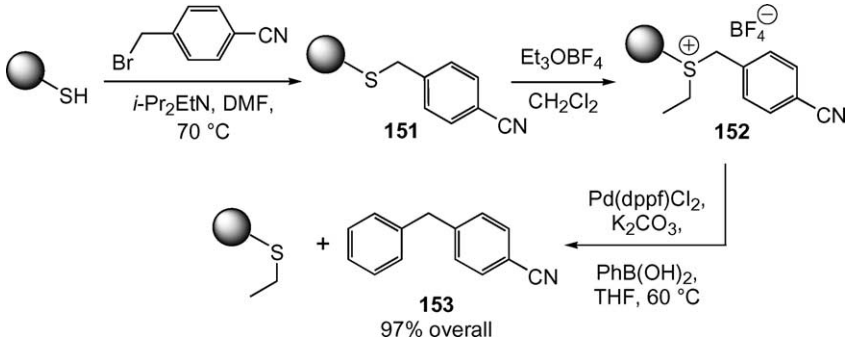
Scheme 34.

bearing a cyclopropane moiety (Scheme 35).[49] Resin-bound sulfonium ions such as **149**, bearing a tethered Michael acceptor group were prepared, from **144**, via **148**. Treatment with base led to sulfur ylide formation and subsequent 1,4-addition of the ylide to the Michael acceptor was followed by the formation of a cyclopropane ring and concomitant cleavage of macrocycle **150** from resin. An advantage of carrying out this type of macrocyclisation reaction on solid support is that it mimics the high-dilution conditions that would normally be necessary in solution to avoid competing intermolecular reactions. The yield of macrocycle is better when an AgroGel-based thiol resin was used in place of a Merrifield-based resin (Scheme 35).[49]

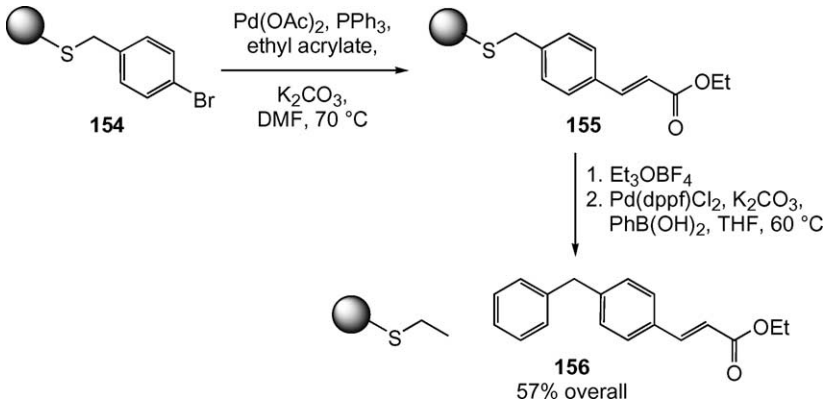


Scheme 35.

Wagner, Mioskowski et al. have developed a sulfide linker that, when activated as an a alkylsulfonium salt, can be cleaved with concomitant carbon–carbon bond formation (Scheme 36).[50] Alkylthiol resin, prepared from Merrifield resin, was alkylated with a benzyl bromide to give a benzyl sulfide resin such as **151**. The sulfide linkage formed is stable to a wide range of reactions involving nucleophiles, electrophiles, acids and bases. Activation of the link was achieved upon treatment with triethyloxonium tetrafluoroborate to give an alkylsulfonium salt **152**. The sulfonium salt readily underwent palladium-catalysed cross-coupling reactions with a variety of boronic acids. The benzylic carbon–sulfur bond couples selectively in preference to the alkyl-carbon sulfur bond, therefore cleaving the compound from the polymer support whilst simultaneously constructing a new carbon–carbon bond. Biarylmethanes, such as **153**, bearing electron-donating, electron-withdrawing and



Scheme 36.



Scheme 37.

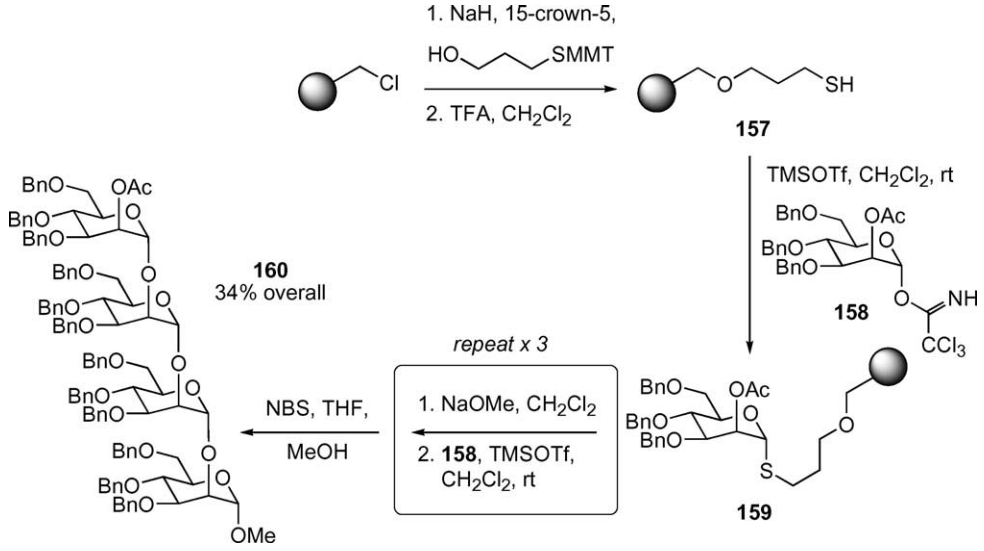
heterocyclic groups were synthesised in yields between 24 and 99% (Scheme 36).[50]

In order to demonstrate that the linker was stable to palladium-catalysed coupling conditions prior to activation, a Heck reaction was carried out on the polymer-supported aryl bromide **154**. No cleavage was observed during the Heck reaction. Upon activation and palladium cross-coupling, the biarylmethane cinnamate derivative **156** was obtained from **155** in 57% overall yield (Scheme 37).[50]

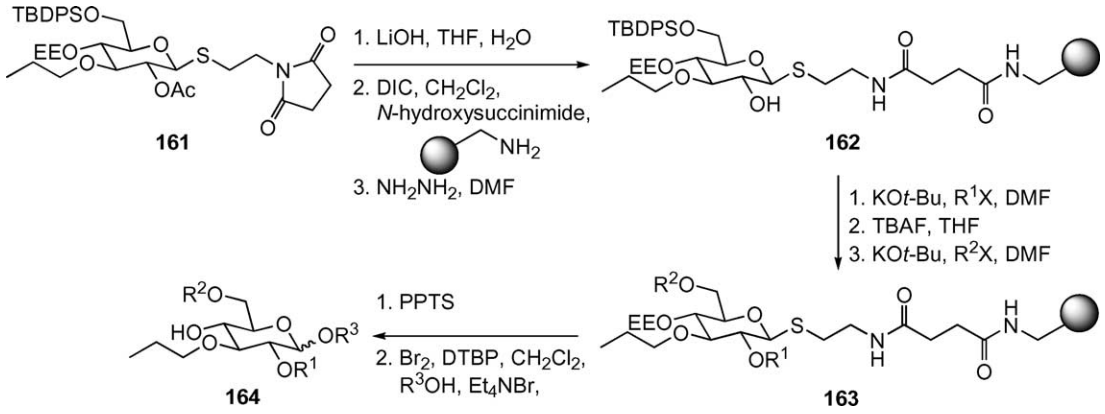
2.1.7. Sulfide linkers in carbohydrate chemistry. The solid-phase synthesis of complex oligosaccharides remains an important challenge in organic synthesis. Schmidt and Rademann have used a sulfide linker system in a solid-phase approach to oligosaccharides.[51,52] Thiol **157** was prepared from Merrifield resin and used to immobilise trichloroacetimidate donor **158**. 1,2-*trans* Thioglycoside **159** was obtained, due to the directing effect of the 2-*O*-acetyl group (Scheme 38).[52] Cycles involving deprotection and glycosylation with donor **158**, allowed oligomannosides such as **160** to be constructed efficiently. Cleavage of the sulfide linkage at the end of the sequence was achieved using *N*-bromosuccinimide (Scheme 38).[52]

In a series of disclosures, Kunz and co-workers have utilised sulfide linkers in the development of carbohydrates as multifunctional scaffolds for solid-phase and combinatorial synthesis.[53–57] The first sulfide linker design used by Kunz was prepared by opening of the imide group of thioglycoside **161** and coupling with aminomethyl polystyrene (Scheme 39).[53] Combinatorial modification of the immobilised glucose scaffold **162** by alkylations of the 2- and 6-hydroxyl groups allowed a series of modified sugars **163** to be prepared. Cleavage of the sulfide link and glycosylation was achieved using bromine in the presence of an alcohol acceptor. A library of 25 glycosides **164** was prepared using this approach in overall yields between 30 and 80% (Scheme 39).[53]

Carbamoyl glucosides have been similarly prepared by reaction of the supported intermediates with a range of



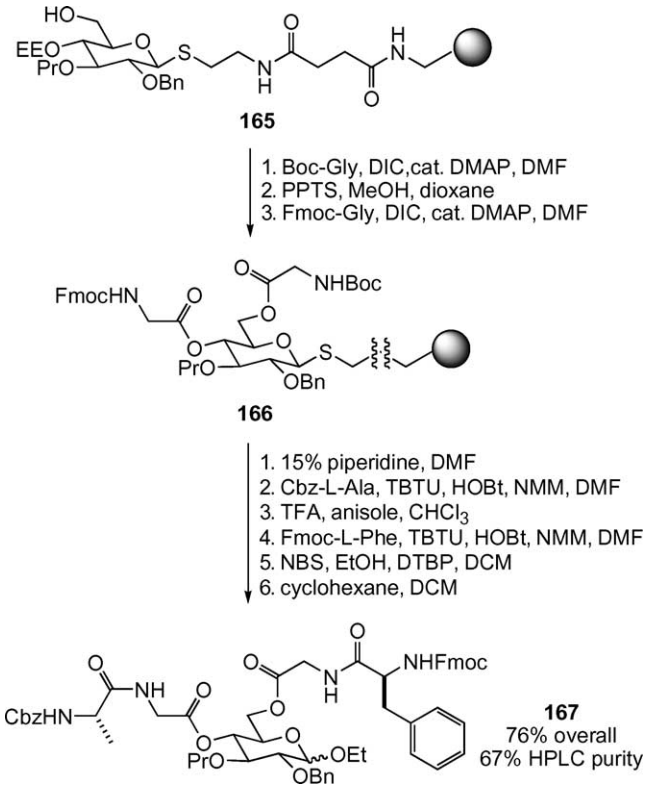
Scheme 38.



Scheme 39.

isocyanates.[55,56] The use of a galactose scaffold has also been reported by Kunz.[54]

Finally, Kunz et al. have used a sulfide linker and a glucose scaffold in a combinatorial synthesis of peptide conjugates.[57] Amino acids can be attached to the carbohydrate scaffold **165** via either the *N*- or the *C*-terminus. Elongation at the *N*-terminus can be achieved without affecting the scaffold or the linker. By utilising two amino acids carrying orthogonal *N*-protecting groups, as in **166**, selective manipulation of the side chains is possible (Scheme 40). At the end of the sequence, the sulfide linkage was cleaved by the previously reported transglycosylation process to give peptide-carbohydrate conjugates such as **167** (Scheme 40).[57]



Scheme 40.

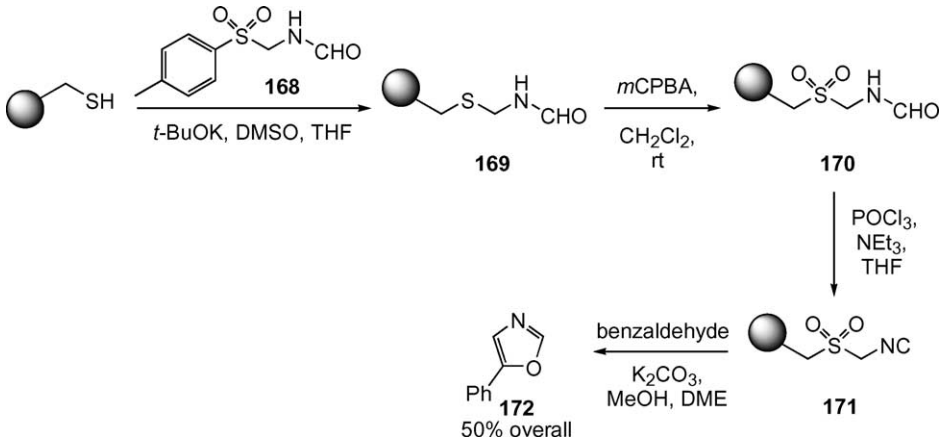
2.1.8. Miscellaneous sulfide linkers. Ganesan and Kulkami have developed a solid-phase equivalent of *p*-tolylsulfonylmethylisocyanide for the synthesis of oxazoles (Scheme 41).[58] Formamide **168** was immobilised using a thiol resin to give **169**. After oxidation to **170** and conversion into isocyanide **171**, treatment with aromatic aldehydes such as benzaldehyde triggered cyclative cleavage and release of 5-aryloxazole **172** in good overall yield. The use of a range of aromatic aldehydes gave 5-aryloxazoles in yields ranging from 25 to 50% (Scheme 41).[58]

Nicolaou et al. have employed a sulfide linkage activated by oxidation to the sulfone in a solid-phase synthesis of 3-arylbenzofurans via a novel cyclofragmentation-release pathway.[59] A thiophenol resin was alkylated with bromochloromethane in the presence of DBU to give **173** (Scheme 42). Chloromethylsulfide resin **173** was then alkylated with salicylaldehyde derivatives such as **174** to give resin-bound aldehydes **175**. Addition of a functionalised arylmagnesium bromide, oxidation of the resultant benzyhydrol **176**, and treatment with trimethylsulfonium iodide gave epoxide **177**, which, after oxidation of the sulfur link to the sulfone **178**, was ready for application in the cyclofragmentation-release strategy. Treatment of sulfone **178** with base resulted in deprotonation α to the sulfone followed by a 5-*exo* tet epoxide opening, loss of formaldehyde, and elimination of the sulfone linkage (Scheme 42). A range of 3-arylbenzofurans such as **179** was synthesised by this route using a variety of salicylaldehydes and aryl Grignard reagents in overall yields between 6 and 29%.[59]

In Nicolaou's approach, the cleaved products were obtained in high purity, as only the desired benzofuran product can undergo release from the resin, for example, if any unwanted reactions such as 6-*endo* cyclisation occur, the resulting by-product cannot undergo a fragmentation-release pathway and therefore remains bound to the resin.[59]

2.2. Sulfoxide linkers

Many sulfide linkers are oxidised to the corresponding sulfoxide to enable a particular cleavage strategy, such as eliminative cleavage. These linkers have been described in



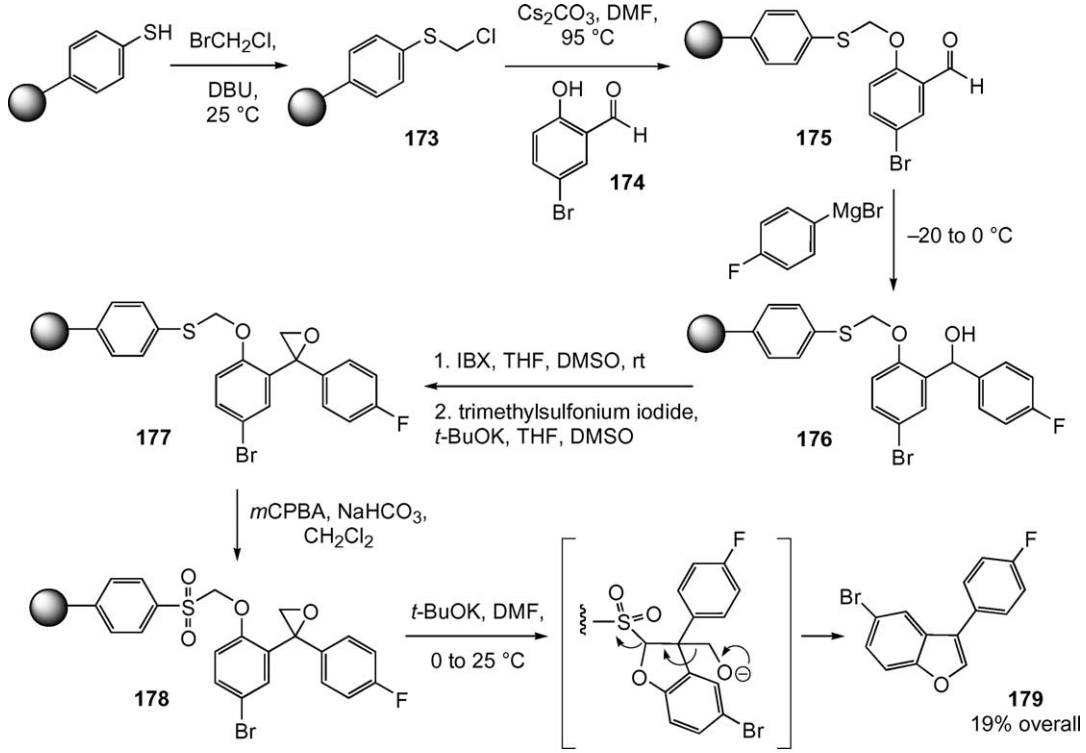
Scheme 41.

the previous section. Several of these sulfide linker systems, however, utilise the sulfoxide oxidation state for purposes other than to enable cleavage, for example, Procter et al. have utilised sulfoxide intermediates in Pummerer cyclisations to form heterocycles (Section 2.1.3, Scheme 12).[23] In addition, Solladié and co-workers have utilised a linking sulfoxide group to control the diastereoselectivity of reduction of a β-carbonyl group (Section 2.1.3, Scheme 13).[25]

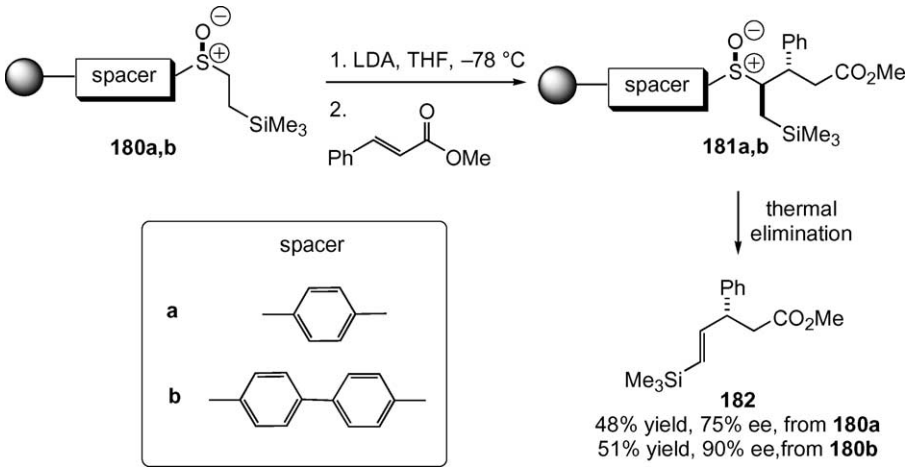
There are few reports of the use of chiral, sulfur- and selenium-based linkers in asymmetric synthesis. In one of these studies, Toru et al. have used polymer-supported enantiomerically enriched sulfoxides, such as **180a**,**b**, in asymmetric conjugate additions to methyl cinnamate (Scheme 43).[60] Thermal eliminative cleavage of the sulfoxide linker occurred regioselectively to give the product **182** in good yield and enantiomeric excess. The stereoselectivity of the conjugate addition was found to be dependent on the nature of the spacer group. When the spacer was a simple phenyl group, as in **180a**, the product was obtained in 48% yield and 75% ee from the cleavage of **181a**. If a biphenyl spacer was used, as in **180b**, a 51% yield of product was obtained in 90% ee. In an alternative cleavage strategy, treatment of **181b** with TBAF gave (*R*)-methyl-3-phenylpent-4-enoate in 56% yield and 90% ee (Scheme 43).[60]

2.3. Sulfone linkers

2.3.1. Sulfone linkers from sulfinate resins. Kurth et al. first reported the synthesis of a sulfone linker from a



Scheme 42.

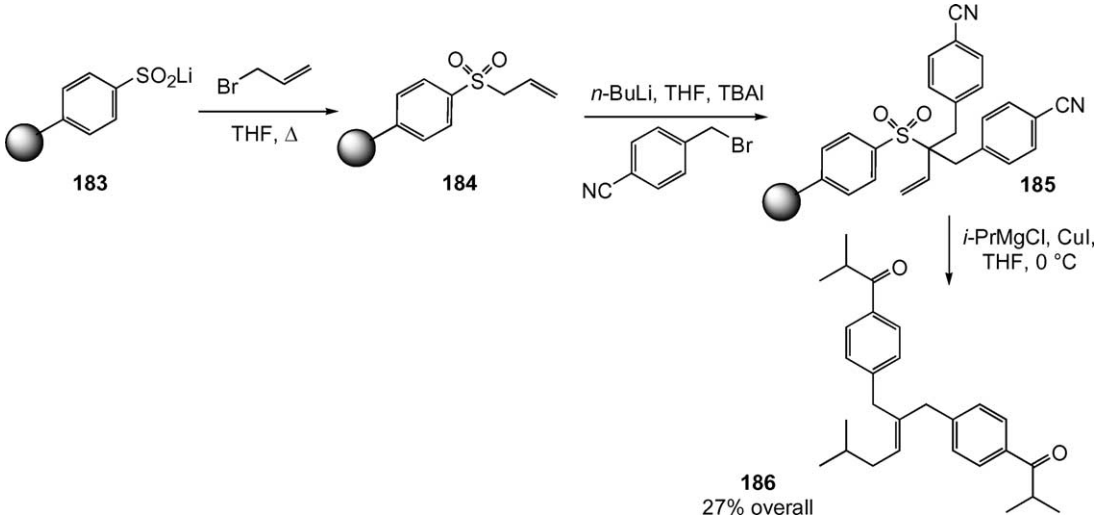


Scheme 43.

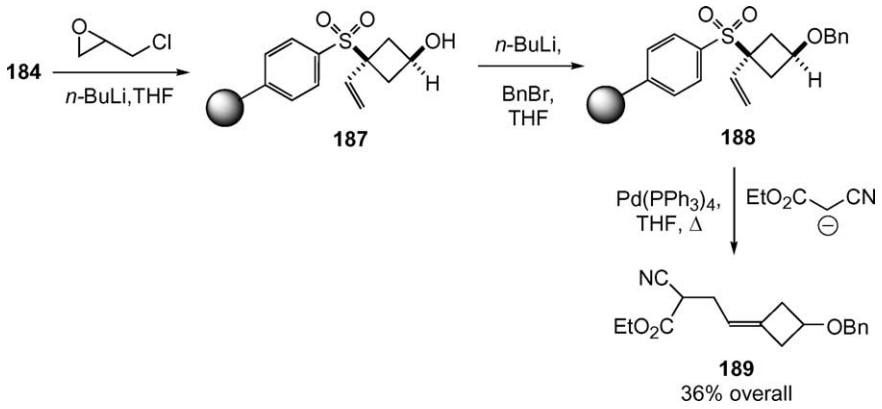
sulfinate resin and applied the transformation in a solid-phase synthesis of trisubstituted olefins.[61] This was the first of many publications from Kurth and others in what has become an important area of linker chemistry. Lithium sulfinate resin **183** was prepared from polystyrene resin by lithiation followed by treatment with sulfur dioxide. Allylation with allyl bromide gave allyl phenyl sulfone **184**, which underwent dialkylation upon treatment with *n*-butyllithium and alkylating agents to give sulfones such as **185** (Scheme 44).[61] Addition of an organocopper species, derived from isopropylmagnesium chloride and copper iodide, resulted in S_N2' displacement of the linking sulfone group and converted the cyano groups into isopropyl ketones. Trisubstituted olefin **186** was released from the resin in good yield. Employing different alkylating agents and displacement with other dialkyl cuprates or Grignard reagents gave a range of trisubstituted olefins in overall yields between 20 and 30% (Scheme 44).[61]

Kurth and co-workers also employed the allyl sulfone **184** in a synthesis of cyclobutylidenes (Scheme 45).[62] Alkylation with epichlorohydrin gave resin-bound cyclobutanol **187**. S_N2' displacement with a Grignard or cuprate to release the cyclobutanol from the resin at this stage was not successful and alternative methods of cleavage were therefore investigated. After protection of the hydroxyl group as a benzyl ether, it was discovered that cyclobutylidene **189** could be cleaved from the sulfone linkage in **188** by palladium-catalysed S_N2' displacement with a nucleophile. Purification after cleavage was necessary to remove excess nucleophile and triphenylphosphine. Several cyclobutylidenes were synthesised in overall yields between 30 and 38% (Scheme 45).[62]

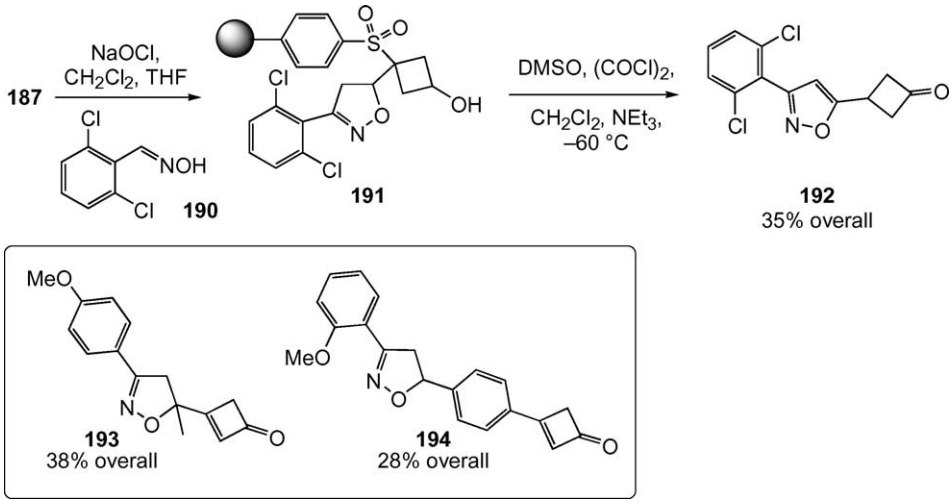
Intermediate cyclobutanol **187** also undergoes 1,3-dipolar cycloaddition upon treatment with oxime **190** and NaOCl.[63] Exposure of the resultant resin **191** to Swern oxidation conditions performed several functions; the cyclobutanol was converted into the cyclobutanone, and the basic reaction conditions effected the release of the compound



Scheme 44.



Scheme 45.

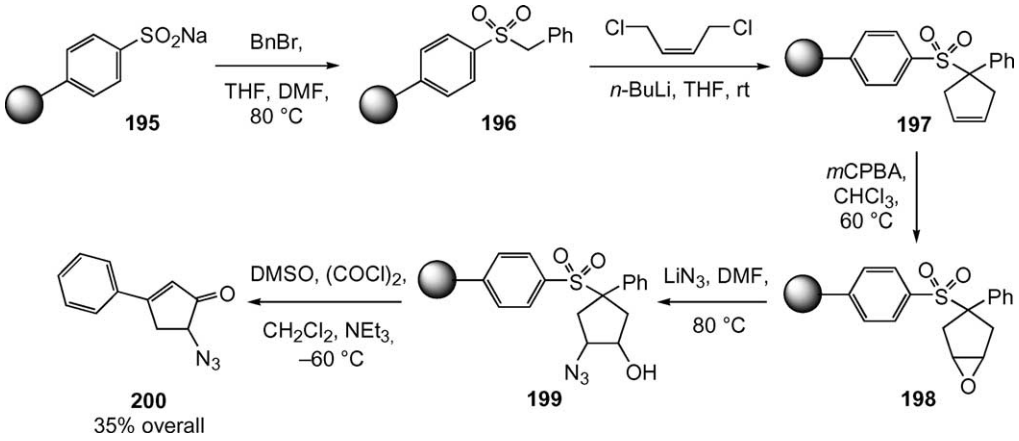


Scheme 46.

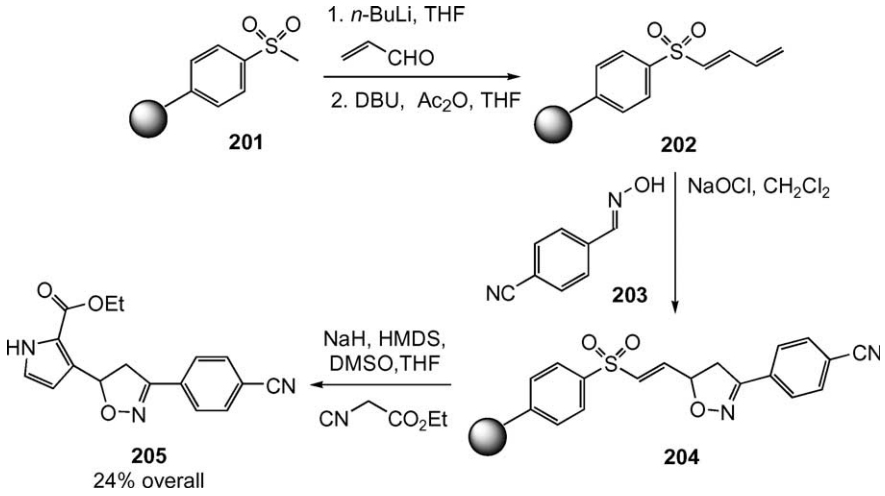
from the resin by sulfone elimination, leading to an isoxazolinocyclobutenone, which finally underwent isomerisation to isoxazolocyclobutanone **192** (Scheme 46).[63]

Isoxazolinocyclobutenones **193** and **194** were prepared by alkylation of sulfur with 3-chloro-2-methylpropene or 4-vinylbenzyl chloride in the initial alkylation step. In these examples, the final isomerisation was not possible and sulfinate elimination yields isoxazolinocyclobutenones as the final products (Scheme 46).[63]

Kurth has also applied his sulfone linker system in a synthesis of cyclopentenones (Scheme 47).[64] Alkylation of the sodium sulfinate resin **195** and further alkylation of the



Scheme 47.



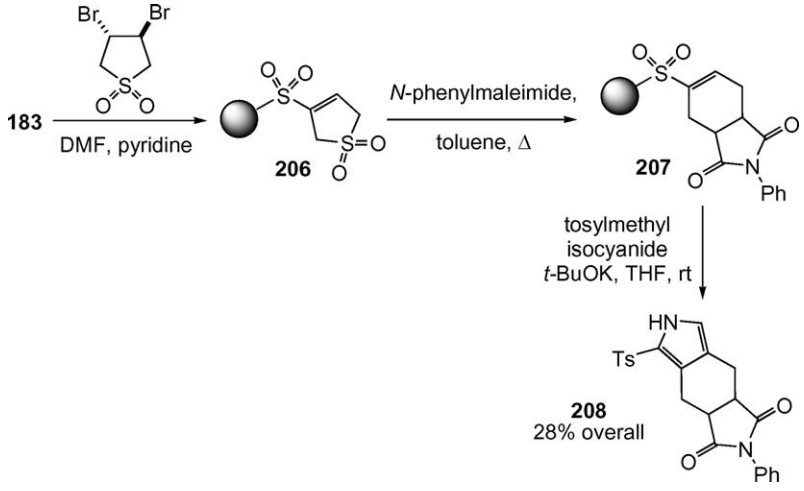
Scheme 48.

resultant sulfone **196** with 1,4-dichloro-2-butene gave cyclopentene **197**. Epoxidation then gave **198** that underwent ring opening to **199** with a variety of nucleophiles such as azide, Grignard reagents, cuprates and piperidines. Kurth's Swern oxidation/elimination strategy was employed to release cyclopentenones, such as **200**, in overall yields between 18 and 40% (Scheme 47).[64]

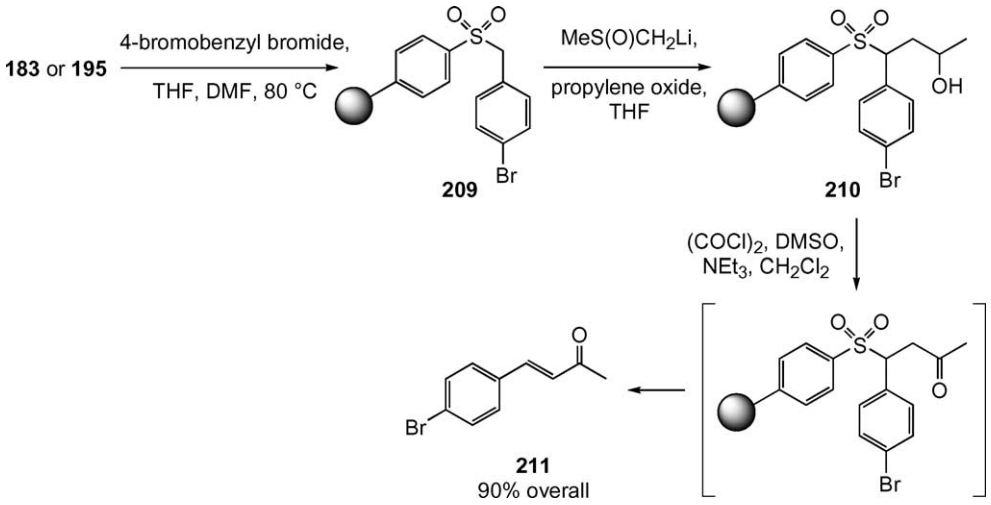
Isoxazolinopyrroles were also synthesised by Kurth.[65] Polymer-supported diene **202** was synthesised by 1,2-addition of the anion of methyl sulfone resin **201** to acrolein, followed by dehydration (Scheme 48). 1,3-Dipolar cycloaddition of **202** with the nitrile oxide derived from oxime **203** gave isoxazoline **204**. Upon treatment with ethyl isocyanoacetate, cyclative cleavage gave pyrrole **205** (Scheme 48). A small library of isoxazolinopyrrole 2-carboxylates was prepared using this method. In some cases, yields for the sequence were low, but products were obtained in good purity.[65]

4,5,6,7-Tetrahydroisoindole derivatives are also accessible using Kurth's sulfone system.[66] Alkylation of lithium sulfinate resin **183** with *trans*-3,4-dibromosulfolane gave sulfolene **206**, which extruded SO_2 upon refluxing in toluene (Scheme 49). The resulting resin-bound 1,3-butadiene underwent cycloadditions with *N*-phenylmaleimide and other dienophiles to give cycloadducts such as **207**. Treatment with tosylmethyl isocyanide under basic conditions resulted in cleavage of the sulfone linkage with concomitant generation of a pyrrole ring. The cleaved 4,5,6,7-tetrahydroisoindole **208** was obtained in 28% overall yield. Varying the dienophile allowed a range of functionalised 4,5,6,7-tetrahydroisoindoles to be produced in yields ranging from 32 to 41% (Scheme 49).[66]

The alkylation of sulfones is usually achieved using bases such as *n*-butyllithium or LDA at low temperature. There are problems, however, when applying these bases to the deprotonation of polymer-supported sulfones. Excess reagent can lead to dialkylation and it is difficult to achieve accurate stoichiometry in polymer-supported systems. Kurth has reported the use of dimsyl anion as a base for the monoalkylation of polymer-supported sulfones in a solid-phase synthesis of enones (Scheme 50).[67] Crucially,



Scheme 49.



Scheme 50.

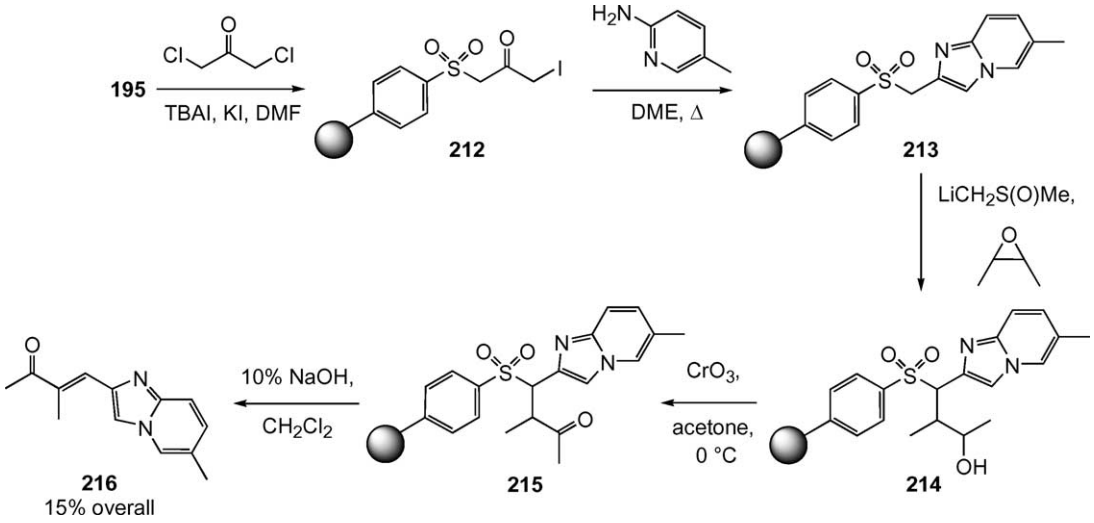
no dialkylation was observed in the reaction of **209** with propylene oxide, even when an excess of base was employed. It is also important to note that this base can be used at room temperature, making it more convenient for use in solid-phase chemistry. A small library of enones, for example, **211**, was synthesised in excellent yield by oxidation of adducts such as **210**, and elimination of the sulfone linker (Scheme 50).[67]

In a similar approach, Lam et al. have used a sulfone linker for the synthesis of imidazo[1,2-*a*]pyridines.[68] Alkylation of sodium sulfinate resin **195** with 1,3-dichloro-propan-2-one and treatment of the resultant α-haloketone resin **212** with an aminopyridine gave **213** (Scheme 51). Dimsyl anion was used to ensure that dialkylation did not occur in subsequent reactions with epoxides. Jones oxidation of **214** followed by base-promoted elimination of the sulfone linker in **215** gave imidazo[1,2-*a*]pyridines such as **216** in 15% overall yield (Scheme 51). A small library of imidazo[1,2-*a*]pyridines was synthesised with overall yields between 15 and 26%.[68]

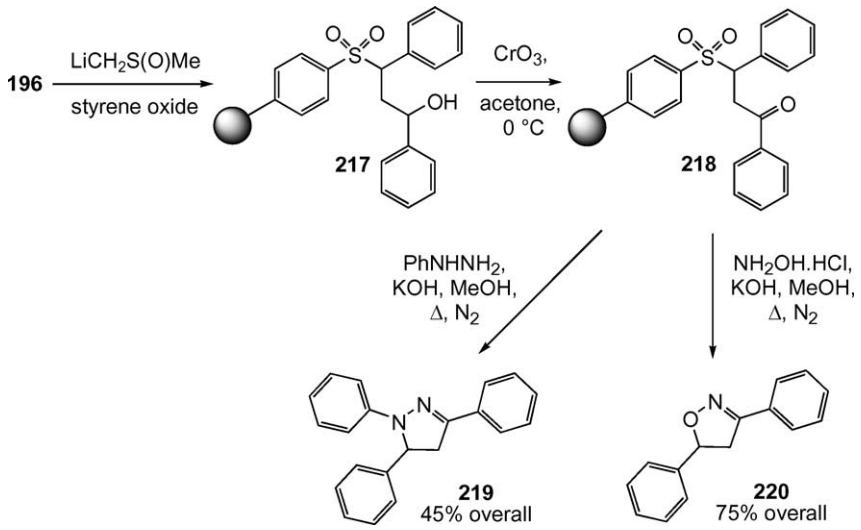
Lam also used a sulfone linker for the synthesis of pyrazolines and isoxazolines (Scheme 52).[69] Monoalkylation of sulfone **196** with styrene oxide, again using dimsyl anion as base, gave γ-hydroxy sulfone **217**. Subsequent Jones oxidation to form **218** and treatment with a hydrazine or hydroxylamine led to eliminative cleavage and in situ cyclisation to give pyrazolines **219** and isoxazolines **220**, respectively (Scheme 52). A collection of pyrazolines and isoxazolines were synthesised in good overall yields, ranging from 25 to 45%, and with >95% purity by NMR.[69]

A similar approach has been used by Lam to prepare 3,6-disubstituted pyridazine derivatives.[70] Alkylation of sodium sulfinate resin **195** with α-bromoketones affords 1,4-diketosulfones such as **221**. On treatment with hydrazine, condensation reactions trigger spontaneous cleavage from the resin in **222** to afford the 3,6-disubstituted pyridazine derivatives such as **223** (Scheme 53).[70]

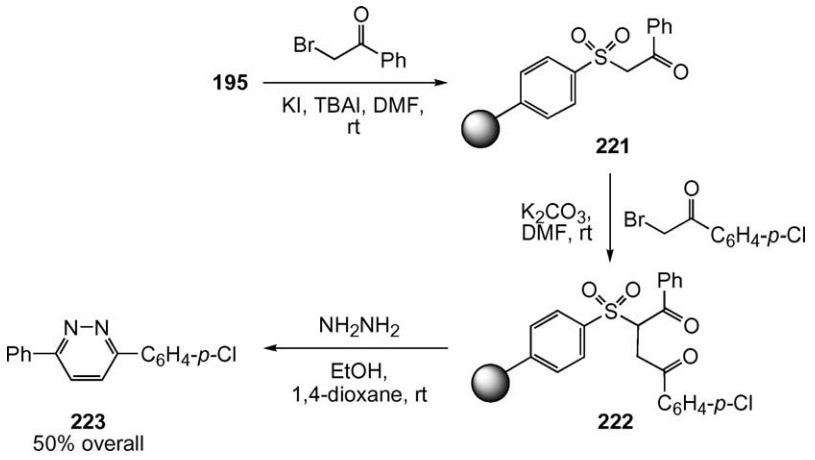
Lam has described the synthesis of other *N*-heterocycles, **225** to **229**,[71,72] using the release of α,β-unsaturated ketones by eliminative cleavage of a sulfone linker system in **224**, and in situ cyclisation using nucleophiles such as



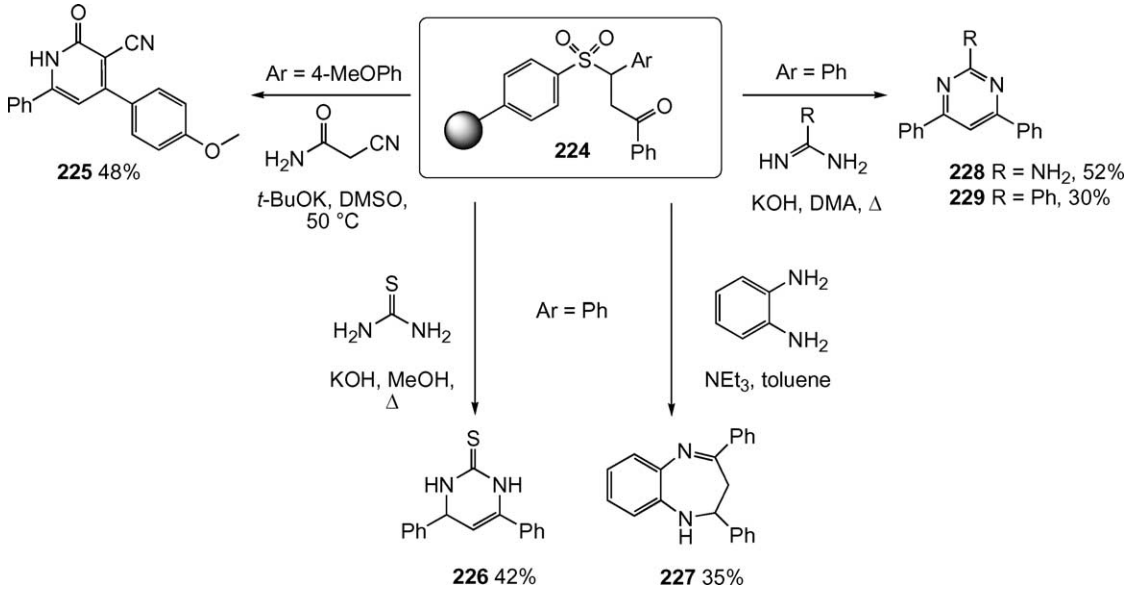
Scheme 51.



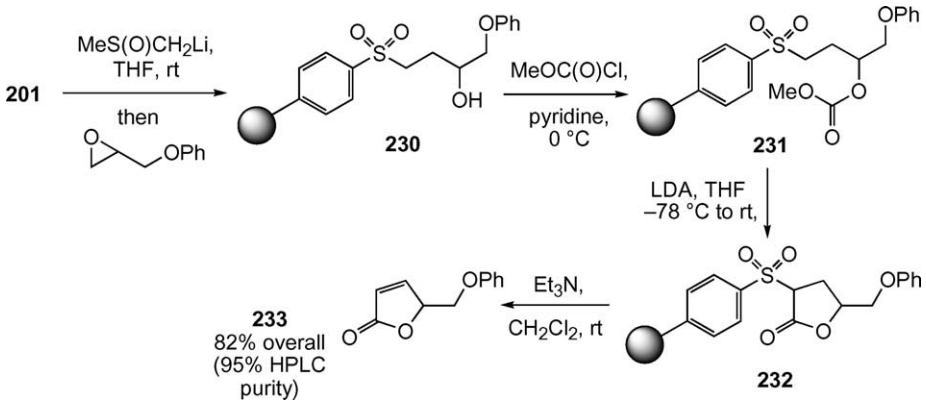
Scheme 52.



Scheme 53.



Scheme 54.



Scheme 55.

cyanoacetamide,[72] thiourea, urea, guanidine, benzamidine and *ortho*-phenylenediamine[71] (Scheme 54).

Sheng and co-workers have reported a solid-phase approach to substituted butenolides that employs a sulfone linker.[73] Methyl sulfone resin **201** can be lithiated and alkylated using a range of epoxides (Scheme 55). Conversion of the resultant γ-hydroxy sulfones, such as **230**, into the corresponding methyl carbonate **231** and cyclisation via a sulfone anion gave immobilised γ-butyrolactones **232**. Eliminative cleavage from the support then gave the butenolide product **233** in high yield and purity (Scheme 55).[73]

Finally, Huang et al. have reported a solid-phase route to hydantoins **236a** and **236b** using a sulfone linker (Scheme 56).[74] Alkylation of sodium sulfinate resin **195** with 2-chloroethanol followed by coupling with *N*-Boc-protected glycine and deprotection gave sulfone resin **234**. Treatment with phenyl isocyanate or isothiocyanate gave polymer-supported ureas **235a** and **235b**, respectively. Cyclative cleavage on treatment with acid gave hydantoin **236a** in 29% yield and thiohydantoin **236b** in 20% yield (Scheme 56).[74]

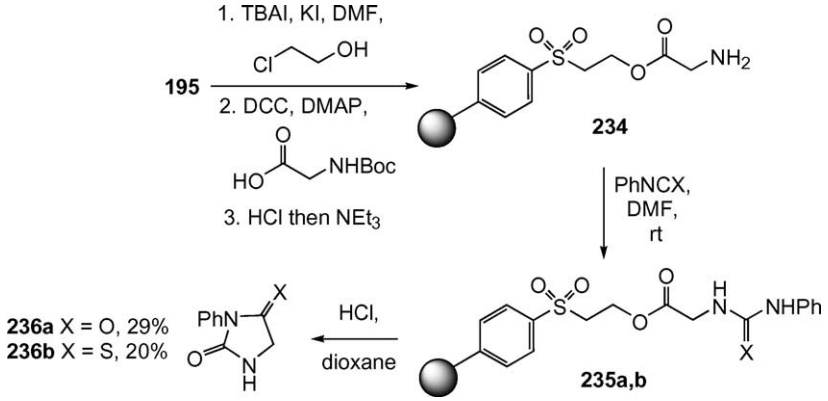
2.3.2. Sulfone linkers from additions to vinyl sulfones. A sulfone linker constructed by addition to vinyl sulfones has been applied by Heinonen and Lönnberg in an approach to tertiary amines.[75] Treatment of hydroxymethylpolystyrene with divinyl sulfone and DBU gave vinyl sulfone resin **237** (Scheme 57). Substituted tetrahydroisoquinolines were then added by Michael addition to give amines such as **238**. Deprotection of the THP group and subsequent Mitsunobu reaction gave immobilised tetrahydroisoquinolines **239**. Treatment with alkyl halides gave quaternary ammonium salts **240** and exposure to base released the tertiary amine **241** from the support (Scheme 57).[75]

An almost identical approach to tertiary amines was reported by Gani and co-workers.[76] A benzyl vinyl sulfone resin and an aryl vinyl sulfone resin **242** were used to immobilise secondary amines by conjugate addition (Scheme 58). Quaternisation of nitrogen in **243** using allyl or benzyl bromide gave quaternary ammonium salts such as **244**. Eliminative cleavage under basic conditions released the tertiary amine product **245** and regenerated the vinyl sulfone resin **242**, ready for re-use (Scheme 58).[76]

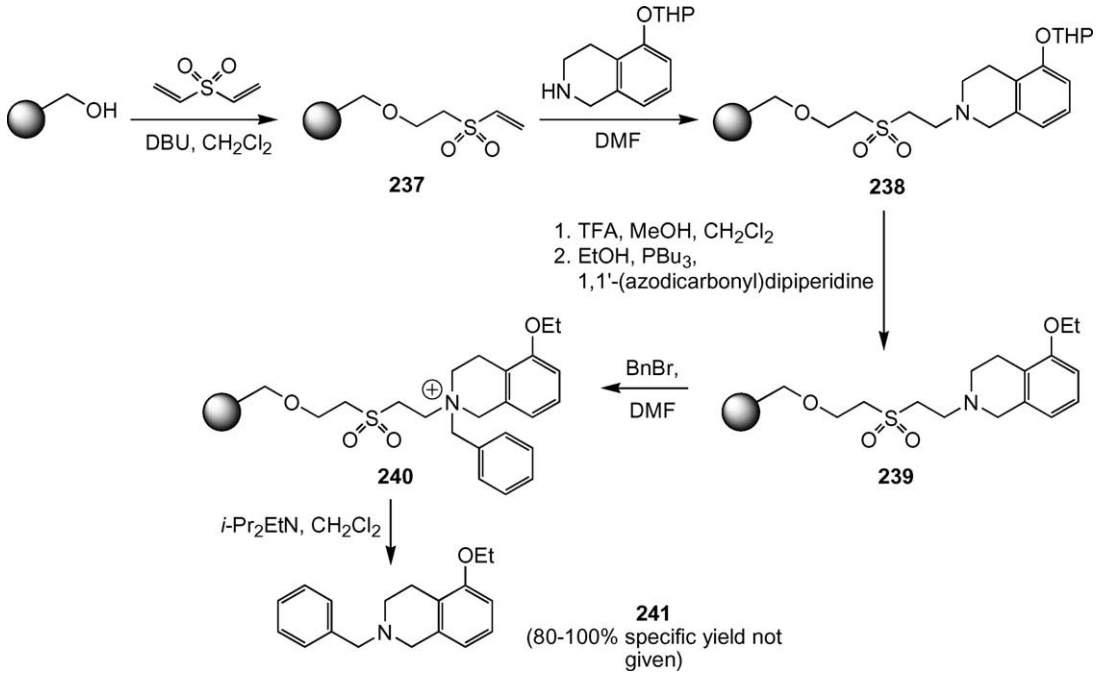
3. Selenium-based linkers

3.1. Selenide linkers

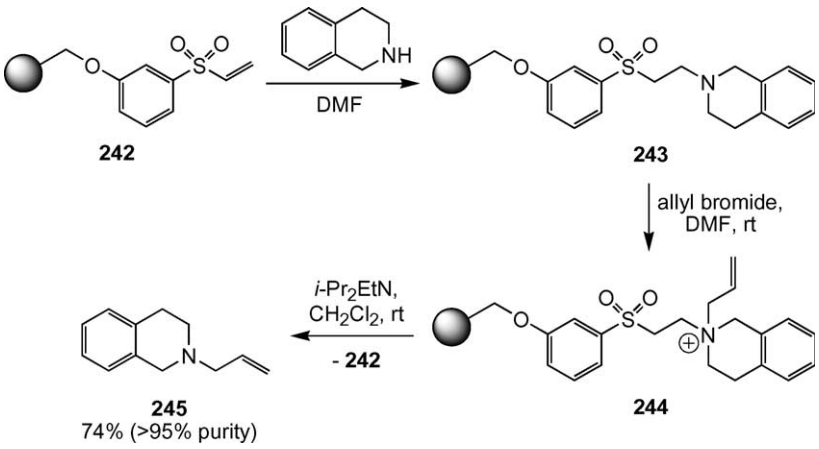
3.1.1. Early selenide linkers. Michels et al. reported the first preparation and use of selenium-containing polymers.[77] Selenol resin **246** was prepared by modification of bromopolystyrene and by polymerisation of *p*-vinylphenyl-selenol. Conversion into the selenenyl chloride resin **247** and immobilisation of 4-methylcyclohexanone gave selenide **248**. Oxidation to the selenoxide and eliminative



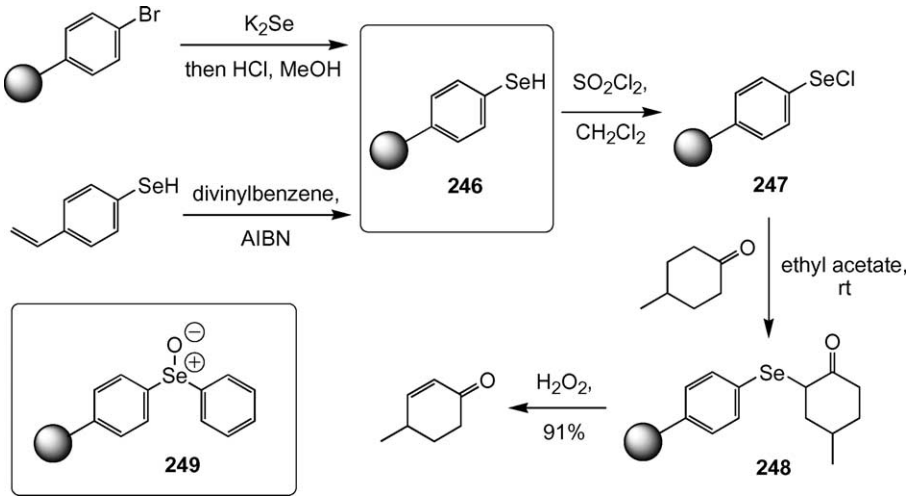
Scheme 56.



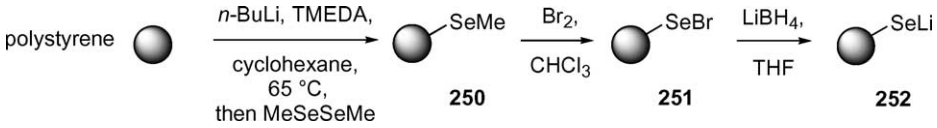
Scheme 57.



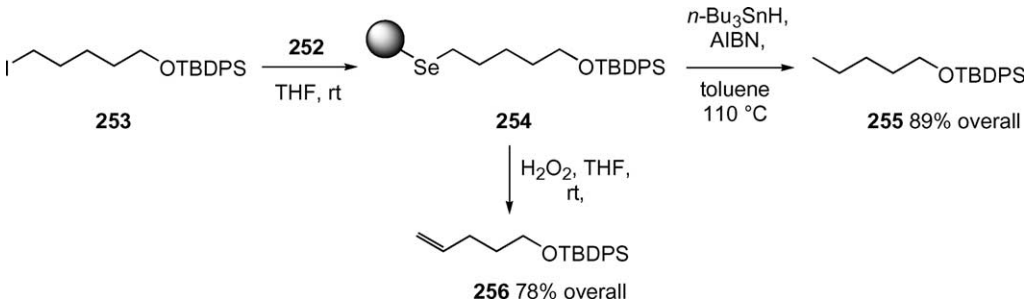
Scheme 58.



Scheme 59.



Scheme 60.



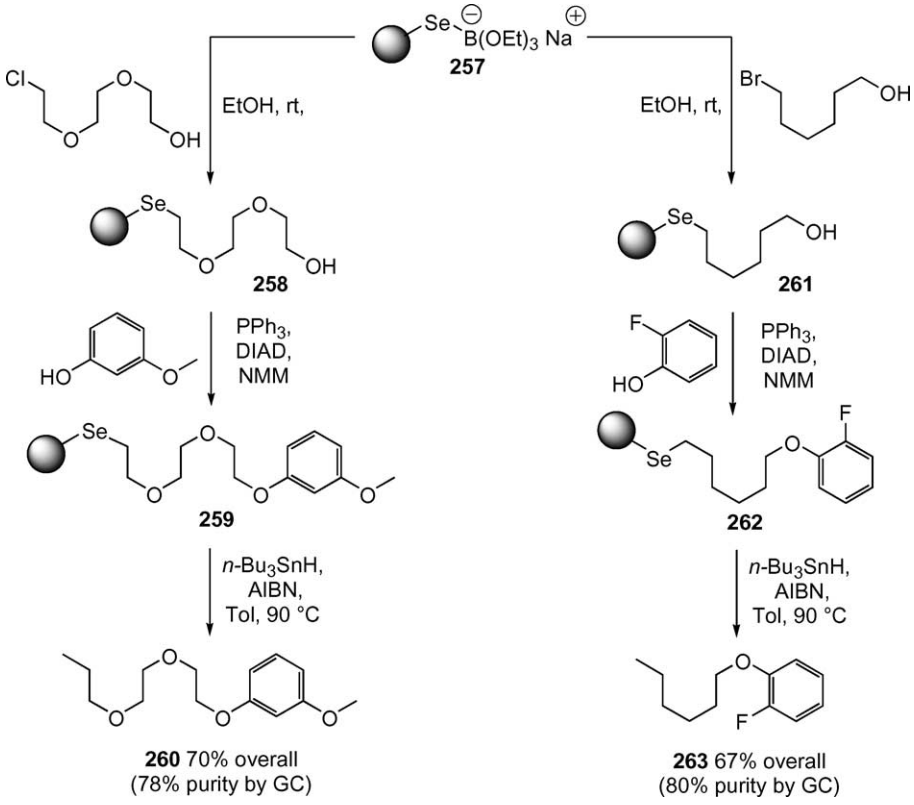
Scheme 61.

cleavage then gave 4-methylcyclohexenone in excellent yield (Scheme 59).[77] An alternative loading strategy using selenol resin **246** and ethyl 2-bromopropionate was also investigated. Oxidation and eliminative cleavage of the resultant selenide resin gave ethyl acrylate in high yield.[77]

In this seminal report, Michels also described the first preparation and use of a selenium-containing polymeric reagent. Polymer-supported diphenylselenoxide **249** (Scheme 59) was prepared and utilised in the dihydroxylation of 2-methyl-2-heptene with H_2O_2.[77]

In later studies, Nicolaou and co-workers were amongst the first to prepare a series of selenium resins for linker construction.[78] Treatment of polystyrene with *n*-butyllithium followed by dimethyldiselenide gave selenide resin **250**. Treatment of **250** with bromine gave selenenyl bromide resin **251**, that was reduced with $LiBH_4$ to give lithium selenide resin **252** (Scheme 60).[78]

With convenient access to selenium resins **251** and **252**, Nicolaou explored the utility of selenium linker systems. Alkyl iodide **253** was efficiently immobilised using lithium selenide resin **252** (Scheme 61).[78] The selenide linkage in **254** could be cleaved in one of two ways: radical cleavage with tributyltin hydride resulted in release of alkane **255** in



Scheme 62.

89% overall yield, whereas oxidation of the selenide to the corresponding selenoxide and eliminative cleavage gave **256** in 78% overall yield (Scheme 61).[78]

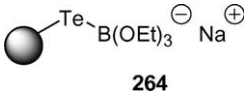
Concurrently with Nicolaou, Ruhland's group developed a selenium linker that also allows the formation of aliphatic C–H bonds upon cleavage (Scheme 62).[79] Sodium seleno(triethylborate)resin **257** was prepared from bromopolystyrene resin by lithium–bromine exchange, treatment of the resulting lithiated resin with selenium powder and, finally, treatment with sodium borohydride in ethanol, thus reducing any diselenide present and eliminating Se–Se cross-linking. Selenolate resin **257** underwent alkylation upon treatment with alkyl chlorides and bromides to give products such as **258** and **261**, respectively. The resin-bound alcohols were then subjected to Mitsunobu reactions with phenols to give **259** and **262**. Reductive cleavage was then carried out using tributyltin hydride. Cleavage of the aliphatic C–Se bond occurs preferentially to the aromatic C–Se bond, to give aryl ethers **260** and **263** in good yields and high purity by GC (Scheme 62). The selenide linker system was used to prepare a 2×3 array of alkyl aryl ethers in yields between 57 and 83% and GC purities between 78 and 88%.[79]

Recently, Ruhland has reported the first use of a tellurium-based linker.[80] A library of simple aryl alkyl ethers was prepared using an analogous, polystyrene-bound telluride complex **264** (Fig. 2). As the homolysis of carbon–tellurium bounds is known to occur at lower temperatures than the homolysis of the corresponding selenides, it was proposed that tellurium linkers might prove valuable for the solid-phase synthesis of temperature-sensitive targets. The cleavage of analogous tellurium and selenium systems at a range of temperatures was therefore studied. The yields were, however, found to be consistently lower using the tellurium linker system.[80]

3.1.2. Selenide linkers formed by cyclative capture. The addition of polymer-bound selenium electrophiles to alkene substrates bearing tethered nucleophiles allows substrates to be immobilised via a selenium linkage whilst simultaneously building a cyclic system. Nicolaou was the first to illustrate this principle in his early report on the utility of selenium linkers,[78] and used a selenenyl bromide resin for the efficient conversion of PGI_{2a} methyl ester **265** into the PG_{I2} analogue **266** (Scheme 63). Treatment of substrate **265** with selenenyl bromide resin **251** resulted in cyclative capture via seleniranium ion formation and cyclisation of the pendant hydroxyl group. Oxidation of the selenide link and eliminative cleavage released **266** in excellent overall yield (Scheme 63).[78]

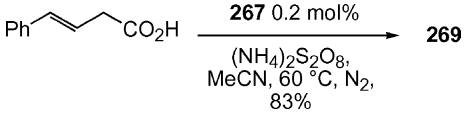
Fujita et al. have prepared selenocyanate resins for use in solid-phase oxyselenenylation–deselenenylation reactions.[81] Selenocyanate resin **267** was prepared by treatment of Merrifield resin with potassium selenocyanate (Scheme 64). Treatment of **267** with copper(II) chloride and *E*-4-phenyl-3-butenoic acid led to cyclative capture via intramolecular oxyselenenylation to give polymer-supported selenolactone **268**. *m*CPBA oxidation to the selenoxide followed by eliminative cleavage gave butenolide **269** (Scheme 64).[81] Interestingly, the use of a Wang-based selenocyanate resin gave only a trace of product.

More recently, Fujita has reported the first example of a solid-phase, selenolactonisation–deselenenylation sequence using a catalytic amount of a selenocyanate resin.[82] Efficient conversion of *E*-4-phenyl-3-butenoic acid into butenolide **269** was achieved using 0.2 mol% of **267** (Scheme 65).

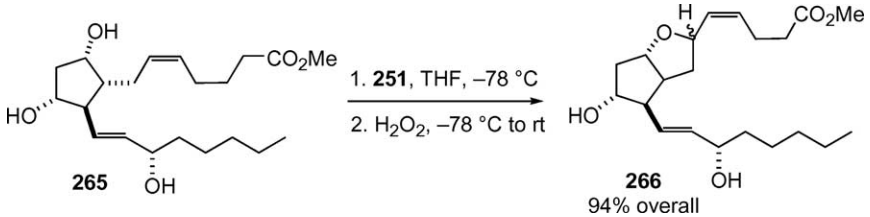


264

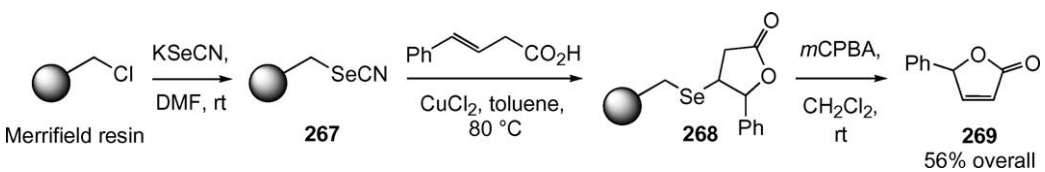
Figure 2.



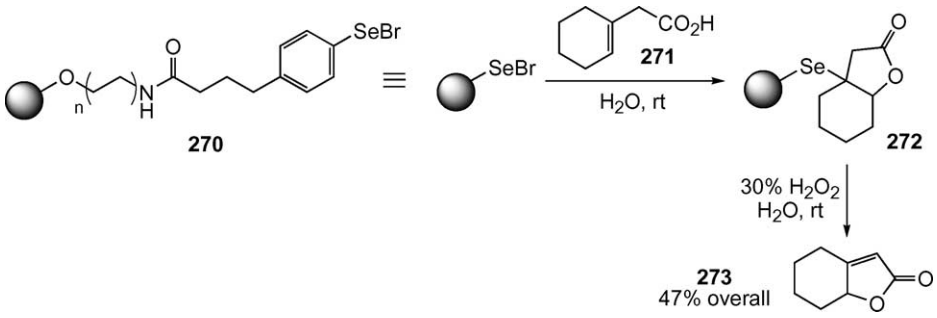
Scheme 65.



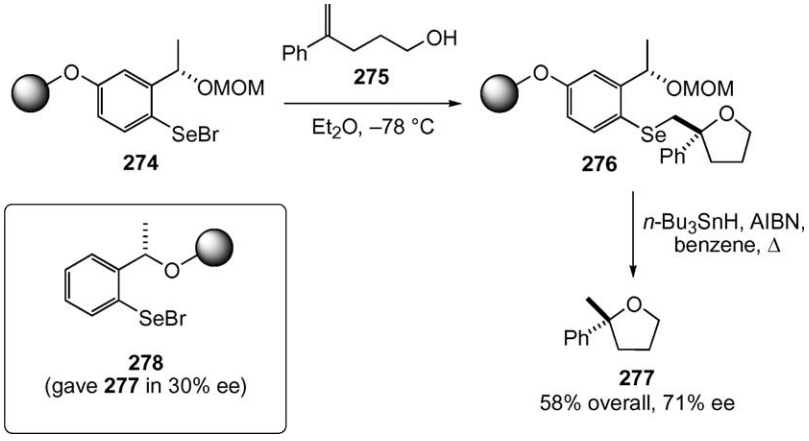
Scheme 63.



Scheme 64.



Scheme 66.



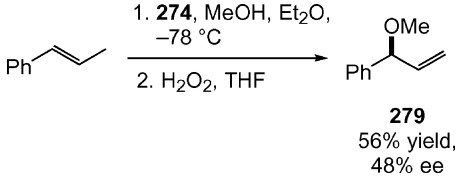
Scheme 67.

Turnover numbers on solid-phase were comparable to those achieved in solution.[82]

Organoselenium reagents are normally incompatible with water due to insolubility or instability. Fujita has carried out the first oxyselenenylation and deselenenylation reactions in water using an amphiphilic polymer-supported selenenyl bromide **270** (Scheme 66),[83] for example, on treatment with resin **270** in water, unsaturated carboxylic acid **271** gave immobilised lactone **272**. Oxidation and eliminative cleavage gave **273** in moderate overall yield (Scheme 66).[83]

Wirth and Uehlin have reported the first polymer-bound, enantiomerically pure, electrophilic selenium reagents for asymmetric addition of nucleophiles to alkenes (Scheme 67).[84] Chiral selenenyl bromides were prepared and immobilised on polystyrene, Tentagel and mesoporous silica supports. Polystyrene-based, selenenyl bromide resin **274** was found to be most effective in asymmetric selenenylation reactions. Treatment of unsaturated alcohol **275** with chiral resin **274** led to diastereoselective seleniranium ion formation and intramolecular, nucleophilic addition of the tethered alcohol, to give tetrahydrofuran **276** immobilised via a chiral selenium linkage. Radical cleavage gave tetrahydrofuran **277** in 58% yield and 71% ee (Scheme 67).[84] Alternative chiral selenenyl bromide **278**, where immobilisation is achieved through the side-chain hydroxyl group, was found to give lower selectivities (Scheme 67).[85]

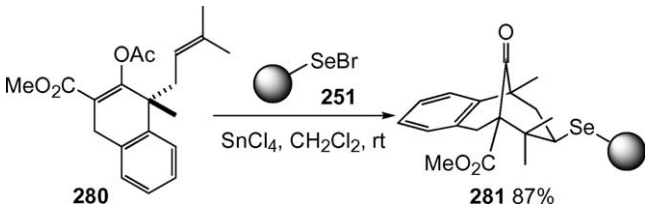
The asymmetric intermolecular addition of nucleophiles to acyclic alkenes mediated by chiral resin **274** has also been studied (Scheme 68),[84] for example, treatment of *E*-phenylpropene with **274** in the presence of methanol results in immobilisation by a methoxyselenenylation reaction. Cleavage of the selenide linker is achieved by oxidation to the selenoxide followed by elimination to give chiral, allylic ether **279** in 56% yield and 48% ee (Scheme 68).[84]



Scheme 68.

The non-asymmetric, methoxyselenenylation reaction has been used by Huang and Sheng to prepare allylic methyl ethers.[86]

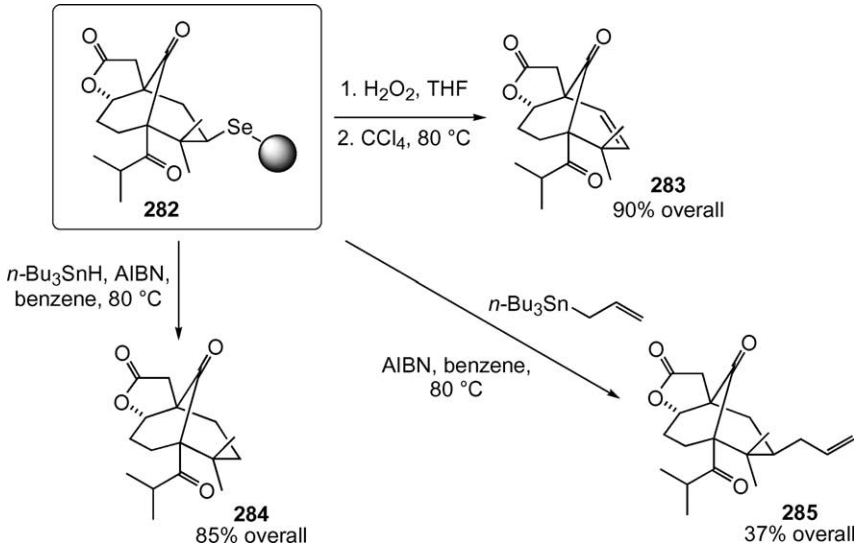
In a series of ground-breaking disclosures, Nicolaou took the use of polymeric selenium electrophiles and cyclative capture from the development stage through to application in the synthesis of large libraries of biologically relevant, structurally complex, targets.



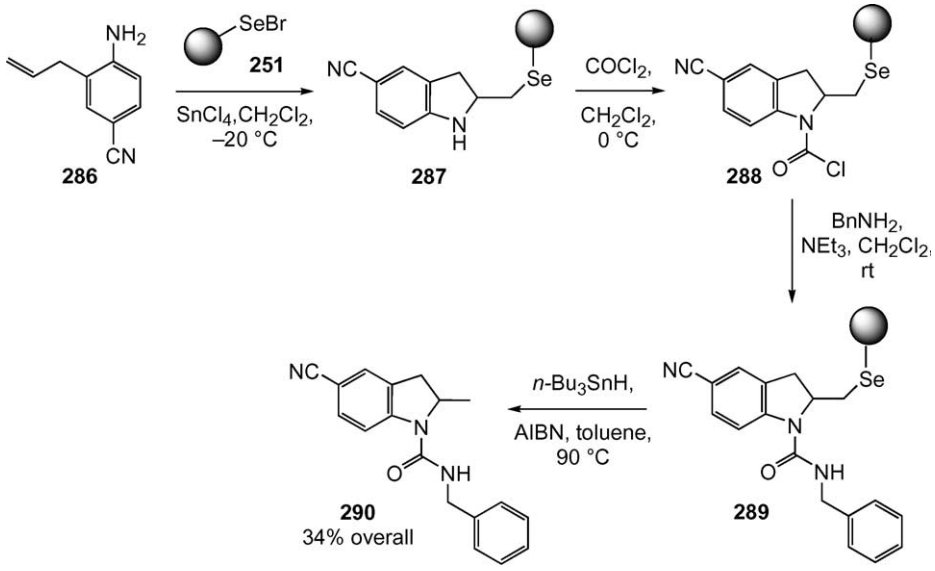
Scheme 69.

Building on his initial work in the area,[78] Nicolaou applied polystyrene-based selenenyl bromide resins in the cyclative capture of substituted cyclohexane-based enol acetates such as **280** to give immobilised, substituted [3.3.1] bicyclic systems **281** (Scheme 69).[87]

Several methods for the cleavage of the selenium linker were investigated and are shown in Scheme 70:[87] oxidation of **282** to give the selenoxide followed by eliminative cleavage gave functionalised bicycle **283**, reductive cleavage to give **284** was achieved by treatment with tributyltin hydride and, finally, radical cleavage using allyltributyltin resulted in cleavage of the selenium link and introduction of an allyl group at the point of attachment to form **285**. Although low yielding, compared to the other cleavage methods, this strategy, in principle, allows further diversification to be introduced via C–C bond formation in the cleavage step (Scheme 70).[87]



Scheme 70.



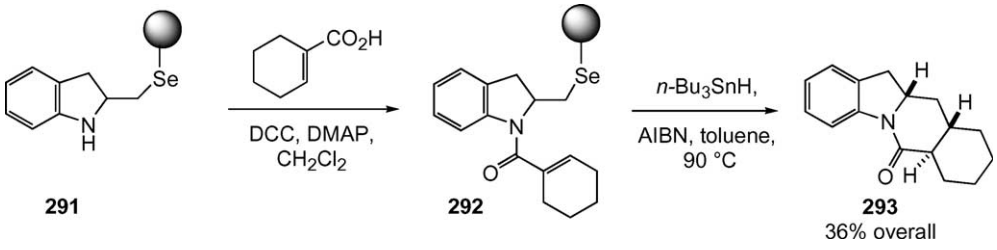
Scheme 71.

Nicolaou has also developed a selenium-based cyclative capture strategy for the solid-phase synthesis of indolines (Scheme 71).[88] Treatment of *o*-allylanilines, such as **286**, with selenenyl bromide resin in the presence of a Lewis acid results in cyclative capture to give immobilised indoline **287**. Further functionalization was then carried out prior to cleavage, acylation of nitrogen with phosgene gave **288** and further reaction with amines giving **289**. Reductive cleavage with tributyltin hydride gave functionalised 1-methylindoline **290** in good yield (Scheme 71). A range of 1-methylindolines were synthesised using this approach in overall yields between 14 and 34%. Unfortunately, purification of the products after cleavage was necessary to remove tin residues.[88]

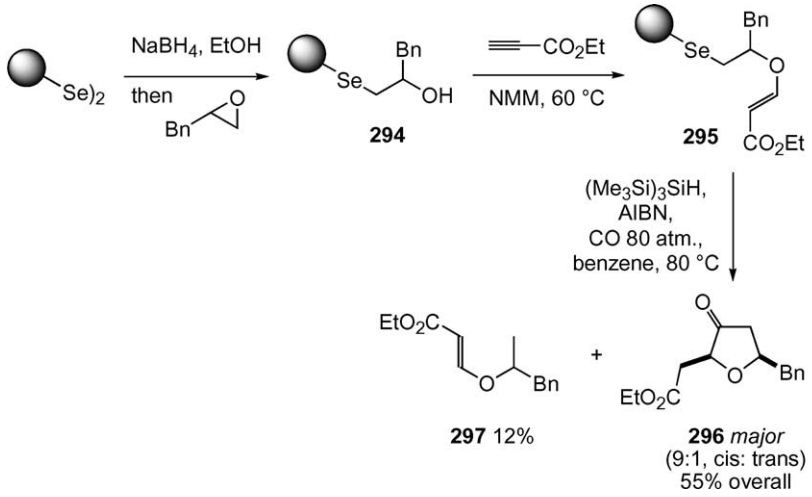
Further complexity can be introduced in the cleavage step when a suitably positioned radical acceptor is present (Scheme 72),[88] for example, resin-bound indoline **291** was coupled with 1-cyclohexenecarboxylic acid. Cleavage of the selenium linker in **292** with tributyltin hydride gave a carbon-centred radical that underwent cyclisation onto the tethered double bond to give indoline **293**, thus illustrating the feasibility of accessing polycycles using this methodology (Scheme 72).[88]

Building on the precedent of Nicolaou, Engman and coworkers have reported the first example of the homolytic cleavage of a selenide linker followed by carbonylation of the resultant radical and cyclisation.[89] Treatment of substrate **295**, prepared from hydroxyselenide **294**, with tri(trimethylsilyl)silane and AIBN under an atmosphere of CO gave lactone **296** in good overall yield and with good diastereoselectivity. Acyclic reduction product **297** was also isolated as a by-product (Scheme 73).[89]

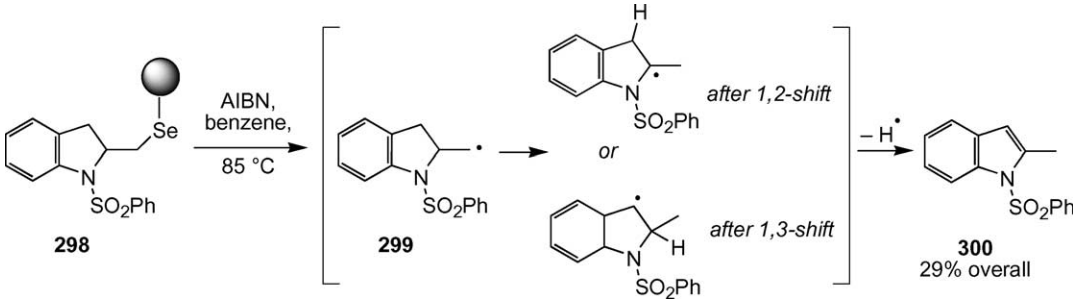
Returning to the studies of Nicolaou, in an attempt to introduce more diversity upon cleavage, exposure of resin-bound indoline **298** to a radical initiator in the absence of a



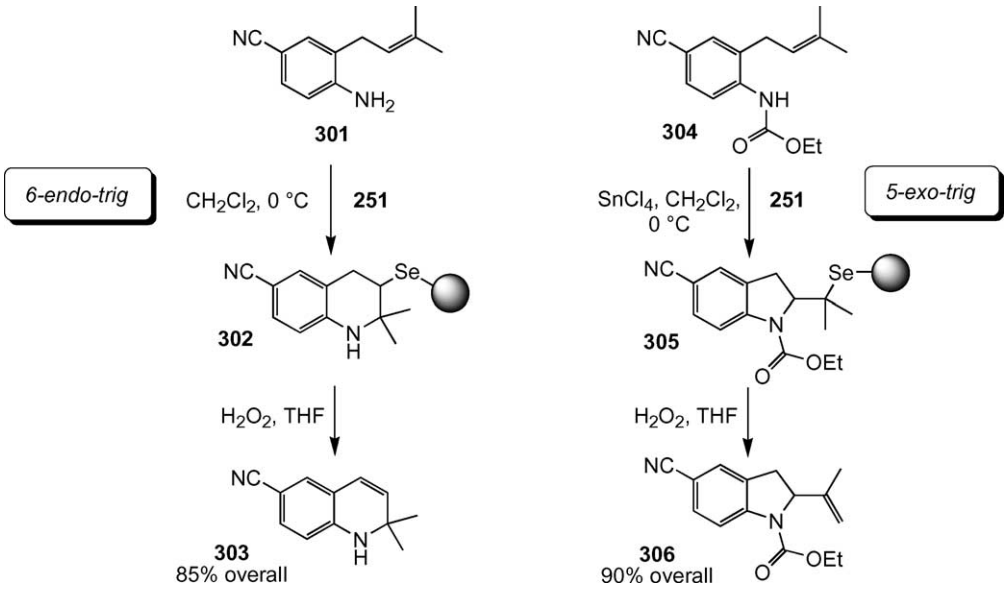
Scheme 72.



Scheme 73.



Scheme 74.



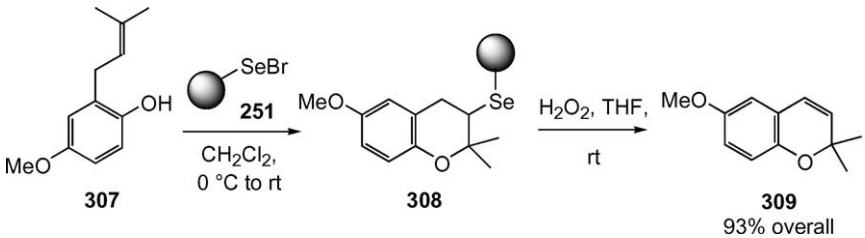
Scheme 75.

radical quench led to cleavage and radical rearrangement allowing access to 2-methylindoles,[90] for example, treatment of **298** with AIBN initially gave primary radical **299** that rearranged to give 2-methylindole **300** in good overall yield (Scheme 74).[90]

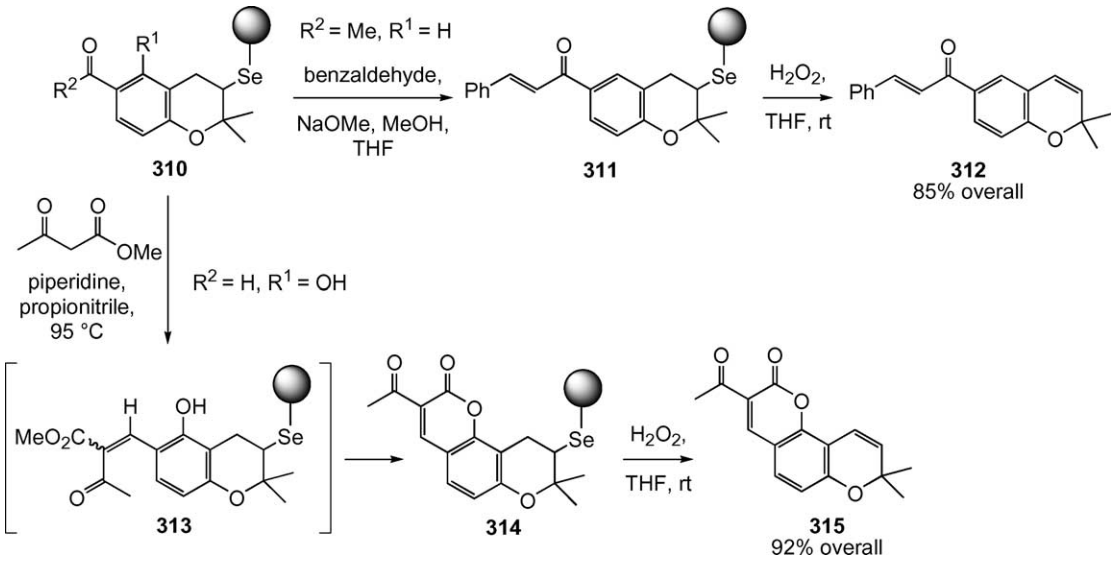
The analogous cyclative loading of *ortho*-prenylanilines was also investigated by Nicolaou.[90] The mode of cyclisation was found to depend upon the electronic properties of the aniline ring, for example, treatment of **301** with selenenyl bromide resin **251** gave dihydroquinoline **302** after 6-*endo-trig* cyclisation. In contrast, ethyl carbamate **304**, in the presence of Lewis acid, gave indoline **305**. Efficient oxidation and eliminative cleavage in both series then gave quinoline **303** and indoline **306**, respectively (Scheme 75).[90]

Nicolaou et al. have also applied cyclative capture and the formation of selenium linkers in the synthesis of compounds containing the 2,2-dimethylbenzopyran moiety, a common structural motif in many natural products and biologically active compounds.[91,92] The key benzopyran-forming, cyclative capture step in the approach involves treatment of a selenenyl bromide resin **251** with *o*-prenylated phenols such as **307** to give immobilised 2,2-dimethylbenzopyran **308**. Oxidation and eliminative cleavage released 2,2-dimethylbenzopyran **309** in excellent yield (Scheme 76). The generality of the cyclative capture step was investigated using *o*-prenylated phenols bearing neutral, electron-rich and electron-poor substituents. Overall yields and product purities were high, regardless of the electronic nature of the aromatic ring.[91,92]

Nicolaou has applied this methodology in the production of focused libraries of benzopyran natural products and bioactive compounds.[91,92] After cyclative capture, further modification of the resin-bound 2,2-dimethylbenzopyran core can be carried out to access a diverse array of compounds, for example, resin-bound benzopyran methyl ketone **310** (R^1=H, R^2=Me) can undergo condensation with aldehydes to give **311** followed by cleavage to give chalcone **312** in 85% overall yield (Scheme 77).[91,92] A library of 24 chalcone analogues was synthesised in this way, with overall yields between 25 and 90%. In a similar manner, a library of pyranocoumarin analogues was produced from resin-bound *o*-hydroxy aldehydes, such as **310** (R^1=OH, R^2=H) (Scheme 77).[91,92] Pyranocoumarins are natural products that show antiviral, cytotoxic and antiplatelet activity. Knoevanagel condensation with a β-ketoester gave intermediate **313** that underwent lactonisation to give pyranocoumarin **314**. Oxidation and eliminative cleavage then gave **315** in 92% overall yield (Scheme 77).[91,92]



Scheme 76.



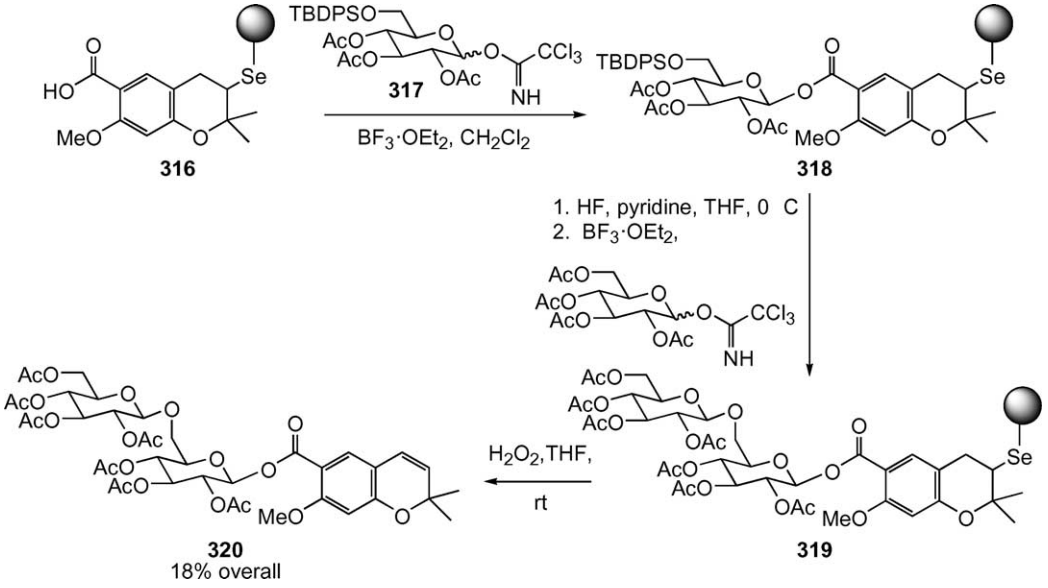
Scheme 77.

The carbohydrate-containing benzopyran, macrophylloside heptaacetate **320**, has been synthesised from resin-bound benzopyran **316** (Scheme 78).[91,92] Glycosylation of **316** with trichloroacetimidate **317** gave glycoside **318**. Silyl group deprotection and a second glycosylation gave **319** as a single anomer. Oxidation and eliminative cleavage gave heptaacetate macrophylloside **320** in 18% overall yield (Scheme 78).[91,92]

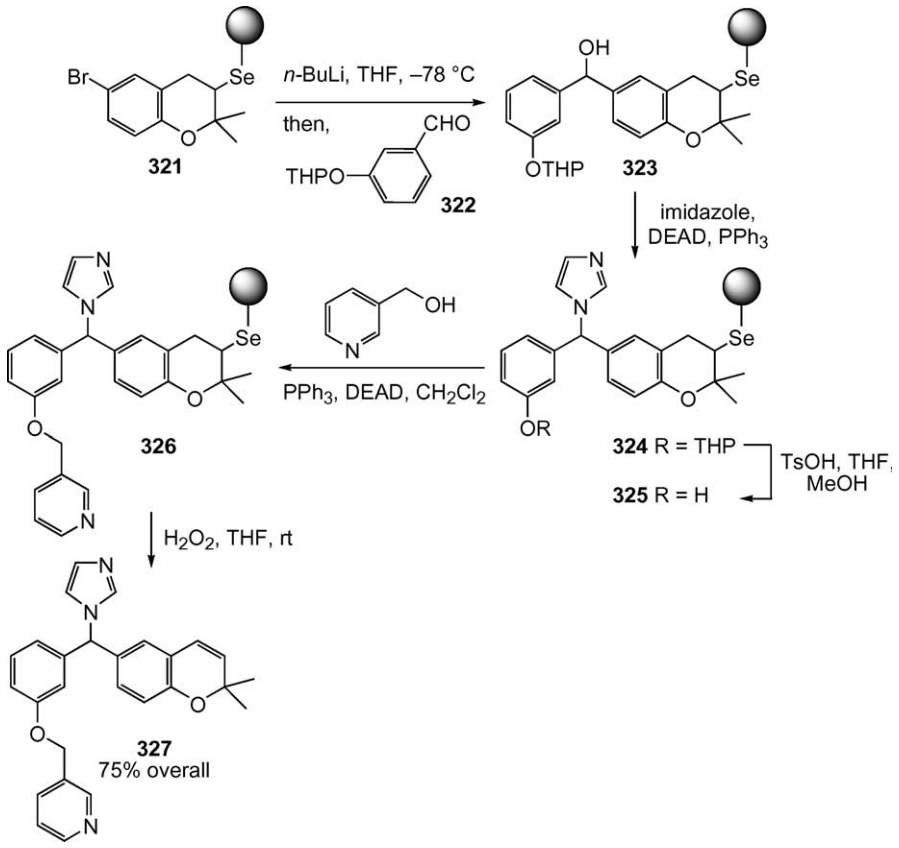
Nicolaou has also used this selenium linker approach for the synthesis of a small library of aldosterone biosynthesis inhibitors.[93] Aryl bromide **321** underwent lithium–halogen exchange and the resultant aryllithium added to benzaldehyde **322**. Benzhydrol **323** then underwent Mitsunobu reaction with imidazole to give **324**. Deprotection to **325**, coupling with 3-pyridylmethanol to give **326**, oxidation and eliminative cleavage released the aldosterone biosynthesis inhibitor **327** (Scheme 79).[93] Variation of the aldehyde and the imidazole components in the approach allowed a range of analogues to be prepared.

Similarly, a library of phosphodiesterase inhibitors was synthesised using this approach.[92] Aryl bromide **328** underwent lithium–halogen exchange and the resultant aryllithium added to DMF to give aldehyde **329**. Condensation with 4-pyridylacetonitrile gave resin-bound stilbene **330**. Cleavage gave phosphodiesterase inhibitor **331** in 72% overall yield (Scheme 80).[92]

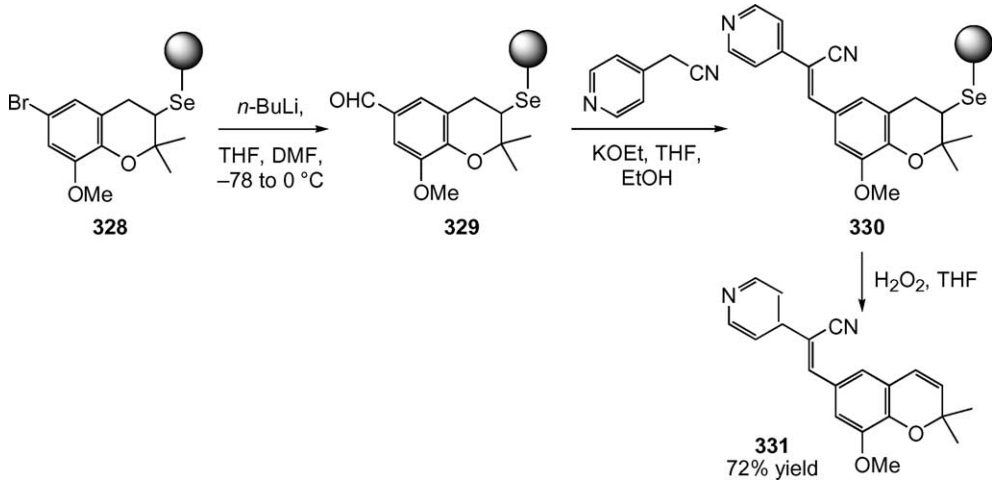
Finally, Nicolaou has used this strategy for the synthesis of



Scheme 78.



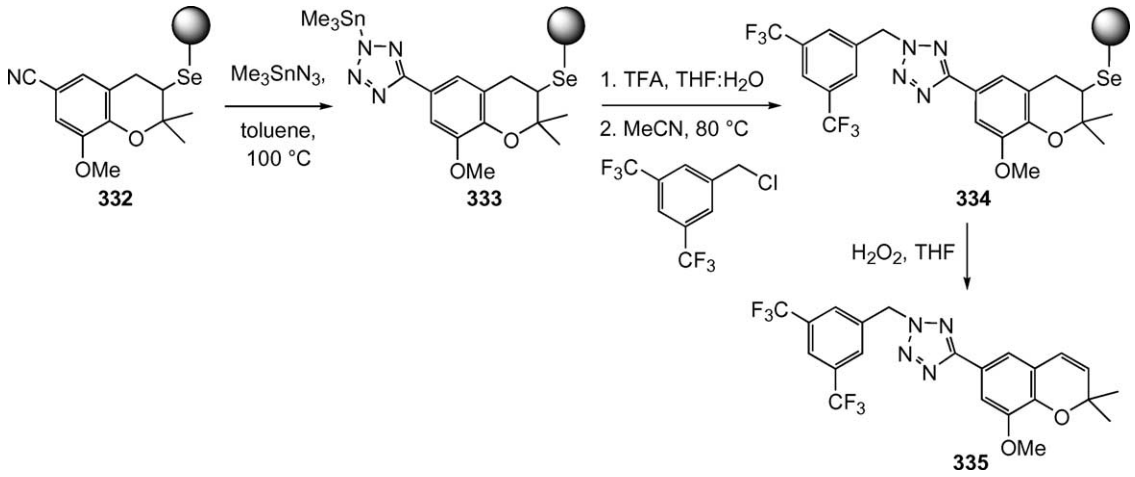
Scheme 79.



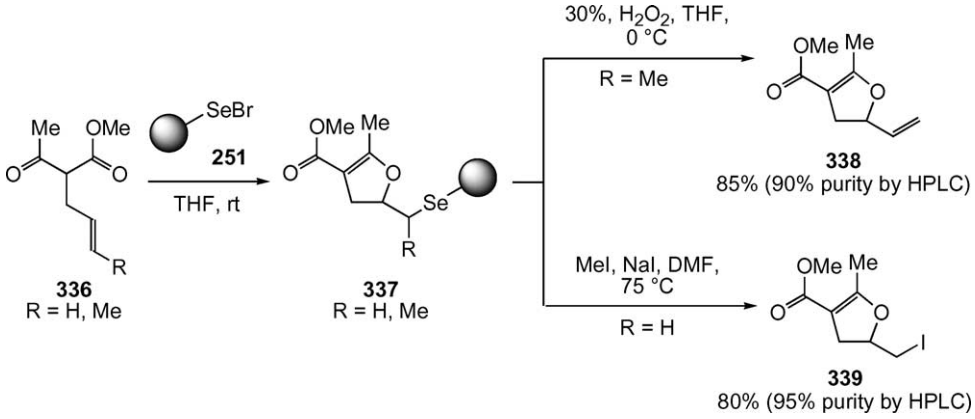
Scheme 80.

tetrazole-containing targets, such as **335**, that are known to be potassium channel activators (Scheme 81).[92] Resin-bound benzopyran **332** bearing a nitrile group was treated with azidotrimethyltin to generate tetrazole **333**. Treatment with aqueous TFA removed the trimethylstannyl group allowing alkylation of nitrogen to form **334**, before cleavage in the usual way to give the desired product (Scheme 81).[92]

Having developed a versatile solid-phase approach to natural and non-natural products based on the benzopyran template, Nicolaou described the construction of a 10,000-membered natural product-like library of compounds using nanokans and optical encoding to tag and sort library members during split-and-mix synthesis.[94,95] A similar combinatorial approach has also been used to identify a



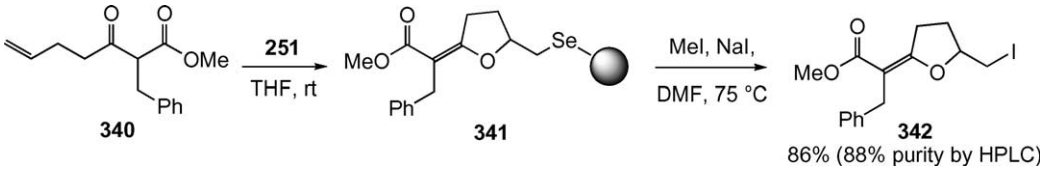
Scheme 81.



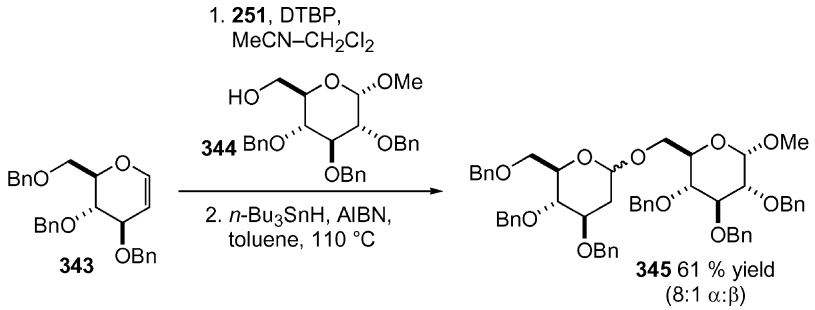
Scheme 82.

family of potent benzopyran-based inhibitors of NADH: ubiquinone oxidoreductase.[96]

Huang and co-workers have also utilised a cyclative capture approach using a polymer-supported selenenyl bromide in a synthesis of substituted dihydrofurans and tetrahydrofurans.[97] Treatment of 2-allyl- and 2-crotyl-substituted 1,3-dicarbonyl compounds **336** with selenenyl bromide resin **251** gave dihydrofurans **337** (Scheme 82). Oxidation and eliminative cleavage of **337** (R=Me) gave dihydrofuran **338**, while nucleophilic substitution using MeI/NaI in DMF was used to cleave the selenium linkage in **337**



Scheme 83.



Scheme 84.

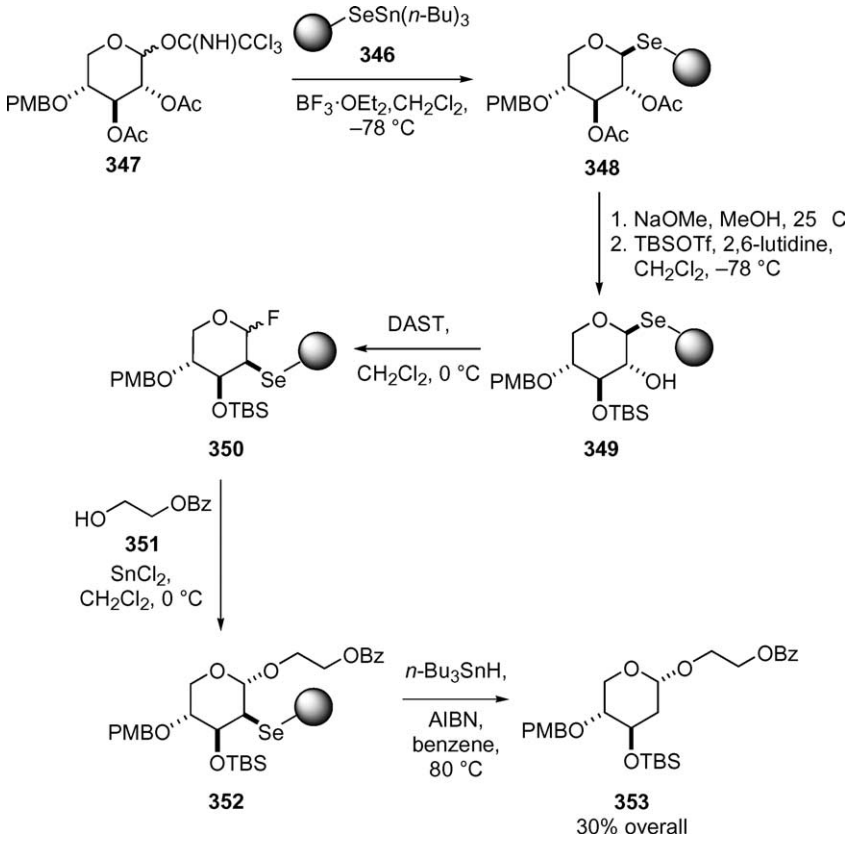
(R=H) and release the iodomethyl-substituted dihydrofuran **339** from the resin in good yield and high purity (Scheme 82).[97]

Alternative substrates, such as **340**, gave excellent yields of iodomethyltetrahydrofurans such as **342** after cyclative capture to give **341** and nucleophilic cleavage from the resin (Scheme 83).[97]

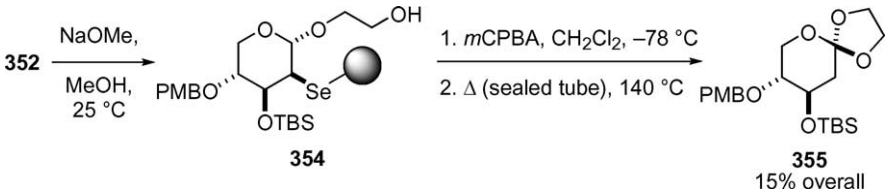
3.1.3. Selenide linkers in carbohydrate chemistry. Nicolaou and co-workers have used a selenenyl bromide resin in the synthesis of 2-deoxyglycosides (Scheme 84).[78] Treatment of tri-*O*-benzylglucal **343** with **251** in the presence of an alcohol such as **344** resulted in glycosylation and immobilisation of the product through a selenide linkage at the 2-position of the sugar ring. Radical cleavage of the C–Se bond using tributyltin hydride released the 2-deoxyglycoside **345** in 61% yield, as an 8:1 mixture of α and β anomers (Scheme 84).[78]

Nicolaou has also exploited a 1,2-selenomigration reaction of selenoglycosides in the solid-supported synthesis of 2-deoxyglycosides, 2-deoxyorthoesters and 2,3-allyl orthoesters.[98] Resin-bound tributyltin selenolate **346** was conveniently synthesised in two steps from a selenenyl bromide resin. Treatment of **346** with trichloroacetimidate donor **347** gave resin-bound selenoglycoside **348** (Scheme 85). Removal of the C2 and C3 acetate protecting groups and reprotection of the C3 hydroxyl gave **349**, ready for the 1,2-selenomigration reaction. Treatment of **349** with DAST promoted 1,2-migration of the selenium link to resin in a stereospecific manner to give **350**, now linked to resin at the C2 position of the sugar, whilst simultaneously generating an anomeric fluoride leaving group.[98] Glycosyl fluoride donor **350** then underwent glycosylation with alcohols or sugars, for example, exposure to Lewis acid and monoprotected diol **351** selectively gave the α-glycoside **352**. Reductive cleavage of the selenium linker with tributyltin hydride gave the desired 2-deoxyglycoside **353** in excellent yield. A 3×3 library of 2-deoxyglycosides was synthesised using this approach in overall yields between 11 and 32% (Scheme 85).[98]

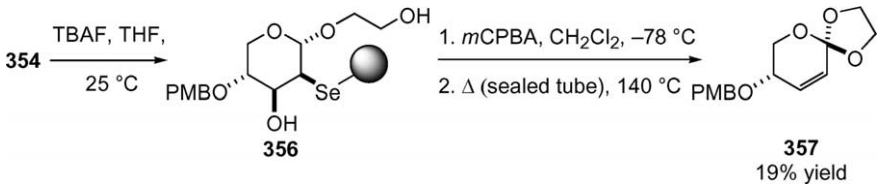
2-Deoxyorthoesters can also be synthesised using the 1,2-selenomigration approach (Scheme 86).[98] Following glycosylation with a monoprotected diol, removal of the



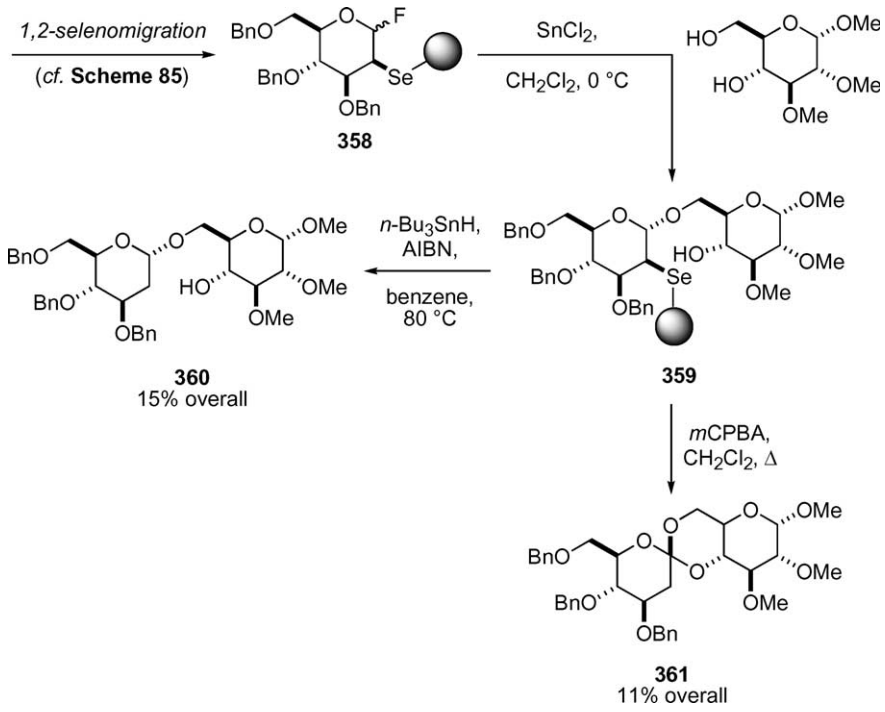
Scheme 85.



Scheme 86.



Scheme 87.



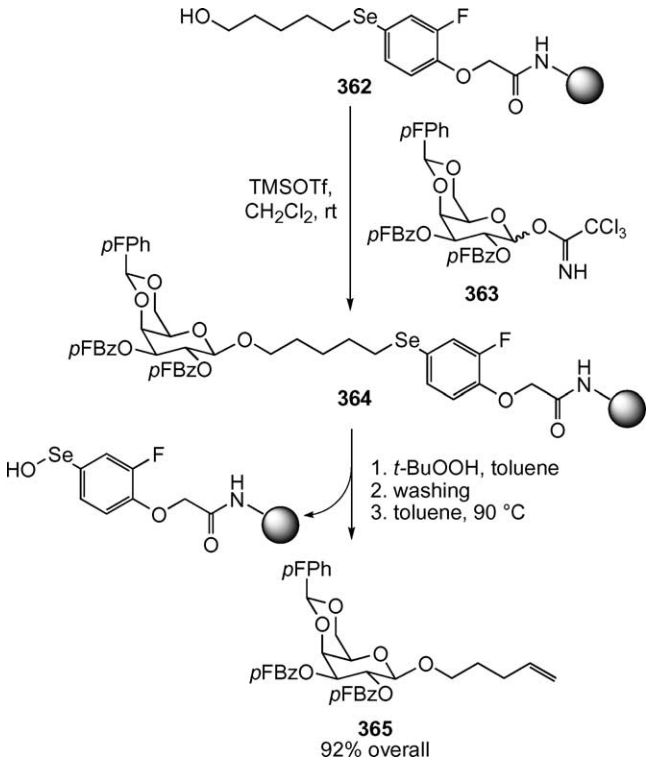
Scheme 88.

benzoate protection gave **354**. Oxidation of the selenium link, eliminative cleavage and cyclisation gave 2-deoxy-orthoester **355** in 15% overall yield (Scheme 86).[98]

2,3-Allyl orthoesters were also made by a modification of this route.[98] Removal of the C3 silyl protecting group from **354**, gave **356**. Oxidation to the selenoxide followed by heating in a sealed tube triggered a sequence involving eliminative cleavage, elimination of the C3 hydroxy group and attack of the tethered alcohol at C1 to give the 2,3-allyl orthoester **357** in 19% overall yield (Scheme 87).[98]

Nicolaou has applied this methodology in synthetic studies towards the antibiotic, everinomicin 13,384-1.[99] Glycosylation of immobilised fluoride donors such as **358**, prepared by 1,2-selenomigration, with sugar acceptors allowed the synthesis of disaccharides such as **359** (Scheme 88). In accord with model studies, radical cleavage allowed access to 2-deoxyglycosides **360**. Oxidation and eliminative cleavage provided the expected orthoesters **361**, while allylic orthoesters were accessible by oxidation and eliminative cleavage of immobilised disaccharides possessing a free hydroxyl group at the C3 position, adjacent to the linking selenium atom (Scheme 88, cf. Schemes 86 and 87).[99]

Kihlberg and co-workers have developed a solid-phase strategy for the synthesis of 4-pentenyl glycosides using a

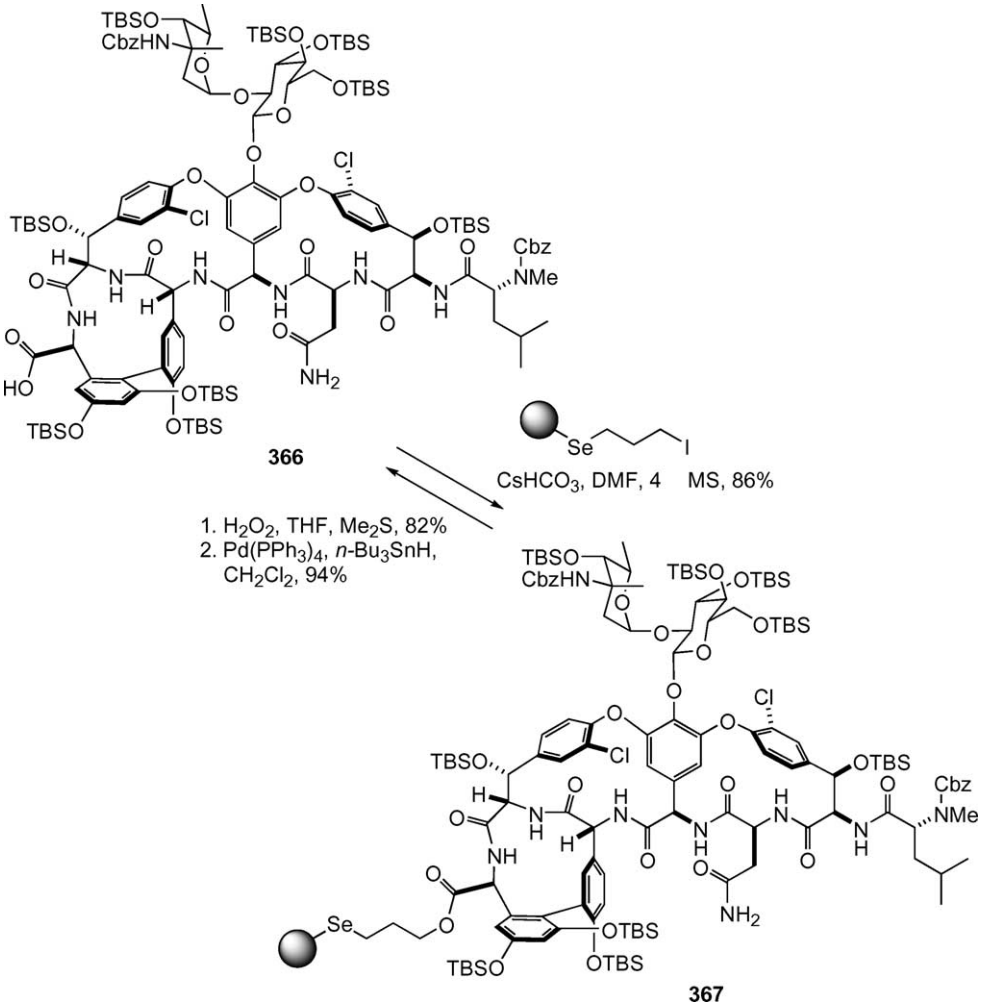


Scheme 89.

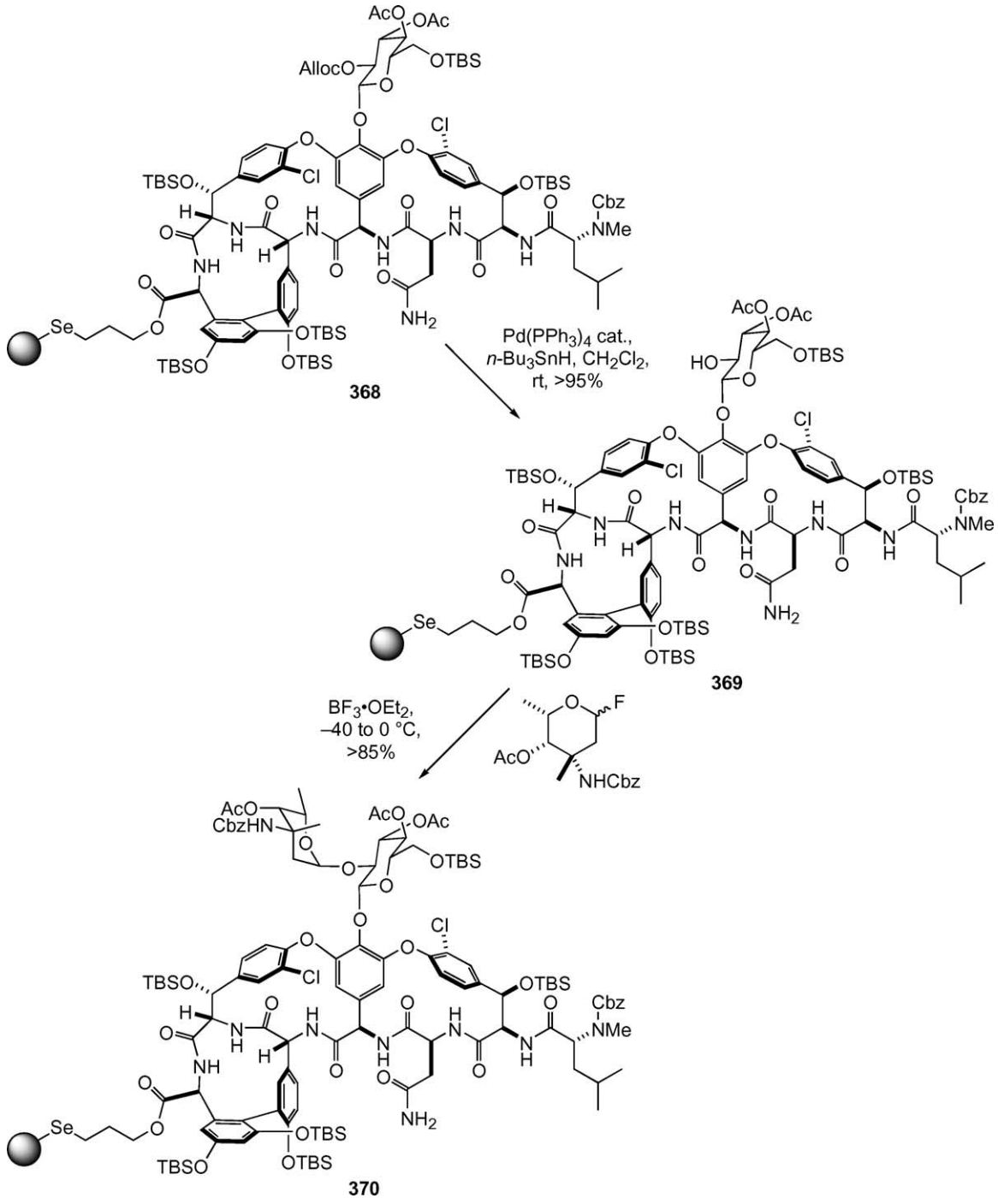
fluorinated, selenium linker.[100] 4-Pentenyl glycosides are useful glycosyl donors for the preparation of complex oligosaccharides. A selenium linkage was used, due to its compatibility with a broad range of reagents and protecting-group protocols regularly utilised in oligosaccharide synthesis. The preparation of a fluorine-labelled selenium-based linker and the use of fluorine-labelled glycosyl donors allowed immobilisation reactions to be monitored using gel-phase ^{19}F NMR spectroscopy.[100] Hydroxyl resin **362** was treated with galactosyl trichloroacetimidate **363** using TMSOTf as a promoter to give polymer-bound galactoside **364** (Scheme 89). ^{19}F NMR of **364** revealed that the glycosidic linkage was formed with high efficiency. Attempted immobilisation of a glycosyl fluoride and sulfoxide were unsuccessful. Oxidation of the selenium link with anhydrous *t*BuOOH led to eliminative cleavage upon heating to give *n*-pentenyl galactoside **365** in excellent yield (Scheme 89). ^{19}F NMR was again used to confirm that complete cleavage had occurred.[100]

3.1.4. Selenide linkers for the synthesis of peptide derivatives. Nicolaou et al. have utilised a selenium-based safety-catch linker for the solid-phase semi-synthesis of the glycopeptide antibiotic, vancomycin.[101] It is known that varying the oligosaccharide moiety of vancomycin significantly increases the activity of analogues against resistant bacterial strains. Production of a library of analogues of targets such as vancomycin is synthetically very challenging and solid-phase synthesis has proved useful for the manipulation of this molecule. Vancomycin contains a diverse array of functionality, making the choice of linker system crucial. In order to demonstrate that a selenium linker would allow the molecule to be efficiently loaded and cleaved from solid support, protected vancomycin derivative **366** was linked to resin through the carboxylic acid group to give pro-allyl ester **367** (Scheme 90).[101] Oxidation of the selenium linkage and eliminative cleavage released alloc-protected vancomycin. Treatment with catalytic Pd(0) and tributyltin hydride removed the alloc group to return protected vancomycin **366** (Scheme 90).[101]

Nicoloau has demonstrated that the glycoside moiety in immobilised vancomycin **368** can be manipulated using this linker strategy (Scheme 91).[101] In the example shown, the alloc protecting group of the glycoside unit of **368** was removed to afford **369**, leaving the selenium pro-alloc linker intact. Glycosidation with a fluoride glycosyl donor then gave **370** (Scheme 91). Performing these manipulations on solid support was found to greatly assist the purification of compounds en route to vancomycin.[101]



Scheme 90.

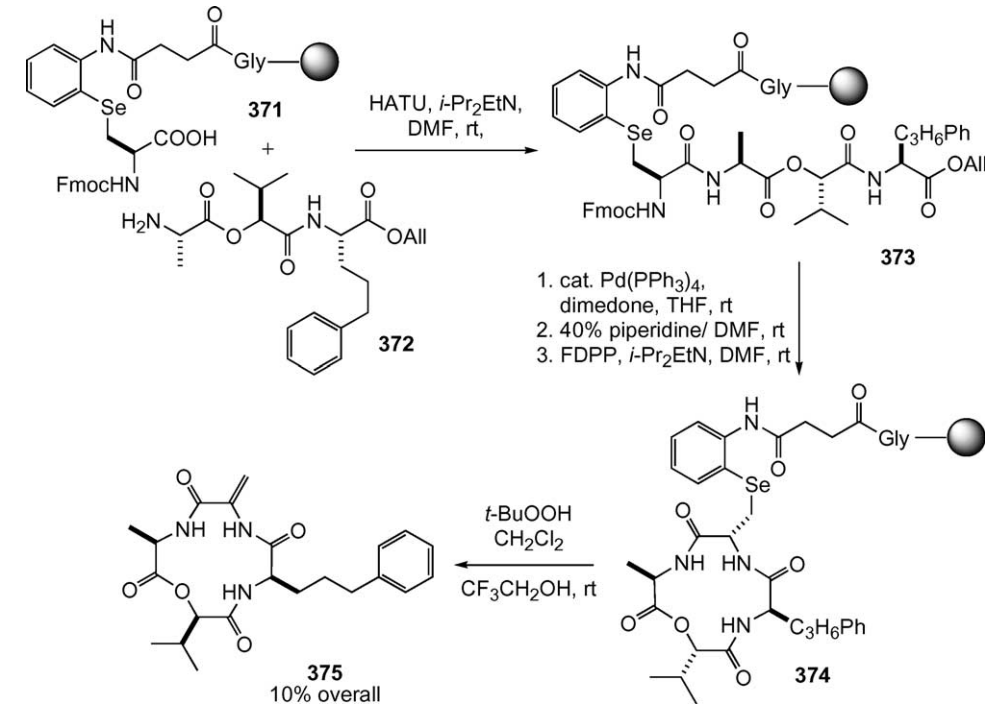


Scheme 91.

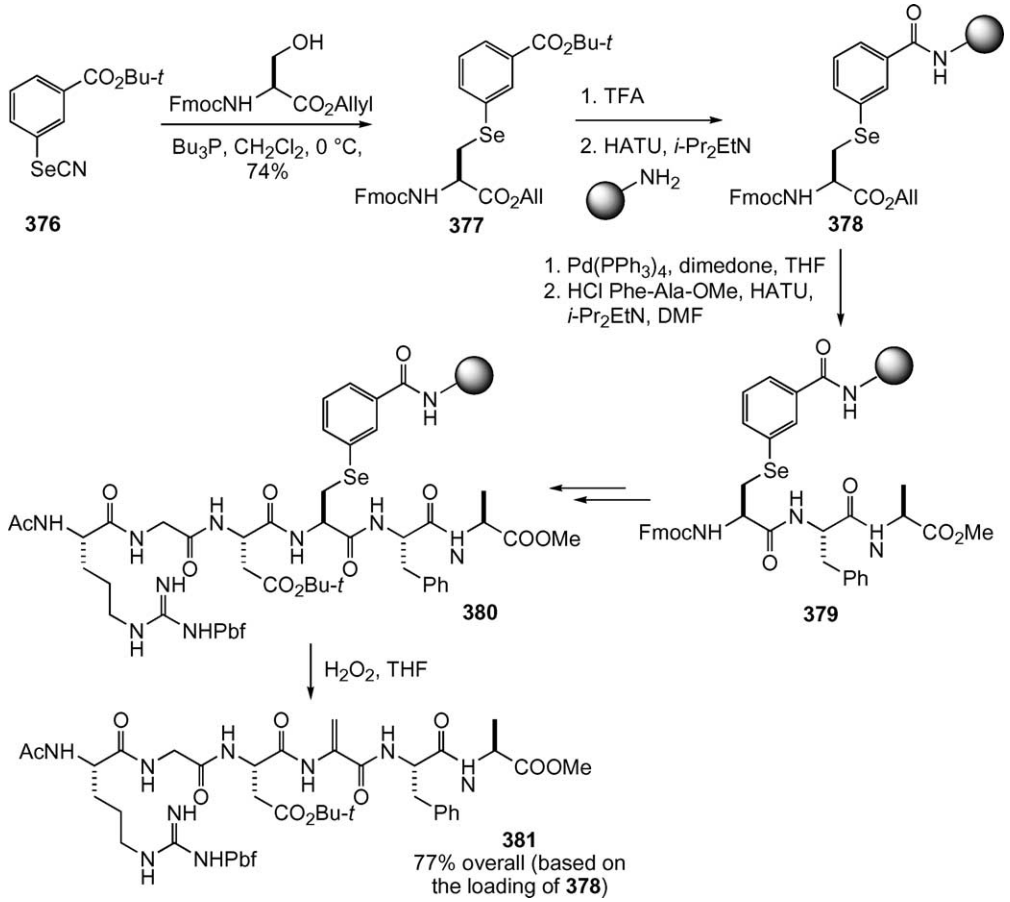
More recently, Nicolaou has prepared vancomycin analogues, using the selenium pro-alloc linker, by removal and replacement of the natural carbohydrate component of the natural product with a variety of monosaccharide units.[102]

Nakamura and co-workers have developed a selenium linker for the synthesis of dehydropeptides.[103] Alanine derivative **371**, immobilised through a selenium link to resin, was coupled with tripeptide allyl ester **372** under standard conditions to give **373**. Upon deprotection, cyclisation was induced to give **374** using pentafluorophenyl diphenylphosphinate (FDPP). Finally, oxidation of **374** to the selenoxide and eliminative cleavage gave AM-toxin II **375** (Scheme 92). Unfortunately, peptide degradation products were also obtained, explaining the low overall yield of AM-toxin II.[103]

Nakamura has subsequently developed an improved selenium linker dehydropeptide synthesis.[104] The selenium linker was constructed via the reaction of selenocyanate **376** with a protected serine derivative in the presence of



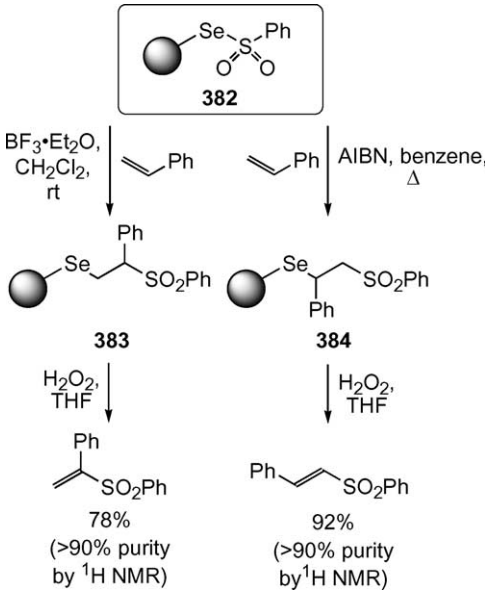
Scheme 92.



Scheme 93.

tributylphosphine to give **377**, followed by deprotection and immobilisation using a commercially available amino resin (Scheme 93). Deprotection of the allyl ester in **378** and coupling with phenylalanylalanine methyl ester hydrochloride gave the immobilised protected tripeptide **379**. N-terminal elongation was then carried out using a peptide synthesiser to give acetylated hexapeptide **380**. Finally, the desired dehydropeptide **381** was obtained after oxidation of selenium and eliminative cleavage from the resin (Scheme 93).[104]

3.1.5. Selenide linkers formed by radical addition. While most selenium linkers are formed by the addition of selenenyl halides to alkenes or the addition of selenolate anions to electrophiles such as alkyl halides, Huang et al. have reported studies on immobilisation via the addition of selenium radicals to alkenes and alkynes.



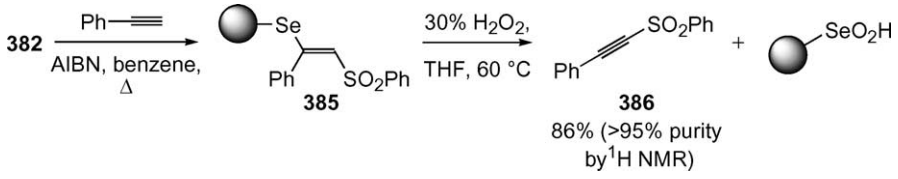
Scheme 94.

Huang has utilised selenosulfonate resin **382** in a solid-phase, regio- and stereocontrolled synthesis of vinyl sulfones.[105] Treatment of olefins with resin **382**, in the presence of a Lewis acid, gave Markovnikov addition product **383**. Under radical conditions, anti-Markovnikov addition occurred to give immobilised sulfone **384**. Oxidation to the corresponding selenoxides and eliminative cleavage gave vinyl sulfones in high yields (Scheme 94).[105]

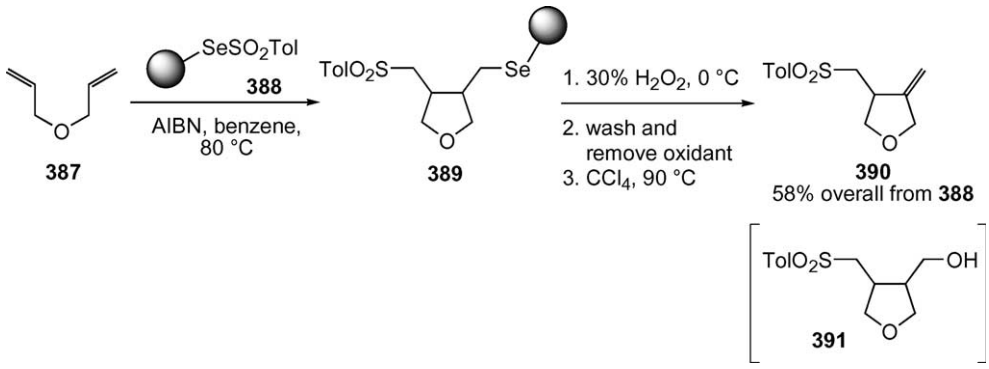
Similarly, radical selenosulfonation of phenylacetylene using **382** proceeded well to provide immobilised vinyl selenides such as **385** (Scheme 95).[106] Alkynyl sulfone **386** was obtained in excellent yield and purity upon oxidation and stereoselective eliminative cleavage. Attractively, resin **382** can be regenerated from the seleninic acid resin by-product and re-used (Scheme 95).[106]

Finally, Huang and Qian have employed the selenosulfonate resin **388** in the radical, cyclative capture of 1,6-dienes such as **387**.[107] Oxidation and eliminative cleavage of **389** provided methylenecyclopentane **390** in moderate yield (Scheme 96).[107] Interestingly, it proved important to wash the resin thoroughly after the low-temperature oxidation step before heating with CCl_4 at 90 °C to bring about the elimination. If the resin was heated directly after oxidation, alcohols such as **391** were found to be major by-products. Alcohol by-products were not formed in the corresponding solution-phase oxidation–elimination reactions, indicating this process is unique to solid-phase.[107]

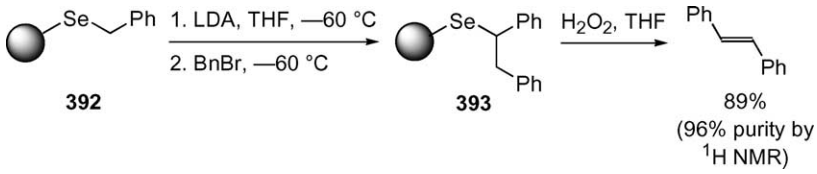
3.1.6. Miscellaneous selenide linkers cleaved by oxidation and eliminative cleavage. Huang has utilised a benzyl selenide resin to prepare olefins and allylic alcohols.[108] Resin **392** was prepared by reaction of lithiated polystyrene with benzylselenenyl bromide. Subsequent



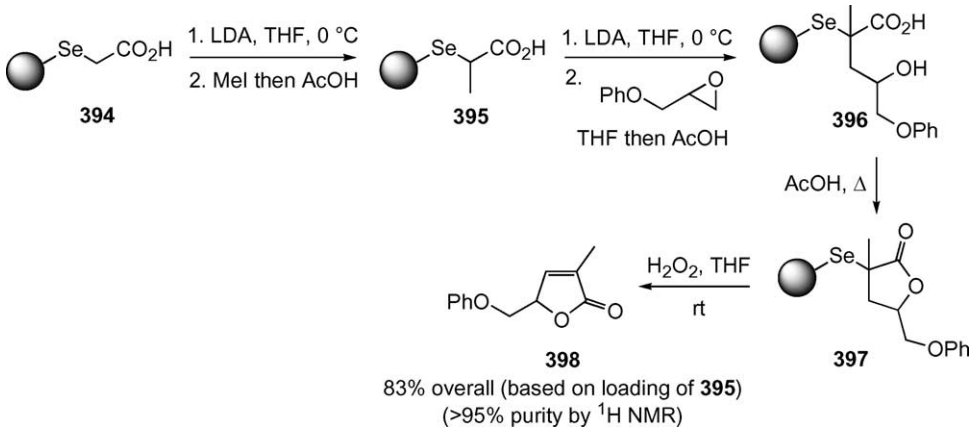
Scheme 95.



Scheme 96.



Scheme 97.

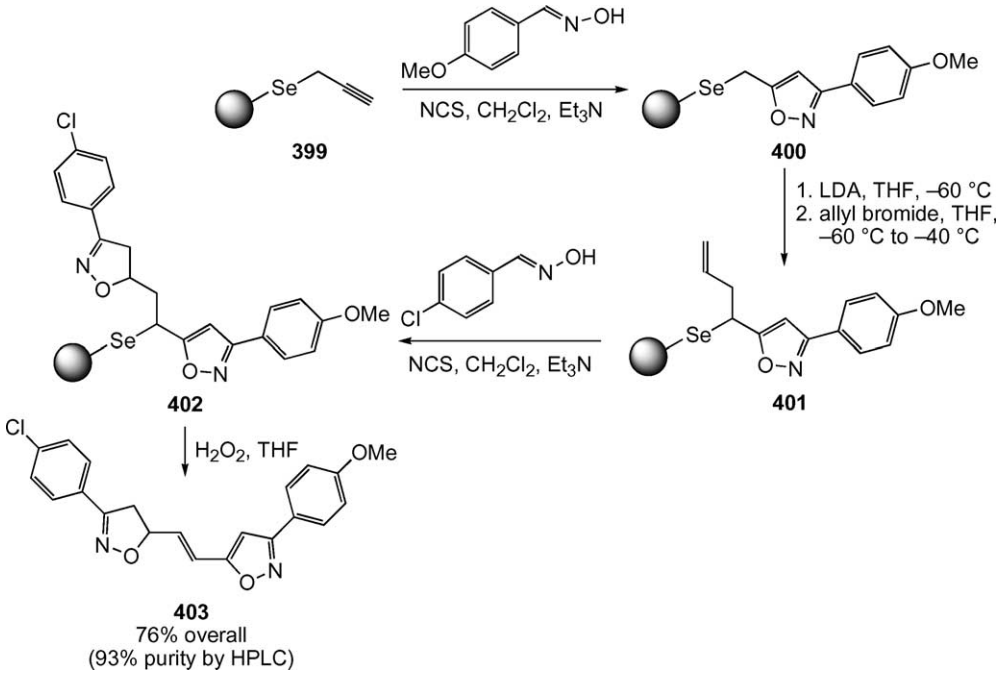


Scheme 98.

deprotonation and alkylation gave immobilised selenides such as **393**. Oxidation and stereoselective eliminative cleavage gave (*E*)-1,2-diphenylethene in high yield and purity. The seleninic acid resin by-product from the reaction could be re-used (Scheme 97).[108]

Huang has also developed a solid-phase approach to butenolides using resin-bound α-selenocarboxylic acids.[109] Supported α-selenopropionic acid **395** was prepared from α-selenoacetic acid resin **394** by treatment with LDA and methyl iodide. Dianion formation using LDA and reaction with racemic epoxides gave intermediates **396** that underwent lactonisation to **397** upon heating. Oxidation and eliminative cleavage afforded butenolides **398** in good yield and high purity (Scheme 98).[109] Employing (*R*)-styrene oxide in the sequence gave the expected non-racemic butenolides.

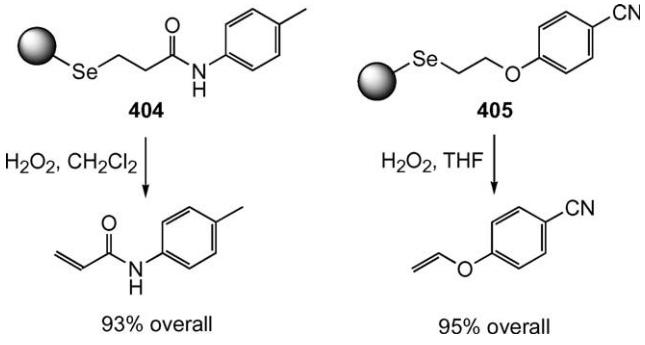
Huang has also reported the preparation of isoxazolyl- and isoxazolinyl-substituted olefins using a selenium linker system.[110] Reduction of a selenenyl bromide resin with $NaBH_4$ and alkylation with propargyl bromide gave selenide resin **399**. Nitrile oxide 1,3-dipolar cycloaddition then gave isoxazole **400** (Scheme 99). Alkylation α to the



Scheme 99.

selenium linking atom using LDA and a range of allyl bromides gave substrates such as **401** for a further nitrile oxide cycloaddition to give **402**. For some substrates, retreatment of the resin was required to achieve satisfactory conversion in the cycloaddition step. Oxidation and eliminative cleavage of **402** gave isoxazolyl-substituted olefins such as **403** in good yield and purity (Scheme 99).[110]

Sheng and co-workers have employed a selenium linker for the synthesis of acrylamides[111] and aryl vinyl ethers[112] via the oxidation and eliminative cleavage of immobilised amide and ether intermediates, such as **404** and **405**, respectively (Scheme 100).[111,112]



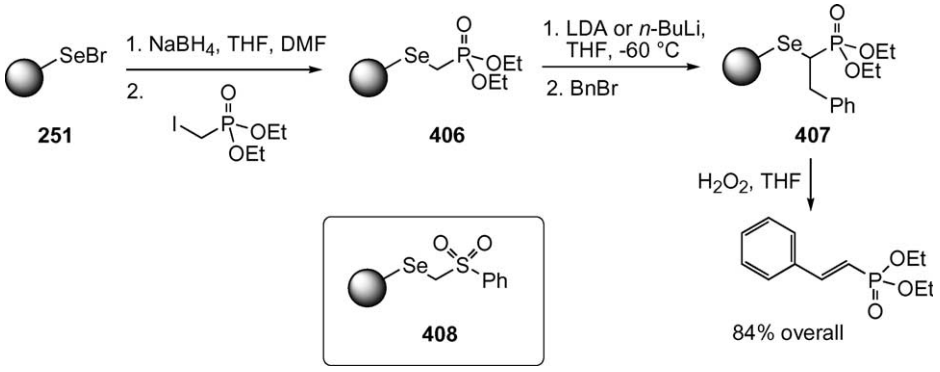
Scheme 100.

In independent studies, Huang[113] and Sheng[114] have reported the synthesis of vinyl phosphonates using a selenium linker. Selenomethyl-phosphonate resin **406** was deprotonated and alkylated with a range of carbon electrophiles to give adducts such as **407**. Oxidation and eliminative cleavage gave *E*-vinyl phosphonates in good yield (Scheme 101). Huang has also reported an analogous synthesis of *E*-vinyl sulfones using sulfonate resin **408**.[113]

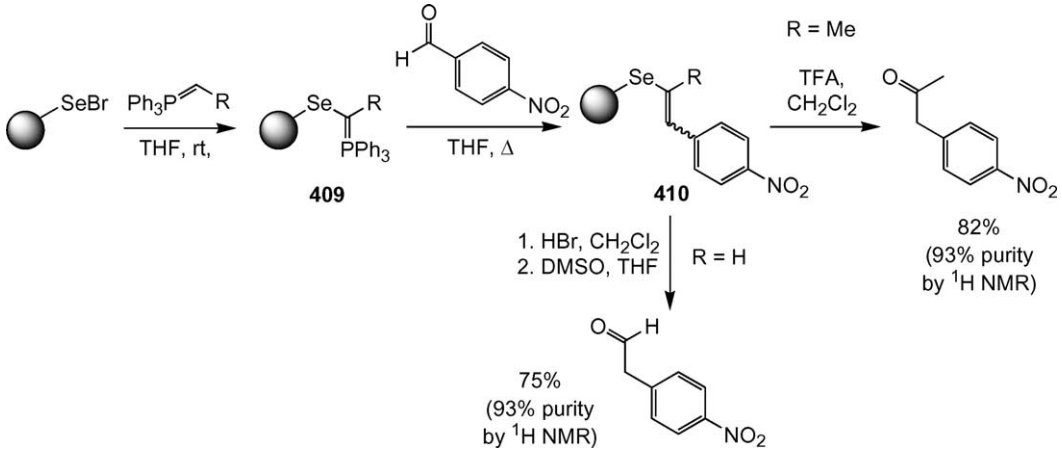
3.1.7. Selenide linkers cleaved using alternative methods. Huang and Sheng have reported a solid-phase approach to aldehydes and ketones using resin-bound selenoalkylidene-triphenylphosphoranes.[115] Treatment of selenenyl bromide resin with alkylidenetriphenylphosphoranes gave resins **409**. Subsequent reaction with aldehydes gave vinyl selenide resins **410**. Cleavage by treatment of **410** (R=H) with HBr and subsequent solvolysis with DMSO gave aldehydes, while cleavage of **410** (R=Me) with TFA gave ketones (Scheme 102). The use of more elaborate phosphoranes in the loading step gave poor overall yields of ketones after cleavage.[115]

4. Sulfur and selenium linkers in fluorous-phase synthesis

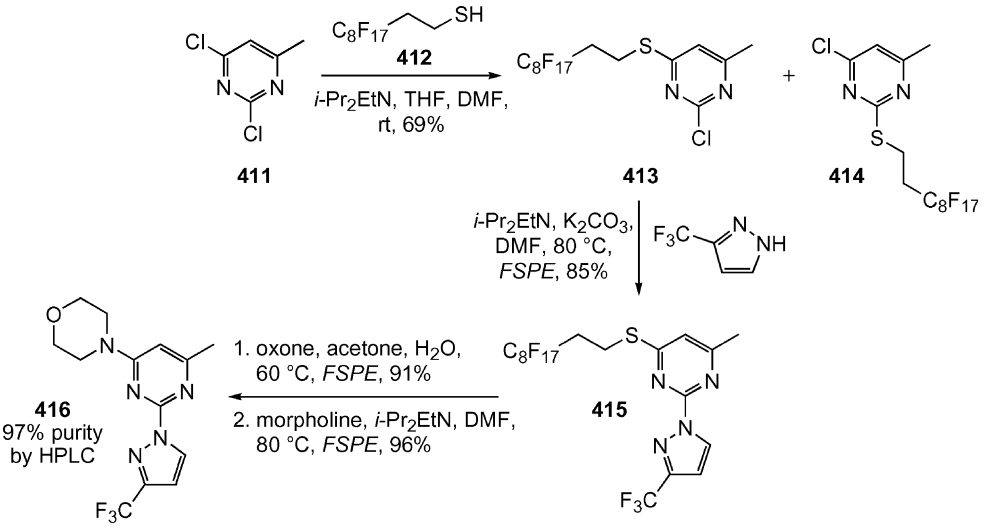
In recent years, sulfur linkers have begun to find application in fluorous-phase synthesis, where perfluoroalkyl phase tags replace polymeric supports.



Scheme 101.



Scheme 102.

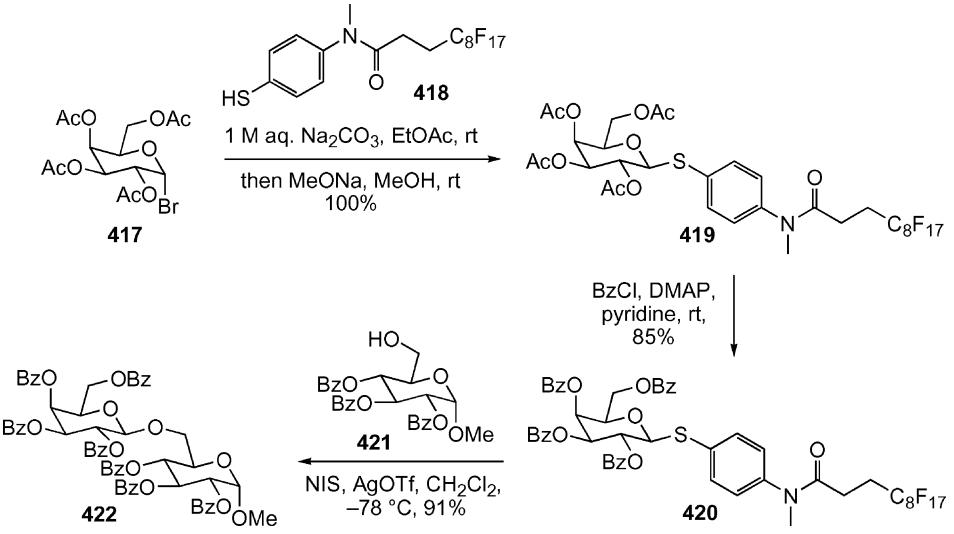


Scheme 103.

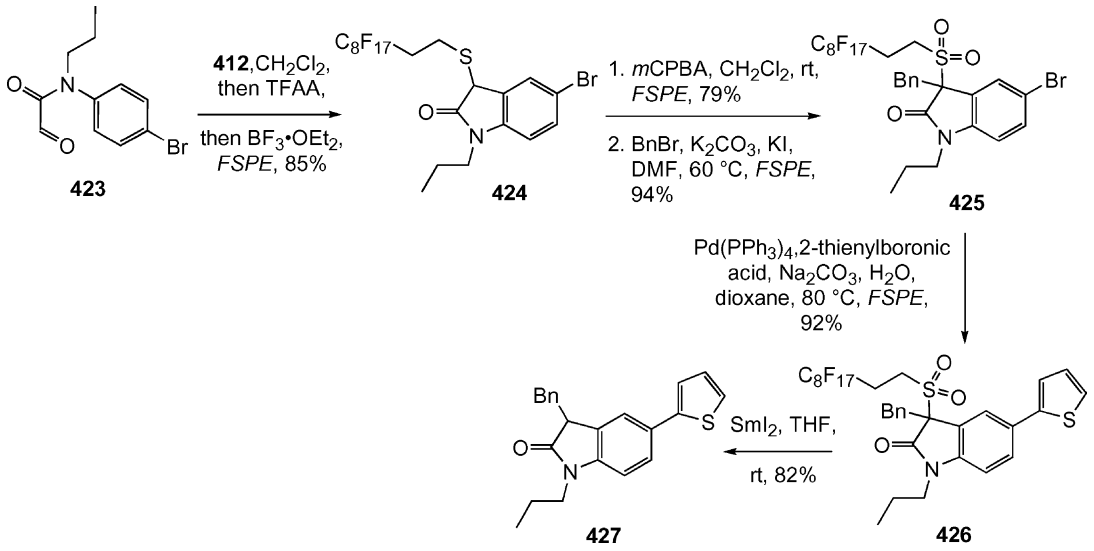
Zhang has developed a fluorous, catch-and-release approach for the synthesis of disubstituted pyrimidines. Fluorous solid-phase extraction (FSPE) was used for the rapid purification of intermediates and products.[116] The fluorous phase tag was introduced by treatment of 2,4-dichloro-6-methylpyrimidine **411** with 1*H*,1*H*,2*H*,2*H*-perfluorodecanethiol **412** (Scheme 103). The use of a solution phase tag allowed the major regioisomer **413** (the minor regioisomer was **414**) to be isolated using conventional chromatography, before nucleophilic substitution with 3-(trifluoromethyl)pyrazole to give **415**. The linking sulfur atom was then activated by oxidation to the sulfone before cleavage by nucleophilic substitution to give **416** (Scheme 103).[116]

Huang and Jing have developed a fluorous-phase synthesis of oligosaccharides using a sulfur linker system.[117] Fluorous thiol **418** was glycosylated with galactosyl bromide **417** to give thioglycoside donor **419** (Scheme 104). Switching of the protecting groups then gave benzoylated thioglycoside **420**. Simultaneous glycosylation and cleavage using acceptor **421** proceeded smoothly to form protected disaccharide **422** in excellent yield (Scheme 104). The sulfide link to the fluorous tag was found to be compatible with esterification, etherification, deacetylation, and glycosylation conditions and the fluorous thiol was recycled from the disulfide byproduct.[117]

Finally, Procter and co-workers have reported a fluorous-phase extension to his Pummerer approach to *N*-heterocycles (see Section 2.1.3, Scheme 12).[118] The improved approach allows rapid access to tagged heterocycles via a cyclative-capture step and is based on the addition of the fluorous thiol **412** to glyoxamides such as **423** (Scheme 105). The glyoxamide moiety in the substrate acts as a 'trigger' that, when attacked by the thiol, captures the fluorous phase tag in a Pummerer cyclisation embedding the tag in the framework of the resultant heterocycle **424**. FSPE can be used for rapid purification of the tagged heterocycles. The sulfide linkage in **425** was shown to be compatible with a variety of conditions including palladium-catalysed cross-couplings. The linkage in **426** was cleaved using SmI_2 to give product oxindoles, such as **427**, in high yield (Scheme 105).[118]



Scheme 104.



Scheme 105.

Variation of the glyoxamide substrate allows access to other *N*-heterocycles,[118] for example, cyclative capture of glyoxamide **428** with fluorous thiol **412** gave tagged tetrahydrobenzazepinone **429**. Modification of the heterocyclic framework by alkylation and cleavage of **430** using SmI_2 gave **431** in good yield (Scheme 106).[118]

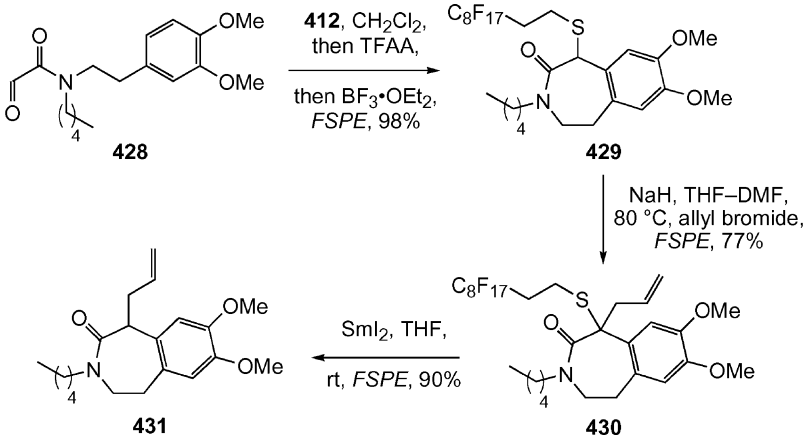
5. Conclusions

The emergence of solid-phase and combinatorial chemistry has led to renewed interest in sulfur and selenium chemistry. Classical reactions such as selenoxide elimination, the selenenylation of alkenes, sulfone alkylation, the epoxidation and cyclopropanation reactions of sulfonium ions, Pummerer reactions and the radical chemistry of selenides have found new roles in linker chemistry.

The scope and utility of sulfur and selenium in linker systems for solid-phase organic synthesis is now well established. The versatility of these linker systems lies in their ability to exist in several oxidation states. This is important for the development of safety-catch linkers, where activation of the linking sulfur or selenium atom is a prerequisite for cleavage, therefore reducing the risk of premature release of the substrate. In addition, sulfur and selenium can mediate a diverse range of chemical transformations in their different oxidation states and can thus be used to enable chemistry on, and allow further diversification of, resin-bound molecules. Attractively, selenium- and, to a lesser extent, sulfur-based linkers allow the use of cyclative capture strategies, where starting materials are immobilised with simultaneous generation of structural complexity.

In both sulfur and selenium linker systems, it has been shown that a variety of cleavage strategies are possible and many of these strategies allow different types of functionality to be introduced in the cleavage step, thus allowing diversity to be introduced at the end of a synthesis. Sulfur- and selenium-based linkers are robust and have been used in the successful synthesis of a large variety of compound types. Libraries varying in size from 10 to 10,000 have now been prepared using sulfur and selenium linker systems.

It seems clear that sulfur- and selenium-based linker systems will continue to play a major role as the area of high-throughput synthesis continues to evolve.



Scheme 106.

Acknowledgements

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Syntheses of per-^{15}N labeled etioporphyrins I–IV and a related tetrahydrobenzoporphyrin for applications in organic geochemistry and vibrational spectroscopy ☆

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Abstract—Nitrogen-15 labeled pyrroles have been prepared from commercially available ^{15}N glycine or sodium nitrite using the Barton-Zard, Knorr, and Kleinspehn approaches. These pyrroles were used as intermediates in the synthesis of per-^{15}N labeled porphyrins needed for the analysis and assignment of vibrational spectra for sedimentary porphyrins. Etioporphyrin-I was prepared via pyrromethene intermediates, while etioporphyrins II–V and a related tetrahydrobenzoporphyrin were synthesized via stepwise routes involving the copper(II) mediated cyclization of a,c-biladienes as the key step. Detailed analyses of both the proton and carbon-13 NMR spectra provide nitrogen-15 coupling constants for these important structures.
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1. Introduction

Metalloporphyrins are commonly found in organic-rich sediments such as oil shales and petroleum.[1,2] These materials are believed to be degradation products, or molecular fossils, of biological pigments such as the chlorophylls.[1,2] The distribution of porphyrin structural types can have diagnostic value in relation to determining thermal maturity and the origins of fossil fuels,[2] and may have applications in chemical prospecting.[1–3] Furthermore, analyses of petroporphyrins can also have value in environmental investigations.[4] The porphyrin distributions in oil shales and petroleum differ considerably from one source to another and hence can provide a characteristic fingerprint for specific sedimentary materials. As the metalloporphyrins found in petroleum are fairly resistant to weathering and biodegradation, chromatographic fingerprints for these compounds can be used in determining the origins of crude oils and tar balls resulting from oil spills. Resonance Raman spectroscopy has shown considerable promise in the analysis of petroporphyrins,[5–11] and for this reason a data base of vibrational spectra for metalloporphyrins has been developed and the characteristic modes of vibration for various structural types have been analyzed. These analyses can be aided by selective isotopic substitutions, such as the incorporation of deuterium, carbon-13, and nitrogen-15, but in many cases the isotopomers can only be obtained by total synthesis.

Several families of porphyrins are found in oil shales and petroleum, generally in the form of nickel(II) or vanadyl chelates, including cycloalkanoporphyrins (CAPs; **1**) with five-, six- or seven-membered exocyclic rings.[1,2] The best known of these geoporphyrins is deoxophylloerythroetioporphyrin (DPEP; **2**), a structure that is closely related to the chlorophylls (Chart 1).[1,2] Indeed, all of the known sedimentary porphyrins are believed to be derived from the chlorophylls and to a lesser extent the hemes, although the origins of some petroporphyrins are poorly understood.[1,2,12] In addition to cycloalkanoporphyrins, etioporphyrins **3** and **4** have been isolated and characterized by ^{1}H NMR spectroscopy and mass spectrometry.[13] There are four etio 'type isomers' where each pyrrole subunit has one ethyl and one methyl substituent: etioporphyrins-III (**3**), I (**5**), II (**6**), and IV (**7**).[14] However, virtually all naturally occurring porphyrins are structurally related to etioporphyrin-III, and while **3** and its 13-desethyl analogue **4** are present in sedimentary materials there is no evidence for the occurrence of etioporphyrins-I, II or IV (**5**–**7**).[2] The absence of the latter type isomers provides a degree of confirmation for the biological origins of petroporphyrins.[2] On the other hand, the four etioporphyrin isomers provide an important group of structures for spectroscopic analysis.[5–11]

☆ Part 18 in the series porphyrins with exocyclic rings. For part 17, see: Manley, J. M.; Roper, T. J.; Lash, T. D. *J. Org. Chem.* **2005**, *70*, 874.

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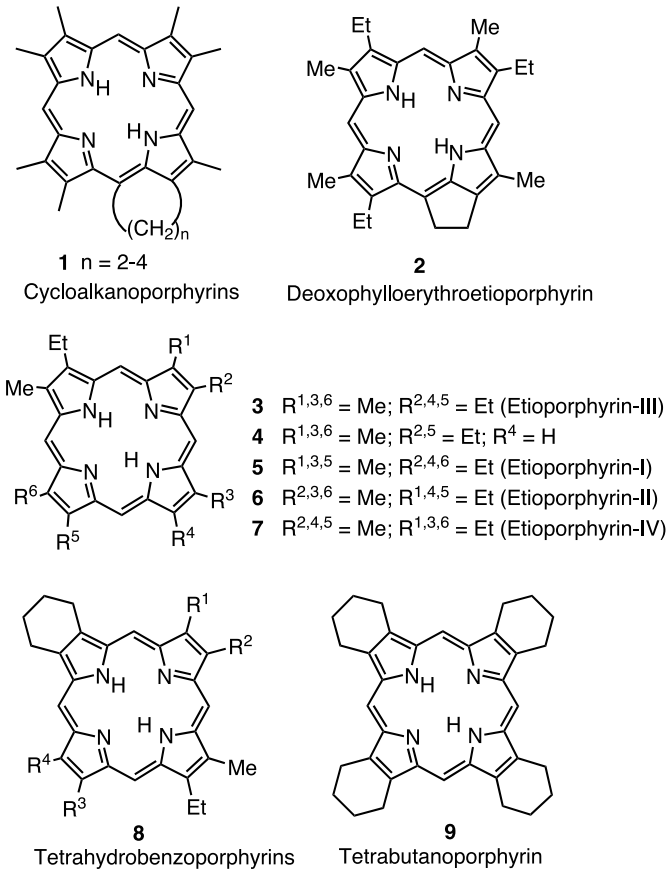


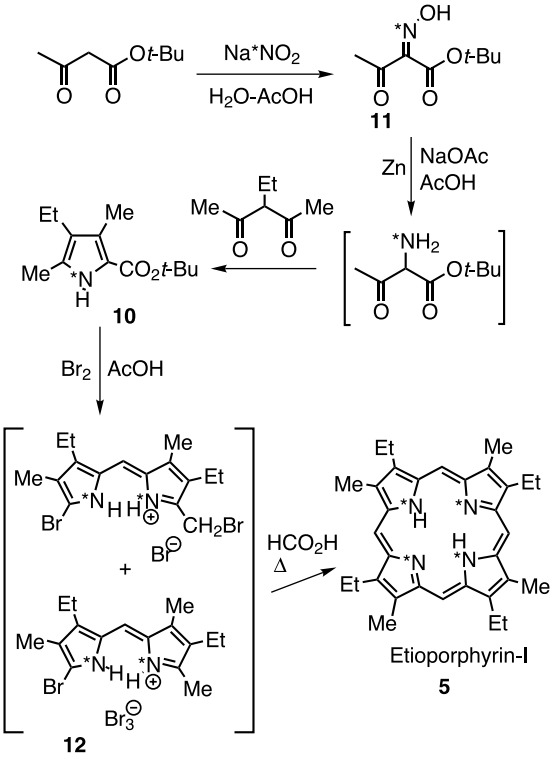
Chart 1.

In addition to the CAPs and etioporphyrins, other families of sedimentary porphyrins include tetrahydrobenzoporphyrins[13,15] (e.g., **8**) and the related benzoporphyrins.[13,16] A series of four isomeric nickel(II) chelates of **8**, together with the symmetrical tetrabutanoporphyrin **9**, have been studied in detail using resonance Raman spectroscopy[7] and labeled versions of **9** with 4 ^{13}C *meso*-carbons and/or 4 ^{15}N's were synthesized as spectroscopic models.[17] per-^{15}N labeled samples of etioporphyrins I–IV and a related tetrahydrobenzoporphyrin were also required as spectroscopic standards in order to more fully assign the vibrational spectra. These compounds represent a considerably greater synthetic challenge due to their relative lack of symmetry, particularly in the case of etioporphyrin-III and butanoporphyrin **8**. In this paper, syntheses of per-^{15}N labeled porphyrins **3** and **5**–**8** are reported.[18,19] In addition, we took the opportunity to investigate ^{15}N coupling in both the proton and carbon-13 NMR spectra for these important parent structures.[20]

2. Results and discussion

2.1. Etioporphyrin-I

The four etioporphyrin type isomers have varying degrees of symmetry and this led us to consider a number of methods for their synthesis.[21] Etioporphyrin-I represented the most symmetrical of these systems and hence was the easiest to synthesize. In this case, we adapted the classical Fischer pyrromethene methodology using conditions



Scheme 1.

reported earlier by Smith (Scheme 1).[22,23] Initially, a labeled pyrrole **10** was required and this was prepared by the Johnson modified Knorr pyrrole condensation.[24] Treatment of *tert*-butyl acetoacetate with ^{15}N labeled sodium nitrite and acetic acid afforded the labeled oxime **11**. This was taken on in crude form and reduced with zinc dust and acetic acid in the presence of 3-ethyl-2,4-pentanedione. Reduction affords an unstable aminoketone

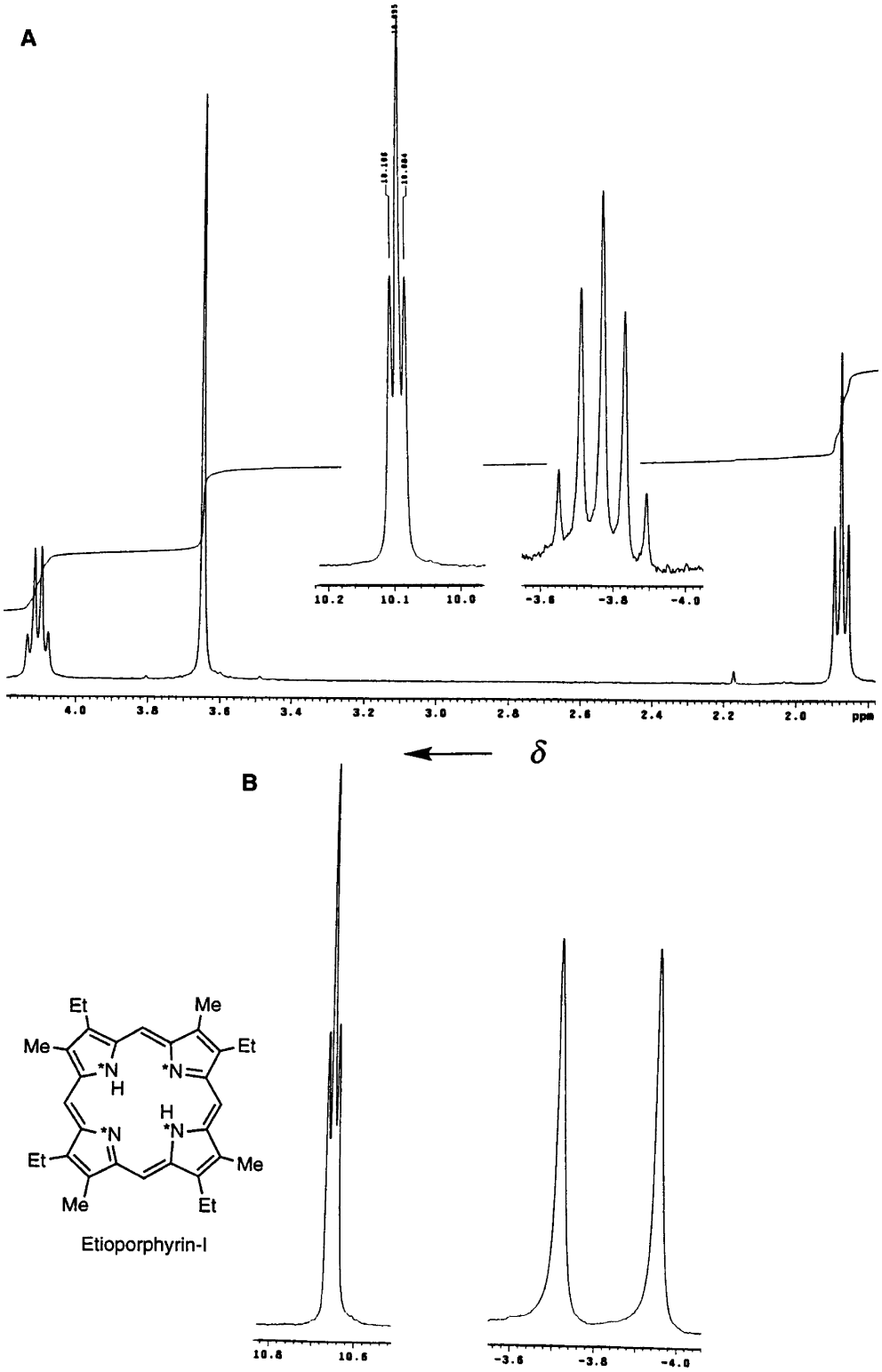


Figure 1. 400 MHz proton NMR spectra of per-^{15}N etioporphyrin-I. (A). Free base in $CDCl_3$. The NH protons appear as a quintet at −3.8 ppm from coupling ($^1J_{NH}=-24$ Hz) from all four nitrogen-15's due to rapid intramolecular exchange within the macrocyclic cavity. The *meso*-protons produce a triplet at 10.6 ppm due to coupling from 2 equiv nitrogen-15 nuclei. (B). Dication in TFA–$CDCl_3$. Only the upfield and downfield regions are shown. The *meso*-protons again appear as a triplet due to ^{15}N coupling but the internal protons are now fixed and only couple to the directly attached nitrogen to give a doublet ($J=-94.4$ Hz).

and this further condenses with the β-diketone to give the required pyrrole **10** in an overall 38% yield from sodium nitrite (Scheme 1). The proton NMR spectrum for the ^{15}N labeled pyrrole showed a characteristic $^{1}J_{NH}$ coupling (for ^{15}N, $I=½$) for the pyrrole proton of -97 Hz.[25] In some spectra, broadened or distorted peaks were observed for the NH resonance and this phenomenon was also seen for other pyrrolic intermediates in our studies. This was due

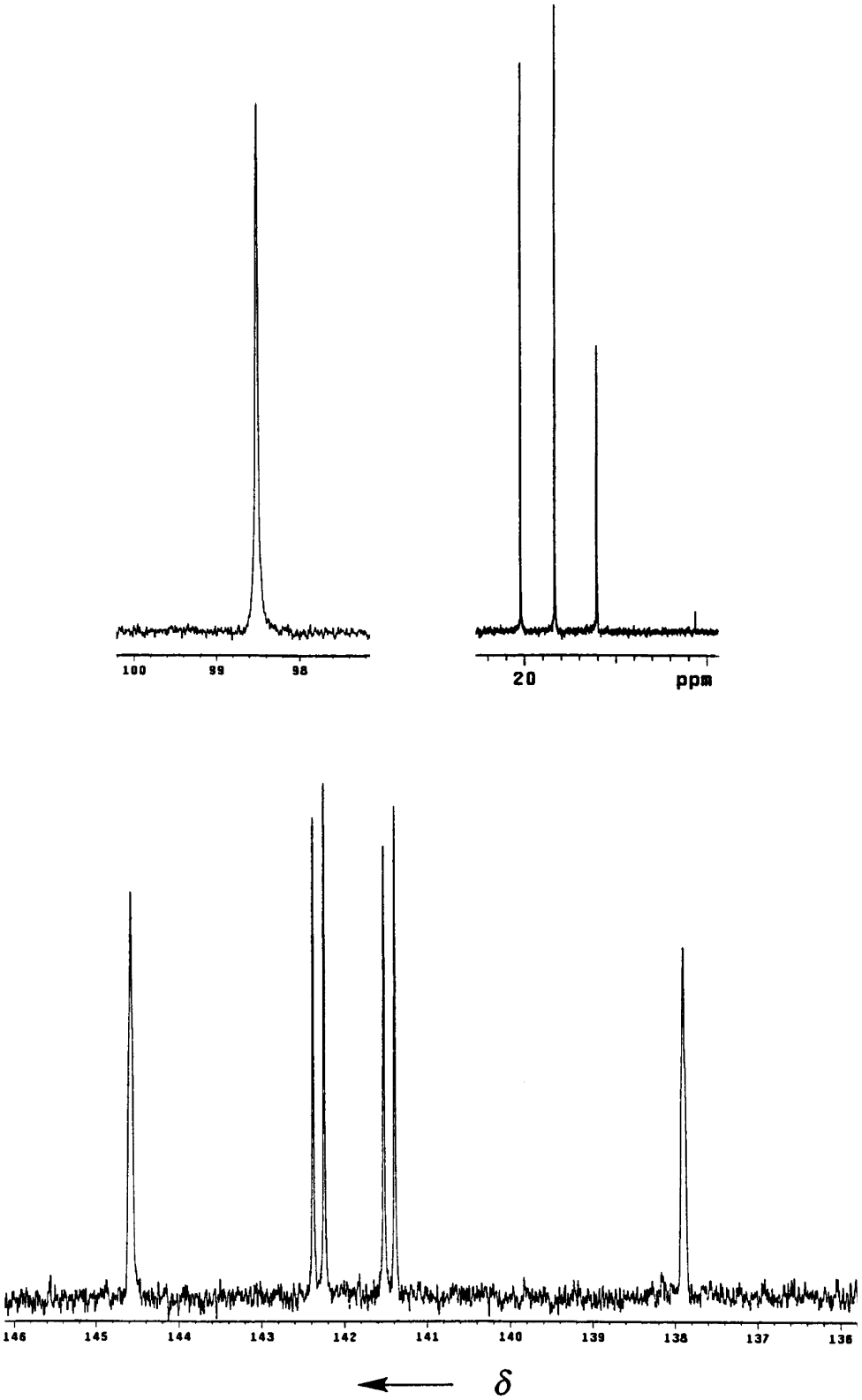


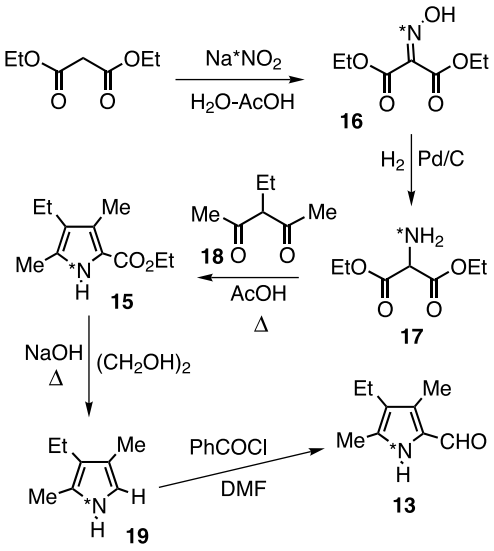
Figure 2. 100 MHz carbon-13 NMR spectrum of per-^{15}N etioporphyrin-I in TFA–$CDCl_3$. Due to the high degree of symmetry in etioporphyrin-I, there should only be three alkyl resonances, one *meso*-resonance (observed at 98.5 ppm) and four pyrrole resonances (137–145 ppm). The α-pyrrole carbons give doublets ($^{1}J_{NC}=13$ Hz) at 141.4 and 142.3 ppm, while the β-carbons show broadened resonances at 137.9 and 144.6 ppm.

to slight temperature changes after the NMR tube was introduced into the probe and was caused by minor shifts occurring during the data acquisition. This effect is not observed for unlabeled pyrroles due to quadrupole broadening and in any case the problem is easily dealt with by allowing the temperature of the sample to equilibrate for a few minutes prior to running the spectrum. Coupling was also observed to the 5-methyl unit, which appeared as a doublet ($^3J_{NH}$=2.8 Hz). In the carbon-13 NMR spectrum, the α carbons gave $^1J_{NC}$ coupling of 14–15 Hz, while the β-carbons afforded more weakly coupled doublets ($^2J_{CN}$= 4 Hz). Weak couplings to two of the β-aliphatic resonances were also noted.

Treatment of **10** with bromine in acetic acid afforded the pyrromethenes **12** and these were treated with refluxing formic acid to give etioporphyrin-I (**5**) in an overall 21% yield (Scheme 1). The 400 MHz proton NMR spectrum in $CDCl_3$ (Fig. 1A) showed a triplet for the 4 equiv *meso*-protons at 10.1 ppm due to coupling from 2 equiv nitrogen-15 nuclei with a coupling constant of 4.4 Hz. The four methyl units are equivalent and give rise to a singlet at 3.67 ppm, the downfield shift being due to the expected strong diamagnetic ring current for the porphyrin nucleus, while the 4 equiv ethyl moieties afford a 12H triplet and an 8H quartet at 1.87 and 4.10 ppm, respectively. Finally, the internal NH protons give a quintet with a coupling constant $J=-24$ Hz.[25] This arises from rapid intramolecular NH exchange such that both protons are coupled equally to all four nitrogen-15 nuclei. The data also demonstrates that intermolecular exchange is not occurring on the NMR timescale. This phenomenon has been reported previously for other porphyrins and is, of course, temperature dependent.[26] Addition of TFA affords the corresponding dication $\mathbf{5}H_2^{2+}$. The *meso*-protons still give a triplet ($^3J_{NH}$= 5 Hz) but the four NH protons now appear as a strongly coupled doublet ($^1J_{NH}=-94.4$ Hz) at −3.8 ppm (Fig. 1B). In the diprotonated species, the individual NH's are fixed and do not exchange, so only one bond coupling to a single nitrogen-15 is observed. The carbon-13 NMR spectrum for per-^{15}N etioporphyrin-I in TFA–$CDCl_3$ showed the presence of three alkyl carbons between 12 and 21 ppm, a single *meso*-carbon at 99 ppm and four sets of resonances for the 4 equiv pyrrole units between 137 and 145 ppm (Fig. 2). The α-carbons afforded two doublets ($^1J_{NC}$= 13 Hz) near 142 ppm, while the β-carbons gave broadened resonances at 137.9 and 144.6 ppm (the 2 bond NC coupling did not resolve in this case, although this can be observed for some of the examples noted below).

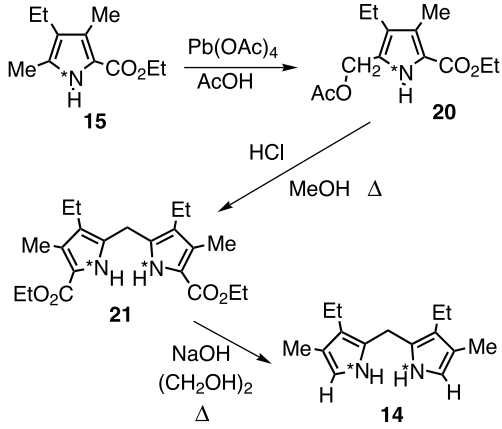
2.2. Etioporphyrin-II

Synthesis of the remaining etioporphyrins requires the use of a more stepwise approach for generating the porphyrin nucleus. A number of routes have been developed for synthesizing structures of this type,[21] but it is critically important that the method chosen would not give rise to any isomeric impurities. Due to our previous experience in this area,[27,28] we selected the well established a,c-biladiene methodology[29–31] for these studies. The synthesis of etioporphyrin-II required the pyrrole aldehyde **13** (Scheme 2) and the symmetrical dipyrrylmethane **14** (Scheme 3). Both of these compounds were prepared from



Scheme 2.

the pyrrole ethyl ester **15**. This key pyrrolic intermediate could be synthesized by the methodology used in preparing the related *tert*-butyl ester **10**, but in this case we selected the use of Kleinspehn's pyrrole synthesis[32] using the modified conditions reported by Paine and Dolphin.[27,33] Treatment of a slight excess of diethyl malonate with aqueous sodium nitrite in acetic acid afforded the oxime **16** and this was further reduced with hydrogen over 10% palladium–charcoal to give diethyl aminomalonate (**17**) (Scheme 2). In this work, a slight excess of diethyl malonate was used over literature procedures. While this resulted in residual diethyl malonate contaminating both **16** and **17**, the presence of this material did not interfere with the subsequent chemistry. Reaction of crude **17** with 3-ethyl-2,4-pentanedione (**18**) in refluxing acetic acid afforded the pivotal pyrrole **15** in 33–38% overall yield. The 400 MHz proton NMR spectrum for ^{15}N labeled **15** in $CDCl_3$ (Fig. 3) showed the NH as a strongly coupled doublet ($^1J_{NH}=-96$ Hz) at 8.8 ppm. In addition, the 5-methyl substituent gave a doublet ($^3J_{NH}$=2.8 Hz) at 2.20 ppm. In the carbon-13 NMR spectrum (Fig. 4), all four pyrrole carbons appeared as doublets and some long range coupling to two of the alkyl resonances was also noted. Treatment of **15** with sodium hydroxide in ethylene glycol at 180–190 °C cleaved



Scheme 3.

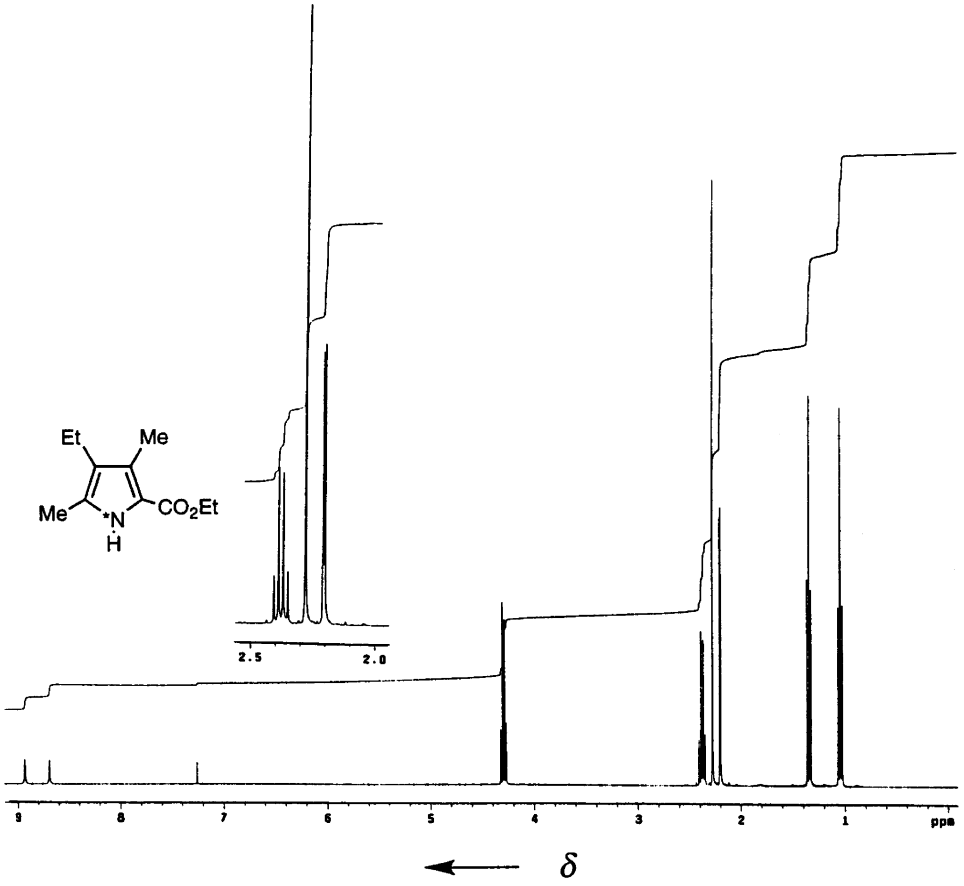


Figure 3. 400 MHz proton NMR spectrum of ^{15}N labeled pyrrole ethyl ester **15**. The doublet at 8.8 ppm corresponds to the pyrrole NH ($^{1}J_{NH}=-96$ Hz). Insert: the ^{15}N coupled doublet at 2.2 ppm corresponds to the 5-methyl substituent ($^{3}J_{NH}=2.8$ Hz).

the ester moiety to give the α-unsubstitued pyrrole **19** and subsequent formylation using Clezy's modified Vilsmeier–Haack conditions[34] afforded the required pyrrole aldehyde **13**. The proton NMR spectrum for ^{15}N labeled **19** gave a doublet of doublets at 7.47 ppm for the NH ($^{1}J_{NH}=-94.5$ Hz, $^{3}J_{HH}=2.7$ Hz) due to coupling from the ^{15}N and the adjacent CH. The pyrrole CH gave a broadened multiplet from these interactions. In the case of the aldehyde **13**, the proton NMR showed the formyl proton as a doublet ($^{3}J_{NH}=2.4$ Hz) and the 5-methyl group also demonstrated ^{15}N coupling. The NH showed up as the usual strongly coupled doublet at 9.9 ppm ($^{1}J_{NH}=-97$ Hz).

Reaction of pyrrole **15** with lead tetraacetate in acetic acid afforded the related acetoxymethyl derivative **20** and subsequent self-condensation in refluxing HCl–methanol gave the symmetrical dipyrrylmethane **21** (Scheme 3). The 400 MHz proton NMR spectrum of labeled **21** showed the usual NH doublet and a triplet for the bridging methylene ($^{3}J_{NH}=2.4$ Hz) at 3.86 ppm due to coupling from the 2 equiv nitrogen-15 nuclei (Fig. 5). Treatment of **21** with sodium hydroxide in refluxing ethylene glycol afforded the required diunsubstituted dipyrrylmethane **14**. Condensation of **14** with 2 equiv of pyrrole aldehyde **13** in the presence of HBr afforded the corresponding a,c-biladiene **22** in 67–76% yield (Scheme 4). The plane of symmetry in this structure allows the coupling interactions in the proton NMR spectrum to be assessed with ease. Of particular note, the terminal methyl produced a doublet ($^{3}J_{NH}=$ 2.8 Hz) at 2.7 ppm, the bridging CH_2 afforded a broadened partially resolved triplet at 5.2 ppm and the 2 equiv bridging methines gave a triplet ($^{3}J_{NH}=5.2$ Hz) at 7.1 ppm. Naturally, the NH protons appeared as two overlapping strongly coupled doublets ($^{1}J_{NH}=-95$ Hz) near 13.2 ppm. Cyclization of a,c-biladiene **22** with copper(II) chloride in DMF at room temperature[30] afforded the copper(II) complex of **6** and subsequent demetallation with 15% sulfuric acid–TFA gave etioporphyrin-II (Scheme 4) in 31–48% yield. The 400 MHz proton NMR spectrum of per-^{15}N-labeled **6** in $CDCl_3$ (Fig. 6A) again showed a quintet for the internal NH protons ($^{1}J_{NH}=-23$ Hz). Two different types of *meso*-protons are present in this type isomer and these produce two overlapping triplets at 10.1 ppm. In the presence of TFA (Fig. 6B), the dication $\mathbf{6}H_2^{2+}$ again gave two overlapping triplets for the *meso*-protons, albeit shifted downfield to 10.6 ppm, and the 4 equiv NH protons afforded a doublet at −3.4 ppm ($^{1}J_{NH}=-94$ Hz). The carbon-13 NMR spectrum for $\mathbf{6}H_2^{2+}$ in TFA–$CDCl_3$ (Fig. 7) gave two singlets between 98 and 99 for the *meso*-carbons and four doublets for the four types of pyrrolic carbons in this structure. The α-carbons appear as clear doublets near 142 ppm ($^{1}J_{NC}=13$ Hz), while the β-carbons resonated at 137.4 and 144.0 ppm ($^{2}J_{CN}=2$ Hz).

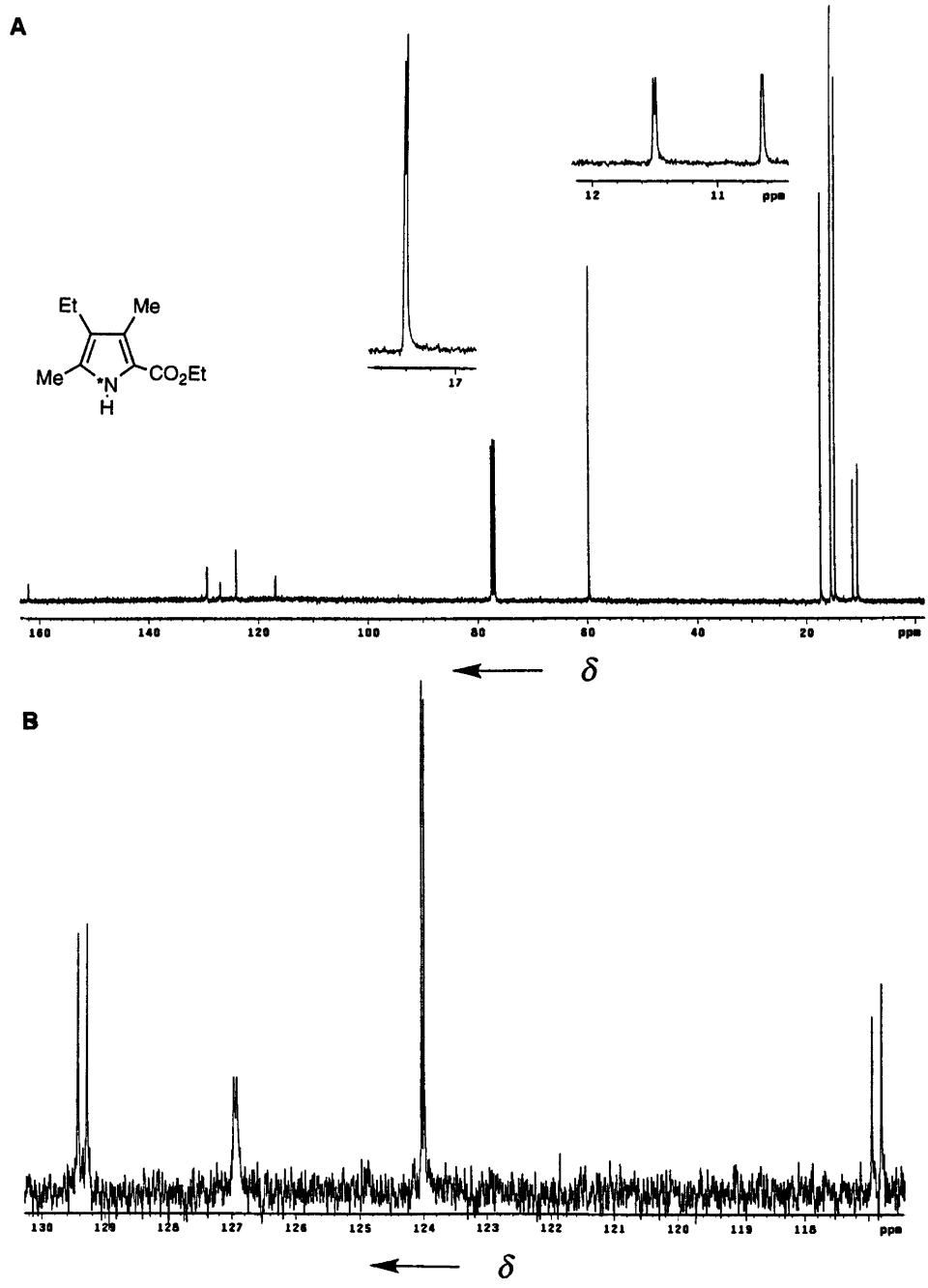


Figure 4. (A). 100 MHz carbon-13 NMR spectrum of ^{15}N labeled pyrrole ethyl ester **15**. Inserts: long range coupling to the resonances at 11.5 and 17.4 ppm is evident. (B). The aromatic region shows four doublets for the pyrrolic carbons.

2.3. Etioporphyrin-III

The synthesis of per-^{15}N etioporphyrin-III presented more of a challenge due to the complete asymmetry of this type isomer. This required the availability of an α-unsubstituted pyrrole **23**, (Scheme 5) which was needed for the synthesis of an unsymmetrical dipyrrylmethane **24** (Scheme 6). Previously, we reported[17] the synthesis of a nitrogen-15 labeled α-unsubstituted tetrahydroisoindole **25** from commercially available ^{15}N-glycine using the Barton-Zard pyrrole condensation[35] (Scheme 5). Due to solubility considerations, the pyrroles were synthesized as *n*-butyl rather than ethyl esters.[17] Treatment of glycine with *n*-butyl alcohol and 1 equiv of *p*-toluenesulfonic acid gave the ester **26** and this condensed with methyl formate in the presence of triethylamine to give the formamide **27**. Dehydration with $POCl_3$–Et_3N then afforded the isocyanoacetate **28**. Condensation of **28** with 1-nitrocyclohexene in the presence of DBU and THF gave **25**, while the nitroacetate **29** reacted under these conditions to give the required pyrrole *n*-butyl ester **23** (Scheme 5). In the latter case, an extra equivalent of DBU was used. This aids in eliminating the elements of acetic acid from **29**[35] to generate the reactive nitroalkene **30** in situ and this further reacts to afford pyrrole **23** in an overall 36% yield from glycine. The proton NMR spectrum of **23** in $CDCl_3$ shows a doublet of doublets for the NH ($^1J_{NH}=-98$ Hz, $^3J_{HH}=3$ Hz) and a triplet for the α-proton at 6.65 ppm (Fig. 8). The triplet results from coupling due to

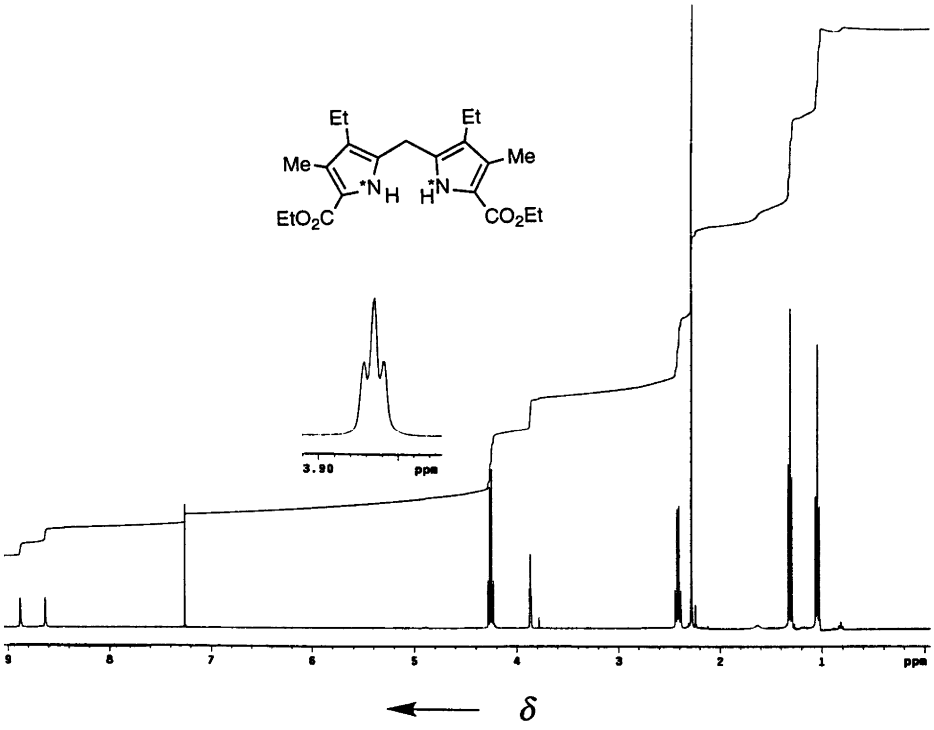
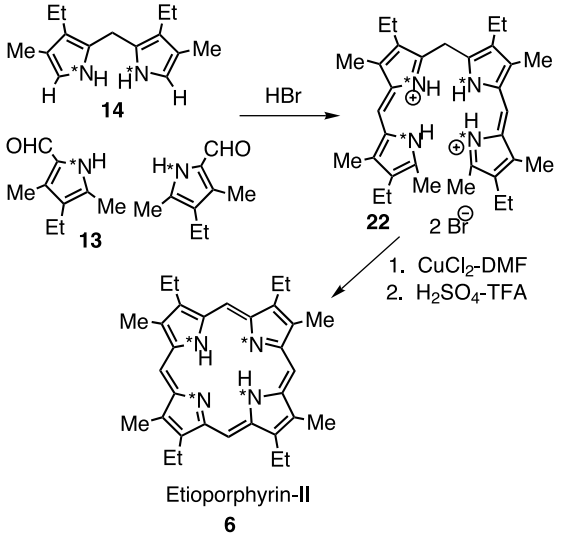


Figure 5. 400 MHz proton NMR spectrum of ^{15}N labeled dipyrrylmethane **21**. The doublet at 8.7 ppm corresponds to the pyrrole NH's ($^{1}J_{NH}=-96.8$ Hz). Insert: the ^{15}N coupled triplet at 3.86 ppm corresponds to the bridging methylene substituent ($^{3}J_{NH}=2.4$ Hz).

the ^{15}N (2 bond) and the NH (3 bond), as both of these interactions have coupling constants of approximately 3 Hz.

Condensation of acetoxymethylpyrrole **20** with **23** in the presence of Montmorillonite clay[36] in dichloromethane gave the asymmetrical dipyrrylmethane **24** (Scheme 6). Purification by flash chromatography and recrystallization from methanol gave **24** as a white powder in 81% yield. The 400 MHz proton NMR spectrum for labeled **24** in $CDCl_3$ showed two overlapping doublets for the NH protons and the bridging methylene gave a triplet ($^{3}J_{NH}=2.6$ Hz) at 3.86 ppm (Fig. 9). Cleavage of the ester moieties with NaOH in refluxing ethylene glycol afforded the deprotected dipyrrylmethane **31** and this condensed with 2 equiv of formylpyrrole **13** to give the a,c-biladiene **32**. The per-^{15}N labeled a,c-biladiene showed up to four overlapping doublets below 13 ppm for the four nonequivalent NH protons. Cyclization with $CuCl_2$–DMF and demetallation with 15% H_2SO_4–TFA gave etioporphyrin-III in good overall yields. The proton NMR spectra (Fig. 10) for per-^{15}N etioporphyrin-III showed many of the expected features, although the decreased symmetry made the resolution of coupled interactions more difficult. The 400 MHz proton NMR spectrum of **3** in $CDCl_3$ showed a quintet for the NH's at -3.75, but the four nonequivalent *meso*-protons afforded a multiplet near 10.1 ppm (Fig. 10A). The dication $\mathbf{3}H_2^{2+}$ in TFA–$CDCl_3$ showed strong ^{15}N coupling to the internal NH's (two overlapping doublets) and the *meso*-protons were better resolved, although they still produced a complex multiplet from 10.63–10.68 ppm (Fig. 10B). The carbon-13 NMR spectrum in TFA–$CDCl_3$ was poorly resolved due to the number of very similar carbons in this structure, but the *meso*-carbons showed four well resolved resonances between 98 and 99 ppm (Fig. 11).



Scheme 4.

2.4. Etioporphyrin-IV

The fourth etioporphyrin type isomer has a higher degree of symmetry and can be prepared from the same pyrrolic intermediates used in the previous studies. Condensation of pyrrole **23** with paraformaldehyde in the presence of *p*-toluenesulfonic acid gave the symmetrical dipyrrylmethane **33** in good yields (Scheme 7). Cleavage of the ester protective groups with sodium hydroxide in refluxing ethylene glycol gave the α-unsubstituted dipyrrylmethane

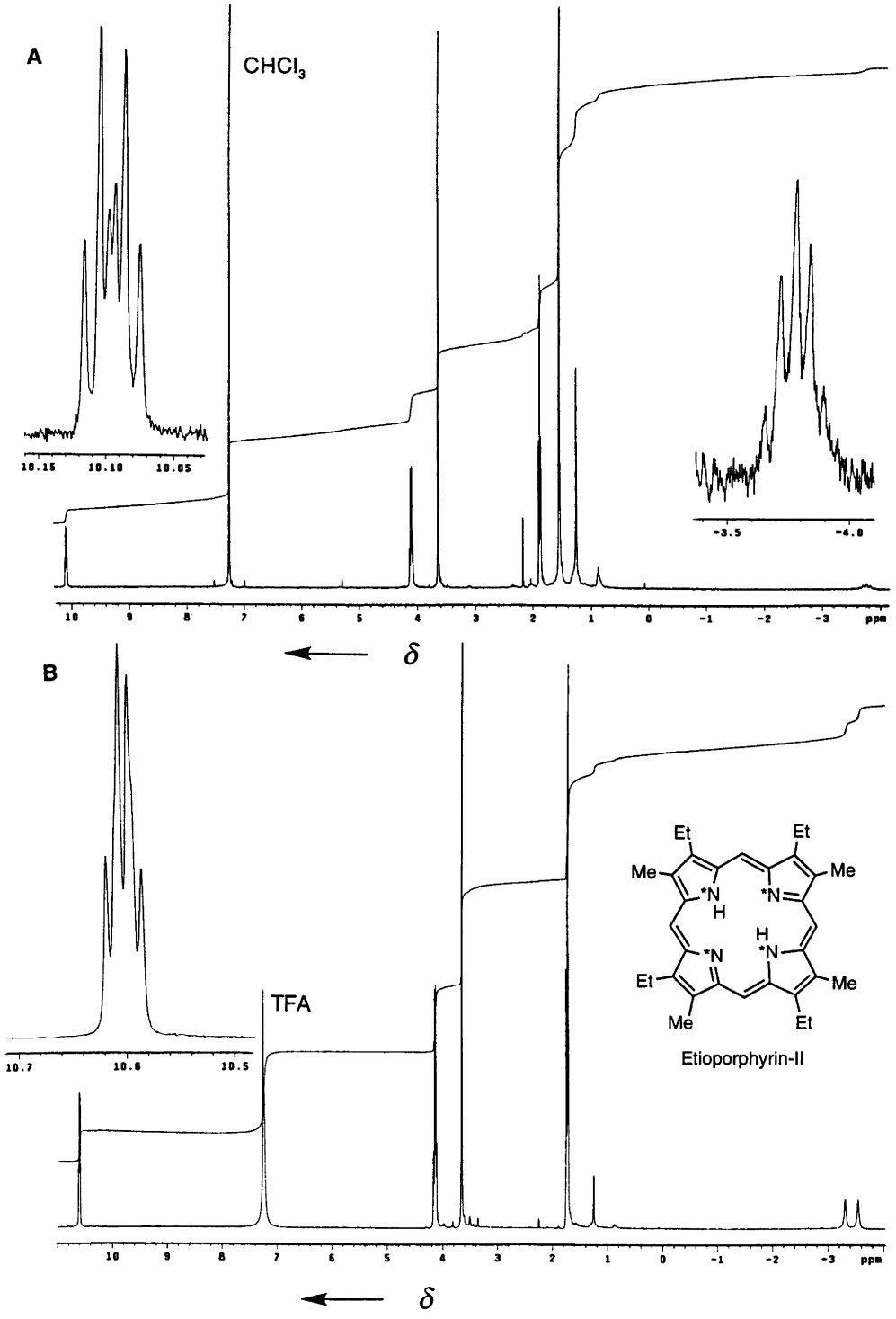


Figure 6. 400 MHz proton NMR spectra of per-^{15}N etioporphyrin-II. (A). Free base in $CDCl_3$. The NH protons appear as a quintet at -3.75 ppm from coupling ($^1J_{NH}=-23.2$ Hz) from all four nitrogen-15's due to rapid intramolecular exchange within the macrocyclic cavity. The two types of *meso*-protons produce two overlapping triplets at 10.1 ppm due to coupling from 2 equiv nitrogen-15 nuclei. (B). Dication in TFA–$CDCl_3$. The *meso*-protons again appear as a two overlapping triplets (10.6 ppm), while the internal protons now produce a doublet at -3.4 ppm ($J=-94$ Hz).

34 and this condensed with 2 equiv of pyrrole aldehyde **13** to give the a,c-biladiene **35**. The unlabeled a,c-biladiene showed two broad 2H singlets for the chemically distinct NH protons at 13.1–13.3 ppm, the methine bridge protons appeared as a singlet at 7.06 ppm and the central methylene bridge gave a singlet at 5.2 ppm (Fig. 12A). In the labeled a,c-biladiene, the NH protons produced two overlapping doublets ($^1J_{NH}=-95$ Hz), the methine bridges were coupled to two nitrogen-15's each to give a triplet ($^3J_{NH}=5$ Hz) and the methylene bridge afforded a broadened partially resolved triplet near 5.2 ppm (Fig. 12B). In addition, the terminal methyls afforded

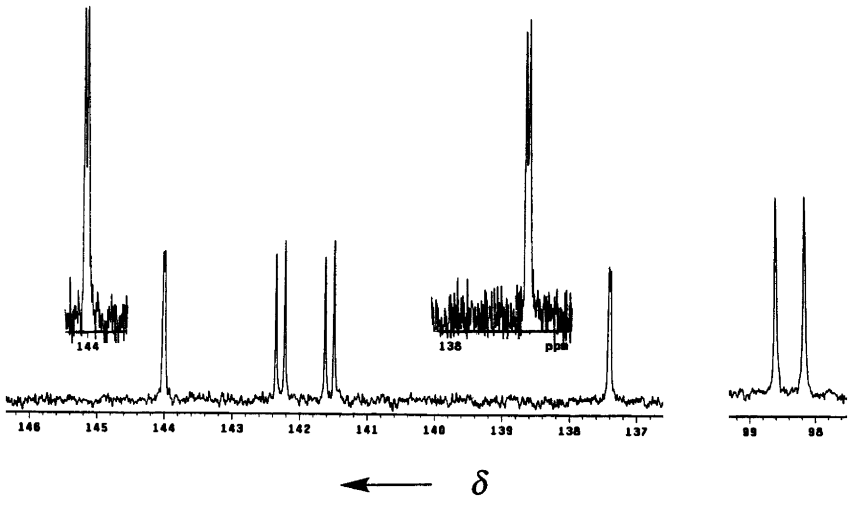
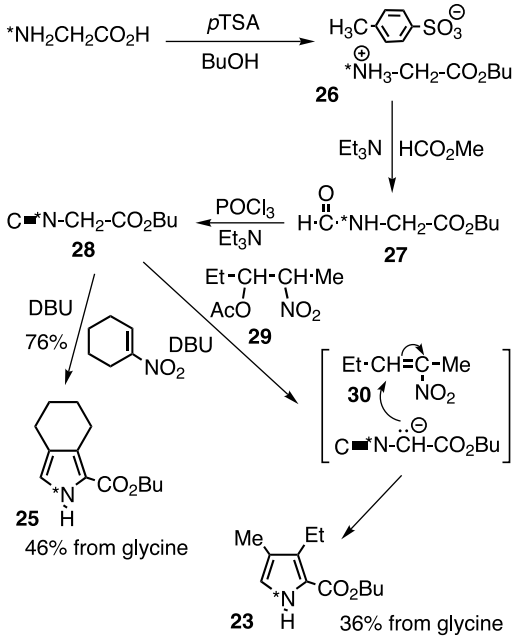


Figure 7. Downfield region for the 100 MHz carbon-13 NMR spectrum of per-^{15}N etioporphyrin-II in TFA–$CDCl_3$. Due to the symmetry in etioporphyrin-II, there are only two *meso*-resonances (observed at 98–99 ppm) and four pyrrole resonances (137–144 ppm). The α-pyrrole carbons give doublets ($^1J_{NC}$=13 Hz) at 141.5 and 142.3 ppm, while the β-carbons show more weakly coupled doublets at 137.4 and 144.0 ppm.

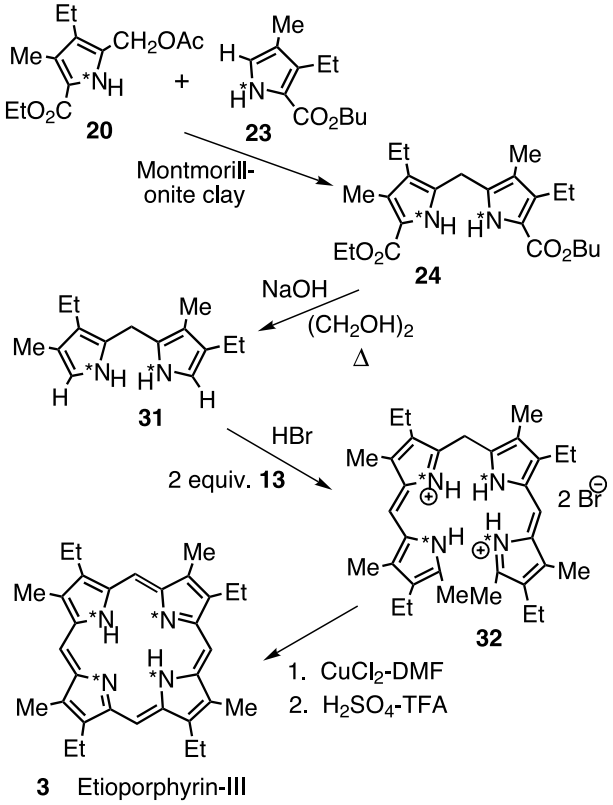
a 6H doublet ($^3J_{NH}$=2.4 Hz) at 2.7 ppm. Cyclization of **35** with copper(II) chloride in DMF, following by demetallation with 15% H_2SO_4–TFA gave etioporphyrin-IV (**7**) in 32–40% yield. Etioporphyrin has three types of *meso*-protons, although these are poorly resolved for either the free base or dication proton NMR spectra (Fig. 13). The NH protons give the usual quintet at −3.8 ppm for the free base ($CDCl_3$) and two overlapping doublets for the four internal protons in the dication (TFA–$CDCl_3$). The carbon-13 NMR spectrum for per-^{15}N **7** in TFA–$CDCl_3$ (Fig. 14) was reasonably well resolved. The four types of α-carbons gave rise to four doublets near 142 ppm, while the four different β-pyrrolic carbons gave two unresolved multiplets at 137.7–137.9 and 144.3–144.4 ppm. In addition, the three types of *meso*-protons afforded three resonances between 98 and 99 ppm that superficially resemble a triplet (ratio 1:2:1).



Scheme 5.

2.5. Tetrahydrobenzoporphyrin 8

The synthesis of tetrahydrobenzoporphyrin **8** was accomplished (Scheme 8) in a similar fashion to labeled etioporphyrin-III. Condensation of acetoxymethylpyrrole **20** with tetrahydroisoindole **25** in the presence of Montmorillonite clay gave, following careful flash chromatography to remove dipyrrylmethane biproducts, the required dipyrrole **36** in good yields. This was treated with NaOH in refluxing ethylene glycol to afford **37** and subsequent condensation with 2 equiv of **13** in the



Scheme 6.

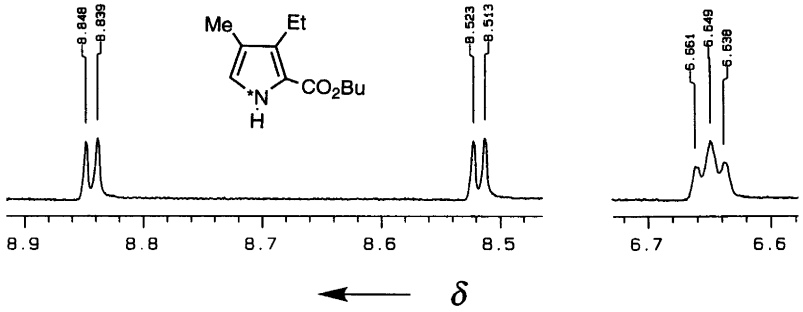


Figure 8. Downfield region for the 300 MHz proton NMR spectrum of ^{15}N labeled pyrrole butyl ester **23**. The pyrrole NH produces a doublet of doublets centered on 8.7 ppm ($^{1}J_{NH}=-96.8$ Hz; $^{3}J_{HH}=3$ Hz). The triplet at 6.65 ppm corresponds to the pyrrole CH, which is coupled to both the nitrogen-15 and the NH; the coupling constants for these two interactions are coincidentally both 3 Hz).

presence of HBr gave the a,c-biladiene **38**. Of note, the 400 MHz proton NMR spectrum showed four overlapping doublets for the NH protons between 12.9 and 13.5 ppm, while the two methine bridges gave two triplets near 7 ppm (Fig. 15). Cyclization with $CuCl_2$–DMF and demetallation as before gave the targeted tetrahydrobenzoporphyrin **8** (Scheme 8). This asymmetrical system gave well resolved proton NMR data (Fig. 16). The free base of per-^{15}N **8** in $CDCl_3$ gave a quintet at −3.8 ppm while the *meso*-protons produced four overlapping triplets near 10 ppm (Fig. 16A). The dication $\mathbf{8}H_2^{2+}$ in TFA–$CDCl_3$ gave three overlapping doublets for the inner NH protons, while the *meso*-protons again gave rise to four overlapping triplets (Fig. 16B). The carbon-13 NMR data for the per-^{15}N dication $\mathbf{8}H_2^{2+}$ in TFA–$CDCl_3$ was too complex to allow for complete analysis, although the *meso*-carbons gave four resonances in the 98–99 ppm range (Fig. 17).

3. Conclusions

By applying several methods, a series of nitrogen-15 labeled pyrroles have been prepared from commercially available ^{15}N sodium nitrite and ^{15}N-labeled glycine. These intermediates have been used to synthesize all four per-^{15}N labeled etioporphyrin type isomers, as well as a butano- or tetrahydrobenzoporphyrin. Etioporphyrin-I was prepared from a single pyrrole precursor via pyrromethene intermediates, but the remaining porphyrins were synthesized in a rational stepwise fashion via a,c-biladiene intermediates. The nitrogen-15 labeled intermediates and porphyrin products were carefully analyzed by proton and carbon-13 NMR spectroscopy and ^{15}N coupling constants for these important systems are reported. The labeled porphyrins will provide important information for the assignment of vibrational spectra and these results will have value in the analysis of fossil fuels and in environmental applications.[5–11,37]

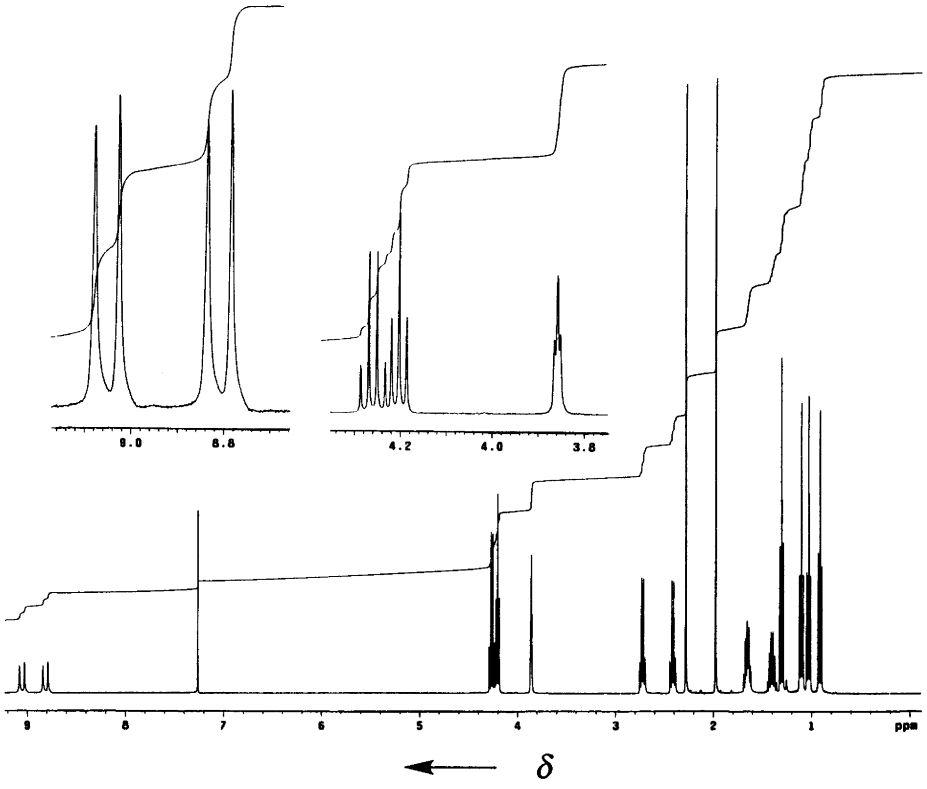


Figure 9. 400 MHz proton NMR spectrum of ^{15}N labeled dipyrrylmethane **24**. The two nonequivalent NH's produce two overlapping doublets at 8.8–9.0 ppm (left insert), while the ^{15}N coupled triplet at 3.86 ppm (right insert) corresponds to the bridging methylene substituent ($^{3}J_{NH}=2.6$ Hz).

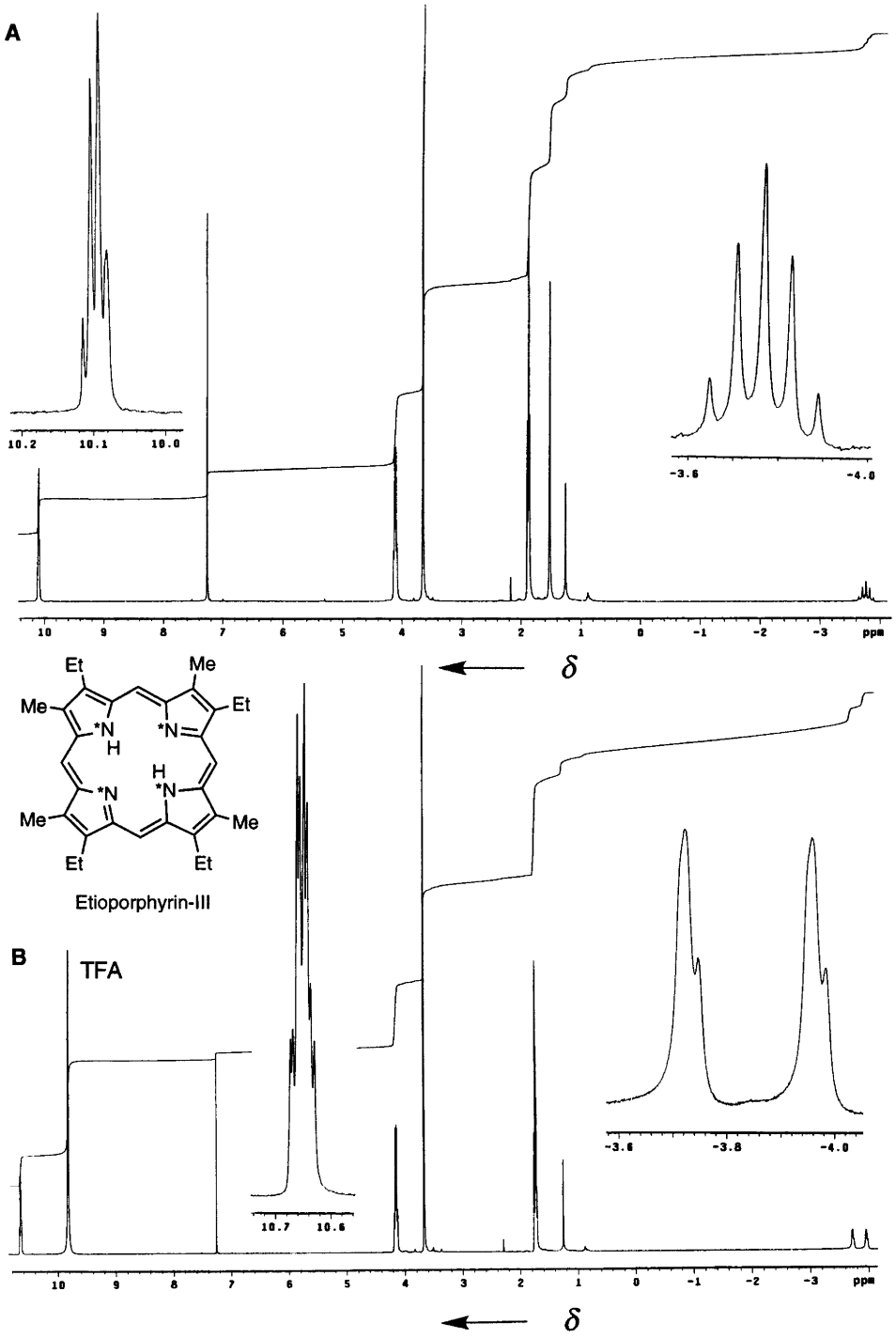


Figure 10. 400 MHz proton NMR spectra of per-^{15}N etioporphyrin-III. (A). Free base in $CDCl_3$. The NH protons appear as a quintet at -3.77 ppm from coupling ($^1J_{NH} = -24$ Hz) from all four nitrogen-15's due to rapid intramolecular exchange within the macrocyclic cavity. The *meso*-protons are all nonequivalent for this type isomer and produce a multiplet at 10.1 ppm. (B). Dication in TFA–$CDCl_3$. The *meso*-protons again appear as a complex multiplet, although more detail can be discerned. The internal NH protons are all nonequivalent and produce two overlapping nitrogen-15 coupled doublets -3.8 ppm.

4. Experimental

4.1. General

Nitrogen-15 labeled glycine (99%) and sodium nitrite (98%) were purchased from Cambridge Isotope Laboratories, Inc. All other reagents were purchased from Aldrich Chemical Co. and were used without further purification. Hydrogenations were carried out using a Parr hydrogenator at 30–40 psi. Chromatography was performed using Grade 3 neutral alumina or 70–230 mesh silica gel. Melting points were determined on a Thomas Hoover capillary melting point apparatus and are uncorrected; unless otherwise noted, the nitrogen-15 labeled compounds afforded the same mp values as the unlabeled materials. IR spectra were recorded on a Perkin-Elmer 1600 Series FT-IR Spectrometer and only selected absorptions (in reciprocal centimeters) are listed, while UV–vis spectra were obtained on a Beckmann

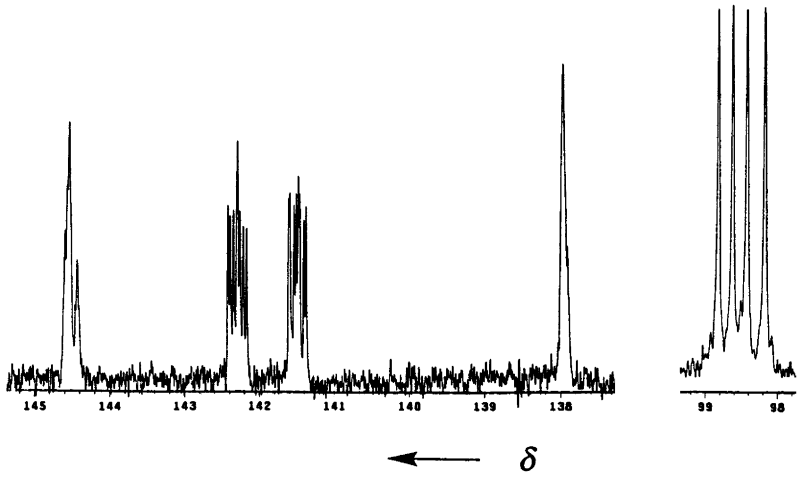
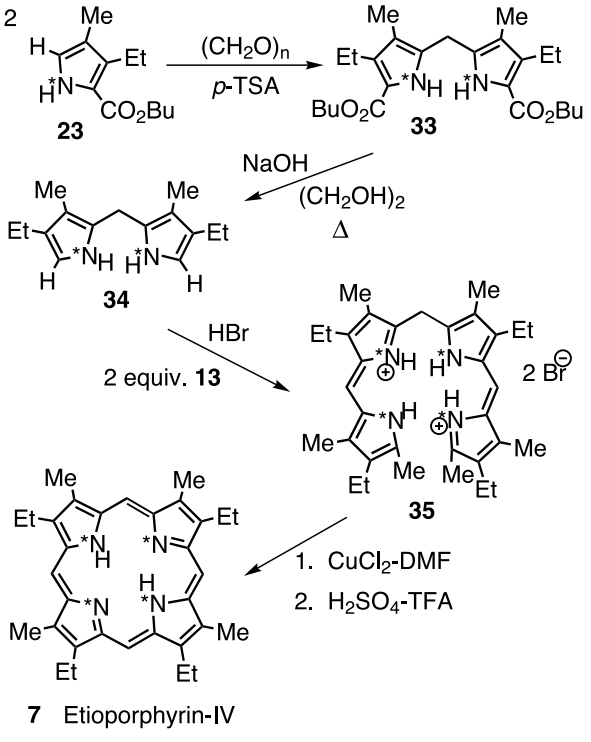


Figure 11. Downfield region for the 100 MHz carbon-13 NMR spectrum of per-^{15}N etioporphyrin-III in TFA–$CDCl_3$. Due to the lack of symmetry in etioporphyrin-III, there are four *meso*-resonances (observed at 98–99 ppm). In principle, there are 16 different pyrrolic carbons all of which may be coupled to the nitrogen-15 nuclei. These resonances fall into four regions but there is insufficient resolution to allow for further analysis.

DU-40 spectrophotometer. Proton and carbon-13 NMR spectra were recorded on Varian Gemini 300 or 400 MHz NMR spectrometers using in the solvent $CDCl_3$ as a standard (7.26 ppm of residual $CHCl_3$ in proton NMR and 77.23 ppm for the middle line of $CDCl_3$ in carbon-13 NMR spectroscopy). The deuteriochloroform used to record the spectra of free base porphyrins was purified by running it through basic alumina. Mass spectral determinations were made at the Mass Spectral Laboratory, School of Chemical Sciences, University of Illinois at Urbana-Champaign, supported in part by a grant from the National Institute of General Medical Sciences (GM 27029). Elemental analyses were obtained from the School of Chemical Sciences Microanalysis Laboratory at the University of Illinois.



Scheme 7.

4.2. Synthetic procedures

4.2.1. Synthesis of the pyrrolic precursors.

4.2.1.1. Ethyl 4-ethyl-3,5-dimethylpyrrole-2-carboxylate (15). Sodium hydroxide (0.35 g) was added to stirred glacial acetic acid (2.5 mL) in a 25 mL round bottom flask. Once the sodium hydroxide was dissolved, 3.0 mL of diethyl malonate were added followed by the dropwise addition of a solution of sodium nitrite (1.340 g; 0.020 mol) in water (2.2 mL) over a period of 1 h. The resulting mixture was stirred at room temperature overnight to allow the complete evolution of nitrogen dioxide. A solution of sodium hydroxide (0.633 g) in water (2.0 mL) was added to the mixture and an oily layer separated. Cold ether was added to the solution and gently swirled to allow the organic layers to mix together. The aqueous phase was removed and the organic layer washed with a solution of sodium bicarbonate (0.40 g) in water (2.5 mL), followed by water (2×3.5 mL). The organic layer was dried with magnesium sulfate and evaporated under reduced pressure to give the oxime **16** as an oil. The oil was dissolved in ethanol (25 mL) and placed in a hydrogenation vessel and washed in with additional ethanol (25 mL). After purging the flask with nitrogen gas, 10% palladium–charcoal (100 mg) was added, and the mixture was shaken under an atmosphere of hydrogen (45 psi) at room temperature overnight. The catalyst was filtered off and the solvent evaporated under reduced pressure to give diethyl aminomalonate (**17**) as a pale yellow oil. A solution of the crude aminomalonate in acetic acid (3 mL) was added to 3-ethyl-2,4-pentanedione (**18**) (1.90 g; 0.015 mol) in a gently boiling solution of glacial acetic acid (10 mL). After the evolution of carbon dioxide had subsided, the mixture was gently boiled for an additional 2 h. The light brown solution was diluted with ice/water and allowed to stand overnight. The resulting precipitate was filtered and recrystallized with ethanol/water to yield the title pyrrole as white crystals (1.260 g; 6.46 mmol; 33% from $NaNO_2$), mp 87.5–88 °C (lit. mp[32] 88–89 °C). IR (nujol mull): ν 3302 (st, sh, NH), 1665 cm^{-1} (st, sh, C=O); ^{1}H NMR ($CDCl_3$): δ 1.05 (3H, t), 1.35 (3H, t), 2.21 (3H, s), 2.28 (3H, s), 2.38 (2H, q), 4.29 (2H, q), 8.55 (1H, br s). *Nitrogen-15 labeled* **15**. Using the same

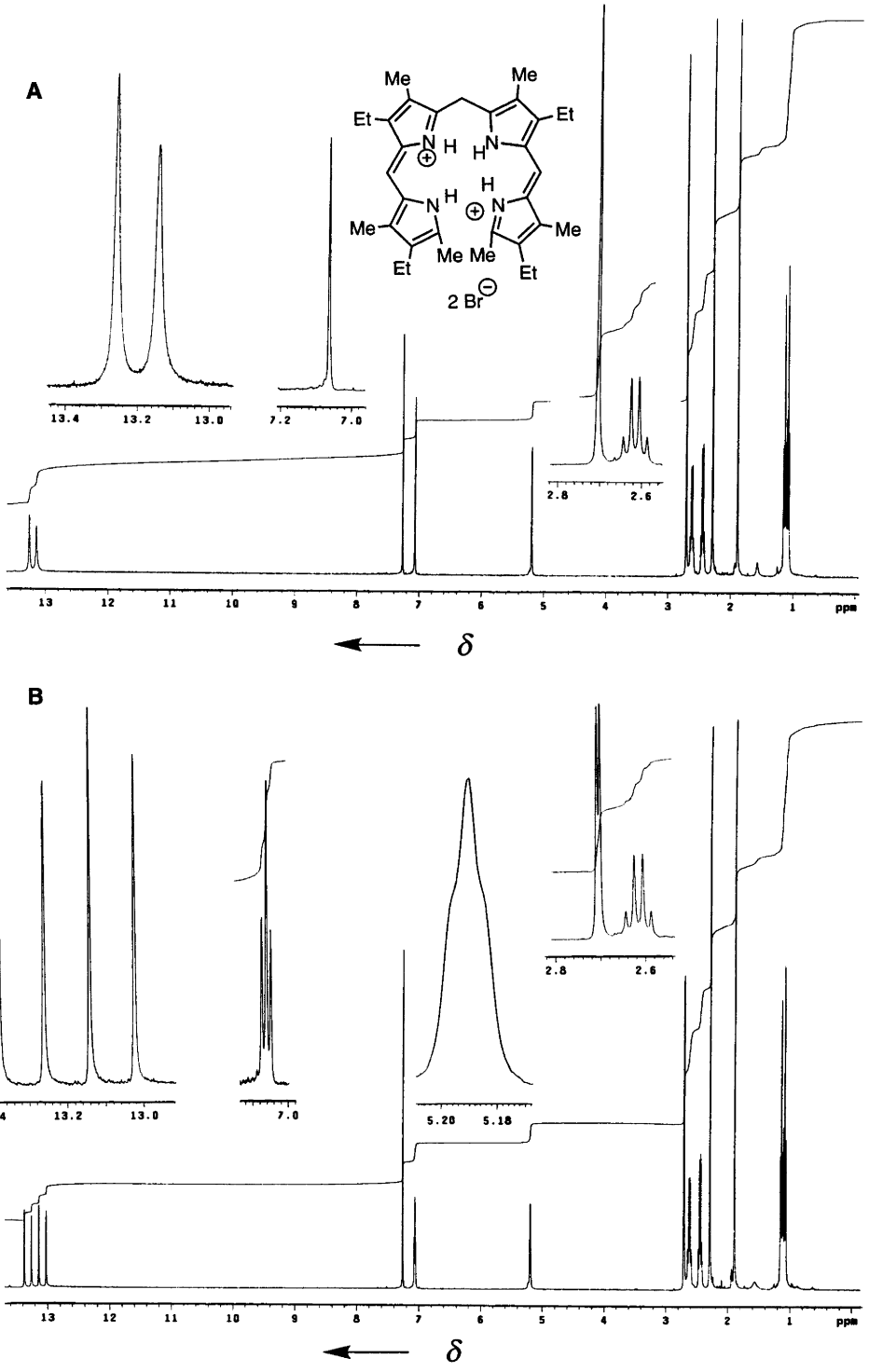


Figure 12. 400 MHz proton NMR spectra of a,c-biladiene **35**. (A). Unlabeled a,c-biladiene in $CDCl_3$. The plane of symmetry in **35** leads to two environments for the NH protons and these appear as two broadened singlets near 13.2 ppm. (B). per-^{15}N labled **35**. The NH protons now show up as two overlapping doublets between 13.0 and 13.4 ppm ($^1J_{NH}=-95$ Hz). The 2 equiv methine bridges give a triplet at 7.06 ppm ($^3J_{NH}=5$ Hz) while the bridging methylene produces an unresolved triplet at 5.2 ppm. The terminal methyl units are also coupled to the nearby nitrogen-15 nuclei and give a doublet at 2.7 ppm ($^3J_{NH}=$ 2.4 ppm).

procedure, 3.80 g of sodium nitrite (5.43 mmol; >98% nitrogen-15 labeled) gave the required pyrrole **15** (4.138 g; 21.1 mmol; 38%) as white crystals. IR (nujol mull): ν 3292 (st, sh, NH), 1665 cm^{-1} (st, sh, C=O). ^1H NMR ($CDCl_3$): δ 1.05 (3H, t, $^3J_{HH}=7.2$ Hz), 1.35 (3H, t, $^3J_{HH}=7.2$ Hz), 2.20 (3H, d, $^3J_{NH}=2.8$ Hz), 2.28 (3H, s), 2.38 (2H, q, $^3J_{HH}=7.2$ Hz), 4.29 (2H, q, $^3J_{HH}=7.2$ Hz), 8.81 (1H, d, $^1J_{NH}=-96$ Hz); ^{13}C NMR ($CDCl_3$): δ 10.6, 11.5 (d, $^2J_{NC}=1.9$ Hz), 14.8, 15.5, 17.4 (d, $^3J_{NC}=1.2$ Hz), 59.7, 116.8 (d, $^1J_{NC}=14.8$ Hz), 124.0 (d, $^2J_{NC}=3.8$ Hz), 127.0 (d, $^2J_{NC}=4$ Hz), 129.3 (d, $^1J_{NC}=14.0$ Hz), 162.0; MS (EI, 70 eV): *m/z* (%): 196 (61) [M^+], 181 (51) ([$M-CH_3$]$^+$),

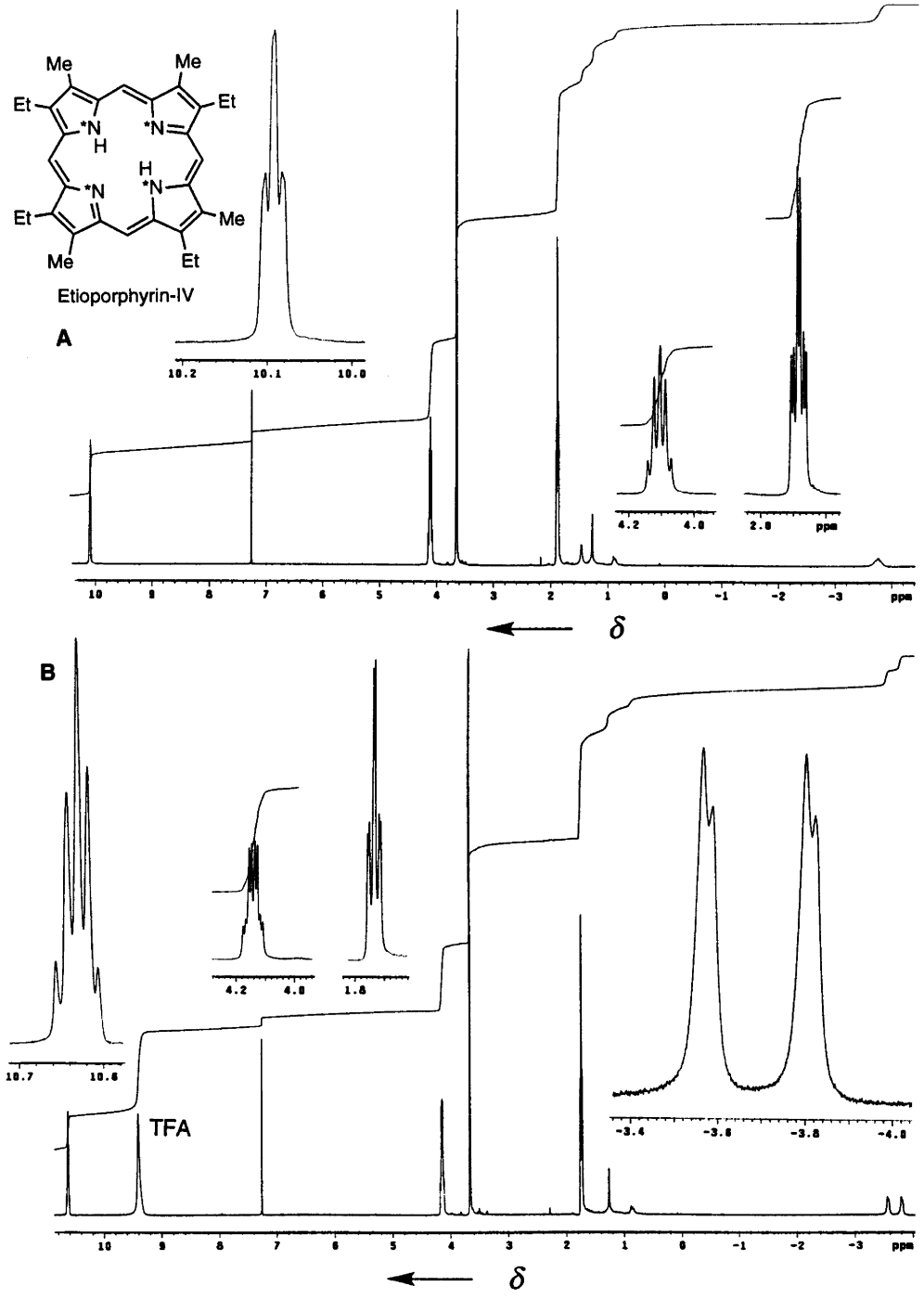


Figure 13. 400 MHz proton NMR spectra of per-^{15}N etioporphyrin-IV. (A). Free base in $CDCl_3$. The NH protons appear as a broadened quintet at −3.77 ppm while the two types of nonequivalent *meso*-protons produce a broadened triplet at 10.1 ppm. (B). Dication in TFA–$CDCl_3$. The *meso*-protons again appear as two overlapping triplets at 10.6–10.7 ppm. There are two types of internal NH protons and these produce two overlapping nitrogen-15 coupled doublets at −3.8 ppm ($^1J_{NH}=-94$ Hz).

167 (6) ($[M-C_2H_5]^+$), 151 (20) ($[M-OC_2H_5]^+$), 135 (100) ($[M-CH_3$ and $EtOH]^+$).

4.2.1.2. Ethyl 5-acetoxymethyl-4-ethyl-3-methyl-pyrrole-2-carboxylate (20). Ethyl 3,5-dimethyl-4-ethyl-pyrrole-2-carboxylate (**15**) (0.500 g; 2.56 mmol) were dissolved in a mixture of glacial acetic acid (8 mL) and acetic anhydride (4 mL). Lead tetraacetate (95%; 1.20 g; 2.56 mmol) was added and the resulting mixture was stirred overnight at room temperature. The red solution was poured into ice/water and the resulting precipitate was collected by filtration and washed with water until the washings remained neutral. The product was dried under vacuum and recrystallized from chloroform/petroleum ether to give the product as white crystals (0.504 g; 1.99 mmol; 78%), mp 128–129.5 °C; (lit. mp[38] 128 °C). ^{1}H NMR ($CDCl_3$): δ 1.09 (3H, t), 1.36 (3H, t), 2.08 (3H, s), 2.29 (3H, s), 2.46 (2H, q), 4.31 (2H, q), 5.03 (2H, s), 8.95 (1H, br s). *^{15}N labeled* **20**.^{1}H NMR ($CDCl_3$): δ 1.08 (3H, t, $^3J_{HH}=7.6$ Hz), 1.37 (3H, t, $^3J_{HH}=7$ Hz), 2.07 (3H, s), 2.28 (3H, s), 2.46 (2H, q,

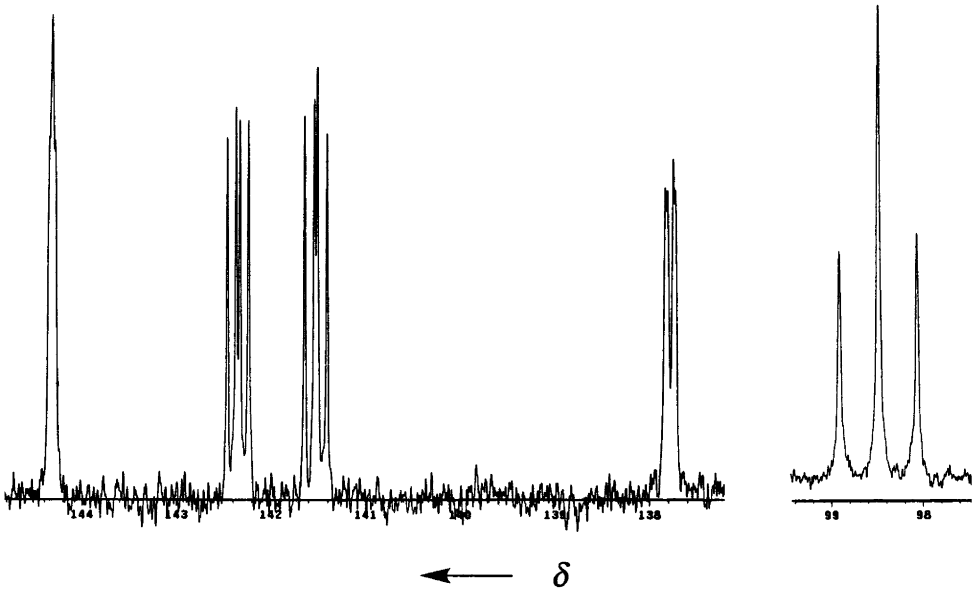
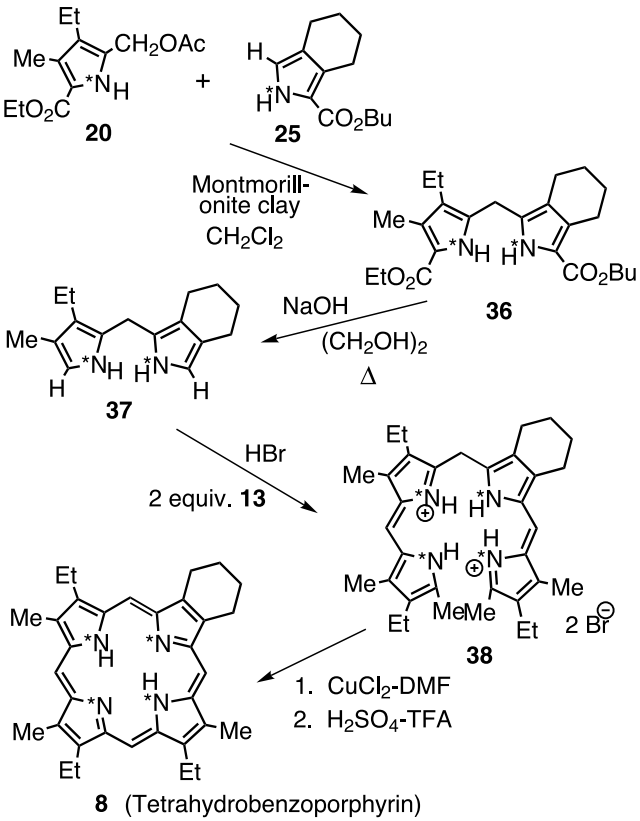


Figure 14. Downfield region for the 100 MHz carbon-13 NMR spectrum of per-^{15}N etioporphyrin-IV in TFA–$CDCl_3$. Due to the plane of symmetry in etioporphyrin-IV, there are three *meso*-resonances observed between 98 and 99 ppm. There are potentially a total of eight pyrrole resonances. The four α-pyrrole carbons give four doublets ($^1J_{NC}=13$ Hz) near 142 ppm, while the β-carbons show two multiplets near 138 and 144 ppm.

$^3J_{HH}=7.6$ Hz), 4.31 (2H, q, $^3J_{HH}=7.2$ Hz), 5.05 (2H, d, $^3J_{NH}=2.8$ Hz), 8.97 (1H, d, $^1J_{NH}=-98$ Hz); ^{13}C NMR ($CDCl_3$): δ 10.4, 14.7, 16.2, 17.3 (d, $^3J_{NC}=1.5$ Hz), 21.1, 57.1 (d, $^2J_{NC}=1.9$ Hz), 60.1, 88.68, 119.4 (d, $^1J_{NC}=14.8$ Hz), 126.2 (d, $^2J_{NC}=4.5$ Hz), 126.5 (d, $^1J_{NC}=14.4$ Hz), 127.1 (d, $^2J_{NC}=3.8$ Hz), 161.6, 171.8.

4.2.1.3. 4-Ethyl-3,5-dimethylpyrrole (19). Ethyl 4-ethyl-3,5-dimethylpyrrole-2-carboxylate (**15**) (0.500 g; 2.56 mmol) was heated with sodium hydroxide (1.00 g) and ethylene glycol (10 mL) under reflux for 1 h. The mixture was diluted with water and extracted with hexane. The hexane solution was dried over sodium sulfate and the solvent evaporated under reduced pressure to give a yellow oil (315 mg; quantitative). ^{1}H NMR ($CDCl_3$): δ 1.08 (3H, t), 2.02 (3H, s), 2.17 (3H, s), 2.39 (2H, q), 6.34 (1H, br), 7.5 (1H, br s). *^{15}N labeled* **19**. ^{1}H NMR ($CDCl_3$): δ 1.08 (3H, t, $^3J_{HH}=7.5$ Hz), 2.02 (3H, d, $^4J_{NH}=1.2$ Hz), 2.17 (3H, d, $^3J_{NH}=2.1$ Hz), 2.39 (2H, q, $^3J_{HH}=7.5$ Hz), 6.34 (1H, m), 7.47 (dd, $^1J_{NH}=94.5$ Hz, $^3J_{HH}=2.7$ Hz).



Scheme 8.

4.2.1.4. 4-Ethyl-3,5-dimethylpyrrole-2-carboxaldehyde (13). The foregoing oil (315 mg; 2.56 mmol) was dissolved in DMF (3 mL) and cooled to 0 °C on a salt/ice

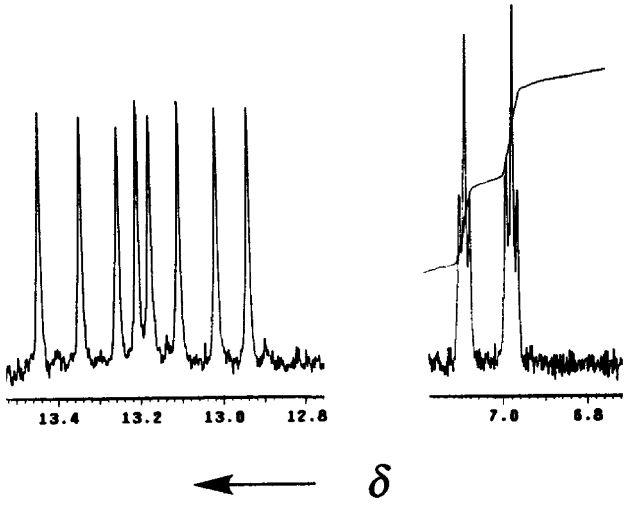


Figure 15. Downfield region for the 400 MHz proton NMR spectrum for a,c-biladiene **38**. The four nonequivalent NH protons produce a series of four overlapping doublets between 12.9 and 13.5 ppm. The two nonequivalent methine protons give triplets ($^3J_{NH}=5$ Hz) at 6.98 and 7.07 ppm.

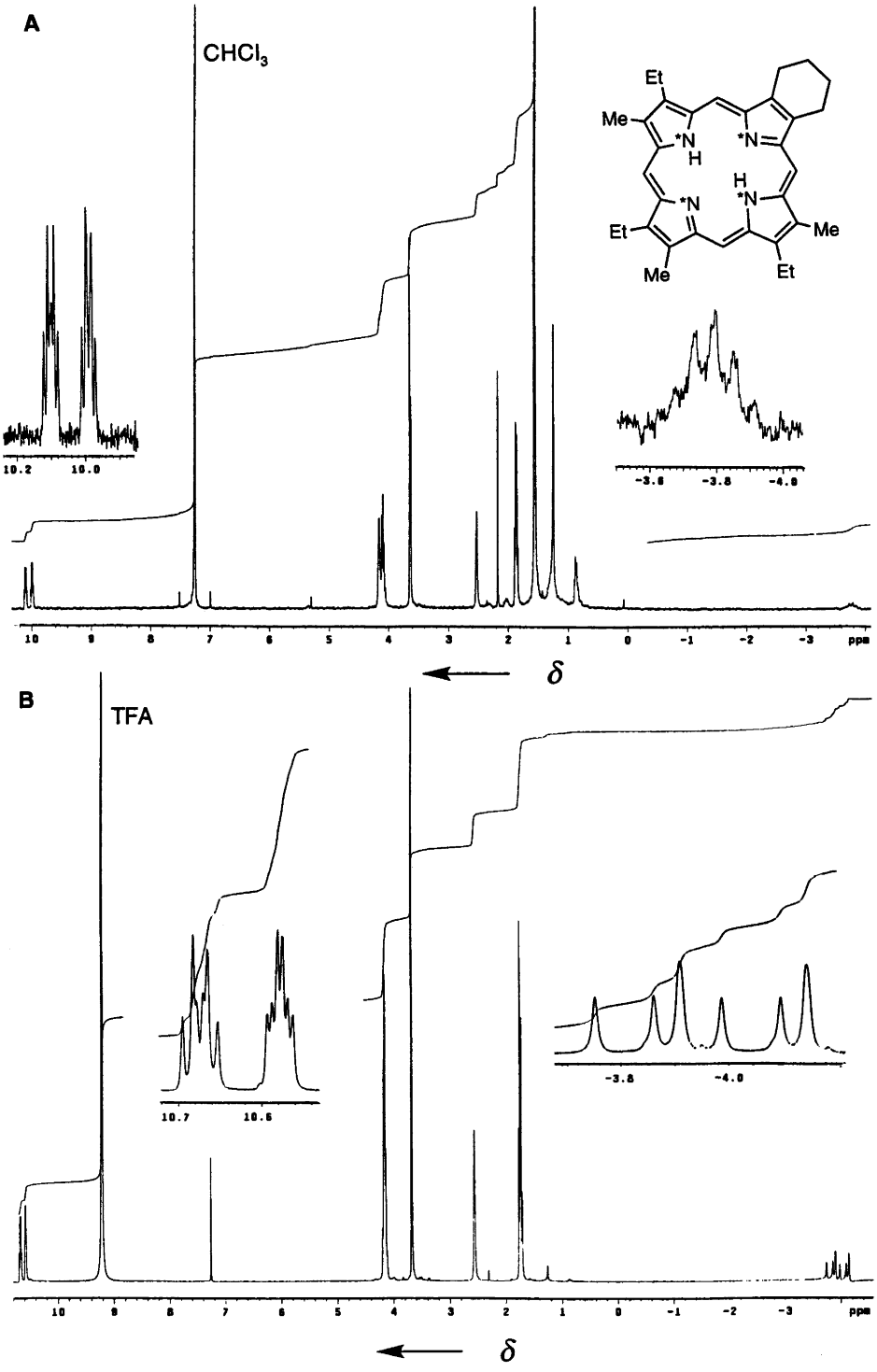


Figure 16. 400 MHz proton NMR spectra of per-^{15}N tetrahydrobenzoporphyrin **8**. (A). Free base in $CDCl_3$. The NH protons appear as a quintet at −3.8 ppm from coupling ($^1J_{NH}$ = −23 Hz) from all four nitrogen-15's due to rapid intramolecular exchange within the macrocyclic cavity. The *meso*-protons are all nonequivalent and produce two sets of two overlapping triplets at 10.0 and 10.1 ppm, respectively. (B). Dication in TFA–$CDCl_3$. The *meso*-protons again appear as two sets of two overlapping while the four nonequivalent internal NH protons give rise three overlapping nitrogen-15 coupled doublets between −3.7 and −4.2 ppm.

bath. Benzoyl chloride (1 mL) was added dropwise while the temperature was maintained below 0 °C. When the addition was complete, the salt/ice bath was removed and the mixture was stirred for an additional 15 min to allow the temperature to gradually rise to room temperature. Toluene (10 mL) was added and the resulting mixture was cooled on the salt/ice bath and stirred for 1 h. The imine salt was filtered and washed with cold toluene (10 mL). The solid and sodium carbonate (1.00 g) were dissolved in 50% ethanol/water (16 mL), and the resulting solution stirred on a boiling water bath for 15 min. Water was then added to the mixture and the solution was either cooled on an ice bath or left at room temperature overnight. The resulting precipitate was filtered and recrystallized from ethanol/water to give

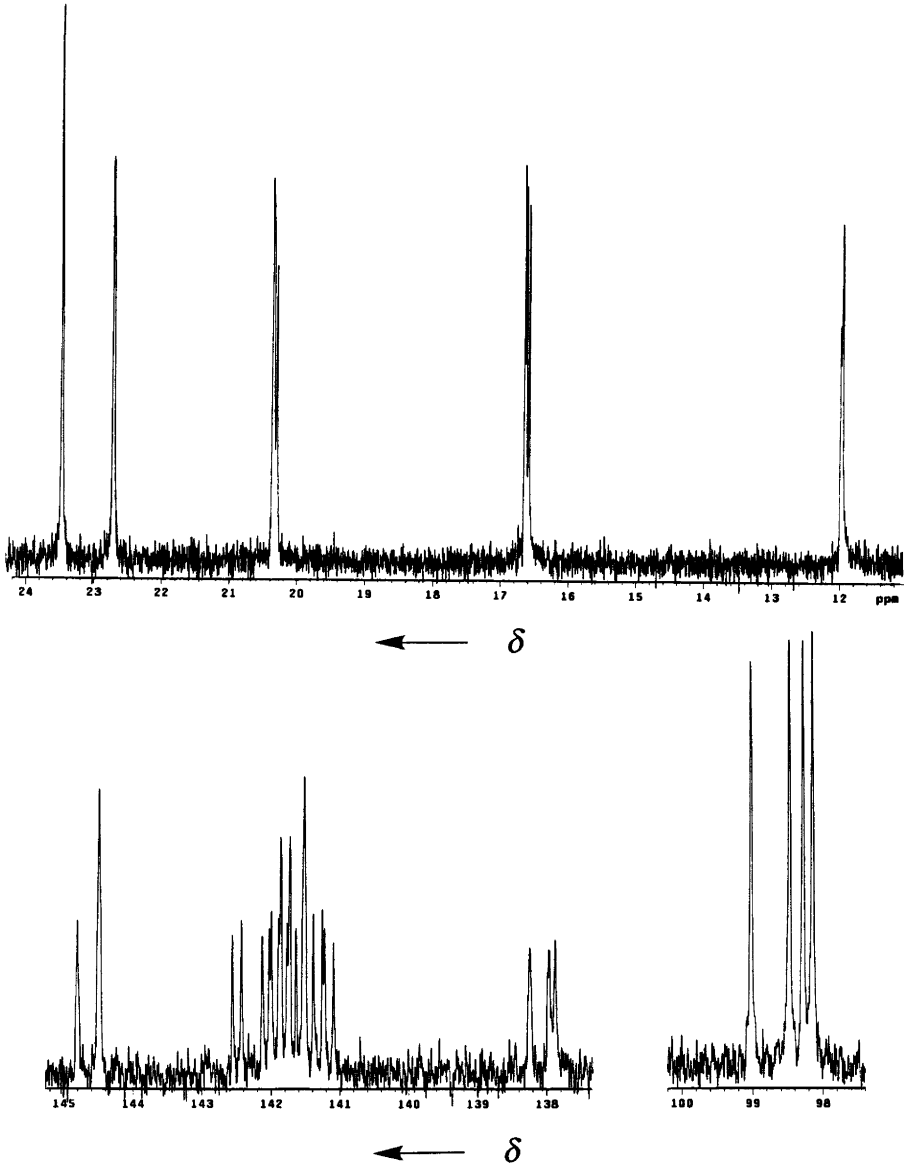


Figure 17. 100 MHz carbon-13 NMR spectrum of per-^{15}N tetrahydrobenzoporphyrin **8** in TFA–$CDCl_3$. Due to the lack of symmetry in **8**, there are four *meso*-resonances (observed between 98 and 99 ppm). In principle, there are 16 different pyrrolic carbons all of which may be coupled to the nitrogen-15 nuclei, but the region between 137 and 145 ppm is too complex for analysis. The alkyl group region from 11 to 24 ppm is also only partially resolved.

the pyrrole aldehyde as light yellow crystals (290 mg; 1.92 mmol; 75%), mp 101–102 °C (lit.[39] mp 106 °C). ^{1}H NMR ($CDCl_3$): δ 1.06 (3H, t, CH_2CH_3), 2.26 (6H, s, CH_3), 2.42 (2H, q, CH_2CH_3), 9.40 (1H, s, CHO), 9.62 (1H, br s, NH). *^{15}N labeled* **13**. ^{1}H NMR ($CDCl_3$): δ 1.05 (3H, t, $^3J_{HH}=7.5$ Hz), 2.25–2.27 (6H, overlapping singlet and doublet), 2.38 (2H, q, $^3J_{HH}=7.5$ Hz), 9.43 (1H, d, $^3J_{NH}=2.4$ Hz), 9.89 (1H, d, $^1J_{NH}=-97$ Hz); ^{13}C NMR ($CDCl_3$): δ 8.9, 11.7 (d, $^2J_{NC}=1.9$ Hz), 15.2, 17.1 (d, $^3J_{NC}=1.6$ Hz), 125.1 (d, $^2J_{NC}=4$ Hz), 128.0 (d, $^1J_{NC}=14.5$ Hz), 132.4 (br), 136.0 (d, $^1J_{NC}=14.4$ Hz), 175.6; MS (EI, 70 eV): *m/z* (%): 152 (41) [M^+], 151 (6.5), 137 (51) ([$M-CH_3$]$^+$), 135 (4).

4.2.1.5. Butyl 3-ethyl-4-methylpyrrole-2-carboxylate (23). DBU (3.5 g) was added dropwise to a stirred solution of 2-acetoxy-3-nitropentane[35] (**29**) (2.536 g; 14.5 mmol) and *n*-butyl isocyanoacetate[17] (**28**) (1.584 g; 11.2 mmol) in THF (10 mL), maintaining the temperature of the reaction mixture between 20 and 30 °C throughout. The reaction flask was placed in an oil bath that had been preheated to 75 °C, keeping the liquid level in the flask slightly above the level of the oil, and the reaction mixture was stirred under reflux for 16 h. The mixture was diluted with chloroform (25 mL) and washed with 5% HCl (25 mL). The aqueous phase was extracted with chloroform and the combined organic phases evaporated under reduced pressure. The residue was chromatographed on silica gel, eluting with toluene, and the product fractions crystallized from hexane to yield the title pyrrole (1.370 g; 6.55 mmol; 36% from glycine) as pale yellow crystals, mp 33.5–35 °C. IR (nujol mull): ν 3322 (st, br, NH), 1672 cm^{-1} (st, sh, C=O); ^{1}H NMR ($CDCl_3$): δ 0.97 (3H, t), 1.13 (3H, t), 1.47 (2H, m), 1.72 (2H, m), 2.04 (3H, s), 2.76 (2H, q), 4.27 (2H, t), 6.66 (1H, d, $^3J_{HH}=3$ Hz), 8.65 (1H, br s); ^{13}C NMR ($CDCl_3$): δ 9.7, 13.7, 15.2, 18.3, 19.4, 31.0, 63.8, 118.9, 120.0, 120.7, 133.2, 162.2. Anal. Calcd for $C_{12}H_{19}NO_2$: C, 68.86; H, 9.15;

N, 6.69. Found: C, 68.47; H, 8.24; N, 6.72. *^{15}N labeled* **23**. IR (nujol mull): ν 3315 (st, br, NH), 1673 cm^{-1} (st, sh, C=O); ^{1}H NMR ($CDCl_3$): δ 0.96 (3H, t, $^3J_{HH}$=7.3 Hz), 1.12 (3H, t, $^3J_{HH}$=7.5 Hz), 1.39–1.52 (2H, m), 1.66–1.75 (2H, m), 2.03 (3H, d, $^4J_{NH}$=0.6 Hz), 2.75 (2H, q, $^3J_{HH}$=7.5 Hz), 4.26 (2H, t, $^3J_{HH}$=6.6 Hz), 6.65 (1H, 't', $^2J_{NH}$=$^3J_{HH}$=3 Hz, pyrrole-H), 8.68 (1H, dd, $^1J_{NH}$=−98 Hz, $^3J_{HH}$=3 Hz, NH); ^{13}C NMR ($CDCl_3$): δ 9.7, 13.7, 15.2, 18.3, 19.4, 31.0, 63.8, 118.9 ($^1J_{NC}$=15 Hz), 120.0 ($^2J_{NC}$=4 Hz), 120.7 (d, $^1J_{NC}$=15 Hz), 133.2, 162.2.

4.2.1.6. Nitrogen-15 labeled *tert*-butyl 4-ethyl-3,5-dimethylpyrrole-2-carboxylate (10). A solution of ^{15}N labeled sodium nitrite (1.00 g; 14.3 mmol) in water (2.0 mL) was added dropwise to a stirred solution of *tert*-butyl acetoacetate (1.80 g; 11.4 mmol) in acetic acid (2 mL) while maintaining the temperature of the reaction mixture below 15 °C with the aid of a salt/ice bath. The resulting crude oxime solution was stirred at room temperature overnight. A solution of 3-ethyl-2,4-pentanedione (1.50 g; 11.7 mmol) in acetic acid (3.0 mL) was heated to 70 °C on a water bath. The crude oxime solution was then added dropwise over a period of 30 min while simultaneously adding a mixture of zinc powder (2.0 g) and anhydrous sodium acetate (1.0 g) in small portions and maintaining the temperature of the reaction mixture between 80 and 90 °C throughout. Once the addition was completed, the mixture was stirred on a boiling water bath for 2 h. The mixture was poured into ice/water (100 mL) and the resulting precipitate suction filtered and washed with liberal amounts of water. Recrystallization from ethanol–water gave the pyrrole (1.21 g; 5.40 mmol; 38% from $NaNO_2$; 47% from *tert*-butyl acetoacetate) as white crystals, mp 134–135 °C (lit. mp[40] 134–135 °C); ^{1}H NMR ($CDCl_3$): δ 1.04 (3H, t, $^3J_{HH}$=7.5 Hz), 1.55 (9H, s), 2.19 (3H, d, $^3J_{NH}$=2.8 Hz), 2.25 (3H, s), 2.37 (2H, q, $^3J_{HH}$=7.5 Hz), 8.42 (1H, d, $^1J_{NH}$=−96.8 Hz); ^{13}C NMR ($CDCl_3$): δ 10.7, 11.5 (d, $^2J_{NC}$=1.5 Hz), 15.6, 17.4 (d, $^3J_{NC}$=1.5 Hz), 28.8, 80.2, 118.2 ($^1J_{NC}$=14.8 Hz), 123.9 (d, $^2J_{NC}$=3.8 Hz), 126.1 (d, $^2J_{NC}$=4 Hz), 128.4 (d, $^1J_{NC}$=14.4 Hz), 161.5; MS (EI, 70 eV): *m/z* (%): 224 (35) [M$^+$], 168 (80) ([M−CH$_2$=C(CH$_3$)$_2$]$^+$), 153 (100) ([M−CH$_2$=C(CH$_3$)$_2$ and CH$_3$]$^+$), 151 (26) ([M−O*t*-Bu]$^+$), 150 (15) ([M−*t*-BuOH]$^+$), 135 (65) ([M−CH$_3$ and *t*-BuOH]$^+$).

4.2.2. Synthesis of nitrogen-15 labeled 2,7,12,17-tetraethyl-3,8,13,18-tetramethylporphyrin (^{15}N$_4$-etioporphyrin-I, 5). Per-^{15}N-etioporphyrin-I was prepared using the method previously described by Smith.[22,23] A solution of bromine (2.17 g) in acetic acid (3.0 mL) was added to a stirred solution of ^{15}N-labeled *tert*-butyl 4-ethyl-3,5-dimethylpyrrole-2-carboxylate (**10**; 1.00 g; 4.46 mmol) in acetic acid (3.5 mL) and the mixture stirred at room temperature overnight. The dark precipitate was filtered, washed with petroleum ether (60–90°) and dried in vacuo. The resulting red-brown pyrromethene (0.92 g) was heated under reflux with formic acid (2.2 mL) for 3 h. The excess formic acid was removed on a rotary evaporator and the residue dissolved in chloroform and washed with water, 5% aqueous sodium bicarbonate solution and water. The solvent was evaporated under reduced pressure and the residue recrystallized from chloroform–methanol to give the porphyrin (111 mg; 0.23 mmol; 21%) as purple crystals, mp >300 °C; ^{1}H NMR ($CDCl_3$): δ −3.77 (2H, quintet, $^1J_{NH}$=−24 Hz), 1.87 (12H, t, $^3J_{HH}$=7.6 Hz), 3.65 (12H, s), 4.10 (8H, q, $^3J_{HH}$=7.6 Hz), 10.09 (4H, t, $^3J_{NH}$=4.4 Hz); ^{1}H NMR (TFA–$CDCl_3$): δ −3.84 (4H, d, $^1J_{NH}$=−94.4 Hz), 1.75 (12H, t, $^3J_{HH}$=7.8 Hz), 3.67 (12H, s), 4.15 (8H, q, $^3J_{HH}$=7.7 Hz), 10.65 (4H, t, $^3J_{NH}$=4.8 Hz); ^{13}C NMR (TFA–$CDCl_3$): δ 12.0, 16.6, 20.3, 98.5, 137.9 (br), 141.4 (d, $^1J_{NC}$=12.9 Hz), 142.3 (d, $^1J_{NC}$=13.3 Hz), 144.6 (br).

4.2.3. Synthesis of etioporphyrin-II.

4.2.3.1. Diethyl 3,3′-diethyl-4,4′-dimethyl-2,2′-dipyrrylmethane-5,5′-dicarboxylate (21). Concentrated hydrochloric acid (16 drops) was added to ethyl 5-acetoxymethyl-4-ethyl-3-methylpyrrole-2-carboxylate (**20**) (494 mg; 1.95 mmol) in methanol (8 mL) and the mixture was heated under reflux for 3 h. The mixture was cooled and stored in a freezer overnight. The resulting crystals were collected by suction filtration. Recrystallization from absolute ethanol gave the title compound (297 mg; 0.794 mmol; 81%) as white needles, mp 126.5 °C (lit.[38] mp 126 °C). ^{1}H NMR ($CDCl_3$): δ 1.05 (6H, t), 1.33 (6H, t), 2.30 (6H, s), 2.40 (4H, q), 3.88 (2H, s), 4.27 (4H, q), 8.50 (2H, br s). *^{15}N labeled* **21**. ^{1}H NMR ($CDCl_3$): δ 1.04 (6H, t, $^3J_{HH}$=7.4 Hz), 1.31 (6H, t, $^3J_{HH}$=7.2 Hz), 2.28 (6H, s), 2.41 (4H, q, $^3J_{HH}$=7.4 Hz), 3.86 (2H, t, $^3J_{NH}$=2.4 Hz), 4.25 (4H, q, $^3J_{HH}$=7.1 Hz), 8.75 (2H, d, $^1J_{NH}$=−96.8 Hz); ^{13}C NMR ($CDCl_3$): δ 10.7 (d, $^3J_{NC}$=0.7 Hz), 14.7, 15.7, 17.4 (d, $^2J_{NC}$=1.5 Hz), 23.2 (t, $^2J_{NC}$=1.8 Hz), 60.0, 118.1 ($^1J_{NC}$=15.1 Hz), 124.5 (d, $^2J_{NC}$=4.1 Hz), 127.1 (d, $^2J_{NC}$=4.9 Hz), 128.8 (d, $^1J_{NC}$=14.8 Hz), 162.0.

4.2.3.2. 2,8,12,18-Tetraethyl-1,3,7,13,17,19-hexamethyl-10,23-dihydrobilin dihydrobromide (22). Dipyrrylmethane **21** (150 mg; 0.40 mmol) was heated with sodium hydroxide (1.00 g) and ethylene glycol (10 mL) under reflux for 1 h. The mixture was diluted with water and extracted with hexane. The hexane solution was dried over sodium sulfate and the solvent evaporated under reduced pressure to give a dark oil **14**. A solution of 4-ethyl-3,5-dimethylpyrrole-2-carboxaldehyde (**13**) (110 mg; 0.73 mmol) in 1.7 mL methanol was added, and residual material was washed into the reaction flask with additional methanol (0.7 mL). Hydrogen bromide in acetic acid (30%, 0.6 mL) was immediately added and the mixture was stirred for 30 min at room temperature. Anhydrous ether (17 mL) was added dropwise after which the mixture was stirred at room temperature overnight. The resulting precipitate was filtered and rinsed with anhydrous ether to give the title a,c-biladiene dihydrobromide as a purple-red solid (162 mg; 2.46 mmol; 67%), mp 227 °C, dec. UV–vis ($CHCl_3$): λ_{max} 374, 458, 528 nm; ^{1}H NMR ($CDCl_3$): δ 0.63 (6H, t, $^3J_{HH}$=7.6 Hz), 1.09 (6H, t, $^3J_{HH}$=7.6 Hz), 2.22 (6H, s), 2.29 (6H, s), 2.44 (4H, q, $^3J_{HH}$=7.6 Hz), 2.55 (4H, q, $^3J_{HH}$=7.5 Hz), 2.71 (6H, s), 5.19 (2H, s), 7.09 (2H, s), 13.20 (2H, s), 13.27 (2H, s). Anal. Calcd for $C_{33}H_{46}N_4Br_2\cdot\frac{1}{2}H_2O$: C, 59.37; H, 7.09; N, 8.39. Found: C, 59.39; H, 6.89; N, 8.36. *per-^{15}N labeled* **22**. Nitrogen-15 labeled dipyrrylmethane **21** (85 mg; 0.226 mmol) and 4-ethyl-3,5-dimethylpyrrole-2-carboxaldehyde (**13**; 63 mg; 0.42 mmol) gave the ^{15}N$_4$-a,c-biladiene (106 mg; 0.16 mmol; 76%). ^{1}H NMR ($CDCl_3$): δ 0.64 (6H, t, $^3J_{HH}$=7.4 Hz), 1.09 (6H, t, $^3J_{HH}$=7.4 Hz), 2.22 (6H, s), 2.29 (6H, s), 2.45 (4H, q, $^3J_{HH}$=7.5 Hz), 2.55

(4H, q, $^3J_{HH}$=7.3 Hz), 2.71 (6H, d, $^3J_{NH}$=2.8 Hz), 5.19 (2H, broad unresolved triplet), 7.09 (2H, t, $^3J_{NH}$=5.2 Hz), 13.20 (2H, d, $^1J_{NH}$=−95.2 Hz), 13.28 (2H, d, $^1J_{NH}$=−94.8 Hz).

4.2.3.3. 3,7,13,17-Tetraethyl-2,8,12,18-tetramethylporphyrin (etioporphyrin-II; 6). a,c-Biladiene **22** (62 mg; 0.094 mmol) was added to a stirred solution of copper(II) chloride (197 mg) in DMF (34 mL). The flask was covered with aluminum foil and the solution stirred at room temperature for 4 h. The solution was diluted with chloroform (30 mL) and washed with water (3×30 mL). Each of the aqueous solutions were back extracted with chloroform (30 mL each). The combined organic layers were dried over sodium sulfate, filtered and the solvent evaporated on a rotatory evaporator, first under water aspirator pressure and then using a vacuum pump. The dark purple residue was taken up in 15% (v/v) sulfuric acid/trifluoroacetic acid (6 mL), and stirred at room temperature for 45 min. The solution was diluted with water, extracted with chloroform and washed with 5% aqueous sodium bicarbonate solution, and the solvent evaporated under reduced pressure to give a dark solid. The residue was chromatographed on grade III alumina, eluting with dichloromethane, and recrystallized from chloroform/methanol to give the title porphyrin (14 mg; 0.029 mmol; 31%) as a purple solid, mp>300 °C. ^{1}H NMR ($CDCl_3$): δ −3.75 (2H, s, NH), 1.87 (12H, t), 3.65 (12H, s), 4.10 (8H, q), 10.09 (2H, s), 10.10 (2H, s); EI MS: *m/z* (rel int.) 480 (6), 479 (36), 478 (100) (M^+), 464 (11), 463 (31), 448 (7), 240 (6), 239 (18), 224 (10), 217 (6). *$^{15}N_4$-etioporphyrin-II (**6**).* Using the same procedure, 70 mg (0.10 mmol) of nitrogen-15 labeled a,c-biladiene dibromide **22** gave 23 mg (0.048 mmol; 48%) of the title porphyrin. ^{1}H NMR ($CDCl_3$): δ −3.77 (2H, quintet, $^1J_{NH}$=−23.2 Hz), 1.88 (12H, t, $^3J_{HH}$=7.8 Hz), 3.64 (12H, s), 4.10 (8H, q, $^3J_{HH}$=7.7 Hz), 10.09 (2H, t, $^3J_{NH}$=4.8 Hz), 10.11 (2H, t, $^3J_{NH}$=4.6 Hz); ^{1}H NMR (TFA–$CDCl_3$): δ −3.43 (2H, d, $^1J_{NH}$= −94 Hz), 1.73 (12H, t, $^3J_{HH}$=7.8 Hz), 3.65 (12H, s), 4.14 (8H, q, $^3J_{HH}$=7.7 Hz), 10.58–10.62 (4H, two overlapping triplets, $^3J_{NH}$=5 Hz); ^{13}C NMR (TFA–$CDCl_3$): δ 12.0, 16.7, 20.3, 98.2, 98.6, 137.4 (d, $^2J_{NC}$=2.6 Hz), 141.5 (d, $^1J_{NC}$=13.3 Hz), 142.3 (d, $^1J_{NC}$=13.3 Hz), 144.0 ($^2J_{NC}$=1.9 Hz); EI MS *m/e* (rel int.): 484 (6), 483 (35), 482 (100) (M^+), 481 (9), 468 (9), 467 (27), 452 (6), 241 (21), 234 (5), 226 (10), 219 (5).

4.2.4. Synthesis of etioporphyrin-III.

4.2.4.1. Butyl 5′-Ethoxycarboxyl-3′,4-diethyl-3,4′-dimethyl-2,2′-dipyrrylmethane-5-carboxylate (24). Montmorillonite clay (K-10; 2.70 g) was added to a stirred solution of acetoxymethylpyrrole **20** (0.253 g; 1.00 mmol) and α-free pyrrole **23** (0.209 g, 1.00 mmol) in dichloromethane (40 mL). The mixture was stirred vigorously overnight at room temperature. The clay was removed by suction filtration and the solvent evaporated under reduced pressure to give a solid. The residue was purified by flash chromatography, eluting with 5% acetone/hexane and recrystallized from 95% methanol to give the title dipyrrylmethane (0.325 g; 0.81 mmol; 81%) as white crystals, mp 105.5–106.5 °C. ^{1}H NMR ($CDCl_3$): δ 0.93 (3H, t, $^3J_{HH}$=7.4 Hz), 1.03 (3H, t, $^3J_{HH}$=7.4 Hz), 1.11 (3H, t, $^3J_{HH}$=7.4 Hz), 1.32 (3H, t, $^3J_{HH}$=7.0 Hz), 1.38–1.47 (2H, m), 1.63–1.71 (2H, m), 1.97 (3H, s), 2.29 (3H, s), 2.40 (2H, q, $^3J_{HH}$=7.4 Hz), 2.73 (2H, q, $^3J_{HH}$=7.6 Hz), 3.85 (2H, s), 4.21 (2H, t, $^3J_{HH}$=6.6 Hz), 4.27 (2H, q, $^3J_{HH}$=7 Hz), 8.55 (1H, br s), 8.58 (1H, br s); ^{13}C NMR ($CDCl_3$): δ 8.9, 10.8, 13.9, 14.7, 15.4, 15.7, 17.5, 18.8, 19.5, 23.1, 31.1, 60.1, 64.0, 116.5, 117.2, 118.0, 124.3, 127.2, 129.6, 130.2, 134.2, 162.4. Anal. Calcd for $C_{23}H_{34}N_2O_4$: C, 68.62; H, 8.51; N, 6.96. Found: C, 68.32; H, 8.16; N, 6.79. *^{15}N labeled* **24**. ^{1}H NMR ($CDCl_3$): δ 0.92 (3H, t, $^3J_{HH}$=7.4 Hz), 1.03 (3H, t, $^3J_{HH}$=7.8 Hz), 1.10 (3H, t, $^3J_{HH}$=7.6 Hz), 1.31 (3H, t, $^3J_{HH}$=7.2 Hz), 1.35–1.45 (2H, m), 1.62–1.69 (2H, m), 1.97 (3H, s), 2.28 (3H, s), 2.41 (2H, q, $^3J_{HH}$=7.5 Hz), 2.72 (2H, q, $^3J_{HH}$=7.5 Hz), 3.86 (2H, t, $^3J_{NH}$=2.6 Hz), 4.20 (2H, t, $^3J_{HH}$=6.6 Hz), 4.26 (2H, q, $^3J_{HH}$=7.2 Hz), 8.90 (1H, d, $^1J_{NH}$=−97 Hz), 8.95 (1H, d, $^1J_{NH}$=−97 Hz); ^{13}C NMR ($CDCl_3$): δ 8.8, 10.7, 13.9, 14.7, 15.4, 15.7, 17.5 (d, $^3J_{NC}$=1.1 Hz), 18.8, 19.5, 23.3 (t, $^2J_{NC}$=1.5 Hz), 31.1, 60.1, 64.0, 116.7 (d, $^2J_{NC}$=4.2 Hz), 117.4 (d, $^1J_{NC}$=15.1 Hz), 118.1 (d, $^1J_{NC}$=15.2 Hz), 124.4 (d, $^2J_{NC}$=3.8 Hz), 127.1 ($^2J_{NC}$=4 Hz), 129.0 (d, $^1J_{NC}$=15.2 Hz), 129.7 (d, $^1J_{NC}$=15.2 Hz), 134.2 ($^2J_{NC}$=4.5 Hz), 162.2.

4.2.4.2. 2,7,12,18-Tetraethyl-1,3,8,13,17,19-hexamethyl-10,23-dihydrobilin dihydrobromide (32). Dipyrrylmethane **24** (146 mg; 0.363 mmol) was heated with sodium hydroxide (1.00 g) and ethylene glycol (10 mL) under reflux conditions for 1 h. The mixture was diluted with water and extracted with hexane. The hexane solution was dried over sodium sulfate and the solvent evaporated under reduced pressure to give **31** as a dark oil. A solution of 4-ethyl 3,5-dimethylpyrrole-2-carboxaldehyde (**13**) (0.118 g, 0.78 mmol) in methanol (2.0 mL) was added and any residual material was washed into the reaction flask with additional methanol (2.0 mL). Hydrogen bromide in acetic acid (30%, 0.8 mL) was immediately added and the mixture was stirred for 30 min at room temperature. Anhydrous ether (17 mL) was added dropwise after which the mixture was stirred at room temperature for a further 2 h. The resulting precipitate was filtered and rinsed with anhydrous ether to give the title a,c-biladiene dihydrobromide **32** as a brick red solid (123 mg, 0.19 mmol; 51%), mp 237 °C dec. UV–is ($CHCl_3$): λ_{max} 374, 458, 528 nm; ^{1}H NMR ($CDCl_3$): δ 0.63 (6H, t, $^3J_{HH}$=7.4 Hz), 1.06–1.12 (6H, two overlapping triplets), 1.93 (3H, s), 2.24 (3H, s), 2.29 (6H, s), 2.41–2.51 (6H, three overlapping quartets), 2.60 (2H, q, $^3J_{HH}$=7.6 Hz), 2.71 (6H, s), 5.19 (2H, s), 7.06 (1H, s), 7.09 (1H, s), 13.16 (1H, s), 13.18 (1H, s), 13.24 (1H, s), 13.30 (1H, s). Anal. Calcd for $C_{33}H_{46}N_4Br_2\cdot\frac{1}{2}H_2O$: C, 59.37; H, 7.09; N, 8.39. Found: C, 59.34; H, 6.81; N, 8.33. *^{15}N labeled* **32**. Nitrogen-15 labeled dipyrrylmethane **24** (100 mg; 0.25 mmol) and pyrrole aldehyde **13** (81 mg; 0.53 mmol) gave the required labeled a,c-biladiene (107 mg; 0.16 mmol; 65%): ^{1}H NMR ($CDCl_3$): δ 0.65 (6H, t), 1.09 (6H, t), 1.91 (3H, s), 2.21 (3H, s), 2.27 (6H, s), 2.42 (6H, m), 2.52 (2H, q), 2.73 (6H, s), 5.19 (2H, br), 7.09 (2H, br), 13.24 (2H, d, $^1J_{NH}$=−96 Hz), 13.26 (1H, d, $^1J_{NH}$=−96 Hz), 13.34 (1H, d, J=−96 Hz).

4.2.4.3. 3,8,13,17-Tetraethyl-2,7,12,18-tetramethylporphyrin (etioporphyrin-III, 3). a,c-Biladiene **32** (104 mg; 0.158 mmol) was added to a stirred solution of copper(II) chloride (0.26 g) in dimethylformamide (45 mL). The flask was covered with aluminum foil and the solution

stirred at room temperature for 2.5 h. The solution was diluted with chloroform (50 mL) and washed with water (3×50 mL). Each of the aqueous solutions was back extracted with chloroform (50 mL each). The combined organic layers were dried over sodium sulfate, filtered and the solvent evaporated on a rotatory evaporator, first under water aspirator pressure and then using a vacuum pump. The dark purple residue was taken up in 15% (v/v) sulfuric acid/trifluoroacetic acid (6 mL) and stirred at room temperature for 45 min. The solution was diluted with water, extracted with chloroform and washed with 5% aqueous sodium bicarbonate solution, and the solvent evaporated under reduced pressure to give a dark solid. The residue was chromatographed on grade III alumina, eluting with dichloromethane, and recrystallized from chloroform/methanol to give the title porphyrin (40 mg; 0.084 mmol; 53%) as a purple solid, mp >300 °C. ^{1}H NMR ($CDCl_3$): δ −3.75 (2H, s), 1.85 (12H, t), 3.65 (12H, s), 4.10 (8H, m), 10.09 (4H, s); EI MS *m/z* (rel int.): 480 (4), 479 (37), 478 (100) (M^+), 463 (24), 448 (6), 240 (7), 240 (7), 239 (20), 224 (9). *$^{15}N_4$-etioporphyrin-III (**3**).* ^{1}H NMR ($CDCl_3$): δ −3.77 (2H, quintet, $^1J_{NH}=-24$ Hz), 1.85 (12H, two overlapping triplets), 3.64 (6H, s), 3.65 (6H, s), 4.10 (8H, q, $^3J_{HH}=7.6$ Hz), 10.08–10.12 (4H, m); ^{1}H NMR (TFA–$CDCl_3$): δ −3.86 (1H, d, $^1J_{NH}=-95$ Hz), −3.84 (3H, d, $^1J_{NH}=-94$ Hz), 1.71–1.77 (12H, m), 3.67 (9H, s), 3.68 (3H, s), 4.12–4.18 (8H, m), 10.63–10.68 (4H, m); ^{13}C NMR (TFA–$CDCl_3$): δ 12.0, 16.6 (3), 20.3, 98.2, 98.4, 98.6, 98.8, 138 (unresolved multiplet), 141.3–141.6 (m), 142.1–142.5 (m), 144.4–144.6 (m); EI MS *m/e* (rel int.): 484 (6), 483 (35), 482 (100) (M^+), 481 (6), 467 (25), 452 (6), 241 (18), 226 (8).

4.2.5. Synthesis of etioporphyrin-IV.

4.2.5.1. Dibutyl 4,4′-diethyl-3,3′-dimethyl-2,2′-dipyrrylmethane-5,5′-dicarboxylate (33). Butyl 3-methyl-4-ethylpyrrole-2-carboxylate (**23**) (0.23 g; 1.10 mmol) and paraformaldehyde (0.13 g, 4.3 mmol) were dissolved in 1.25 mL ethanol in a 10 mL round bottom flask. Concentrated hydrochloric acid (0.025 mL) was added, and the mixture heated under reflux for 30 min under nitrogen. After it had cooled to room temperature, the reaction flask was kept in the freezer overnight. The resulting crystals were filtered off and recrystallized from absolute ethanol to give the required dipyrrylmethane (148 mg; 0.344 mmol; 63%) as white crystals, mp 113–114 °C. ^{1}H NMR ($CDCl_3$): δ 0.87 (6H, t, $^3J_{HH}=7.4$ Hz), 1.11 (6H, t, $^3J_{HH}=7.4$ Hz), 1.33–1.42 (4H, m), 1.59–1.66 (4H, m), 1.99 (6H, s), 2.72 (4H, q, $^3J_{HH}=7.4$ Hz), 3.87 (2H, s), 4.19 (4H, t, $^3J_{HH}=6.6$ Hz), 9.58 (2H, br s); ^{13}C NMR ($CDCl_3$): δ 8.8, 13.8, 15.3, 18.8, 19.5, 23.1, 31.0, 64.0, 116.5, 117.1, 130.4, 134.2, 162.5. *^{15}N labeled* **33**. ^{1}H NMR ($CDCl_3$): δ 0.94 (6H, t), 1.12 (6H, t), 1.40 (4H, m), 1.65 (4H, m), 1.97 (6H, s), 2.71 (4H, q), 3.83 (2H, t, $^3J_{NH}=3$ Hz), 4.19 (4H, t), 8.48 (2H, d, $^1J_{NH}=-96$ Hz).

4.2.5.2. 2,7,13,18-Tetraethyl-1,3,8,12,17,19-hexamethyl-10,23-dihydrobilin dihydrobromide (35). Dipyrrylmethane **33** (116 mg; 0.27 mmol) was heated with sodium hydroxide (1.00 g) and ethylene glycol (10 mL) under reflux conditions for 1 h. The mixture was diluted with water and extracted with hexane. The hexane solution was dried over sodium sulfate and the solvent evaporated under reduced pressure to give **34** as a dark brown oil. A solution of 4-ethyl-3,5-dimethylpyrrole-2-carboxaldehyde (**13**) (81 mg; 0.54 mmol) in 1 mL of methanol was added, and residual material was washed into the reaction flask with additional methanol (1 mL). Hydrogen bromide in acetic acid (30%, 0.8 mL) was immediately added and the mixture was stirred for 30 min at room temperature. Anhydrous ether (17 mL) was added dropwise after which the mixture was stirred at room temperature overnight. The resulting precipitate was filtered and rinsed with anhydrous ether to give the title a,c-biladiene dihydrobromide as a purple-red solid (93 mg; 0.14 mmol; 52%), mp 227 °C dec. UV–vis ($CHCl_3$): λ_{max} ($\log_{10}\varepsilon$) 372, 458, 528 nm; ^{1}H NMR ($CDCl_3$): δ 1.08 (6H, t), 1.13 (6H, t), 1.89 (6H, s), 2.29 (6H, s), 2.44 (4H, q), 2.61 (4H, q), 2.70 (6H, s), 5.19 (2H, s), 7.06 (2H, s), 13.14 (2H, br s), 13.26 (2H, br s). *$^{15}N_4$-labeled* **35**. Using the same procedure, 120 mg (0.28 mmol) of nitrogen-15 labeled dipyrrylmethane **33** and 83 mg (0.55 mmol) of ^{15}N pyrrole aldehyde **13** gave the a,c-biladiene (103 mg; 0.155 mmol; 56%) as a red solid. ^{1}H NMR ($CDCl_3$): δ 1.08 (6H, t, $^3J_{HH}=7.6$ Hz), 1.13 (6H, t, $^3J_{HH}=7.6$ Hz), 1.89 (6H, s), 2.29 (6H, s), 2.44 (4H, q, $^3J_{HH}=7.5$ Hz), 2.62 (4H, q, $^3J_{HH}=7.6$ Hz), 2.70 (6H, d, $^3J_{NH}=2.4$ Hz), 5.19 (2H, broad unresolved triplet), 7.06 (2H, t, $^3J_{NH}=5.0$ Hz), 13.14 (2H, d, $^1J_{NH}=-95.2$ Hz), 13.26 (2H, d, $^1J_{NH}=-94.8$ Hz).

4.2.5.3. 2,8,13,17-Tetraethyl-3,7,12,18-tetramethylporphyrin (etioporphyrin-IV; 7). a,c-Biladiene dihydrobromide **35** (70 mg; 0.11 mmol) was added to a stirred solution of copper(II) chloride (150 mg) in DMF (26 mL). The flask was covered with aluminum foil and the solution stirred at room temperature for 4 h. The solution was diluted with chloroform (30 mL) and washed with water (3×30 mL). Each of the aqueous solutions were back extracted with chloroform (30 mL each). The combined organic layers were dried over sodium sulfate, filtered and solvent evaporated on a rotatory evaporator, first under aspirator pressure and then using a vacuum pump. The purple residue was taken up in 15% (v/v) sulfuric acid/trifluoroacetic acid (5 mL) and stirred at room temperature for 45 min. The solution was diluted with water, extracted with chloroform and washed with 5% aqueous sodium bicarbonate solution, and the solvent evaporated under reduced pressure to give a dark solid. The residue was chromatographed on grade III alumina, eluting with dichloromethane, and recrystallized from chloroform /methanol to give the title porphyrin (17 mg; 0.036 mmol; 32%) as a purple solid, mp >300 °C. ^{1}H NMR ($CDCl_3$): δ −3.75 (2H, s, NH), 1.87 (12H, t), 3.64 (12H, s), 4.10 (8H, q), 10.09 (4H, s); EI MS *m/z* (rel int.): 480 (6), 479 (35), 478 (100) (M^+), 464 (9), 463 (25), 448 (6), 239 (21), 224 (9), 217 (5). *per-^{15}N-etioporphyrin-IV*. Using the same procedure, 92 mg (0.14 mmol) of nitrogen-15 labeled a,c-biladiene **35** gave $^{15}N_4$-etioporphyrin-IV (27 mg; 0.056 mmol; 40%). ^{1}H NMR ($CDCl_3$): δ −3.77 (2H, br quintet, $^1J_{NH}=-23$ Hz), 1.88 (6H, t, $^3J_{HH}=7.6$ Hz), 1.89 (6H, t, $^3J_{HH}=7.8$ Hz), 3.64 (6H, s), 3.65 (6H, s), 4.07–4.14 (8H, two overlapping quartets), 10.09 (4H, br t, $^3J_{NH}=4$ Hz); ^{1}H NMR (TFA–$CDCl_3$): δ −3.70 (2H, d, $^1J_{NH}=-94$ Hz), −3.68 (2H, d, $^1J_{NH}=-94$ Hz), 1.73 (6H, t, $^3J_{HH}=7.6$ Hz), 1.74 (6H, t, $^3J_{HH}=7.6$ Hz), 3.66 (6H, s), 3.67 (6H, s), 4.11–4.18 (8H, two overlapping quartets), 10.60–10.66 (4H, m); ^{13}C NMR (TFA–$CDCl_3$): δ 11.9–12.1 (m), 16.6, 16.7, 20.3, 20.4, 98.1, 98.5, 98.9, 137.7–137.9

(m), 141.5 (d, $^1J_{NC}$=13.3 Hz), 141.6 (d, $^1J_{NC}$=13.3 Hz), 142.3 (d, $^1J_{NC}$=13.2 Hz), 142.4 (d, $^1J_{NC}$=13.3 Hz), 144.3–144.4 (m); EI MS: *m/z* (rel int.): 484 (6), 483 (34), 482 (100) (M^+), 468 (8), 467 (25), 452 (6), 241 (22), 226 (9), 219 (6).

4.2.6. Synthesis of butanoporphyrin 8.

4.2.6.1. Butyl 3,4-butano-5′-ethoxycarbonyl-4′-ethyl-3′-methyl-2,2′-dipyrrylmethane-5-carboxylate (36). Montmorillonite clay (K-10, 2.70 g) was added to a stirred solution of acetoxymethylpyrrole **20** (253 mg; 1.00 mmol) and *n*-butyl 4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylate (**25**)[17] (221 mg, 1.00 mmol) in dichloromethane (40 mL). The mixture was stirred vigorously overnight at room temperature. The clay was removed by suction filtration and the solvent evaporated over reduced pressure to give a pink solid. The residue was purified by flash chromatography eluting with 5% ethyl acetate/toluene and recrystallized from ethanol to give the title dipyrrylmethane (260 mg; 0.63 mmol; 63%) as white crystals, mp 114–115 °C. ^{1}H NMR ($CDCl_3$): δ 0.90 (3H, t), 1.03 (3H, t), 1.32 (3H, t), 1.41 (2H, m), 1.66 (2H, m), 1.72 (4H, m), 2.28 (3H, s), 2.40 (2H, m), 2.77 (2H, q), 3.82 (2H, s), 4.23 (4H, m), 8.75 (2H, br s); ^{13}C NMR ($CDCl_3$): δ 10.7, 13.9, 14.7, 15.7, 17.5, 19.5, 21.5, 23.0, 23.4, 23.5, 23.6, 31.1, 60.1, 64.0, 116.7, 118.0, 119.2, 124.3, 127.1, 129.2, 129.3, 129.4, 162.2, 162.5. Anal. Calcd for $C_{24}H_{34}N_2O_4$: C, 69.47; H, 8.26; N, 6.75. Found: C, 69.35; H, 7.77; N, 6.67. *^{15}N labeled* **36**. ^{1}H NMR ($CDCl_3$): δ 0.93 (3H, t, $^3J_{HH}$=7.2 Hz), 1.03 (3H, t, $^3J_{HH}$=7.4 Hz), 1.32 (3H, t, $^3J_{HH}$=7.2 Hz), 1.36–1.47 (2H, m), 1.64–1.71 (2H, m), 1.72–1.76 (4H, m), 2.28 (3H, s), 2.37–2.43 (4H, m), 2.77 (2H, br t), 3.82 (2H, t, $^3J_{NH}$=2.6 Hz), 4.21 (2H, t, $^3J_{HH}$=6.6 Hz), 4.27 (2H, q, $^3J_{HH}$=7.2 Hz), 8.59 (1H, d, $^1J_{NH}$=−97 Hz), 8.62 (1H, d, J_{NH}=−97 Hz); ^{13}C NMR ($CDCl_3$): δ 10.7, 13.9, 14.7, 15.7, 17.5, 19.5, 21.5, 22.9, 23.5, 23.6, 31.1, 60.1, 64.0, 116.7 (d, $^1J_{NC}$=15.5 Hz), 118.0 (d, $^1J_{NC}$=14.8 Hz), 119.1 (d, $^2J_{NC}$=3.8 Hz), 124.2 (d, $^2J_{NC}$=3.8 Hz), 127.1 (d, $^2J_{NC}$=4.6 Hz), 129.3–129.5 (m), 162.3, 162.6.

4.2.6.2. 7,8-Butano-3,12,17-triethyl-1,2,13,18,19-pentamethyl-10,23-dihydrobilin dihydrobromide (38). Dipyrrylmethane (**36**) (91 mg; 0.22 mmol) was heated with sodium hydroxide (1.00 g) and ethylene glycol (10 mL) under reflux conditions for 30 min. The mixture was diluted with water and extracted with hexane. The hexane solution was dried over sodium sulfate and the solvent evaporated under reduced pressure to give **37** as a dark oil. A solution of 4-ethyl-3,5-dimethylpyrrole-2-carboxaldehyde (**13**) (66 mg; 0.44 mmol) in methanol (1.0 mL) was added and all residual material was washed into the reaction flask with additional methanol (0.7 mL). Hydrogen bromide in acetic acid (30%, 0.3 mL) was immediately added and the mixture was stirred for 30 min at room temperature. Anhydrous ether (15 mL) was added dropwise after which the mixture was stirred at room temperature for 2 h. The resulting precipitate was filtered and rinsed with anhydrous ether to give the title a,c-biladiene dihydrobromide as a brick red solid (96 mg; 0.143 mmol; 65%), mp 237 °C, dec. UV–vis: λ_{max}: 374, 458, 528 nm; ^{1}H NMR ($CDCl_3$): δ 0.71 (3H, t), 1.09 (6H, t), 1.69 (4H, m), 2.25–2.31 (11H, m), 2.46 (4H, m), 2.71 (6H, s), 5.15 (2H, s), 7.00 (1H, s), 7.11 (1H, s), 13.04 (1H, s), 13.12 (1H, s), 13.20 (1H, s), 13.31 (1H, s). Anal. Calcd for $C_{34}H_{46}N_4Br_2\cdot H_2O$: C, 59.30; H, 7.02; N, 8.14. Found: C, 59.22; H, 6.77; N, 8.10. *^{15}N labeled* **38**. Using the same procedure, 160 mg (0.38 mmol) of labeled dipyrrylmethane **36** and 108 mg (0.71 mmol) of labeled pyrrole aldehyde **13** gave the required a,c-biladiene (186 mg; 0.276 mmol; 77%) as a brick red solid. ^{1}H NMR ($CDCl_3$): δ 0.70 (3H, t, $^3J_{HH}$=7.4 Hz), 1.08 (6H, two overlapping triplets), 1.68 (4H, m), 2.25 (3H, s), 2.26 (3H, s), 2.29 (3H, s), 2.41–2.49 (4H, m), 2.66 (2H, m), 2.70 (6H, two overlapping doublets, $^3J_{NH}$=3 Hz), 5.15 (2H, s), 6.98 (1H, t, $^3J_{NH}$=5.2 Hz), 7.07 (1H, t, $^3J_{NH}$=5.0 Hz), 13.06 (1H, d, $^1J_{NH}$=−94.8 Hz), 13.14 (1H, d, $^1J_{NH}$=−94.8 Hz), 13.23 (1H, d, $^1J_{NH}$=−95.2 Hz), 13.33 (1H, d, $^1J_{NH}$= −94.4 Hz).

4.2.6.3. 2,3-Butano-7,13,17-triethyl-8,12,18-trimethylporphyrin (8). a,c-Biladiene dihydrobromide (**38**) (70 mg; 0.104 mmol) was added to a stirred solution of copper(II) chloride (0.15 g) in DMF (45 mL). The flask was covered with aluminum foil and the solution stirred at room temperature for 2.5 h. The solution was diluted with chloroform (50 mL) and washed with water (3×50 mL). The combined organic layers were then dried over sodium sulfate, filtered and the solvent evaporated under vacuum. The dark purple residue was taken up in 15% (v/v) sulfuric acid/trifluroacetic acid (6 mL) and stirred at room temperature for 45 min. The solution was diluted with water, extracted with chloroform and washed with 5% aqueous sodium bicarbonate solution, and the solvent evaporated under reduced pressure to give a dark solid. The residue was chromatographed on grade III alumina, eluting with dichloromethane, and recrystallized from chloroform/methanol to give the tetrahydrobenzoporphyrin (20 mg; 0.041 mmol; 40%) as a purple solid, mp >300 °C. ^{1}H NMR ($CDCl_3$): δ −3.76 (2H, s), 1.84–1.90 (9H, three overlapping triplets), 2.53 (4H, m), 3.62 (3H, s), 3.64 (6H, s), 4.05–4.14 (6H, three overlapping quartets), 4.16 (4H, m), 9.98 (1H, s), 9.99 (1H, s), 10.09 (1H, s), 10.11 (1H, s); ^{1}H NMR (TFA–$CDCl_3$): δ −3.89 (1H, br s), −3.84 (2H, br s), −3.73 (1H, br s), 1.70–1.76 (9H, three overlapping triplets), 2.56 (4H, m), 3.66 (3H, s), 3.67 (6H, s), 4.10–4.18 (10H, m), 10.56 (2H, s), 10.65 (1H, s), 10.66 (1H, s); 9.98 (1H, s), 9.99 (1H, s), 10.09 (1H, s), 10.11 (1H, s); ^{13}C NMR (TFA–$CDCl_3$): δ 12.0, 16.6 (2), 16.7, 20.3 (2), 22.7, 23.5, 98.1, 98.2, 98.4, 99.0, 137.6, 137.7, 138.0, 141.2 (2), 141.3, 141.7, 141.8 (2), 142.0, 142.1, 142.5, 144.2, 144.5; EI MS: *m/z* (rel int.) 492 (7), 491 (38), 490 (100) (M^+), 478 (5), 476 (8), 475 (22), 246 (10), 245 (27), 230 (6), 223 (5), 216 (5). *^{15}N labeled* **8**. ^{1}H NMR ($CDCl_3$): δ −3.79 (2H, quintet, $^1J_{NH}$=−23 Hz), 1.83–1.89 (9H, three overlapping triplets), 2.53 (4H, m), 3.62 (3H, s), 3.64 (6H, s), 4.05–4.14 (6H, m), 4.16 (4H, m), 9.97–10.01 (2H, two overlapping triplets, $^3J_{NH}$=4 Hz), 10.07–10.13 (2H, two overlapping triplets, $^3J_{NH}$=4 Hz); ^{1}H NMR (TFA–$CDCl_3$): δ −4.03 (2H, d, $^1J_{NH}$=−94.8 Hz), −3.98 (1H, d, $^1J_{NH}$=−94.0 Hz), −3.87 (1H, d, $^1J_{NH}$= −94.0 Hz), 1.70–1.76 (9H, three overlapping triplets), 2.56 (4H, m), 3.66 (3H, s), 3.67 (6H, s), 4.11–4.19 (10H, m), 10.57 (1H, t, $^3J_{NH}$=5 Hz), 10.58 (1H, t, $^3J_{NH}$=4.8 Hz), 10.67 (1H, t, $^3J_{NH}$=5.2 Hz), 10.68 (1H, t, $^3J_{NH}$=5 Hz); ^{13}C NMR (TFA–$CDCl_3$): δ 12.0, 16.6 (2), 16.7, 20.3 (2), 22.7, 23.5, 98.1, 98.3, 98.5, 99.0, 137.9, 138.0, 138.2, 141.0–142.1 (m), 142.5 (d, $^1J_{NC}$=13.2 Hz), 144.5, 144.8; EI MS: *m/z* (rel int.) 496 (7), 495 (36), 494

(100) (M^+), 493 (6), 482 (8), 480 (9), 479 (24), 251 (5), 247 (27), 240 (6), 232 (7), 218 (6).

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Porphyrins with exocyclic rings. Part 19: Efficient syntheses of phenanthrolinoporphyrins☆

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Abstract—5-Nitro-1,10-phenanthrolines react with isocyanoacetate esters in the presence of DBU in THF to give excellent yields of the corresponding phenanthrolinopyrroles. These were condensed with acetoxymethylpyrroles using catalytic quantities of *p*-toluenesulfonic acid in acetic acid to give dipyrrylmethanes, but these structures proved to be poorly suited for porphyrin synthesis due to the electron-withdrawing nature of the fused phenanthroline unit. However, phenanthrolinopyrrole ethyl esters could be converted to the corresponding α-unsubstituted pyrroles with KOH in ethylene glycol at 180–190 °C, and these condensed with 2 equiv of acetoxymethylpyrroles in refluxing acetic acid-isopropyl alcohol to give tripyrranes. In a one pot procedure, tripyrrane di-*tert*-butyl esters were treated with TFA at room temperature to cleave the protective groups, diluted with dichloromethane, reacted with pyrrole dialdehydes and oxidized to afford phenanthrolinoporphyrins in 52–83% yield. These conditions also allow the synthesis of porphyrins with additional fused acenaphthylene or phenanthrene rings. Although the UV–vis spectra for these porphyrins are unexceptional, the presence of an external coordination site allows many potential applications to be considered. Porphyrins with two phenanthroline units could not be prepared by the '3+1' strategy. Instead, α-unsubstituted phenanthrolinopyrroles were reacted with a bis(dimethylaminomethyl)pyrrole in refluxing acetic acid to give moderate yields of the corresponding *opp*-diphenanthrolinoporphyrins. In one case, a triphenanthrolinoporphyrin and trace amounts of an *adj*-diphenanthrolinoporphyrin were formed as by-products.
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1. Introduction

Porphyrins with fused aromatic rings are of interest in a variety of areas including the development of extended chromophores[1,2] and as models for high molecular weight petroporphyrins.[3,4] In addition, fused porphyrin systems have considerable potential in molecular recognition studies and in the formation of wire or array type structures.[1,5,6] Extensive efforts have been put into the synthesis of benzoporphyrins[3,7–10] but little work has been carried out on porphyrins with other fused aromatic subunits.[11] We have developed a strategy for synthesizing porphyrins of this type from pyrrolic precursors that are generated in turn by the reaction of isocyanoacetate esters with readily available nitroaromatic compounds.[12,13] This approach has allowed the synthesis of porphyrins with fused naphthalene, phenanthrene,[12–14] acenaphthylene,[15–17] benzothiadiazole,[17,18] fluoranthene,[19] isoquinoline and quinoline rings.[20] A particularly interesting example of porphyrins with fused aromatic rings are phenanthrolinoporphyrins **1** (Chart 1). The 9,10-phenanthroline system has been extensively studied as an inorganic ligand[21] and the attachment of this unit allows the possibility of coordinating metals both within the porphyrin cavity and at the external nitrogens.[22] Related structures (e.g., **2**) have been promoted as possible 'alligator clips' for porphyrin molecular wires.[23] A preliminary communication on the synthesis of phenanthrolinoporphyrins **1** was published some time ago.[22] We now report full details on the synthesis of porphyrins **1** and related *opp*-diphenanthrolinoporphyrins.[24,25]

2. Results and discussion

In order to synthesize porphyrins **1**, phenanthrolinopyrroles **3** were required (Scheme 1). Although we had synthesized dihydronaphthopyrroles by a Knorr-type pyrrole condensation,[26] this approach was not readily amenable to the preparation of **3**. In 1985, Barton and Zard had introduced a method for synthesizing pyrroles by reacting nitroalkenes with ethyl isocyanoacetate in the presence of a non-nucleophilic base.[27] This approach has seen widespread application and is particularly useful in the preparation of

☆ For part 18 in the series, see: Lash, T. D.; Chen, S. *Tetrahedron* **2005**, *61*, 11577–11600.

Keywords: Porphyrins; Pyrroles; Phenanthroline; MacDonald condensation; '3+1' Methodology.
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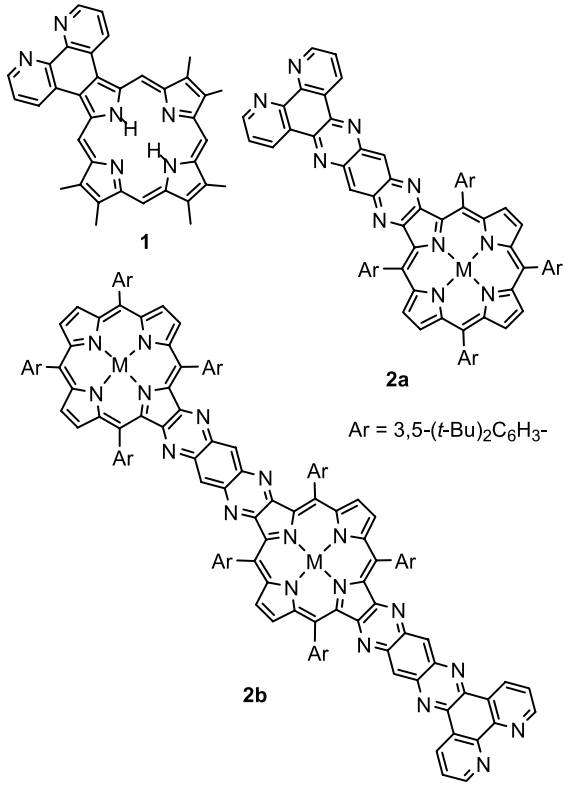
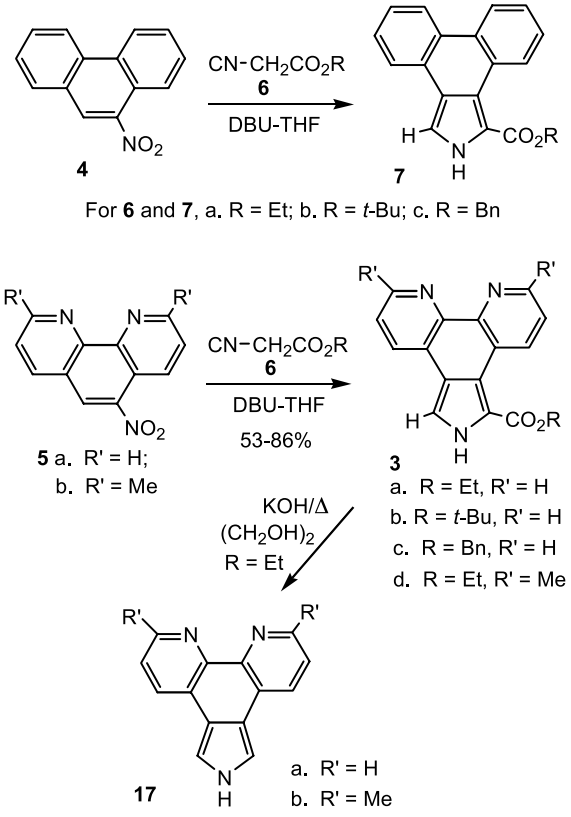
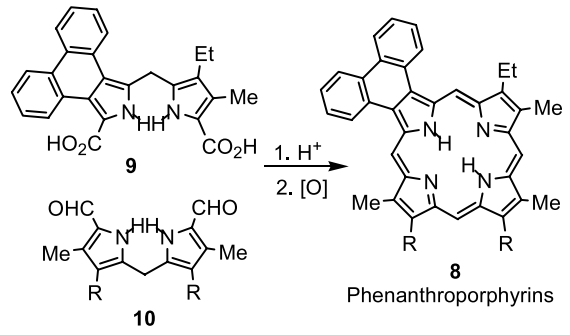


Chart 1.

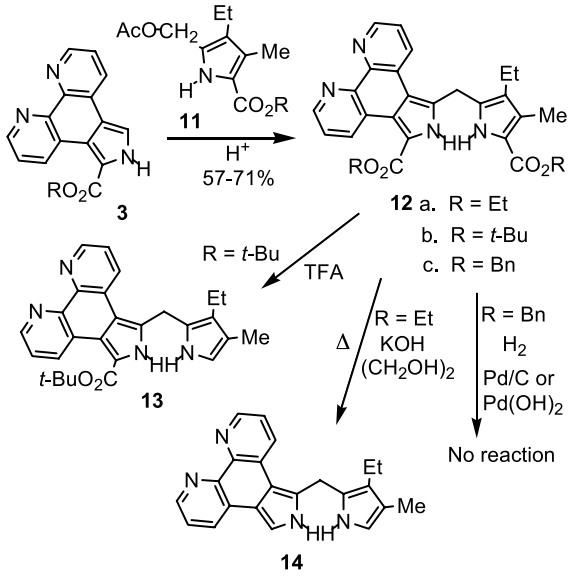


Scheme 1.

pyrrolic precursors to the porphyrins.[28–32] It seemed likely that 9-nitrophenanthrene (**4**) and 5-nitrophenanthroline (**5a**) would have sufficient nitroalkene character to undergo this



Scheme 2.



Scheme 3.

type of chemistry.[33] In fact, reaction of **4** or **5a** with ethyl, *tert*-butyl or benzyl isocyanoacetates **6** in the presence of DBU gave the related pyrroles **7** and **3**, respectively, in excellent yields (Scheme 1).[33] In the initial studies, mixtures of isopropyl alcohol and THF were used as the reaction solvent. However, these conditions often gave rise to partial transesterification to afford isopropyl esters. Subsequently, THF alone was found to give equally good results the formation of these *c*-annelated pyrroles. Reactions with ethyl or *tert*-butyl isocyanoacetate were carried out at room temperature but it was necessary to react benzyl isocyanoacetate **6c** with **4a** under refluxing conditions. Neocuproine (2,9-dimethyl-1,10-phenanthroline) was nitrated with concentrated nitric acid and fuming sulfuric acid to give 5-nitroneocuproine (**5b**) and this also reacted with ethyl isocyanoacetate to give the dimethyl-phenanthrolinopyrrole **3d**. Similar syntheses of **3a** and **7a** were reported in independent work by Ono and co-workers.[34]

In an earlier paper in this series, we reported the synthesis of phenanthroporphyrins **8** using the MacDonald '2+2' condensation (Scheme 2),[14] but this methodology proved not to be viable in the synthesis of phenanthrolinoporphyrins **1**. In the '2+2' approach, a dipyrrylmethane (e.g., **9**) is condensed with a dipyrrylmethane dialdehyde **10** in the presence of an acid catalyst.[35–37] The synthesis of dipyrrylmethanes with fused phenanthroline units was

carried out under conventional conditions. Hence, phenanthrolinopyrroles **3a–c** were condensed with acetoxymethylpyrroles **11** in acetic acid, using *p*-toluenesulfonic acid as a catalyst, and the required dipyrrolic products **12** were isolated in 57–71% yield (Scheme 3). Attempts to cleave the benzyl esters of **12c** with hydrogen over 10% palladium–charcoal or Pearlman's catalyst ($Pd(OH)_2$) were unsuccessful and catalytic transfer hydrogenation using hydrogen donors such as ammonium formate, cyclohexene or cyclohexadiene also gave no reaction. The phenanthroline unit appears to poison the catalyst making benzyl esters unsuitable as protective groups for this series. In related studies, we commonly use *tert*-butyl esters as protective groups. Pyrrole *tert*-butyl esters can generally be cleaved with TFA over a period of 10 min at room temperature. However, when di-*tert*-butyl ester **12b** was treated with TFA for 10 min, only one of the ester moieties was cleaved affording the mono-*tert*-butyl ester **13** (Fig. 1). Even when the reaction was run for 2–4 days at room temperature, little cleavage of the remaining *tert*-butyl ester was noted. Heating simply lead to decomposition. The decreased reactivity cannot be due to steric effects because dipyrrylmethanes with phenanthrene units do react under conventional conditions. Presumably, protonation of the phenanthroline unit exerts a strong electron-withdrawing effect that decreases the reactivity of this ester unit. Attempts to cleave the *tert*-butyl esters with trimethylsilyl iodide[38] were also unsuccessful. It was possible to saponify and decarboxylate diethyl ester **12a** with KOH in refluxing ethylene glycol to give **14**, but attempts to react this product with a dipyrrylmethane dialdehyde under MacDonald conditions analogous to those shown in Scheme 2 gave only trace amounts of phenanthrolinoporphyrin. Hence, even when the ester moieties have been removed, these dipyrrylmethanes appear to be poorly suited for porphyrin synthesis.

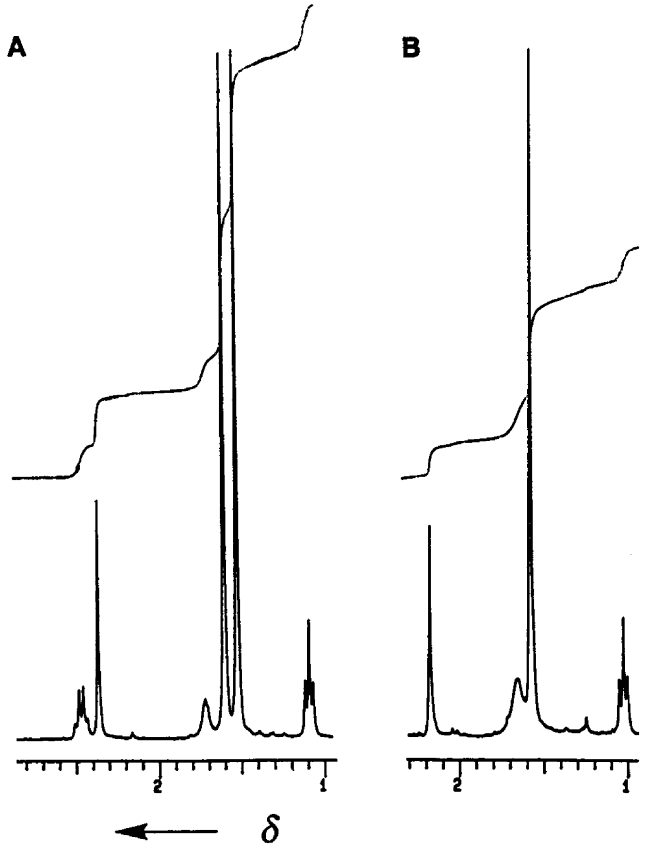
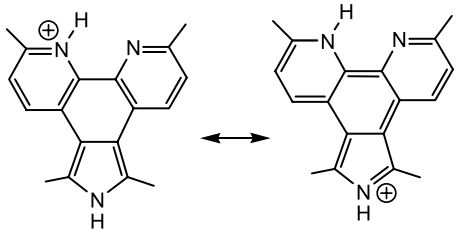
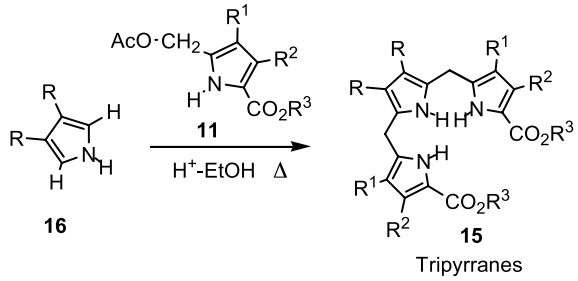


Figure 1. Partial 300 MHz proton NMR spectrum of dipyrrylmethane **12b** showing the alkyl group region. A. Initial NMR spectrum. The two *tert*-butyl ester units give singlets at 1.5 and 1.6 ppm. B. NMR spectrum of **12b** after it had been treated with neat TFA at room temperature for 10 min. The spectrum shows that only one of the *tert*-butyl esters has been cleaved.

Protonation of the phenanthroline unit undoubtedly has a strong electronic effect decreasing the electron density of the fused pyrrole ring (Scheme 4). Porphyrin syntheses such as the MacDonald condensation rely on the electron rich nature of pyrroles to generate carbon–carbon bonds, and the presence of a fused phenanthroline ring appears to sufficiently reduce the ability of the pyrrole units to undergo electrophilic substitution that porphyrin formation is prevented. This is not to say that electrophilic substitution could no longer occur, otherwise it would not have been possible to prepare dipyrrylmethanes **12**. However, intermediates in porphyrin syntheses are unstable and may be prone to acidolysis,[39] and a pronounced decrease in reaction rate would effectively block porphyrin formation. However, if the critical bond forming reaction were to occur at positions sufficiently removed from the phenanthroline system, porphyrin formation should be possible. At the time that these studies were being conducted, the '3+1' variation on the MacDonald condensation had seen little application.[36] In fact, this method had been used by Johnson and co-workers[40] to prepare furan and thiophene analogues of the porphyrins in the early 1970s but this approach had not been put to any further use over the following 20 years.[36] The '3+1' methodology requires the availability of tripyrranes **15**, and these were not conveniently accessible when the initial studies were conducted.[36] However, in 1987 Sessler et al. introduced a straightforward method of preparing tripyrranes by reacting acetoxymethylpyrroles **11** with 2,5-unsubstituted pyrroles **16** in the presence of a strong acid in refluxing ethanol (Scheme 5).[41] In many cases

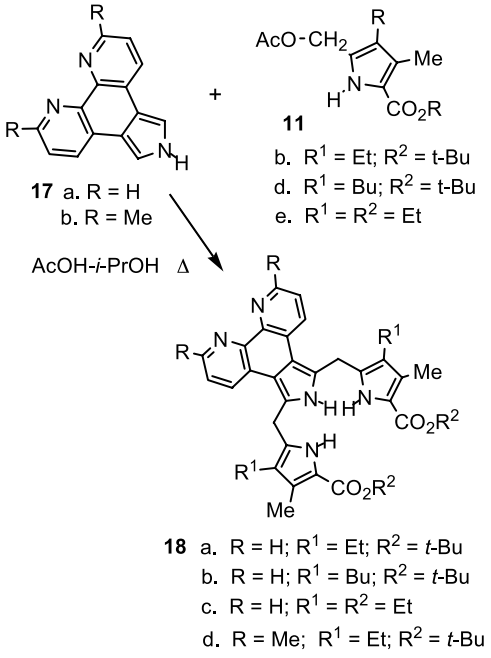


Scheme 4.

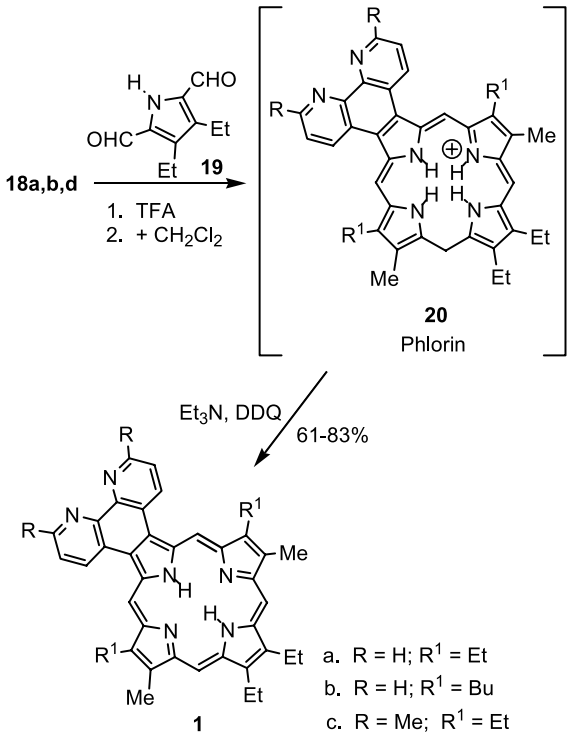


Scheme 5.

the tripyrrolic product precipitates from the solution in pure form, an important consideration due to the poor stability of these compounds in solution. As benzyl esters cannot easily be cleaved in the presence of a phenanthroline unit, we wanted to use *tert*-butyl esters as the terminal protective groups. This in turn necessitated the use of a milder acid catalyst. However, we found that 5% acetic acid in ethanol gave equally good results in the formation of tripyrranes (Scheme 5),[22,42] and unlike the original procedure[41] these conditions worked well with a 2:1 ratio of reactants **11** and **16**. Treatment of ethyl ester **3a** with KOH in ethylene glycol at 180–190 °C gave rise to a one pot saponification–decarboxylation to afford the unsubstituted tetracycle **17a** in excellent yields (Scheme 1). The neocuproine derived ethyl ester **3d** was similarly converted into α-unsubstituted pyrrole **17b** under these conditions. Reaction of **17a** with acetoxymethylpyrrole **11b** in refluxing acetic acid–ethanol gave poor yields of tripyrrane **18a**. However, when the solvent was changed to isopropyl alcohol, **18a** could be isolated in 54% yield (Scheme 6). Reaction of **17a** with **11d** and **11e** gave similar results, affording tripyrranes **18b** and **18c**, while dimethylphenanthrolinopyrrole **17b** reacted with **11b** to give tripyrrane **18d** in 67% yield. At this stage, we needed to cleave the terminal *tert*-butyl ester groups to unmask the electron-rich α-positions of the pyrrolic units. However, the tripyrrane product would be unstable and this necessitates minimal handling prior to porphyrin formation. As the ester cleavage requires the use of TFA, and this is also a potential catalyst for subsequent chemistry, we conceived a one pot strategy for porphyrin synthesis.[22] Hence, the tripyrrane **18a** was treated with TFA at room temperature under nitrogen for 10 min, then diluted to 20 times the volume with dichloromethane and condensed with pyrrole dialdehyde **19** (Scheme 7). After 2 h, the solution was neutralized by the dropwise addition of triethylamine and then oxidized with 1 equiv of DDQ. Following chromatography and recrystallization from chloroform–methanol, the required phenanthrolinoporphyrin was



Scheme 6.



Scheme 7.

obtained in a remarkable 83% yield. Reaction of **18b** and **18d** with **19** under these conditions also gave excellent yields of porphyrins **1b** and **1c**, respectively. Prior to neutralization, the UV–vis spectrum for a diluted sample taken from the reaction mixture showed the presence of a broad peak at λ_{max} 730 nm which was consistent with the formation of a protonated phlorin intermediate (e.g., **20**).[20,42,43] This dihydroporphyrin intermediate must be oxidized to form the aromatic porphyrin system. However, addition of triethylamine lead to an immediate conversion to the porphyrin free base suggesting that this system is remarkably easy to oxidize in the presence of trace amounts of atmospheric oxygen. Although the addition of DDQ was not strictly necessary, this was usually added to ensure complete reaction.

When these studies were being developed,[22,24,25] two independent reports on the use of the '3+1' methodology for porphyrin synthesis appeared in the literature.[44a,45] These methods were developed for rather different applications, and differed from our procedures by using relatively dilute conditions.[44,45] In fact, our conditions are more convenient and provide superior yields in most cases.[22,42] We have applied this chemistry to the synthesis of diverse porphyrin structures,[15–20,22,42] as well as for the synthesis of many new porphyrin analogue systems.[46–49] This version of the '3+1' chemistry also has the advantage of allowing a one pot deprotection, condensation, oxidation sequence that avoids the need to isolate unstable intermediates. For these reasons, this version of the chemistry has seen widespread application by others in the area of porphyrin synthesis.[50–54]

Phenanthrolinoporphyrins **1** are relatively insoluble in organic solvents, but good quality NMR spectra could be

obtained in the presence of TFA. The proton NMR spectra demonstrate that the porphyrins retain a strong diatropic ring current (Fig. 2) where the external *meso*-protons appear near 11 ppm while the internal NHs resonate between −2 and −4 ppm. The phenanthroline protons nearest to the porphyrin nucleus are also strongly deshielded giving rise to resonances at >10 ppm. The UV–vis spectra for **1a–c** were unexceptional showing only minor shifts compared to regular porphyrins.[2] The Soret bands for the free base porphyrins appeared at 422–424 nm while the longest wavelength Q band appeared as a weak absorption at 636 nm. The spectra for the protonated forms were also only slightly red shifted compared to octaalkylporphyrins. These data are similar to the results obtained for porphyrins with fused naphthalene or phenanthrene rings.[12–14,26]

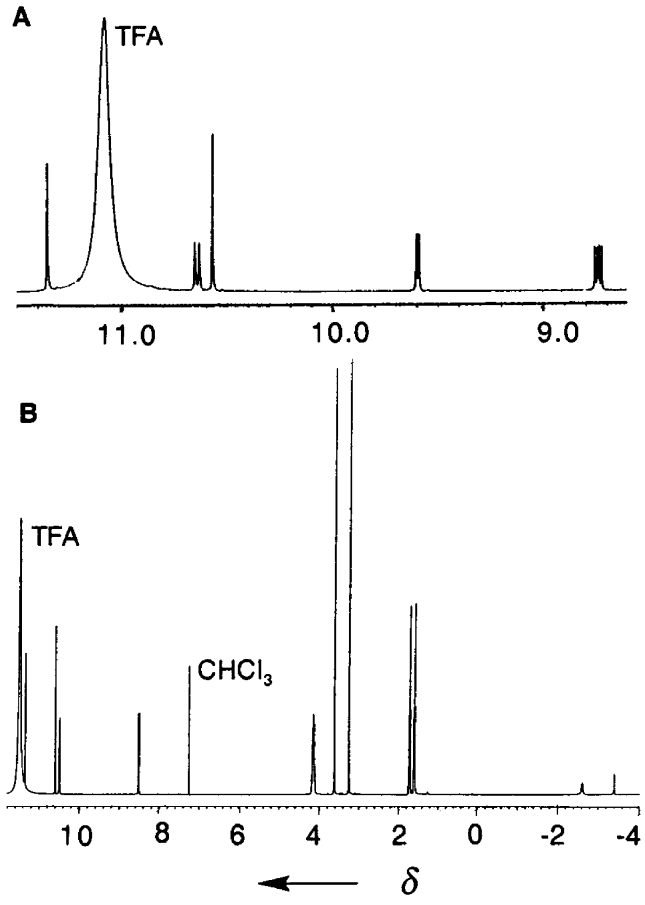
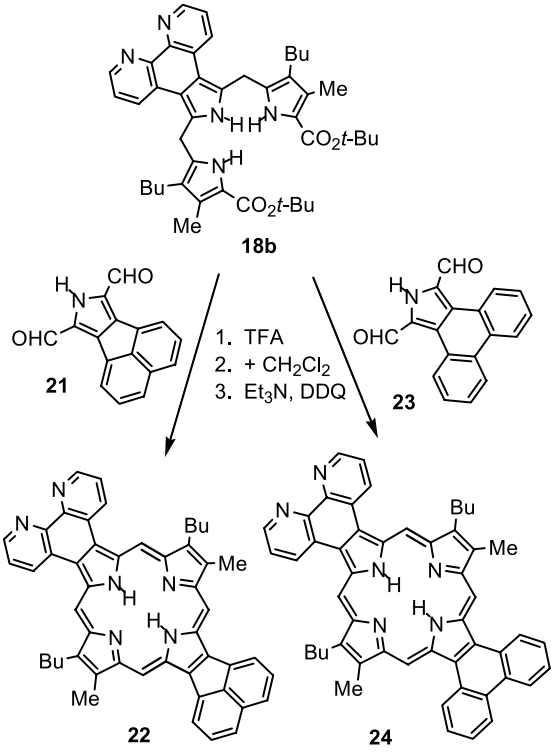


Figure 2. Four hundred mega-hertz proton NMR spectra of phenanthrolinoporphyrins in TFA–DCl_3. A. Downfield region for porphyrin **1a** showing two singlets for the *meso*-protons on either side of the TFA peak. B. Proton NMR spectrum of the neocuproine derived porphyrin **1c**

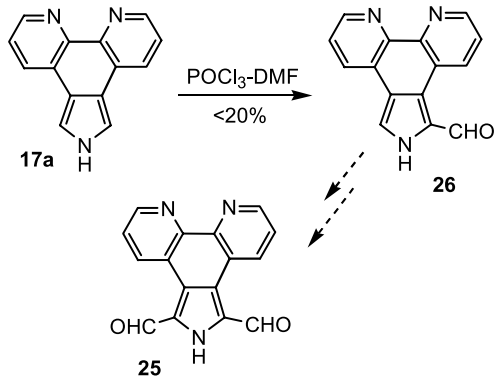
Although naphthalene, phenanthrene and phenanthroline exert only a small effect on the porphyrin chromophore, fused acenaphthylene rings are known to strongly modify the UV–vis spectra.[15–17] In order to access the influence of a fused acenaphthylene on the phenanthrolinoporphyrin system, tripyrrane **18b** was condensed with acenaphthopyrrole dialdehyde **21**[17] under the '3+1' conditions to give the mixed fused porphyrin system **22** in 52% yield (Scheme 8). The tripyrrane was also reacted with phenanthropyrrole dialdehyde **23**[17] to give the mixed phenanthrene/phenanthroline fused porphyrin **24** in 74% yield (Scheme 7). The latter system provides a contrast as phenanthrene usually has a relatively small effect on the porphyrin chromophore compared to acenaphthylene.[2] A comparison of the spectra shows that the 'acenaphthylene effect'[2] is relatively small for this system. The Soret band for **22** in chloroform appears at 435 nm, compared to 431 nm for **24**, although the acenaphthoporphyrin **22** gives a weak absorption at 682 nm, that is not found for **24**. The longest wavelength Q band for **24** is found at 594 nm, but this is best contrasted to the apparently equivalent absorption at 617 nm for **22**. In 5% TFA–chloroform, **22** shows a strong Q band at 662 nm while **24** shows a similar absorption at 651 nm. The shifts are consistently larger for the acenaphthylene fused porphyrin but the effects are considerably smaller than those observed for other acenaphthoporphyrins.[15–17]



Scheme 8.

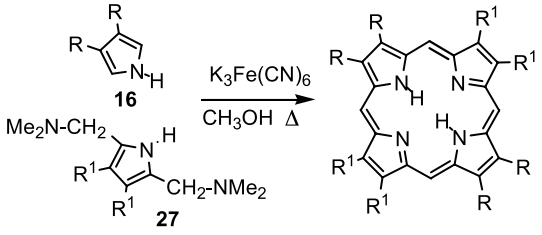
The possibility of fusing more than one phenanthroline unit onto the porphyrin nucleus was also explored. Initially, the synthesis of a phenanthrolinopyrrole dialdehyde **25** was attempted (Scheme 9) so that '3+1' chemistry along the lines used to prepare **22** and **24** could be utilized. However, Vilsmeier formylation of **17a** gave only a low yield of monoaldehyde **26** and attempts to take this on to **25** using a protection–formylation–deprotection methodology[42] were unsuccessful. A direct route to pyrrole dialdehydes using triethyl orthoformate and TFA has been reported,[55] but failed to give any product in this case. Once again, these problems most likely result from deactivation by the electron-withdrawing phenanthroline unit which are exacerbated by the presence of acid.

Nguyen et al. have reported a synthesis of porphyrins from

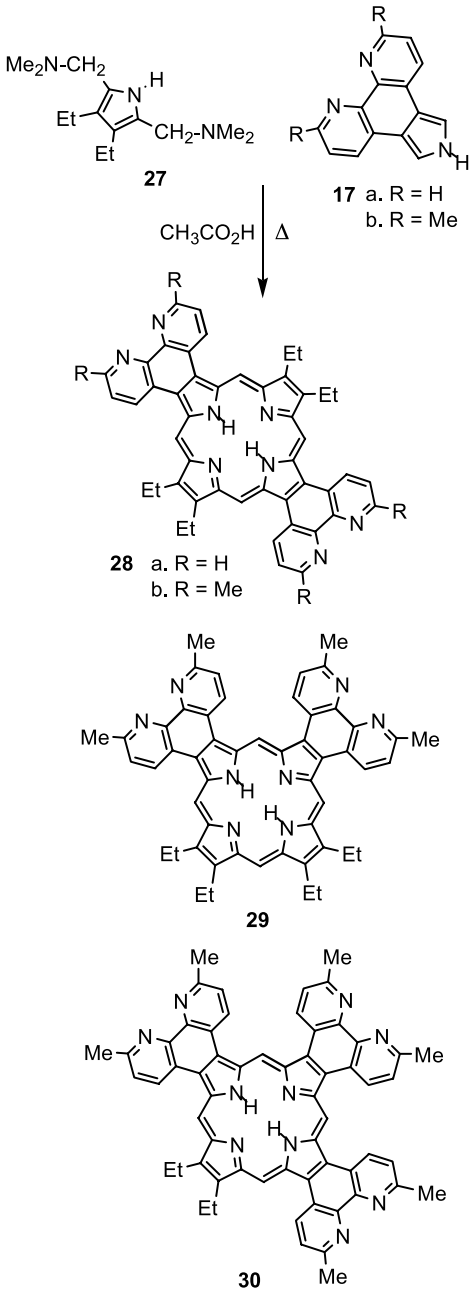


Scheme 9.

two different pyrrolic precursors using non-acidic conditions (Scheme 10),[56] and this method was explored as an alternative entry into diphenanthrolinoporphyrins. In this method, a bis(dimethylaminomethyl)pyrrole **27** is prepared by reacting a 2,5-diunsubstituted pyrrole with Eschenmoser's salt.[56] This can be reacted with a different α-unsubstituted pyrrole **16** in refluxing methanol containing potassium ferricyanide as an oxidant to give porphyrin products (Scheme 10).[56] In some cases, the dimethylaminomethyl moieties were converted to the corresponding quaternary salts with methyl iodide to improve the leaving group ability.[56] The idea was to prevent scrambling by using nonacidic conditions and oxidizing the intermediates to porphyrin before any fragmentation processes could occur.[56] These considerations suggest that this method could be suitable for synthesizing diphenanthrolinoporphyrins. However, initial attempts to prepare *opp*-diphenanthrolinoporphyrins **28** using this approach were unsuccessful (Scheme 11). The bis(dimethylaminomethyl)pyrrole **27** was heated with **17a** or **17b** and $K_3Fe(CN)_6$ in refluxing methanol, but only trace amounts of monophenanthrolinoporphyrins were generated. One of the problems was that the phenanthrolinopyrroles were virtually insoluble in methanol. Using THF as a cosolvent, **17b** reacted with **27** to give trace amounts of mono- and diphenanthrolinoporphyrins but the main product from this chemistry was octaethylporphyrin. After exploring a number of different reaction conditions, we found that the best results were obtained using acetic acid as a solvent under refluxing conditions open to the air. Addition of potassium ferricyanide dramatically reduced yields and the oxidant was not used for our syntheses. Phenanthrolinopyrrole **17a** reacted with **27** to give *opp*-diphenanthrolinoporphyrin **28a** in 11% yield. This porphyrin was poorly soluble in organic solvents but readily dissolved in excess TFA–chloroform. The proton and carbon-13 NMR spectra of **28a** in TFA–DCl_3 confirmed the highly symmetrical



Scheme 10.



Scheme 11.

nature of this compound. The 400 MHz proton NMR spectrum gave a 4H singlet for the *meso*-protons at 11.4 ppm, while the phenanthroline protons gave rise to three sets of resonances at 8.8 (4H, m), 9.6 (4H, d) and 10.7 ppm (4H, d). As expected, the carbon-13 NMR spectrum only gave 12 resonances for the 48 carbons and the four *meso*-carbons showed up as a single peak at 100.6 ppm.

Condensation of **17b** with **27** in refluxing acetic acid gave slightly more complicated results (Scheme 11). After workup, while most of the reaction products were reasonably soluble, a small amount of a green insoluble material was also obtained. Accurate mass data showed that this compound had the molecular formula $C_{52}H_{46}N_8$, and this was assigned as the *adj*-diphenanthrolinoporphyrin **29**.

The soluble material was chromatographed on a grade 3 alumina column. A minor fraction containing monophenanthrolinoporphyrin was initially collected, followed by a major green fraction corresponding to the *opp*-diphenanthrolinoporphyrin **28b** and a third more polar green band. Recrystallization of the major fraction from chloroform-methanol gave **28b** as purple crystals in 6–8% yield. The final fraction was also recrystallized from chloroform-methanol to give a green solid in $<2\%$ yield. Accurate mass data showed that this compound had the molecular formula $C_{60}H_{46}N_{10}$ and together with the proton NMR data, this material could be assigned as the triphenanthrolinoporphyrin **30**. The formation of **29** and **30** is not really surprising given the fact that acetic acid is used as a solvent for the condensation and acid catalyzed fragmentation-recombination processes can therefore take place. However, the scrambling is not as severe as would usually be the case due to the decreased reactivity of the phenanthrolinopyrrole unit. The proton and carbon-13 NMR data for **28b** showed the expected symmetry and the *meso*-carbon resonance was observed at 100.4 ppm. The proton NMR spectrum of **30** in TFA–DCl_3 shows a decreased level of symmetry with two 2H singlets for *meso*-protons at 11.2 and 12.1 ppm, while the phenanthroline protons afforded a series of six 2H doublets at 8.45, 8.50, 8.56, 10.37, 10.40 and 10.44 ppm. The availability of by-products **29** and **30** allowed for comparisons to be made on the cumulative effects of one, two or three phenanthroline units on the porphyrin chromophore. The UV–vis spectra of **1c**, which has one fused dimethylphenanthroline, conveniently completes the set (Fig. 3). Monophenanthrolinoporphyrin **1c** gives a Soret band at 422 nm and Q bands at 520, 558, 579 and 633 nm. The UV–vis spectrum of **28b** shows a sharpened Soret band at 432 nm, and only two significant Q bands at 578 and 597 nm, although weak absorptions are noted at 554 and 645 nm. The *adj*-diphenanthroline system **29** gave a Soret band at 441 nm and a more defined set of four Q bands at 542, 575, 594 and 653 nm. The spectra of dinaphtho- and diphenanthroporphyrins **31**–**34** (Chart 2) showed similar trends, where the *opp*-diannelated systems only showed weak longer wavelength absorptions (Q band I) and the two major Q bands were relatively close together, while the adjacent ring fused structures gave a series of more defined Q bands.[14,57] All three series show longer wavelength absorptions for the *adj*-di-fused porphyrins **29**, **31** and **33** compared to the *opp*-di-fused structures **28**, **32** and **34**. The UV–vis spectrum for triphenanthrolinoporphyrin **30** showed somewhat mixed features but overall gave the largest bathochromic shifts. The Soret band gave a λ_{max} value of 447 nm, while the longest wavelength Q band shifted to 663 nm. Although Q band I is more prominent than is seen for **28b**, Q bands II and III have moved closer together and are now only separated by 11 nm. In the presence of TFA, the protonated porphyrins also show increasing red shifts for the Soret band going from **1c** to **28b** to **29** to **30** with λ_{max} values of 432, 450, 455 and 469 nm, respectively.

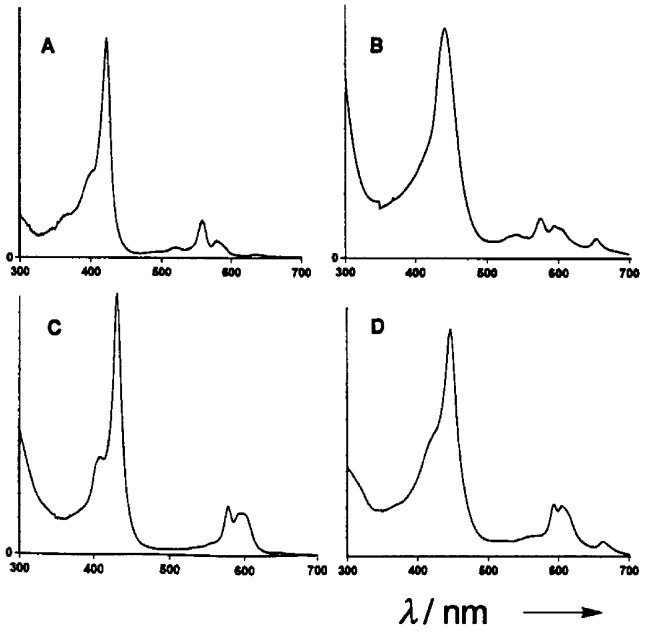


Figure 3. UV–vis spectra for phenanthrolinoporphyrins in 1% Et_3N–chloroform. A. Monophenanthrolinoporphyrin **1c**. B. *adj*-Diphenanthrolinoporphyrin **29**. C. *opp*-Diphenanthrolinoporphyrin **28b**. D. Triphenanthrolinoporphyrin **30**.

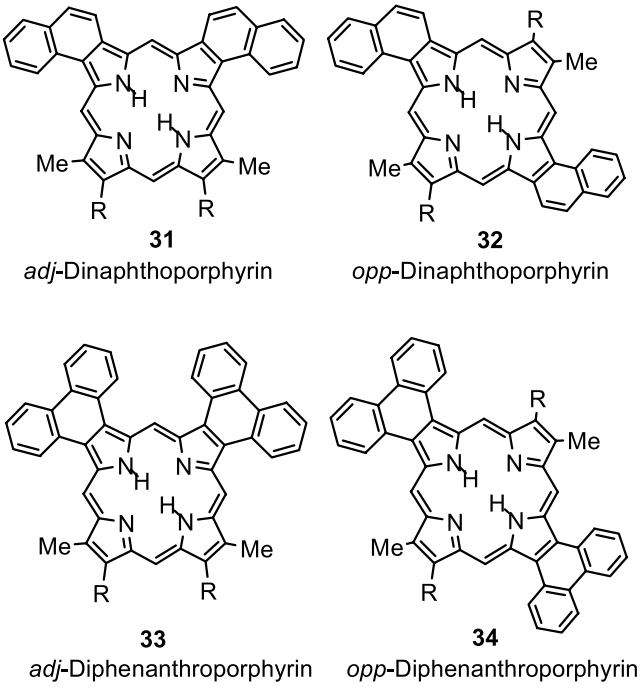


Chart 2. Dinaphtho and diphenanthroporphyrins.

3. Conclusions

The '3+1' methodology provides a superior route to phenanthrolinoporphyrins and these are readily available in 4 steps from nitrophenanthrolines. Although the introduction of multiple phenanthroline units could not be achieved using this strategy, *opp*-diphenanthrolinoporphyrins could easily be prepared by reacting phenanthrolinopyrroles with a bis(dimethylaminomethyl)-pyrrole in refluxing acetic acid. Porphyrin by-products were formed in these reaction, but these were easily removed by column chromatography. Hence, this important family of porphyrins is easily accessible for potential applications in coordination chemistry and molecular recognition studies.

4. Experimental

4.1. General

9,10-Phenanthroline (**6d**), neocuproine, TFA, DDQ and DBU were purchased from Aldrich or Acros, and were used without further purification. THF was distilled from calcium hydride immediately prior to use. Chromatography was performed using Grade III neutral alumina or 70–230 mesh silica gel. Melting points were determined in open capillary tubes on a Mel-Temp apparatus and are uncorrected. UV–vis absorption spectra were run on a Varian Cary Spectrophotometer, and NMR data was obtained on a Varian Gemini 400 MHz FT NMR spectrometer. Mass spectral determinations were conducted at the Mass Spectral Laboratory, School of Chemical Sciences, University of Illinois at Urbana-Champaign, and elemental analyses were obtained from the School of Chemical Sciences Microanalysis Laboratory at the University of Illinois.

4.2. Synthetic procedures

4.2.1. Ethyl 7,8-diazaphenanthro[9,10-*c*]pyrrole-1-carboxylate (3a). DBU (2.02 g) was added dropwise to a solution of ethyl isocyanoacetate[58] (1.50 g) and 5-nitro-1,10-phenanthroline[59] (3.00 g) in THF (20 mL) and the resulting mixture stirred at room temperature overnight. The solution was diluted with chloroform, washed with water, and dried over sodium sulfate. The solvent was evaporated under reduced pressure and the residue recrystallized from chloroform/hexane to give the phenanthrolinopyrrole (2.95 g; 76%) as yellow crystals, mp 277–278 °C, dec; ir (nujol mull): ν 1687 cm^{-1} (C=O str.); ^1H NMR (d_6-DMSO-$CDCl_3$): δ 1.17 (3H, t, J=7.2 Hz), 4.15 (2H, q, J=7.2 Hz), 7.21–7.32 (2H, m), 7.60 (1H, d, J=3.3 Hz), 8.11 (1H, dd, J=1.5, 8.1 Hz), 8.64–8,71 (2H, m), 9.80 (1H, dd, J=1.5, 8.2 Hz), 11.65 (1H, br s); ^{13}C NMR ($CDCl_3$): δ 14.4, 60.7, 116.4, 117.2, 120.1, 121.7, 122.9, 123.4, 124.8, 125.3, 130.6, 136.0, 145.2, 146.6, 148.4, 148.7, 160.8; EI MS (70 eV): *m/z* (% rel. int.) 291 (68) [M^+], 245 (100) [M^+ − EtOH], 217 (33), 191 (16). Anal. Calcd for $C_{17}H_{13}N_3O_2\cdot\frac{1}{4}H_2O$: C, 69.02; H, 4.60; N, 14.20. Found: C, 68.66; H, 4.48; N, 13.97.

4.2.2. *tert*-Butyl 7,8-Diazaphenanthro[9,10-*c*]pyrrole-1-carboxylate (3b). Over a period of 5 min, DBU (0.897 g) was added dropwise to a stirred mixture of 5-nitro-1,10-phenanthroline (1.25 g) and *tert*-butyl isocyanoacetate[14] (0.780 g) in THF (14 mL) and 2-propanol (14 mL). The solution was stirred overnight at room temperature, diluted with chloroform, washed with 25 mL of water and dried over sodium sulfate. The solvent was evaporated and the residue recrystallized from carbon tetrachloride to give the phenanthrolinopyrrole (1.656 g; 83%) as yellow crystals, mp 235–236 °C, dec; ir (nujol mull): ν 1692 cm^{-1} (C=O str.); ^1H NMR ($CDCl_3$): δ 1.70 (9H, s), 7.47 (1H, dd, J=7.6, 4.4 Hz), 7.59 (1H, dd, J=8.0, 4.4 Hz), 7.67 (1H, d, J=2.8 Hz), 8.23 (1H, d, J=8.0 Hz), 8.95 (1H, d, J=4.4 Hz), 9.02 (1H, d, J=4.4 Hz), 10.04 (1H, d, J=8.4 Hz), 10.18 (1H, br s); ^{13}C NMR ($CDCl_3$): δ 28.4, 82.1, 114.6, 118.5, 120.2, 121.1, 122.7, 123.0, 124.4, 125.0, 130.2, 135.9, 145.3, 146.6, 148.3, 148.6, 159.6; EI MS (70 eV): *m/z* (% rel. int.) 319 (17) [M^+], 291 (18), 263 (45) [M^+ − $(CH_3)_2C{=}CH_2$], 245 (100) [M^+ − *t*-BuOH], 219 (62), 191 (18). Anal. Calcd for $C_{19}H_{17}N_3O_2\cdot\frac{1}{2}H_2O$: C, 69.52; H, 5.47; N, 12.60. Found: C, 69.92; H, 5.47; N, 12.60.

4.2.3. Benzyl 7,8-diazaphenanthro[9,10-*c*]pyrrole-5-carboxylate (3c). Benzyl isocyanoacetate[28a] (1.78 g) and 5-nitro-1,10-phenanthroline (1.25 g) were dissolved in 22 mL of THF. DBU (1.66 g) was added dropwise over several min, and the resulting mixture heated under reflux overnight. The mixture was diluted with chloroform, washed with water, and dried over sodium sulfate. The solvent was removed under reduced pressure, and the residue recrystallized from isopropyl alcohol to give the pyrrole (1.40 g; 71%) as tan crystals, mp 234–236 °C, dec; IR (nujol mull): ν 1675 cm^{-1} (C=O str.); ^1H NMR ($CDCl_3$): δ 5.42 (2H, s), 7.36–7.45 (3H, m), 7.49–7.58 (3H, m), 7.76 (1H, d, J=2.8 Hz), 8.29 (1H, dd, J=1.6, 8.4 Hz), 8.99 (1H, dd, J=1.6, 8.4 Hz), 9.04 (1H, dd, J=1.5, 8.2 Hz), 10.06 (1H, dd, J=1.6, 8.0 Hz), 10.36 (1H, br s); ^{13}C NMR ($CDCl_3$): δ 66.9, 116.2, 116.9, 120.6, 122.4, 123.2, 123.5, 124.6, 125.2, 128.8, 128.9, 129.0, 130.7, 135.9, 136.2, 145.6, 147.0, 148.8, 149.2, 160.3; EI MS (70 eV): *m/z* (% rel. int.) 353 (40) [M^+], 309 (23), 291 (7), 245 (41) [M^+ − BnOH], 219 (31), 191 (17), 91 (100). Anal. Calcd for $C_{22}H_{15}N_3O_2\cdot\frac{1}{4}H_2O$: C, 73.83; H, 4.36; N, 11.74. Found: C, 73.71; H, 4.37; N, 11.67.

4.2.4. Ethyl 7,8-diaza-6,9-dimethylphenanthro[9,10-*c*]pyrrole-1-carboxylate (3d). Neocuproine monohydrate (11.50 g) was dissolved in 50 mL of fuming sulfuric acid (50 mL; 30% SO_3) in a 250 mL Erlenmeyer flask and the temperature spontaneously rose to approximately 90 °C. Concentrated nitric acid (27 mL) was immediately added to the hot stirred solution at such a rate that the temperature approached 170 °C, and the resulting mixture stirred for 30 min. The solution was poured over ice (800 g), and the resulting mixture basified with a 30% aqueous sodium hydroxide solution. Dilute nitric acid was added to the mixture until the pH was slightly acidic. The yellow precipitate was filtered, washed well with water, and dried in vacuo to give crude 5-nitrocuproine (8.75 g, 68%) as a light yellow powder. ^1H NMR ($CDCl_3$): δ 3.00 (3H, s), 3.01 (3H, s), 7.64 (1H, d, J=8.4 Hz), 7.68 (1H, d, J=8.8 Hz), 8.29 (1H, d, J=8.4 Hz), 8.62 (1H, s), 8.93 (1H, d, J=8.8 Hz). The nitro compound (3.10 g) was reacted with ethyl isocyanoacetate (1.50 g) under the conditions described for **3d**. The crude product was heated with carbon tetrachloride, cooled, and suction filtered to give the phenanthrolinopyrrole (3.02 g; 77%) as a tan powder, mp >300 °C. An analytical sample was obtained by recrystallization from ethanol as pale yellow crystals, mp >300 °C; IR (nujol mull): ν 1675 cm^{-1} (C=O str.); ^1H NMR (d_6-DMSO): δ 1.38 (3H, t, J=7.0 Hz), 2.70 (3H, s), 2.71 (3H, s), 4.38 (2H, q, J=7.0 Hz), 7.50–7.55 (2H, two overlapping doublets), 8.27 (1H, d, J=3.6 Hz), 8.65 (1H, d, J=8.4 Hz), 9.92 (1H, d, J=8.4 Hz), 12.95 (1H, br s); ^{13}C NMR (d_6-DMSO): δ 14.3, 24.5, 24.6, 60.1, 115.5, 118.0, 119.4, 120.8, 122.3, 122.4, 122.5, 123.4, 131.5, 135.2, 143.3, 145.2, 155.9, 156.2, 160.5. Anal. Calcd for $C_{19}H_{17}N_3O_2\cdot\frac{1}{2}H_2O$: C, 69.50; H, 5.52; N, 12.79; Found: C, 69.63; H, 5.68; N, 12.22.

4.2.5. 7,8-Diazaphenanthro[9,10-*c*]pyrrole (17a). Nitrogen gas was bubbled through a mixture of ethyl

7,8-diazaphenanthro[9,10-*c*]pyrrole-1-carboxylate (**3a**) (1.50 g) and potassium hydroxide (3.00 g) in ethylene glycol (60 mL) for 10 min, and the solution was refluxed on a preheated oil bath at 180 °C for a further 30 min. The product precipitated out of the solution during the reaction. The mixture was poured into ice-water and after suction filtration, the solid was washed well with deionized water and vacuum dried overnight to give the pyrrole (1.04 g; 92%) as a shiny off-white powder, mp >300 °C; ir (nujol mull): ν 3300 cm^{-1} (NH str.); ^1H NMR (d_6-DMSO-$CDCl_3$): δ 7.61 (2H, d, J=4.4, 8.0 Hz), 7.86 (2H, J=2.4 Hz), 8.60 (2H, d, J=8 Hz), 8.78 (2H, dd, J=1, 4 Hz), 12.16 (1H, br s); ^{13}C NMR (d_6-DMSO-$CDCl_3$): δ 111.5, 114.7, 122.3, 125.4, 130.7, 144.3; EI MS (70 eV): *m/z* (% rel. int.) 219 (100) [M^+], 192 (10) [M^+ − HCN]. Anal. Calcd for $C_{14}H_9N_3$: C, 76.71; H, 4.11; N, 19.18. Found: C, 76.39; H, 4.19; N, 18.94.

4.2.6. 7,8-Diaza-6,9-dimethylphenanthro[9,10-*c*]pyrrole (17b). The title compound was prepared by the previous procedure from ethyl ester **3d** (500 mg). Following suction filtration, the sample was dried in vacuo to give **17b** (325 mg; 84%) as a tan powder, mp >300 °C; ^1H NMR (d_6-DMSO): δ 2.70 (6H, s), 7.48 (2H, d, J=8.4 Hz), 7.87 (2H, d, J=2.8 Hz), 8.49 (2H, d, J=8.4 Hz), 12.08 (1H, br s); ^{13}C NMR (d_6-DMSO): δ 24.5, 111.7, 116.5, 122.8, 123.6, 131.1, 143.9, 154.5. Anal. Calcd for $C_{16}H_{13}N_3$: C, 77.71; H, 5.30; N, 16.99. Found: C, 77.33; H, 5.03; N, 17.38.

4.2.7. *tert*-Butyl 4-Butyl-3,5-dimethylpyrrole-2-carboxylate. A solution of sodium nitrite (21.36 g) in water (35 mL) was added dropwise to a solution of *tert*-butyl acetoacetate (36.36 g) in glacial acetic acid (35 mL), maintaining the temperature of the mixture below 10 °C throughout the addition using an ice-salt bath. The resulting oxime solution was stirred at room temperature for 3 h. A mixture of 3-butyl-2,4-pentanedione[35d] (42.66 g) in glacial acetic acid (70 mL) was preheated with the aid of a water bath to 70 °C. The oxime solution was then added dropwise while simultaneously adding a mixture of zinc powder (51.4 g) and sodium acetate (29.52 g), adjusting the rate of addition so that the temperature of the reaction mixture was maintained at 80–90 °C. Once the addition was completed, the mixture was vigorously stirred and heated on a boiling water bath for 1 h. The mixture was cooled to 70 °C, and the solution decanted from the residual zinc into 2 L of ice-water. The resulting precipitate was filtered off, washed well with water, and recrystallized from toluene to give the pyrrole (15.15 g; 26%) as pale yellow crystals, mp 98–100 °C; ir (nujol mull): ν 3322 (NH str.), 1657 cm^{-1} (C=O str.); ^1H NMR ($CDCl_3$): δ 0.93 (3H, t, J=7.0 Hz), 1.30–1.45 (4H, m), 1.58 (9H, s), 2.21 (3H, s), 2.26 (3H, s), 2.36 (2H, t, J=7.3 Hz), 8.80 (1H, br s); ^{13}C NMR ($CDCl_3$): δ 10.9, 11.7, 14.2, 22.7, 24.0, 28.8, 33.4, 80.1, 118.1, 122.3, 126.3, 128.9, 161.5. Anal. Calcd for $C_{15}H_{25}NO_2$: C, 71.67; H, 10.02; N, 5.57. Found: C, 72.05; H, 10.05; N, 5.87.

4.2.8. *tert*-Butyl 5-acetoxymethyl-4-ethyl-3-methylpyrrole-2-carboxylate (11b). Lead tetraacetate (39.28 g) was added to a stirred solution of *tert*-butyl 4-ethyl-3,5-dimethylpyrrole-2-carboxylate[60] (17.84 g) in acetic acid (80 mL) and acetic anhydride (4 mL) and the resulting mixture stirred at room temperature under anhydrous conditions for 3 h. The mixture was poured into ice-water (400 mL) and the resulting precipitate was collected by suction filtration and washed with liberal amounts of water. Recrystallization from chloroform–petroleum ether (60–80°) afforded the acetoxymethylpyrrole (18.85 g; 83%) as white flakes, mp 111.5–113 °C (lit. mp[61] 115–116 °C); ^1H NMR ($CDCl_3$): δ 1.07 (3H, t, J=7.6 Hz), 1.56 (9H, s), 2.06 (3H, s), 2.25 (3H, s), 2.45 (2H, q, J=7.6 Hz), 5.01 (2H, s), 8.99 (1H, br s); ^{13}C NMR ($CDCl_3$): δ 10.4, 16.2, 17.3, 21.2, 28.7, 57.2, 80.8, 120.7, 125.2, 125.9, 127.0, 161.2, 171.7.

4.2.9. *tert*-Butyl 5-acetoxymethyl-4-butyl-3-methylpyrrole-2-carboxylate (11d). The acetoxymethylpyrrole was prepared by the procedure given for **11b** from *tert*-butyl 4-butyl-3,5-dimethylpyrrole-2-carboxylate (5.00 g). Recrystallization from chloroform–petroleum ether (60–80°) gave the acetoxymethylpyrrole (4.50 g, 60%) as fluffy white needles, mp 85–87 °C; ir (nujol mull): ν 3305 (NH str.), 1735 (acetoxy C=O str.), 1655 cm^{-1} (pyrrole C=O str.); ^1H NMR ($CDCl_3$): δ 0.92 (3H, t, J=7.0 Hz), 1.29–1.46 (4H, m), 1.57 (9H, s), 2.06 (3H, s), 2.25 (3H, s), 2.43 (2H, t, J=7.3 Hz), 5.02 (2H, s), 9.02 (1H, br s); ^{13}C NMR ($CDCl_3$): δ 10.6, 14.2, 21.1, 22.7, 23.8, 28.7, 33.8, 57.3, 80.7, 120.7, 125.4 (2), 126.3, 161.1, 171.5. Anal. Calcd for $C_{17}H_{27}NO_4$: C, 65.99; H, 8.79; N, 4.53. Found: C, 66.22; H, 9.04; N, 4.72.

4.2.10. Ethyl 3-(5-ethoxycarbonyl-3-ethyl-4-methyl-2-pyrrolylmethyl)-7,8-diazaphenanthro[9,10-*c*]pyrrole-1-carboxylate (12a). *p*-Toluenesulfonic acid (0.510 g) was added to a solution of ethyl 5-acetoxymethyl-4-ethyl-3-methylpyrrole-2-carboxylate[62] (0.236 g) and **3a** (0.257 g) in acetic acid (6 mL), and the mixture stirred at room temperature for 7 h. The solution was poured into ice-water, neutralized with solid sodium bicarbonate and extracted with chloroform (20 mL). The solvent was removed under reduced pressure and the residue recrystallized from methanol to yield the dipyrrole (0.302 g; 71%) as a pale yellow powder, mp 271–272 °C; ir (nujol mull): ν 3372 (NH str.), 3092 (NH str.), 1691 (C=O str.), 1676 cm^{-1} (C=O str.); ^1H NMR ($CHCl_3$): δ 1.07 (3H, t, J=7.6 Hz), 1.31 (3H, t, J=7.0 Hz), 1.41 (3H, t, J=7.0 Hz), 2.38 (3H, s), 2.44 (2H, q, J=7.5 Hz), 4.27 (2H, q, J=7.2 Hz), 4.34 (2H, q, J=7.2 Hz), 4.50 (2H, s), 7.40 (1H, dd, J=4.4, 8.4 Hz), 7.50 (1H, dd, J=4.4, 8.4 Hz), 8.11 (1H, d, J=8.0 Hz), 8.87 (1H, d, J=4.0 Hz), 8.92 (1H, dd, J=1.8, 4.6 Hz), 9.71 (1H, br s), 9.86 (1H, d, J=8.4 Hz), 10.12 (1H, br s); ^{13}C NMR ($CDCl_3$): δ 10.6, 14.4, 14.5, 15.7, 17.3, 21.8, 26.0, 60.0, 60.8, 114.3, 115.1, 119.6, 122.9, 123.0, 123.1, 124.9, 125.2, 125.5, 126.0, 126.9, 128.8, 130.6, 136.1, 145.3, 145.9, 147.1, 148.7, 159.9, 162.3; EI MS (70 eV): *m/z* (% rel. int.) 484 (100) [M^+], 439 (13), 438 (27) [M^+ − EtOH], 304 (50), 292 (36). Anal. Calcd for $C_{28}H_{28}N_4O_4 \cdot \frac{1}{4}H_2O$: C, 68.78; H, 5.83; N, 11.46. Found: C, 68.71; H, 5.77; N, 11.38.

4.2.11. *tert*-Butyl 3-(5-*tert*-butoxycarbonyl-3-ethyl-4-methyl-2-pyrrolylmethyl)-7,8-diazaphenanthro[9,10-*c*]pyrrole-1-carboxylate (12b). *p*-Toluenesulfonic acid (540 mg) was added to a stirred mixture of *tert*-butyl 7,8-diazaphenanthro[9,10-*c*]pyrrole-1-carboxylate (**3b**; 951 mg) and *tert*-butyl 5-acetoxymethyl-4-ethyl-3-methylpyrrole-2-carboxylate (838 mg) in glacial acetic acid

(25 mL). The mixture was stirred at room temperature for 2 h, poured into 350 mL ice/water and the resulting precipitate collected by suction filtration. Recrystallization from 95% ethanol gave the dipyrrylmethane (1.003 g; 63%) as pale yellow crystals, mp 223–224 °C; ir (nujol mull): ν 3407 (NH str.), 1700 cm^{-1} (C=O str.); ^1H NMR ($CDCl_3$): δ 1.07 (3H, t, J=7.5 Hz), 1.51 (9H, s), 1.59 (9H, s), 2.35 (3H, s), 2.45 (2H, q, J=7.5 Hz), 4.28 (2H, s), 7.34 (1H, dd, J=8.0, 4.2 Hz), 7.51 (1H, dd, J=8.2, 4.4 Hz), 7.97 (1H, d, J=8.4 Hz), 8.84 (1H, d, J=4.0 Hz), 8.93 (1H, d, J=4.4 Hz), 9.88 (1H, br s), 9.92 (1H, d, J=8.0 Hz), 10.02 (1H, br s); ^{13}C NMR ($CDCl_3$): δ 10.8, 16.1, 17.8, 26.2, 29.1, 80.3, 81.9, 115.1, 115.9, 121.9, 122.3, 123.1, 125.4, 125.9, 128.0, 130.1, 135.9, 145.8, 146.0, 147.0, 148.2, 159.1, 161.1. Anal. Calcd for $C_{32}H_{36}N_4O_4\cdot\frac{1}{2}H_2O$: C, 69.92, H, 6.78, N, 10.19. Found: C, 69.53; H, 6.61; N, 10.05.

4.2.12. Benzyl 3-(5-benzyloxycarbonyl-3-ethyl-4-methyl-2-pyrrolylmethyl)-7,8-diazaphenanthro[9,10-*c*]pyrrole-1-carboxylate (12c). A mixture of **3c** (5.00 g), benzyl 5-acetoxymethyl-4-ethyl-3-methylpyrrole-2-carboxylate[63] (4.24 g) and *p*-toluenesulfonic acid (7.67 g) was dissolved in acetic acid (200 mL), and the mixture was stirred at room temperature for 8 h. The solution was poured into ice-water, neutralized with $NaHCO_3$, and extracted into chloroform. The solvent was evaporated under reduced pressure, and the residue recrystallized from methanol to give the dipyrrole (4.94 g; 57%) as off-white crystals, mp 242.5–243.5 °C; ir (nujol mull): ν 3420 (NH str.), 1702 cm^{-1} (C=O str.); ^1H NMR ($CDCl_3$): δ 0.85 (3H, t, J=7.6 Hz), 2.37 (3H, s), 2.38 (2H, q, J=7.5 Hz), 4.41 (2H, s), 5.31 (2H, s), 5.33 (2H, s), 7.16–7.31 (6H, m), 7.37–7.46 (6H, m), 7.95 (1H, d, J=8.0 Hz), 8.70 (1H, d, J=4.0 Hz), 8.78 (1H, d, J=4.0 Hz), 9.67 (1H, br s), 9.81 (1H, d, J=8.0 Hz), 10.65 (1H, br s); ^{13}C NMR ($CDCl_3$): δ 9.2, 13.9, 15.8, 24.8, 63.7, 64.8, 113.1, 114.6, 116.3, 121.8, 121.9, 122.0, 123.0, 123.9, 124.4, 125.8, 126.7, 126.8, 126.9, 127.1, 127.3, 127.5, 128.9, 130.3, 134.7, 135.2, 135.7, 143.9, 145.0, 146.3, 147.3, 159.2, 160.0; EI MS (70 eV): *m/z* (% rel. int.) 608 (3.2) [M^+], 560 (10), 484 (42), 394 (20), 309 (26), 219 (40), 91 (100). Anal. Calcd for $C_{38}H_{32}N_4O_4\cdot\frac{1}{4}H_2O$: C, 74.45; H, 5.31; N, 9.14. Found: C, 74.09; H, 5.26; N, 9.11.

4.2.13. 1,3-Bis(5-*tert*-butoxycarbonyl-3-ethyl-4-methyl-2-pyrrolylmethyl)-7,8-diazaphenanthro[9,10-*c*]pyrrole (18a). A mixture of phenanthrolinopyrrole **17a** (0.25 g) and acetoxymethylpyrrole **11b** (0.67 g) was dissolved in isopropyl alcohol (5 mL) and acetic acid (5 mL), and the mixture refluxed under nitrogen overnight. The solvent was evaporated under the reduced pressure, and the residue recrystallized from isopropyl alcohol to yield the tripyrrane (0.41 g; 54%) as a light yellow powder, mp 248 °C, dec; ir (nujol mull): ν 3378 (NH str.), 1694 cm^{-1} (C=O str.); ^1H NMR ($CDCl_3$): δ 0.95 (6H, t, J=7.5 Hz), 1.51 (18H, s), 2.28 (6H, s), 2.33 (4H, q, J=7.5 Hz), 4.38 (4H, s), 7.48 (2H, dd, J=4.4, 8 Hz), 7.97 (1H, br s), 8.24 (2H, dd, ^3J=8 Hz, ^4J=1 Hz), 8.52 (2H, br s), 8.92 (2H, dd, ^3J=4.4 Hz, ^4J=1.2 Hz); ^{13}C NMR (d_6-DMSO-$CDCl_3$): δ 10.2, 15.1, 16.9, 25.2, 28.2, 79.6, 112.8, 118.9, 121.3, 122.7, 123.5, 125.3, 126.8, 127.4, 130.5, 146.3, 160.8; EI MS: *m/z* (% rel. int.): 661 (4) (M^+), 602 (18), 561 (48), 487 (40), 461 (60), 441 (32), 397 (66), 385 (78), 367 (88), 352 (100), 341 (100), 340 (99); HRMS (EI): Calcd for $C_{40}H_{47}N_5O_4$: 661.3628. Found: 661.3624. Anal. Calcd for $C_{40}H_{47}N_5O_4\cdot\frac{1}{2}H_2O$: C, 72.07; H, 6.61; N, 10.51; Found: C, 72.34; H, 6.81; N, 10.45.

4.2.14. 1,3-Bis(5-*tert*-butoxycarbonyl-3-butyl-4-methyl-2-pyrrolylmethyl)-7,8-diazaphenanthro[9,10-*c*]pyrrole (18b). A mixture of phenanthrolinopyrrole **17a** (0.50 g) and **11d** (1.41 g) was dissolved in isopropyl alcohol (10 mL) and acetic acid (5 mL), and the mixture refluxed under nitrogen overnight. The solvent was evaporated under reduced pressure, and the residue recrystallized from chloroform–hexane to yield the tripyrrane (0.81 g; 51%) as a light yellow powder, mp 231.5–232.5 °C; ir (nujol mull): ν 3438 (NH str.), 3301 (NH str.), 1682 (C=O str.), 1658 cm^{-1} (C=O str.); ^1H NMR ($CDCl_3$): δ 0.84 (6H, t, J=7.5 Hz), 1.22–1.35 (8H, m), 1.47 (18H, s), 2.24 (6H, s), 2.28 (4H, t, J=7.5 Hz), 4.33 (4H, s), 7.42 (2H, dd, J=5, 8 Hz), 8.13 (2H, d, J=8 Hz), 8.58 (1H, br s), 8.83 (2H, d, J=4.8 Hz), 8.94 (2H, br s); ^{13}C NMR ($CDCl_3$): δ 10.7, 13.9, 22.7, 23.8, 25.8, 28.5, 33.1, 80.5, 113.5, 119.9, 121.3, 123.2, 123.3, 126.5, 127.1, 127.2, 131.1, 145.4, 146.0, 161.5; EI MS: *m/z* (% rel. int.): 717 (1) (M^+), 658 (3), 617 (6), 543 (5), 517 (3), 469 (5), 425 (4), 413 (8), 395 (8), 369 (13); HRMS (EI): Calcd for $C_{44}H_{51}N_5O_4$: 717.4254. Found: 717.4254. Anal. Calcd for $C_{44}H_{51}N_5O_4\cdot\frac{2}{5}H_2O$: C, 73.31; H, 7.19; N, 9.72; Found: C, 73.58; H, 7.18; N, 9.72.

4.2.15. 1,3-Bis(5-*tert*-butoxycarbonyl-3-ethyl-4-methyl-2-pyrrolylmethyl)-7,8-diaza-6,9-dimethylphenanthro-[9,10-*c*]pyrrole (18d). The tripyrrane was prepared from 7,8-diaza-6,9-dimethylphenanthro[9,10-*c*]pyrrole (**17b**; 250 mg) and **11b** (569 mg) by the procedure given for **18a**. Recrystallization from chloroform–hexanes gave the title tripyrrole (466 mg; 67%) as a pale brown powder, mp 190 °C, dec (darkens at 150 °C); ^1H NMR ($CDCl_3$): δ 0.95 (6H, t, J=7.5 Hz), 1.47 (18H, s), 2.28 (6H, s), 2.32 (4H, q, J=7.5 Hz), 2.70 (6H, s), 4.23 (4H, s), 7.20 (2H, d, J=8 Hz), 7.95 (2H, d, J=8 Hz), 8.09 (1H, br s), 9.20 (2H, br s); ^{13}C NMR ($CDCl_3$): δ 10.7, 15.6, 17.4, 25.1, 25.8, 28.7, 80.6, 113.7, 119.7, 119.8, 123.1, 124.4, 124.7, 126.2, 126.7, 131.1, 145.3, 155.4, 161.2. Anal. Calcd for $C_{42}H_{51}N_5O_4.2H_2O$: C, 69.49; H, 7.63; N, 9.65; Found: C, 69.58; H, 7.07; N, 9.72.

4.2.16. 1,3-Bis(5-ethoxycarbonyl-3-butyl-4-methyl-2-pyrrolylmethyl)-7,8-diazaphenanthro[9,10-*c*]pyrrole (18c). The tripyrrane was prepared by the procedure given for **18a** from **17a** (0.500 g) and **11e**[35d] (1.34 g), which gave the tripyrrane (0.77 g, 51%) as a pale yellow powder, mp 242–244 °C; ir (nujol mull): ν 3378 (NH str.), 3194 (NH str.), 1710 cm^{-1} (C=O str.); ^1H NMR ($CDCl_3$): δ 0.80 (6H, t, J=7.5 Hz), 0.86 (6H, t, J=7.2 Hz), 1.20–1.36 (8H, m), 2.17 (6H, s), 2.28 (4H, br t), 3.38 (4H, br q), 4.46 (4H, s), 7.48–7.53 (2H, m), 8.47 (2H, d, J=8 Hz), 8.91 (2H, d, J=4 Hz), 9.92 (1H, br s), 10.89 (2H, br s); EI MS: *m/z* (% rel. int.): 661 (47) (M^+), 615 (34), 570 (5), 512 (16), 452 (8), 461 (60), 441 (90), 423 (3), 407 (21), 395 (100); HRMS (EI): Calcd for $C_{40}H_{47}N_5O_4$: 661.3628. Found: 661.3628. Anal. Calcd for $C_{40}H_{47}N_5O_4\cdot\frac{1}{2}H_2O$: C, 71.60; H, 7.06; N, 10.44. Found: C, 71.39; H, 6.89; N, 10.44.

4.2.17. 2^4,3^4-Diaza-8,12,13,17-tetraethyl-7,18-dimethyl-phenanthro[9,10-*b*]porphyrin (1a). Tripyrrane **18a** (100 mg) was dissolved in TFA (1 mL) and stirred at

room temperature for 10 min under nitrogen. The mixture was diluted with dichloromethane (19 mL), followed immediately by the addition of dialdehyde **19**[42,55] (27 mg). The mixture was stirred at room temperature under nitrogen for a further 2 h. Then, the mixture was neutralized by the dropwise addition of triethylamine, DDQ (34 mg) was added and the solution was stirred for a further 1 h. The solution was diluted with chloroform, washed with water, and the solvent evaporated under reduced pressure. The residue was chromatographed on a Grade 3 alumina column, eluting with 1% methanol–chloroform. A green band was collected and the solvent was evaporated under reduced pressure. The residue was recrystallized from chloroform-methanol to give the porphyrin (76 mg; 83%) as purple crystals, mp >300 °C; UV–vis ($CHCl_3$): λ_{max} ($\log_{10}\varepsilon$) 402 (4.83), 424 (5.30), 520 (3.88), 560 (4.47), 580 (4.20), 636 (3.04); UV–vis (10% TFA–$CHCl_3$): λ_{max} ($\log_{10}\varepsilon$) 432 (5.30), 536 (3.45), 556 (3.87), 572 (3.98), 622 (4.34); ^{1}H NMR (TFA–$CDCl_3$): δ −3.15 (2H, br s), −2.30 (2H, br s), 1.64 (6H, t, J=7.6 Hz), 1.74 (6H, t, J=7.6 Hz), 3.63 (6H, s), 4.09–4.21 (8H, 2 overlapping quartets), 8.74 (2H, dd, J=4.8, 8.8 Hz), 9.56 (2H, dd, J=1, 5 Hz), 10.57 (2H, s), 10.64 (2H, d, J=8 Hz), 11.35 (2H, s); ^{13}C NMR (TFA–$CDCl_3$): δ 12.0, 16.3, 17.4, 20.2, 20.4, 23.1, 98.9, 100.1, 125.2, 126.9, 128.1, 135.2, 138.5, 139.7, 140.6, 142.0, 144.3, 145.5, 145.9, 146.2, 147.8; EI MS: *m/z* (% rel. int.): 602 (100) [M^+], 587 (24), 572 (10), 557 (6), 301 (20) [M^{2+}]; HRMS (EI): Calcd for $C_{40}H_{38}N_6$: 602.3158. Found: 602.3164. Anal. Calcd for $C_{40}H_{38}N_6\cdot\frac{3}{8}CHCl_3$: C, 69.42; H, 5.54; N, 11.88. Found: C, 69.38; H, 5.49; N, 11.80.

4.2.18. 2^4,3^4-Diaza-7,18-dibutyl-12,13-diethyl-8,17-dimethylphenanthro[9,10-*b*]porphyrin (1b). The title porphyrin was prepared by the procedure given for **1a** from **18b** (100 mg) and **19** (25 mg), which gave the porphyrin (75 mg; 75%) as a purple powder, mp >300 °C; UV–vis ($CHCl_3$): λ_{max} ($\log_{10}\varepsilon$) 402 (4.85), 424 (5.33), 522 (3.88), 562 (4.47), 582 (4.20), 636 (3.06); UV–vis (10% TFA–$CHCl_3$): λ_{max} ($\log_{10}\varepsilon$) 434 (5.36), 536 (3.73), 556 (4.02), 572 (4.12), 624 (4.43); ^{1}H NMR (TFA–$CDCl_3$): δ −2.89 (2H, br s), −2.11 (2H, br s), 0.97 (6H, t, J=7.4 Hz), 1.55 (4H, sextet), 1.74 (6H, t, J=7.6 Hz), 2.00 (4H, quintet), 3.63 (6H, s), 4.09–4.17 (8H, m), 8.73 (2H, dd, J=5, 8.2 Hz), 9.61 (2H, d, J=4.8 Hz), 10.56 (2H, s), 10.62 (2H, d, J=8.4 Hz), 11.29 (2H, s); ^{13}C NMR (TFA–$CDCl_3$): δ 12.1, 13.7, 17.4, 20.1, 23.1, 26.8, 34.3, 98.9, 100.2, 125.1, 126.9, 128.0, 135.2, 138.8, 139.7, 140.5, 142.3, 144.3, 144.9, 145.5, 145.8, 147.8; EI MS: *m/z* (% rel. int.): 658 (100) [M^+], 643 (4), 585 (4), 572 (2), 542 (4), 329 (16) [M^{2+}]; HRMS (EI): Calcd for $C_{44}H_{46}N_6$: 658.3784. Found: 658.3789.

4.2.19. 2^4,3^4-Diaza-8,12,13,17-tetraethyl-2^3,3^3,7,18-tetramethylphenanthro[9,10-*b*]porphyrin (1c). The porphyrin was prepared by the foregoing procedure from tripyrrane **18d** (100 mg) and pyrrole dialdehyde **19** (26 mg). Recrystallization from chloroform-methanol gave the phenantholinoporphyrin (56 mg, 61%) as purple crystals, mp >300 °C; UV–vis ($CHCl_3$): λ_{max} ($\log_{10}\varepsilon$) 422 (5.37), 520 (3.97), 558 (4.58), 579 (4.23), 633 (3.41); UV–vis (5% TFA–$CHCl_3$): λ_{max} ($\log_{10}\varepsilon$) 432 (5.44), 569 (4.145), 622 (4.52); ^{1}H NMR (TFA–$CDCl_3$): δ −3.42 (2H, br s), −2.63 (2H, br s), 1.60 (6H, t, J=7.8 Hz), 1.72 (6H, t, J=7.8 Hz), 3.25 (6H, s), 3.62 (6H, s), 4.10–4.19 (8H, m), 8.52 (2H, d, J=8.8 Hz), 10.50 (2H, d, J=8.8 Hz), 10.60 (2H, s), 11.36 (2H, s); ^{13}C NMR (TFA–$CDCl_3$): δ 11.9, 16.2, 17.4, 20.2, 20.4, 23.1, 99.1, 99.8, 124.6, 125.3, 129.0, 135.6, 138.6, 138.9, 140.2, 142.0, 143.8, 145.3, 145.5, 146.0, 160.1; HRMS (FAB): Calcd for $C_{42}H_{42}N_6$ +H: 631.3549. Found: 631.3548. Anal. Calcd for $C_{42}H_{42}N_6.0.15\ CHCl_3$: C, 78.03; H, 6.55; N, 12.95. Found: C, 78.00; H, 6.51; N, 12.84.

4.2.20. 12^4,13^4-Diaza-8,17-dibutyl-7,18-dimethylacenaphtho[1,2-*b*]phenanthro[9,10-*l*]porphyrin (22) (with M. L. Thompson). Tripyrrane **18b** (57 mg) was stirred with TFA (1 mL) in a pear shaped flask under nitrogen for 10 min. The solution was diluted with dichloromethane, acenaphthopyrrole dialdehyde **21**[17] (24 mg) was added immediately and the resulting mixture stirred at room temperature under nitrogen for 2 h. The mixture was neutralized by the dropwise addition of triethylamine, DDQ (20 mg) was added and the mixture stirred for an additional 1 h. The mixture was washed with water, the aqueous layer back extracted with chloroform, and the organic solutions combined. Recrystallization from chloroform-methanol gave the porphyrin (30 mg; 52%) as a purple powder, mp >300 °C; UV–vis ($CHCl_3$): λ_{max} ($\log_{10}\varepsilon$) 435 (5.20), 539 (3.84), 588 (4.46), 617 (4.63), 682 (3.62); UV–vis (5% TFA–$CHCl_3$): λ_{max} ($\log_{10}\varepsilon$) 452 (5.04), 468 (5.03), 532 (3.77), 559 (3.90), 580 (4.07), 603 (4.00), 662 (4.79); ^{1}H NMR (TFA–$CDCl_3$): δ −2.42 (2H, br s), −1.48 (2H, br s), 1.01 (6H, t, J=7.4 Hz), 1.59 (4H, sextet), 2.05 (4H, quintet), 3.71 (6H, s), 4.17 (4H, t, J=7.8 Hz), 8.15 (2H, dd, J=8.0, 7.2 Hz), 8.36 (2H, d, J=8.0 Hz), 8.73 (2H, dd, J=8, 5 Hz), 9.15 (2H, d, J=7.2 Hz), 10.60 (2H, dd, ^3J=8.4 Hz, ^4J=1 Hz), 10.97 (2H, s), 11.25 (2H, s); ^{13}C NMR (TFA–$CDCl_3$): δ 12.2, 13.8, 23.2, 26.9, 34.4, 100.3, 101.2, 125.3, 126.9, 127.9, 128.1, 129.6, 130.8, 131.1, 132.2, 135.8, 136.5, 138.1, 139.4, 139.7, 140.5, 142.9, 145.0, 145.1, 145.9, 147.9; HRMS (FAB): Calcd for $C_{50}H_{42}N_6$ +H: 727.3549. Found: 727.3549.

4.2.21. 2^4,3^4-Diaza-7,18-dibutyl-8,17-dimethyldiphenanthro[9,10-*b*:9,10-*l*]porphyrin (24) (with M. L. Thompson). Following the previous procedure, tripyrrane **18b** (69 mg) was condensed with phenanthropyrrole dialdehyde **23**[17] (30 mg) to give the title porphyrin (54 mg; 74%) as purple crystals, mp >300 °C; UV–vis ($CHCl_3$): λ_{max} ($\log_{10}\varepsilon$) 409 (4.74), 431 (5.11), 578 (4.37), 594 (4.28), 598 (4.28); UV–vis (5% TFA–$CHCl_3$): λ_{max} ($\log_{10}\varepsilon$) 445 (5.04), 575 (3.99), 594 (3.95), 651 (4.65); ^{1}H NMR (TFA–$CDCl_3$): δ −2.42 (2H, br s), −1.10 (2H, br s), 0.99 (6H, t, J=7.3 Hz), 1.56 (4H, sextet), 1.98 (4H, quintet), 3.63 (6H, s), 4.11 (4H, t, J=7.6 Hz), 8.23 (2H, t, J=7.5 Hz), 8.36 (2H, d, J=7.5 Hz), 8.74 (2H, dd, J=8.2, 4.9 Hz), 9.30 (2H, d, J=8.2 Hz), 9.65 (2H, d, J=4.9 Hz), 9.89 (2H, d, J=8.2 Hz), 10.59 (2H, d, J=8.5 Hz), 11.21 (2H, s), 11.34 (2H, s); ^{13}C NMR (TFA–$CDCl_3$): δ 12.1, 13.6, 22.9, 26.7, 34.1, 99.9, 100.2, 124.8, 125.1, 126.7, 126.8, 127.1, 127.8, 129.8, 130.1, 130.7, 133.7, 135.2, 138.2, 139.6, 140.4, 140.8, 143.0, 144.4, 146.2, 147.7; HRMS (FAB): Calcd for $C_{52}H_{44}N_6$ + H: 753.3706. Found: 753.3706.

4.2.22. 2^4,3^4,12^4,13^4-Tetraaza-7,8,17,18-tetraethyldiphenanthro[9,10-*b*:9,10-*l*]porphyrin (28a). A mixture

of 7,8-diazaphenanthro[9,10-*c*]pyrrole **17a** (219 mg) and diamine **27**[56] (237 mg) were stirred under reflux in acetic acid (40 mL) for 3 h. The dark greenish solution was diluted with chloroform, and washed sequentially with water, saturated sodium bicarbonate solution (×3) and water (the aqueous solutions were back extracted at each step). The solvent was removed under reduced pressure and the residue treated with methanol and filtered. The insoluble material was taken up in chloroform (20 mL), filtered and the solid further recrystallized from chloroform-methanol to give the porphyrin (28 mg; 7.7%) as a purple powder. The filtrates were combined, evaporated and chromatographed on a Grade III alumina column eluting first with 1.5% methanol–chloroform and then with 4% methanol–chloroform. A small red fraction corresponding to monophenanthroporphyrin was collected initially, followed by a green fraction corresponding to the required product. The product fractions were evaporated and recrystallized from chloroform-methanol to give the *opp*-diphenanthrolinoporphyrin **28a** (total yield 41 mg; 11.3%) as a purple powder, mp >300 °C; UV–vis (1% Et_3N–$CHCl_3$): λ_{max} ($\log_{10}\varepsilon$) 408 (4.65), 432 (5.18), 555 (3.93), 579 (4.43), 595 (3.43), 603 (4.40); UV–vis (1% TFA–$CHCl_3$): λ_{max} ($\log_{10}\varepsilon$) 450 (5.25), 635 (4.19), 686 (4.38); UV–vis (5% TFA–$CHCl_3$): λ_{max} ($\log_{10}\varepsilon$) 420 (4.59), 447 (5.25), 569 (3.88), 597 (3.82), 624 (3.80), 655 (4.50); ^{1}H NMR (TFA–$CDCl_3$): δ −1.12 (2H, br s), 0.93 (2H, br s), 1.67 (12H, t, J=7.6 Hz), 4.18 (8H, q, J=7.6 Hz), 8.81 (4H, m), 9.95 (4H, d, J=4.4 Hz), 10.66 (4H, d, J=8.4 Hz), 11.39 (4H, s); ^{13}C NMR (TFA–$CDCl_3$): δ 16.8, 20.1, 100.6, 126.5, 126.6, 128.3, 137.7, 139.9, 140.7, 145.2, 145.8, 148.3; HRMS (FAB): Calcd for $C_{48}H_{38}N_8$ + H: 727.3298. Found: 727.3296. Anal. Calcd for $C_{48}H_{38}N_8 \cdot {}^3/_4CHCl_3$: C, 71.72; H, 4.78; N, 13.72. Found: C, 72.03; H, 5.02; N, 13.61.

4.2.23. Condensation of 17b with 3,4-diethyl-2,5-bis[(*N*,*N*-dimethylamino)methyl]pyrrole. A mixture of 6,9-dimethyl-7,8-diazaphenanthro[9,10-*c*]pyrrole **17b** (247 mg) and diamine **27** (237 mg) were stirred under reflux in acetic acid (38 mL) for 3.5 h. The dark greenish solution was diluted with chloroform, and washed sequentially with water, saturated sodium bicarbonate solution (×3) and water (the aqueous solutions were back extracted at each step). The solvent was removed under reduced pressure and the residue treated with methanol (40 mL) and filtered. In some cases, but not all, a small amount of insoluble green material corresponding to *adj*-diphenanthrolinoporphyrin **29** (HRMS (FAB): Calcd for $C_{52}H_{46}N_8$ +H: 783.3924. Found: 783.3921) was observed. The filtrate was evaporated under reduced pressure and the residue chromatographed on Grade III neutral alumina, initially eluting with chloroform. A faint red fraction corresponding to monophenanthrolinoporphyrin (<0.05%) was collected. When the eluting solvent was changed to 1.5% methanol–chloroform, a green fraction corresponding to **28b** eluted, and a second green band was subsequently obtained as the proportion of methanol was increased. The first green fraction was recrystallized from chloroform-methanol to give the *opp*-diphenanthrolinoporphyrin **28b** (25 mg; 6.4%) as purple crystals. Alternatively, recrystallization from chloroform–hexanes gave a green powder. The more polar green fraction was evaporated and recrystallized from chloroform–hexanes to give the triphenanthrolinoporphyrin **30** (6 mg; 1.5%) as a green solid.

$2^4,3^4,12^4,13^4$-Tetraaza-7,8,17,18-tetraethyl-$2^3,3^3,12^3,13^3$-tetramethyldiphenanthro[9,10-*b*:9,10-*l*]porphyrin (**28b**). Mp >300 °C; UV–vis (1% Et_3N–$CHCl_3$): λ_{max} ($\log_{10}\varepsilon$) 409 (5.05), 432 (5.48), 554 (4.18), 578 (4.76), 597 (4.70), 645 (3.60); UV–vis (5% TFA–$CHCl_3$): λ_{max} ($\log_{10}\varepsilon$) 422 (4.97), 450 (5.52), 571 (4.03), 595 (3.99), 621 (3.93), 653 (4.74); ^{1}H NMR (TFA–$CDCl_3$): δ −1.38 (2H, br s), 0.95 (2H, br s), 1.67 (12H, t, J=7.6 Hz), 3.33 (12H, s), 4.16 (8H, q, J=7.6 Hz), 8.60 (4H, d, J=8.8 Hz), 10.51 (4H, d, J=8.4 Hz), 11.36 (4H, s); ^{13}C NMR (TFA–$CDCl_3$): δ 16.2, 19.8, 22.2, 100.4, 124.3, 126.4, 129.1, 137.6, 139.0, 140.4, 145.0, 146.2, 161.2; HRMS (FAB): Calcd for $C_{52}H_{46}N_8$ + H: 783.3924. Found: 783.3928. Anal. Calcd for $C_{52}H_{46}N_8 \cdot CHCl_3$: C, 70.55; H, 5.25; N, 12.42. Found: C, 70.60; H, 5.68; N, 11.87.

$2^4,3^4,7^4,8^4,12^4,13^4$-Hexaaza-17,18-diethyl-$2^3,3^3,7^3,8^3,12^3,13^3$-hexamethyltriphenanthro[9,10-*b*:9,10-*g*:9,10-*l*]porphyrin (**30**). Mp >300 °C; UV–vis (1% Et_3N–HCl_3): λ_{max} ($\log_{10}\varepsilon$) 415 (4.59), 447 (4.87), 566 (3.85), 594 (4.23), 605 (4.20), 663 (3.68); UV–vis (5% TFA–HCl_3): λ_{max} ($\log_{10}\varepsilon$) 441 (4.65), 469 (5.13), 553 (3.69), 603 (3.93), 661 (4.25); ^{1}H NMR (TFA–DCl_3): δ −0.93 (4H, br s), 1.65 (6H, t, J=7.6 Hz), 3.29 (6H, s), 3.31 (12H, s), 4.11 (4H, q, J=7.6 Hz), 8.45 (2H, d, J=8.4 Hz), 8.50 (2H, d, J=8.4 Hz), 8.56 (2H, J=8.4 Hz), 10.36–10.42 (4H, 2 overlapping doublets), 10.44 (4H, d, J=8.4 Hz), 11.24 (2H, s), 12.08 (2H, s); HRMS (FAB): Calcd for $C_{60}H_{46}N_{10}$+H: 907.3985. Found: 907.3982.

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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.2005.09.089

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Porphyrins with exocyclic rings. Part 20: Synthesis and spectroscopic characterization of porphyrins with fused 2,1,3-benzoxadiazole and 2,1,3-benzoselenadiazole moieties☆

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Abstract—Porphyrins with fused 2,1,3-benzoxadiazole and 2,1,3-benzoselenadiazole units were prepared by the '3+1' MacDonald-type methodology. 4-Nitro-2,1,3-benzoxadiazole, 6-chloro-4-nitro-2,1,3-benzoxadiazole and 4-nitro-2,1,3-benzoselenadiazole condensed with isocyanoacetates in the presence of the non-nucleophilic base DBU to give tricyclic pyrrole derivatives in excellent yields. Further cleavage of the ester moieties and decarboxylation afforded α-unsubstituted pyrroles and these were further condensed with 2 equiv of an acetoxymethylpyrrole *tert*-butyl ester to give crude preparations of tripyrranes. The *tert*-butyl ester protective groups were cleaved with TFA and following dilution with dichloromethane, '3+1' condensation with a pyrrole dialdehyde, and oxidation with ferric chloride, the heterocyclic ring fused porphyrins were obtained in moderate yields. The yields were lower than expected because of difficulties in preparing required tripyrranes due to the reduced reactivity of the pyrrolic intermediates. The UV–vis spectra of these new porphyrin systems were highly modified showing broadened split Soret bands. In addition, the nickel(II), copper(II) and zinc complexes gave unusual UV–vis spectra with weakened split Soret bands and strong Q-type absorptions above 600 nm. These modified structures show some potential for applications as photosensitizers in photodynamic therapy.
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1. Introduction

Porphyrins with extended chromophores[1,2] have been investigated for applications that range from material science[3] to medicine.[4] Much of this work has concentrated on tetrabenzoporphyrins,[5] but other types of ring fused porphyrin systems have the potential to produce more diverse physical and spectroscopic properties.[1,2] In some cases, porphyrins with fused aromatic units show strongly red shifted absorptions,[1,2] a property that could result in the development of superior photosensitizers for photodynamic therapy (PDT).[4] In PDT, the porphyrin 'drug' is excited by visible light and transfers energy to generate singlet oxygen. As porphyrins commonly show an affinity for tumor cells over normal tissues, the highly toxic effects of singlet oxygen are localized to the malignant tissues. Bodily tissues strongly absorb light through most of the visible region, but red light in the region of 650–800 nm gives much better penetration while providing the necessary energy for singlet oxygen production.[4] Unfortunately, porphyrins usually only have weak absorptions above 600 nm, and for this reason modified chromophores are attracting considerable interest.[1,2] However, fusion of many benzenoid aromatic ring systems to the porphyrin nucleus produces only minor shifts to the UV–vis absorption spectra. Naphthoporphyrins **1a**[6,7] and the related quino- and isoquinoporphyrins **1b** and **1c**[8] produce shifts of less than 10 nm to the Soret and Q bands, and even phenanthro-(**2a**)[9] and phenanthrolinoporphyrins (**2b**)[10] give similar spectroscopic shifts (Chart 1).[11] However, acenaphthoporphyrins **3** have highly modified spectra with three Soret absorptions and a relatively strong Q band at 660 nm.[12–14] Di- and tetraacenaphthoporphyrins give even larger shifts, in some cases showing Soret bands above 600 nm.[13,14] Thiadiazolobenzoporphyrins **4a** also show intriguing UV–vis spectra with broadened split Soret bands and a highly modified Q band region.[13,15] The related nickel(II), copper(II) and zinc complexes also show two Soret bands that are atypically weakened, together with an intense Q-type band near 600 nm.[13] Although the absorptions fall short of the desired wavelength range from 650 to 800 nm, the relatively polar nature of these porphyrins and the presence of strong absorption bands at higher wavelengths makes the study of related chromophores a desirable goal. For this reason we targeted the synthesis of related porphyrins **4b–d** with fused 2,1,3-benzoxadiazole (benzofurazan) and 2,1,3-benzoselenadiazole units.[16]

☆ For part 19 in the series, see: Lash, T. D.; Lin, Y.; Novak, B. H.; Parikh, M. D. *Tetrahedron* **2005**, *61*, 11601–11614.

Keywords: Pyrroles; 2,1,3-Benzoxadiazoles; 2,1,3-Benzoselenadiazoles; '3+1' Methodology; Modified porphyrin chromophores.
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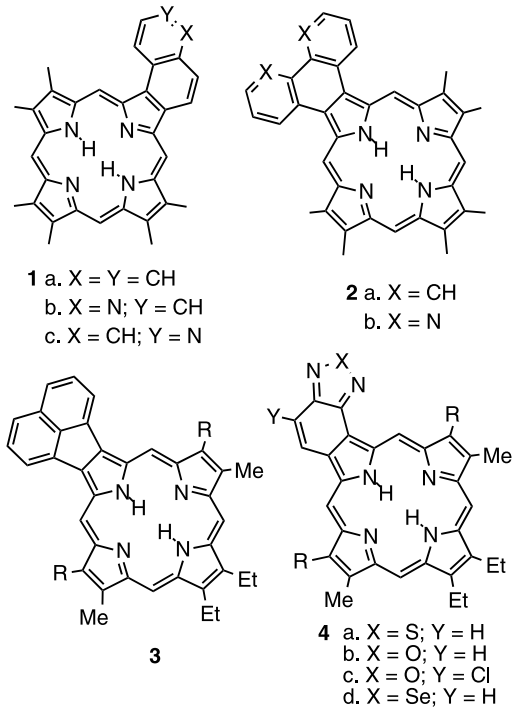


Chart 1. Porphyrins with fused aromatic subunits.

2. Results and discussion

The synthesis of porphyrins with fused heterocyclic rings required the availability of pyrrolic tricycles **5** (Scheme 1). Nitroaromatic compounds with a degree of nitroalkene character can react with isocyanoacetates in the presence of a non-nucleophilic base such as DBU to give *c*-annelated pyrroles.[8–20] This chemistry is in fact a variation on the Barton–Zard pyrrole condensation which was first reported in 1985,[21] and can be applied to the synthesis of diverse fused pyrrole and porphyrin products.[8–17] In previous work, 4-nitro-2,1,3-benzothiadiazole (**6a**) was reacted with ethyl isocyanoacetate (**7a**) in refluxing THF but only poor yields (15%) of the required pyrrole ethyl ester **8a** was

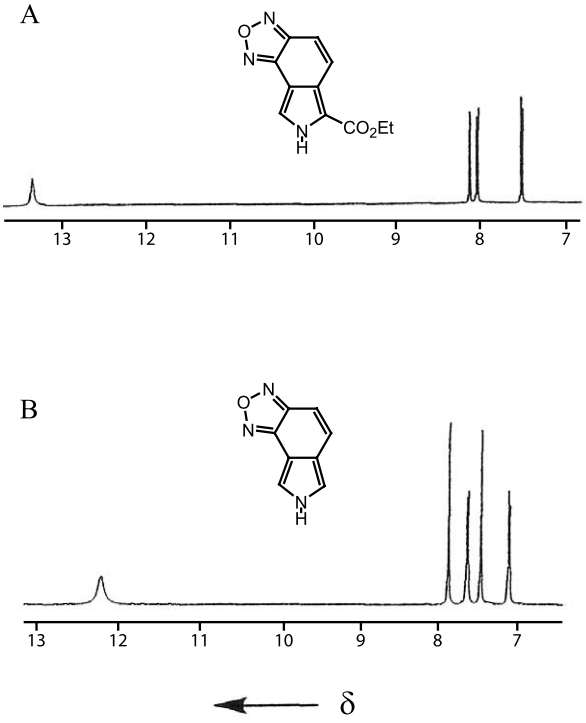
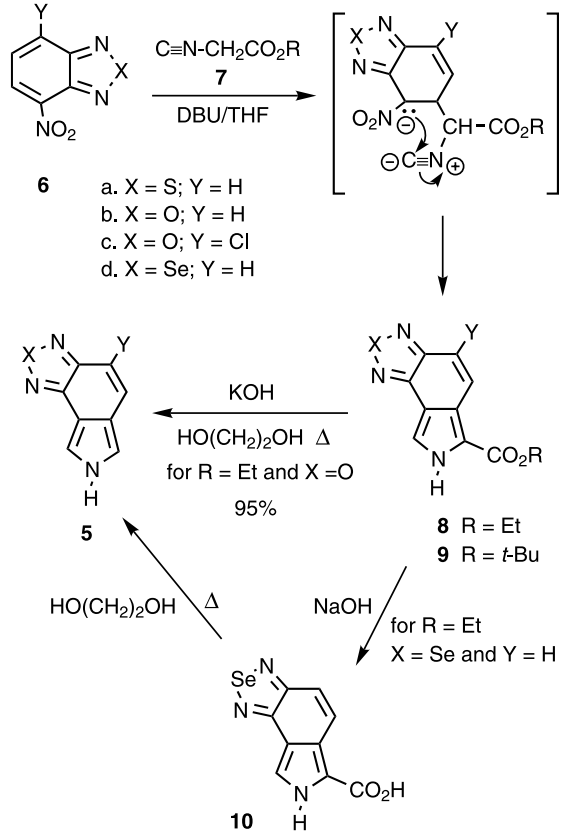
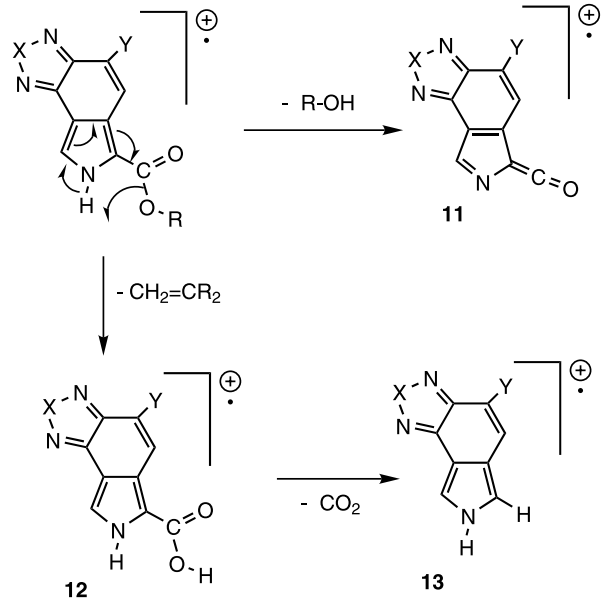


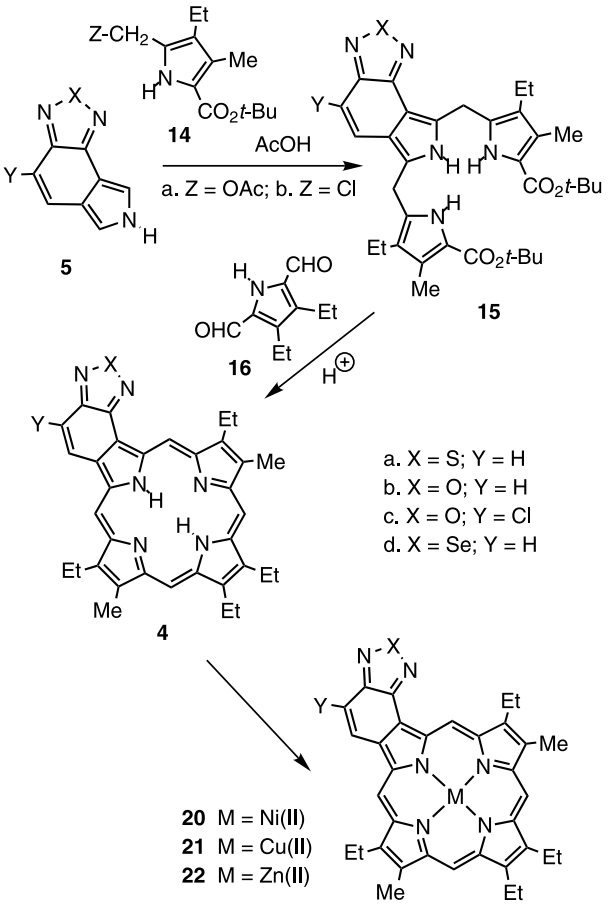
Figure 1. (a) Partial 400 MHz proton NMR spectrum of pyrrole ester **8b** in d_6-DMSO; (b) 400 MHz Proton NMR spectrum of oxadiazolobenzopyrrole **5b** in d_6-DMSO.



Scheme 1.

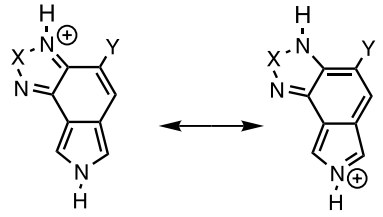


Scheme 2.

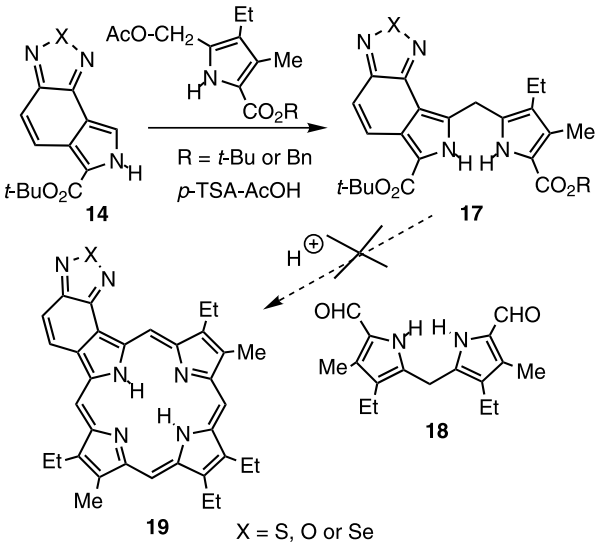


Scheme 3.

obtained.[13,15] However, under highly diluted conditions, where 400 mL of THF solvent was used for every 1 g of nitro compound, a 48% yield of the required tricycle **8a** was obtained, and the related *tert*-butyl ester **9a** was similarly prepared from **6a** and **7b** in 47% yield.[13,15] It was unclear why the dilute conditions were beneficial as better results were usually obtained at higher concentrations for other nitro compounds, but the low solubility of these heterocycles may be a factor.[13] The same approach was used to prepare the new heterocycles **8b–d** and **9b–d**. In all cases, dilute conditions gave superior results. 4-Nitrobenzofurazan **6b** (NBD) is not commercially available, and initial investigations were conducted using the available 7-chloro-derivative NBD chloride (**6c**). Reaction of **6c** with **7a** or **7b** gave the ethyl or *tert*-butyl esters **8c** and **9c**, respectively, in 65–72% yield. The very low solubilities of these products in organic solvents made purification by chromatography impractical, but impurities could be removed by heating the crude products with methanol and this gave the desired pyrroles in pure form. Nitration of



Scheme 4.



Scheme 5.

benzofurazan with concentrated nitric and sulfuric acids at 30 °C gave the nitro-derivative **6b** in 90% yield, and this similarly reacted with **7a** and **7b** in the presence of DBU in refluxing THF to give the pyrrole esters **8b** and **9b** in 65%

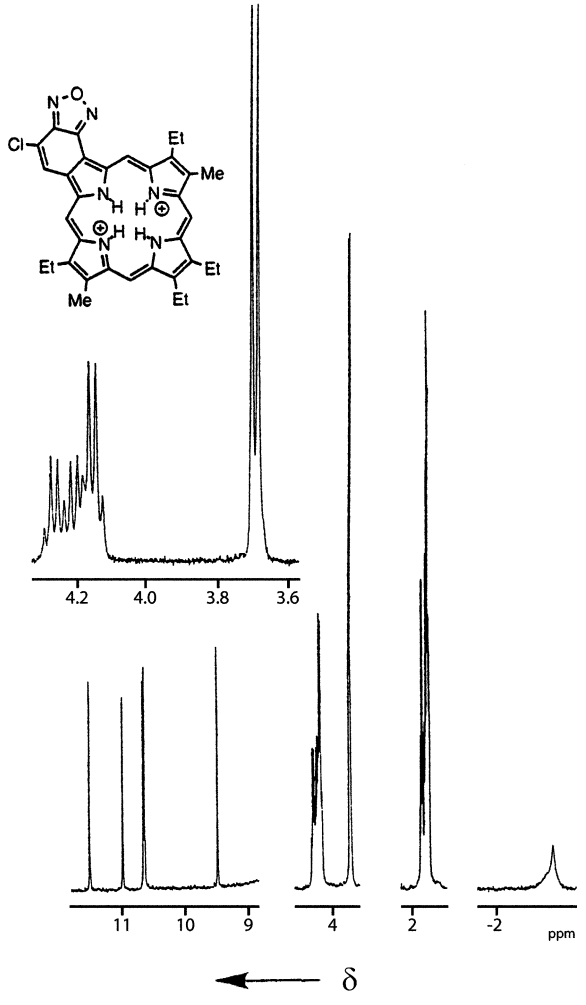


Figure 2. 400 MHz proton NMR spectrum of oxadiazolobenzoporphyrin **4c** in TFA–$CDCl_3$.

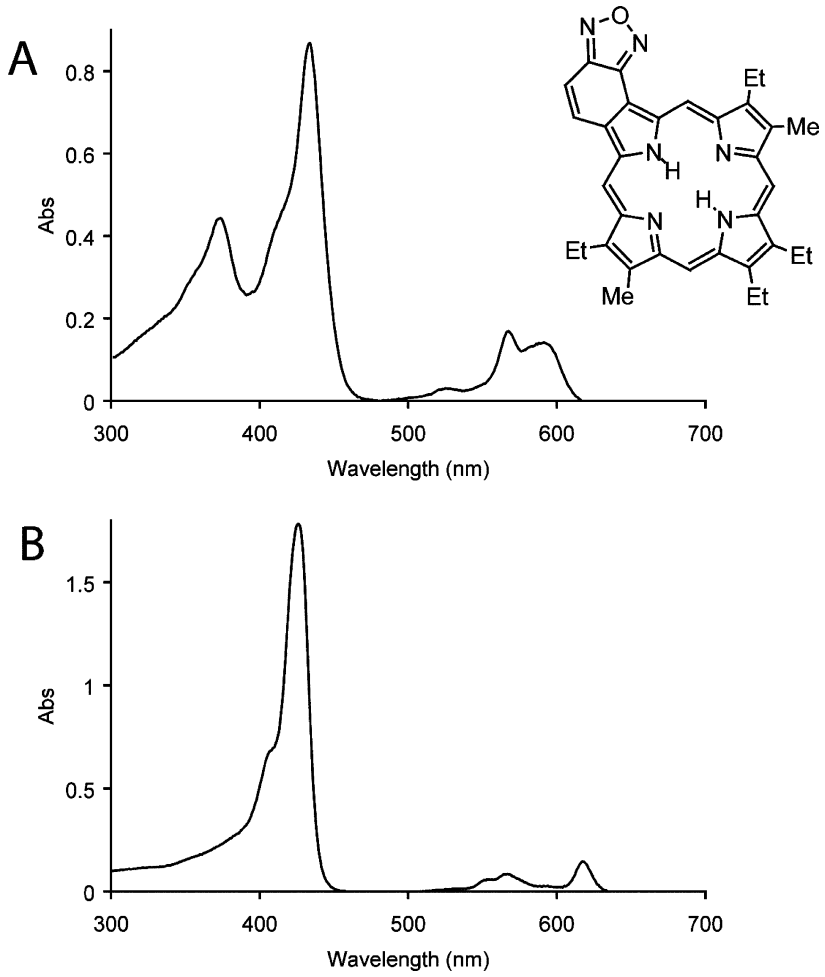


Figure 3. UV–vis spectra for oxadiazolobenzoporphyrin **4b**. (a) Free base in chloroform; (b) Protonated species in 1% TFA–chloroform.

yield. 4-Nitro-2,1,3-benzoselenadiazole (**6d**) similarly reacted with DBU and isocyanoacetates **7** in refluxing DBU to give the related pyrrole esters **8d** and **9d** in 86–89% yield. All of these pyrrole esters had very poor solubility characteristics, but NMR spectra could be obtained in d_6-DMSO. For instance, the proton NMR spectrum of oxadiazolobenzopyrrole **8b** (Fig. 1(a)) confirms the structure of this heterocycle showing the presence of two doublets at 7.5 and 8.0 ppm for the central benzo-unit and a singlet at 8.1 ppm for the pyrrolic CH. The NH is evident as a broad resonance at 13.4 ppm.

Our intent was to prepare the targeted porphyrin systems by MacDonald-type '3+1' condensations using tripyrranes,[22,23] and the unsubstituted pyrroles **5** were required as precursors to these intermediates. Thiadiazolobenzopyrrole ethyl ester **8a** underwent saponification and decarboxylation with KOH in ethylene glycol under nitrogen at 180 °C for 30 min to give excellent yields of **5a**. When the oxygen analogues **8b** and **8c** were reacted under these conditions, very poor yields of the decarboxylation products were obtained. However, when a small amount of hydrazine was added to the reaction mixture, the required tricycles **5b** and **5c** were isolated in 50–76% yield. The hydrazine presumably protects the reactants from oxidative degradation due to the presence of trace amounts of oxygen. However, when the selenium heterocycle **8d** was treated with KOH under these conditions, complete decomposition occurred regardless of reaction time (5–30 min) or whether or not hydrazine was present. Following numerous attempts to modify these conditions, the best results were obtained in a two-step procedure. Ethyl ester **8d** was saponified under mild conditions to give the corresponding carboxylic acid. This underwent decarboxylation in ethylene glycol in the absence of any base at 180 °C to give the required α-unsubstituted pyrrole in 85% yield. However, attempts to convert *tert*-butyl esters **9** to α-unsubstituted pyrroles by treatment with TFA were unsuccessful. The NMR spectra for pyrroles **5** were again generally obtained in d_6-DMSO (e.g., Fig. 1(b)).

The electron impact mass spectra for the new heterocycles were also investigated. For pyrrole esters with fused phenanthrene, phenanthroline, quinoline, isoquinoline, fluoranthene or acenaphthylene rings,[8–11,13] the primary fragmentation pathway was loss of ROH to give species of type **11** (Scheme 2), although loss of an alkene fragment was more prominent for the *tert*-butyl esters resulting in carboxylic acid radical cations like **12**. Similar results were obtained for thiadiazolopyrrole ester **8a** and **9a**. The ethyl ester gave primarily loss of ethanol to give **11** and loss of alkene was a minor pathway, while both fragmentations

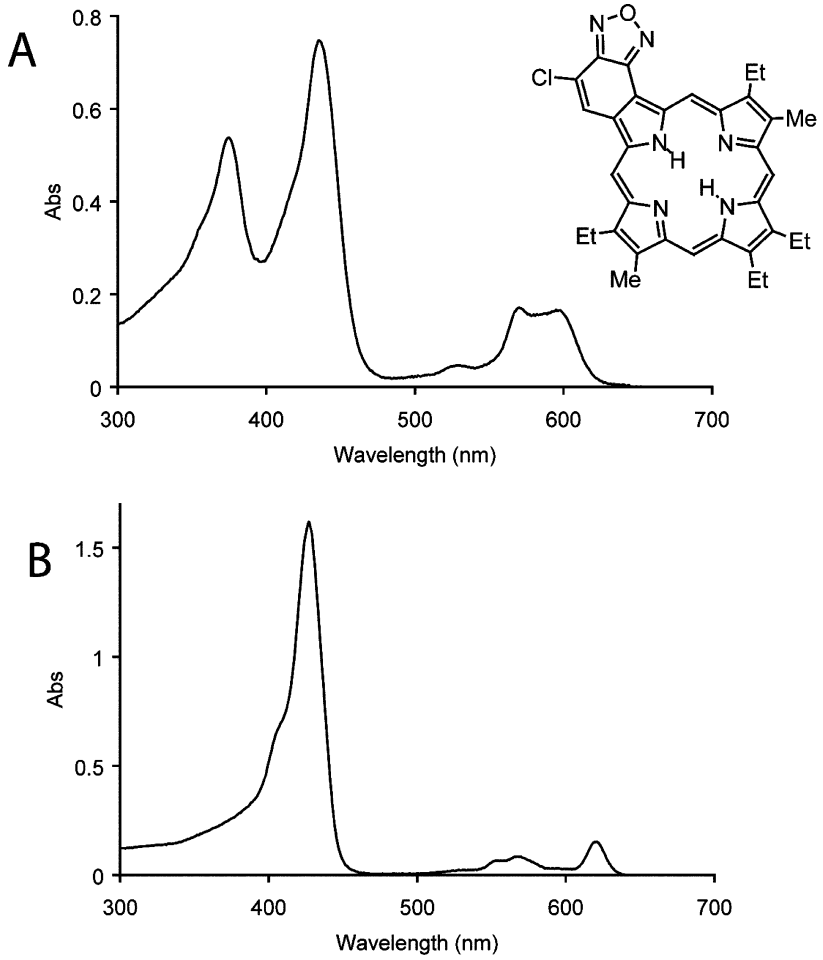


Figure 4. UV–vis spectra for chloro-oxadiazolobenzoporphyrin **4c**. (a) Free base in chloroform. (b) Protonated species in 1% TFA–chloroform.

were about equally favored for the *tert*-butyl ester **9a**.[13] Apart from the presence of multiple isotope peaks for selenium, esters **8d** and **9d** gave similar results. However, the oxadiazolopyrrole ethyl ester **8b** gave loss of EtOH to **11** as a minor fragmentation pathway, and the strongest fragment ion **13** corresponds to loss of ethylene and CO_2. The *tert*-butyl ester **9b** also gave strong fragment ions for **12** and **13**, although it slightly bucks the trend by showing a small increase in the loss of ROH to give **11**. The same types of fragmentation were observed for the chloro-derivatives **8b** and **8c**, although the spectra were not of as high a quality. The unsubstituted tricycle **5a** shows loss of HCN as the main fragmentation pathway.[13] As expected, the two oxadiazolobenzopyrroles **5b** and **5c** primarily gave loss of NO. Unfortunately, selenadiazolobenzopyrrole **5d** did not analyze well by EI MS and its behavior in mass spectrometry could not be determined.

Now that the heterocyclic building blocks **5** were available, the synthesis of the heterocyclic ring fused porphyrins could be investigated. The electron-withdrawing diazole units decrease the reactivity of the fused pyrrole moieties towards electrophilic substitution and this can lead to difficulties. This problem was previously encountered in the synthesis of phenanthrolinoporphyrins **2b** (Chart 1) where the fused pyridine rings exert a similar disruptive influence.[10] The best results for **2b** were obtained when the carbon–carbon bond forming steps were well removed from the fused heterocycle during macrocycle formation, thereby necessitating the use of tripyrrane intermediates.[10] In the earlier synthesis of thiadiazolobenzoporphyrin **4a**, tricycle **5a** was reacted with 2 equiv of an acetoxymethylpyrrole **14a** in refluxing acetic acid–ethanol[23,24] to give a crude preparation of tripyrrane **15a** (Scheme 3).[13,15] This intermediate was taken on without purification and treated with TFA to cleave the *tert*-butyl ester groups. In a one pot sequence, the mixture was diluted with dichloromethane, condensed with pyrrole dialdehyde **16**, and following neutralization with triethylamine the intermediary species were oxidized with DDQ to give **4a** in up to 40% yield. The same approach was used to prepare **4b–d**. Unfortunately, reaction of the oxadiazole derivatives **5b** or **5c** with **14a** in refluxing acetic acid–ethanol afforded virtually no tripyrrane product, and replacement of the alcohol solvent with 2-propanol gave no improvement to the yields. Weak acid is used to catalyze this chemistry but we recognized that protonation of the heterocycle would further decrease the reactivity of the pyrrole subunit (Scheme 4). The oxygen-containing heterocycles appear to have decreased reactivity compared to **5a**, and the major products in the reactions using **5b** and **5c** were ether derivatives of **14a** formed by solvolysis. Attempts to use other acid catalysts such as *p*-toluenesulfonic acid or Montmorillonite clay gave rise to no tripyrrane formation. The possibility of avoiding the use of an acid

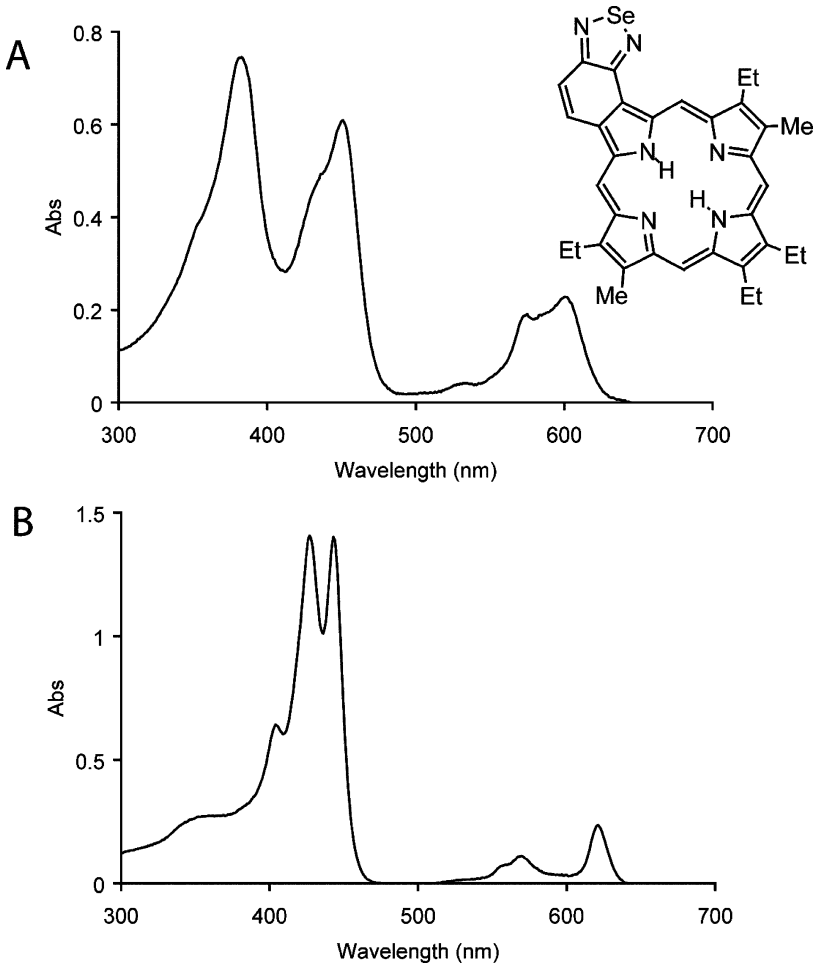


Figure 5. UV–vis spectra for selenadiazolobenzoporphyrin **4d**. (a) Free base in chloroform; (b) Protonated species in 1% TFA–chloroform.

catalyst was also considered using pyridine as a solvent. The chloromethylpyrrole **14b** was reacted with **5b** or **5c** in refluxing pyridine. In principle, pyridine would initially generate a pyridinium salt[25] that could readily eliminate and further react with the pyrrole nucleus. However, only trace amounts of tripyrrane could be detected under these conditions as well. The best results for tripyrrane **15b** involved reacting **5b** with acetoxymethylpyrrole **14a** in refluxing acetic acid-toluene. However, the product was heavily contaminated with impurities and as these tripyrrolic compounds tend to decompose during chromatography this material was taken on through the '3+1' methodology (Scheme 3) in crude form and reacted with TFA and dialdehyde **16**. Subsequent oxidation with DDQ gave poor yields of the porphyrin product, but much better results were obtained using an aqueous ferric chloride solution for the oxidation step,[26,27] and following chromatography and recrystallization from chloroform–methanol the oxadiazolobenzoporphyrin **4b** was isolated as a green powder in 31% yield. The chloro-substituted tricycle **5c** gave the best results when reacted with **14a** in refluxing acetic acid-xylenes. The chloro-group may further decrease the reactivity of **5c** making the use of higher temperatures beneficial. Even so, the tripyrrane intermediate was obtained in very crude form. Following deprotection of the terminal ester groups with TFA, condensation with **16** and oxidation with DDQ, porphyrin **4c** was obtained as a very dark green powder in 17% yield. The selenium heterocycle **4d** was also unreactive toward tripyrrane formation, but the best solvent in this case was ethyl acetate. The poor solubility of **5d** may also exacerbate the problems due to diminished reactivity, and even under the best conditions when **5d** was sonicated for 20–30 min with ethyl acetate prior to the addition of acetic acid to maximize solubilization, the tripyrrane was generated in very crude form. This crude material was taken on through the '3+1' route as before, and selenadiazolobenzoporphyrin **4d** was isolated in 9% yield.

During the course of these studies, the use of dipyrrylmethane intermediates was briefly considered. The *tert*-butyl esters **9** were condensed with acetoxymethylpyrroles in the presence of *p*-toluenesulfonic acid in acetic acid to give moderate yields of dipyrrylmethanes **17** (Scheme 5). The di-*tert*-butyl esters were treated with TFA to cleave the protective groups and condensed with dipyrrylmethane dialdehyde **18** under MacDonald '2+2' reaction conditions[28–30] but no more than trace amounts of the desired porphyrin products **19** were observed. Again, the chemistry appears to be blocked by the reduced reactivity of the ring fused pyrrole unit.

The free base porphyrins **4b–d** were only sparingly soluble in organic solvents, but high quality NMR spectra were easily obtained for the protonated forms in TFA–$CDCl_3$.

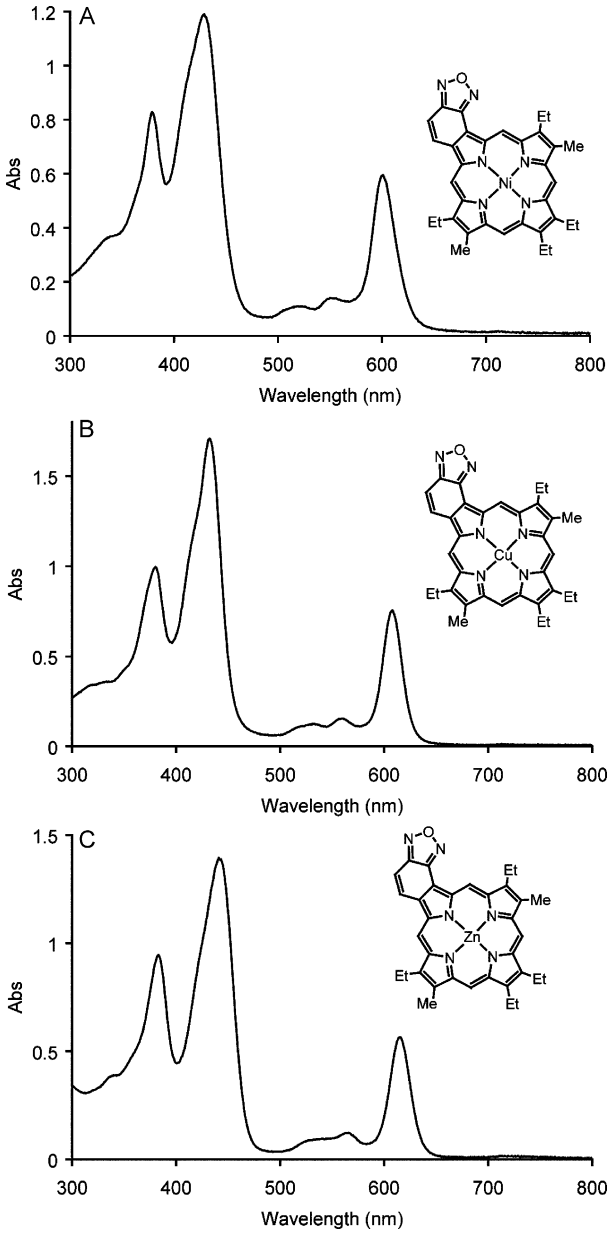


Figure 6. UV–vis spectra for metal complexes of porphyrin **4b** in chloroform. (a) Ni(II) complex **20b**; (b) Cu(II) complex **21b**; (c) Zn complex **22b**.

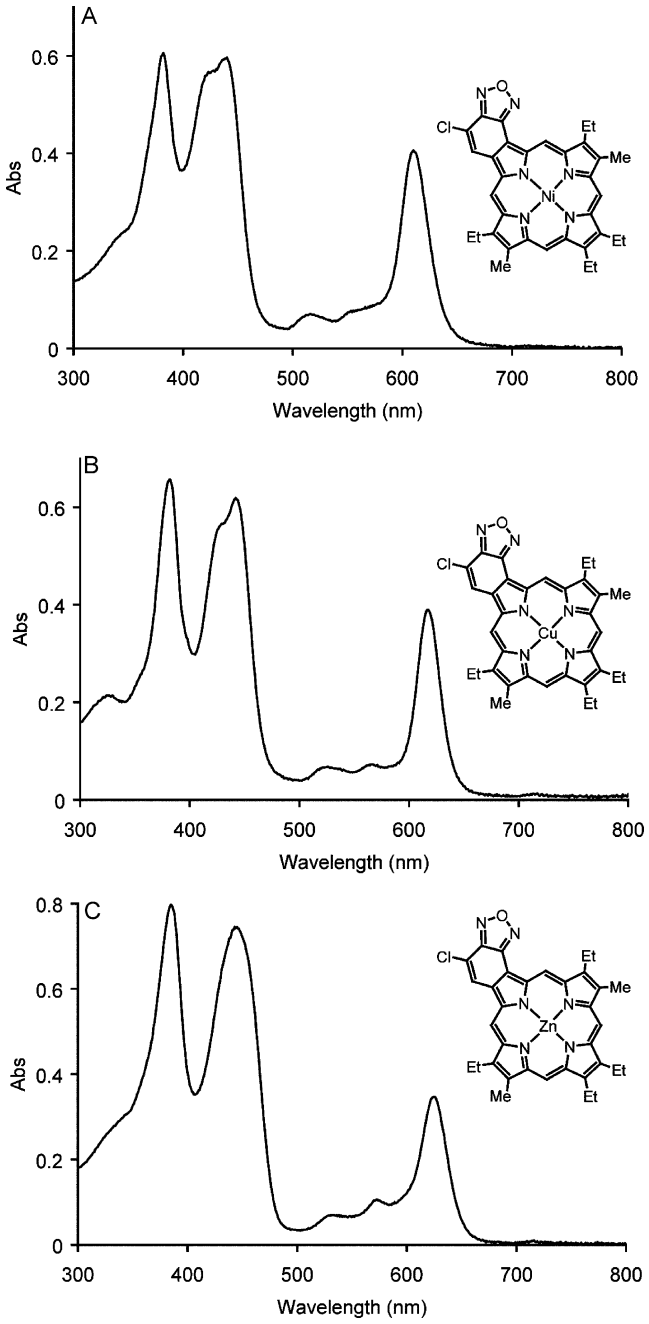


Figure 7. UV–vis spectra for metal complexes of porphyrin **4c** in chloroform. (a) Ni(II) complex **20c**; (b) Cu(II) complex **21c**; (c) Zn complex **22c**.

The proton NMR spectra showed typical diatropic ring currents for the porphyrin macrocycles. For instance, **4c** gives four singlets for the *meso*-protons downfield near 11 ppm, while the internal NHs resonate upfield near −3 ppm (Fig. 2).

The UV–vis spectra for the free base porphyrins **4b–d** (Figs. 3–5) were very different from typical porphyrin spectra, although they were similar to the results previously obtained for **4a**. Porphyrins usually give four Q bands but the longest wavelength band Q I appears to be very weak or absent in the spectra for **4a–d** and the shortest wavelength band (Q IV) is relatively weak. Two major Q bands, tentatively assigned as Q II and Q III, can be seen which are merging in to one another. The longer wavelength band Q II for **4a** shows up at 588 nm for **4a**, but the equivalent bands in the new porphyrins **4b**, **4c** and **4d** are comparatively red shifted and appear at 591, 595 and 600 nm, respectively. All four porphyrins also show split Soret bands, with λ_{max} values of 380 and 438 nm for **4a**, 374 and 434 nm for **4b**, 375 and 436 nm for **4c**, and 383 and 451 nm for **4d**. For both the Soret and Q absorptions, the largest shifts are seen for the selenium system **4d**, but the Soret bands are slightly blue shifted for the oxygen systems **4b** and **4c**. The presence of a chloro-substituent in **4c** induces small bathochromic shifts compared to **4b**. In TFA–chloroform, the protonated forms for the oxadiazolobenzoporphyrins **4b** and **4c** gave a single Soret band, while the sulfur and selenium containing

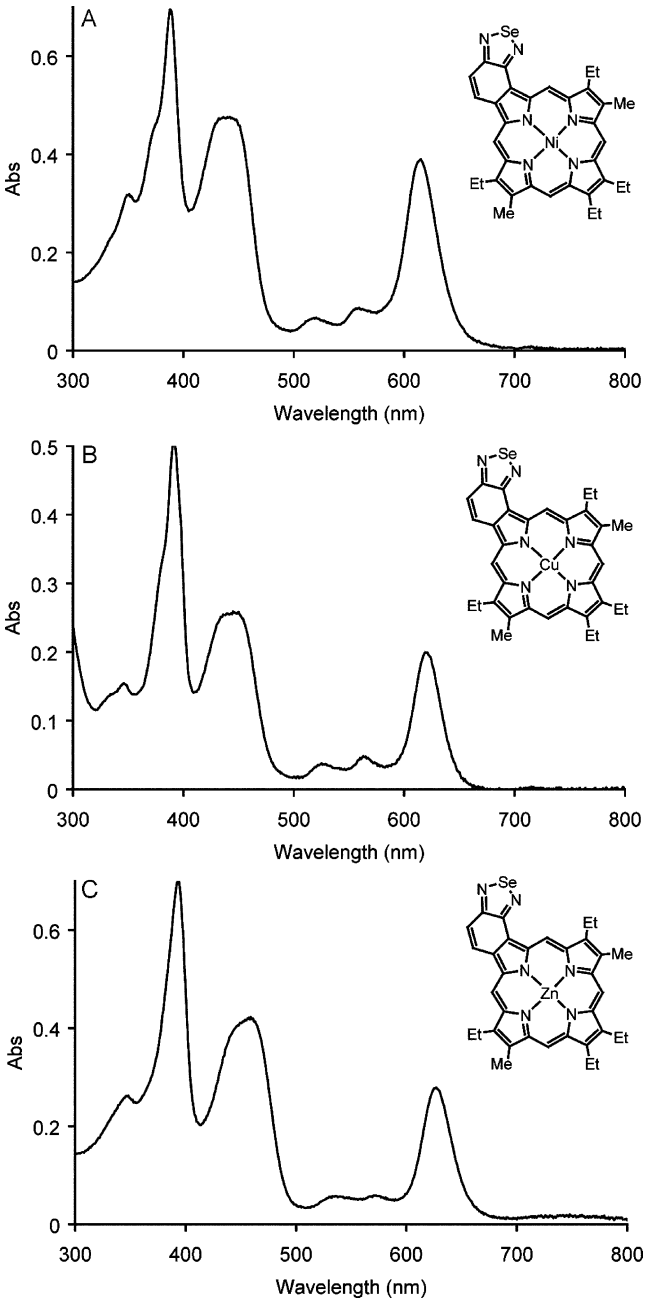


Figure 8. UV–vis spectra for metal complexes of porphyrin **4d** in chloroform. (a) Ni(II) complex **20d**; (b) Cu(II) complex **21d**; (c) Zn complex **22d**.

porphyrins **4a** and **4d** showed split Soret band regions (Figs. 3–5).

Reaction of porphyrins **4b–d** with copper(II), nickel(II) or zinc acetate in DMF or refluxing chloroform–methanol afforded the corresponding metalloporphyrins **20–22** (Scheme 3). These all showed very weak Soret bands and an intense Q band absorption above 600 nm (Figs. 6–8). The shifts for metallo-derivatives derived from **4a** and **4b** were similar, where the longest wavelength band for the 'b series' is red shifted by only 1–3 nm. The chloro-derivatives (or 'c series') showed larger bathochromic shifts with the major Q band having from 8 to 10 nm longer wavelengths than the 'b series'. The selenium chelates, or 'd series', consistently showed the largest effects with the longest wavelength band red shifted compared to the 'b series' by 11–14 nm. As is the case for most porphyrins,[31] the absorption bands for all four series are red shifted going across the period table from nickel(II) porphyrins **20** to copper(II) porphyrins **21** to zinc porphyrins **22**. Focusing on the zinc complexes **22**, the Soret absorptions were observed at 390 and 442 nm for **22a**, 383 and 441 nm for **22b**, 385 and 445 nm for **22c** and 393 and 458 nm for **22d**. The longer wavelength Q band was observed for this series at 612, 615, 625 and 627 nm, respectively, for **22a–d**. These unusually strong longer wavelength bands are approaching values that could be useful for PDT applications, and it is worth noting that the free base porphyrins **4a–d** have all been shown to be photosensitizers for singlet oxygen generation.[32]

3. Conclusions

Novel *c*-annelated pyrroles fused to 2,1,3-benzoxadiazole or 2,1,3-benzoselenadiazole have been synthesized in excellent yields by the Barton–Zard methodology. These pyrroles were taken on to tripyrrane intermediates that were used in crude form to give moderate yields of porphyrins with fused heterocyclic subunits. Oxa- and selenadiazolobenzoporphyrins and their metallo-derivatives have very unusual spectroscopic properties, and these studies may lead to the development of new photosensitizers for applications in PDT. The main limitation in the present study is the diminished reactivity of the ring fused pyrroles that inhibits tripyrrane formation. Further studies are in progress to both improve the yields of porphyrin products and further increase the bathochromic shifts to the intense Q bands for the nickel(II), copper(II) and zinc chelates by extending the fused heterocycles.[33]

4. Experimental

4.1. General

4-Nitro-2,1,3-benzoselenadiazole (**6d**), NBD chloride (**6c**), TFA, and DBU were purchased from Aldrich or Acros, and were used without further purification. THF was distilled from calcium hydride immediately prior to use. Chromatography was performed using Grade III neutral alumina or 70–230 mesh silica gel. Melting points were determined in open capillary tubes on a Mel-Temp apparatus and are uncorrected. Due to the small quantities of porphyrin samples available, high resolution MS data were used in place of CHN analyses, and purity was established by NMR spectroscopy and TLC. UV–vis absorption spectra were run on a Varian Cary Spectrophotometer, and NMR data was obtained on a Varian Gemini 400 MHz FT NMR spectrometer. Mass spectral determinations were conducted at the Mass Spectral Laboratory, School of Chemical Sciences, University of Illinois at Urbana-Champaign, and elemental analyses were obtained from the School of Chemical Sciences Microanalysis Laboratory at the University of Illinois.

4.2. Synthetic procedures

4.2.1. Ethyl oxadiazolobenzo[4,5-*c*]pyrrole-1-carboxylate (8b). Benzofurazan[34] (1.00 g) was taken up in 4.0 mL of concentrated sulfuric acid and the solution cooled in an ice bath. Concentrated nitric acid (1.5 mL) was added dropwise, maintaining the temperature <30 °C, and the resulting mixture stirred at room temperature for a further 20 min. Water was added dropwise, and the resulting precipitate suction filtered to give 4-nitrobenzofurazan (**6b**; 1.25 g; 90%) as a pale yellow powder, mp 84–85 °C (lit. mp[35] 93 °C); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.68 (1H, dd, $J=7.6$, 8.8 Hz), 8.32 (1H, d, $J=8.8$ Hz), 8.52 (1H, d, $J=7.6$ Hz); ^{13}C NMR (d_6-DMSO): δ 124.7, 129.9, 130.1, 137.4, 142.8, 150.6. Ethyl isocyanoacetate[36] (0.63 g) and DBU (0.84 g) were dissolved in freshly distilled THF (300 mL) in a 1000 mL round bottom flask, a solution of **6b** (1.00 g) in THF (200 mL) was added, and the resulting dark mixture was allowed to stir under reflux overnight. The solution was diluted with dichloromethane, washed with water, dried over sodium sulfate, and the solvents evaporated under reduced pressure. Recrystallization from methanol gave **8b** (0.80 g, 57%) as a pale brown solid, mp 261 °C. An analytical sample was obtained by sublimation at 0.05 torr and 210 °C as pale yellow crystals, mp 263 °C. ^{1}H NMR (400 MHz, d_6-DMSO): δ 1.36 (3H, t, $J=7$ Hz), 4.36 (2H, q, $J=7$ Hz), 7.51 (1H, d, $J=9.6$ Hz), 8.05 (1H, d, $J=10$ Hz), 8.14 (1H, s), 13.36 (1H, br s); ^{1}H NMR (400 MHz, $CDCl_3$, downfield region only): δ 7.43 (1H, d, $J=9.6$ Hz), 7.87 (1H, d, $J=3.6$ Hz), 8.07 (1H, d, $J=9.6$ Hz), 10.0 (1H, br s); ^{13}C NMR (d_6-DMSO): δ 15.0, 61.1, 108.0, 112.1, 118.1, 121.5, 125.1, 129.2, 145.3, 149.4, 160.8; EI MS (70 eV): *m/z* (rel. int.) 232 (14), 231 (100, M^+), 203 (5, $[M^+-C_2H_4]$), 186 (13), 185 (17, $[M^+-$EtOH]), 159 (77, $[M^+-C_2H_4–CO_2]$), 158 (13), 157 (22), 156 (3), 155 (15); HRMS (EI): Calcd for $C_{11}H_9N_3O_3$: 231.0641. Found: 231.0644. Anal. Calcd for $C_{11}H_9N_3O_3$: C, 57.14; H, 3.92; N, 18.17. Found: C, 57.01; H, 3.79; N, 17.76.

4.2.2. *tert*-Butyl oxadiazolobenzo[4,5-*c*]pyrrole-1-carboxylate (9b). *tert*-Butyl isocyanoacetate[9c] (0.86 g) and DBU (0.92 g) in THF (300 mL) were reacted with **6b** (1.00 g) in THF (200 mL) under the conditions described above. Recrystallization from methanol gave the title pyrrole (1.12 g, 71%) as a pale brown powder, mp 260 °C, dec. An analytical sample was obtained by sublimation at 0.05 torr and 210 °C as pale yellow crystals, mp 268–269 °C, dec. ^{1}H NMR (400 MHz, d_6-DMSO): δ 1.59 (9H, s), 7.49 (1H, d, $J=9.2$ Hz), 8.00 (1H, d, $J=8.8$ Hz), 8.10 (1H, s), 13.18 (1H, br s); ^{13}C NMR (d_6-DMSO): δ 28.7, 82.3, 107.8, 111.9, 119.4, 121.0, 124.5, 129.3, 145.3, 149.4, 160.2; EI MS (70 eV): *m/z* (rel. int.) 259 (11, M^+), 203 (60, $[M^+-C_4H_8]$), 186 (13), 185 (24, $[M^+-t$-BuOH]), 160 (10), 159 (100, $[M^+-C_4H_8–CO_2]$), 158 (13), 157 (22), 156 (3), 155 (15); HRMS (EI): Calcd for $C_{13}H_{13}N_3O_3$: 259.0964. Found: 259.0957. Anal. Calcd for $C_{13}H_{13}N_3O_3$: C, 60.22; H, 5.05; N, 16.21. Found: C, 59.97; H, 4.86; N, 16.20.

4.2.3. Ethyl 7-chloro-oxadiazolobenzo[4,5-*c*]pyrrole-1-carboxylate (8c). Ethyl isocyanoacetate (0.56 g) and DBU (0.76 g) in THF (300 mL) were reacted with **6c** (1.00 g) in THF (200 mL) under the conditions described above. Recrystallization from methanol gave the title pyrrole (1.12 g, 83%) as a brown powder, mp 262 °C, dec. An analytical sample was obtained by sublimation at 0.05 torr and 175 °C as pale yellow crystals, mp 290 °C. ^{1}H NMR (400 MHz, d_6-DMSO): δ 1.36 (3H, t, $J=7$ Hz), 4.36 (2H, q, $J=7$ Hz), 8.01 (1H, s), 8.13 (1H, s), 13.44 (1H, br s); ^{13}C NMR (d_6-DMSO): δ 14.9, 61.4, 106.8, 116.5, 118.1, 122.0, 124.7, 127.8, 145.3, 148.7, 160.4; EI MS (70 eV): *m/z* (rel. int.) 267 (23), 265 (60, M^+), 237 (3.4, $[M^+-C_2H_4]$), 230 (7, $[M^+-$Cl]), 222 (5), 221 (6), 220 (8), 219 (18, $[M^+-$EtOH]), 205 (39), 203 (84), 195 (16), 194 (8), 193 (47, $[M^+-C_2H_4–CO_2]$); HRMS (EI): Calcd for $C_{11}H_8ClN_3O_3$: 265.0255. Found: 265.0254. Anal. Calcd for $C_{11}H_8ClN_3O_3$: C, 49.73; H, 3.03; N, 15.82. Found: C, 49.51; H, 2.79; N, 15.77.

4.2.4. *tert*-Butyl 7-chloro-oxadiazolobenzo[4,5-*c*]pyrrole-1-carboxylate (9c). *tert*-Butyl isocyanoacetate (0.71 g) and DBU (0.92 g) in THF (300 mL) were reacted with **6b** (1.00 g) in THF (200 mL) under the conditions described above. Recrystallization from methanol gave the title pyrrole (1.18 g, 80%) as a pale brown powder, mp 292 °C, dec. An analytical sample was obtained by sublimation at 0.05 torr and 210 °C as pale yellow crystals, mp 294 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.68 (9H, s), 7.83 (1H, s), 8.05 (1H, s), 10.03 (1H, br s); ^{13}C NMR (d_6-DMSO): δ 28.6, 82.5, 106.6, 116.2, 119.3, 121.5, 124.1, 127.9, 145.3, 148.7, 159.8; EI MS (70 eV): *m/z* (rel. int.) 295 (6), 293 (18, M^+), 239 (34), 238 (11), 237 (100, $[M^+-C_4H_8]$), 221 (17), 220 (18), 219 (50, $[M^+-t$-BuOH]), 203 (13), 202 (19), 195 (2), 193 (7.6, $[M^+-C_4H_8–CO_2]$), 192 (4), 191 (6.8); HRMS (EI): Calcd for $C_{13}H_{12}ClN_3O_3$: 293.0575. Found: 293.0567. Anal. Calcd for $C_{13}H_{12}ClN_3O_3\cdot\frac{1}{8}H_2O$: C, 52.76; H, 4.17; N, 14.20. Found: C, 52.58; H, 3.88; N, 14.19.

4.2.5. Ethyl selenadiazolobenzo[4,5-*c*]pyrrole-1-carboxylate (8d). Ethyl isocyanoacetate (0.63 g) and DBU (0.84 g) in THF (300 mL) were reacted with **6d** (1.00 g) in THF (200 mL) under the conditions described above. Recrystallization from methanol gave the title pyrrole (1.12 g, 85%) as a brown powder, mp 230 °C. An analytical sample was obtained by sublimation at 0.05 torr and 210 °C as pale orange crystals, mp 236 °C. ^{1}H NMR (400 MHz, d_6-DMSO): δ 1.36 (3H, t, $J=7$ Hz), 4.36 (2H, q, $J=7$ Hz), 7.40 (1H, d, $J=9.6$ Hz), 7.98 (1H, s), 7.99 (1H, d, $J=9.6$ Hz), 13.12 (1H, br s); ^{13}C NMR (d_6-DMSO): δ 15.1, 60.9, 116.0, 118.8, 120.8, 122.0, 125.6, 125.9, 155.8, 160.1, 161.2; EI MS (70 eV): *m/z* (rel. int.) 297 (18), 296 (13), 295 (100, M^+), 294 (8), 293 (50), 292 (18), 291 (17), 267 (5.0, $[M^+-C_2H_4]$), 265 (2.7), 259 (97.4), 252 (4.7), 251 (17), 250 (19), 249 (86, $[M^+-$EtOH]), 248 (17), 247 (44), 246 (16), 245 (15), 237 (20), 225 (2.2), 224 (2.7), 223 (8.0, $[M^+-C_2H_4–CO_2]$), 222 (8.1), 221 (15), 203 (41); HRMS (EI): Calcd for $C_{11}H_9N_3O_2Se$: 294.9866. Found: 294.9860. Anal. Calcd for $C_{11}H_9N_3O_2Se\cdot\frac{1}{5}H_2O$: C, 44.37; H, 3.18; N, 14.11. Found: C, 44.19; H, 2.98; N, 14.04.

4.2.6. *tert*-Butyl selenadiazolobenzo[4,5-*c*]pyrrole-1-carboxylate (9d). *tert*-Butyl isocyanoacetate (0.64 g) and DBU (0.87 g) in THF (300 mL) were reacted with **6d** (1.00 g) in THF (200 mL) under the conditions described above. Recrystallization from methanol gave the title pyrrole (1.30 g, 92%) as a pale brown powder, mp 210 °C. An analytical sample was obtained by sublimation at 0.05 torr and 210 °C as pale orange crystals, mp 212 °C. ^{1}H NMR

(400 MHz, d_6-DMSO): δ 1.60 (9H, s), 7.38 (1H, d, J=9.6 Hz), 7.95 (1H, s), 7.99 (1H, d, J=9.6 Hz), 12.94 (1H, br s); ^{13}C NMR (d_6-DMSO): δ 28.8, 81.7, 117.2, 118.7, 120.3, 121.8, 125.0, 126.1, 155.9, 160.1 160.7; EI MS (70 eV): *m/z* (rel. int.) 325 (5.7), 324 (4.0), 323 (100, M^+), 322 (2.1), 321 (13), 320 (4.8), 319 (5.0), 270 (2.4), 269 (20), 268 (11), 267 (100, [$M^+-C_4H_8$]), 266 (62), 265 (48), 264 (17), 263 (18), 252 (4.6), 251 (17), 250 (19), 249 (86, [M^+-t-BuOH]), 248 (9), 247 (45), 246 (17), 245 (17), 224 (2.3), 223 (7.9, [$M^+-C_4H_8$–CO_2]), 222 (7.0), 221 (10), 220 (4.5), 219 (5.7), 218 (2.4); HRMS (EI): Calcd for $C_{13}H_{13}N_3O_2Se$: 323.0182. Found: 323.0172. Anal. Calcd for $C_{13}H_{13}N_3O_2Se$: C, 48.46; H, 4.07; N, 13.04. Found: C, 48.05; H, 3.92; N, 12.82.

4.2.7. Oxadiazolobenzo[4,5-*c*]pyrrole (5b). Nitrogen was bubbled through a mixture of ethyl ester **8b** (1.00 g), potassium hydroxide (2.35 g) and hydrazine (8 drops) in ethylene glycol (48 mL) for 10 min, and the resulting mixture stirred under nitrogen at 180 °C for 30 min. The mixture was poured into ice water, and the precipitate suction filtered and dried in vacuo to give the α-unsubstituted pyrrole (0.34–0.52 g, 50–76%) as a pale brown powder, mp 178–179 °C. An analytical sample was obtained by sublimation at 0.05 torr and 150 °C as pale yellow crystals, mp 180 °C. ^{1}H NMR (400 MHz, d_6-DMSO): δ 7.11 (1H, d, J=9.6 Hz), 7.47 (1H, s), 7.64 (1H, d, J=9.2 Hz), 7.88 (1H, s), 12.32 (1H, br s); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.15 (1H, d, J=9.6 Hz), 7.26 (1H, s), 7.50 (1H, J=9.6 Hz), 7.74 (1H, s), 9.0 (1H, br s); ^{13}C NMR (d_6-DMSO): δ 106.0, 107.2, 116.9, 117.4, 121.5, 130.6, 146.0, 150.0; EI MS (70 eV): *m/z* (rel. int.) 160 (11), 159 (100, M^+), 130 (8), 129 (39, [M^+-NO]), 128 (4.5); HRMS (EI): Calcd for $C_8H_5N_3O$: 159.0434. Found: 159.0433. Anal. Calcd for $C_8H_5N_3O$: C, 60.38; H, 3.17; N, 26.40. Found: C, 60.47; H, 3.00; N, 25.69.

4.2.8. 7-Chloro-oxadiazolobenzo[4,5-*c*]pyrrole (5c). Ethyl ester **8c** (1.00 g), potassium hydroxide (2.35 g) and hydrazine (8 drops) in ethylene glycol (48 mL) was reacted as described for the previous procedure to give **5c** (0.38–0.48 g, 50–65%) as a khaki green powder, mp 274 °C. An analytical sample was obtained by sublimation at 0.05 torr and 150 °C as pale yellow crystals, mp 270 °C. ^{1}H NMR (400 MHz, d_6-DMSO): δ 7.48 (1H, s), 7.83 (1H, s), 7.91 (1H, s), 12.51 (1H, br s); ^{13}C NMR (d_6-DMSO): δ 104.9, 111.2, 117.6, 117.9, 121.2, 129.6, 145.9, 149.2; EI MS (70 eV): *m/z* (rel. int.) 196 (3.5), 195 (35), 194 (11), 193 (100, M^+), 165 (13), 164 (5.2), 163 (36, [M^+-NO]), 162 (3.8); HRMS (EI): Calcd for $C_8H_4ClN_3O$: 193.0043. Found: 193.0043. Anal. Calcd for $C_8H_4ClN_3O\cdot\frac{1}{2}H_2O$: C, 47.43; H, 2.48; N, 20.74. Found: C, 47.67; H, 2.08; N, 20.22.

4.2.9. Selenadiazolobenzo[4,5-*c*]pyrrole-1-carboxylic acid (10). Ethyl ester **8d** (0.80 g) was dissolved in DMSO (20 mL) on a preheated oil bath at 100 °C under a nitrogen atmosphere. A solution of sodium hydroxide (3.2 g) in water (10 ml) was added, and the mixture allowed to reflux under nitrogen for 2 h. The solution was diluted with water (60 mL) and cooled to 0 °C with the aid of a salt-ice bath. The solution was neutralized to litmus paper with acetic acid, maintaining the temperature <5 °C, the resulting mixture was stirred at room temperature for 20 min, and the deep green precipitate suction filtered and washed well with water. Following vacuum drying, the carboxylic acid (0.473 g, 69%) was obtained as a green powder, mp 218–219 °C; ^{1}H NMR (400 MHz, d_6-DMSO): δ 7.36 (1H, d, J=9.6 Hz), 7.92 (1H, s), 8.03 (1H, d, J=9.6 Hz), 13.0 (2H, br); ^{13}C NMR (d_6-DMSO): δ 116.9, 118.7, 120.2, 121.6, 125.5, 126.3, 155.9, 160.2, 162.6; HRMS (EI): Calcd for $C_9H_5N_3O_2Se$: 264.9562. Found: 264.9554.

4.2.10. Selenadiazolobenzo[4,5-*c*]pyrrole (5d). Nitrogen was bubbled through a mixture of the foregoing carboxylic acid (1.00 g), and ethylene glycol (48 mL) for 10 min, and the resulting mixture stirred under nitrogen at 180 °C for 30 min. The mixture was poured into ice water, and the precipitate suction filtered and dried in vacuo to give the α-unsubstituted pyrrole (0.51 g, 60%) as a brown powder. An analytical sample was obtained by sublimation at 0.05 torr and 140 °C as pale orange crystals, mp 180 °C. ^{1}H NMR (400 MHz, d_6-DMSO): δ 7.03 (1H, d, J=9.6 Hz), 7.31 (1H, s), 7.55 (1H, d, J=9.6 Hz), 7.75 (1H, s), 12.07 (1H, br s); ^{13}C NMR (d_6-DMSO): δ 114.9, 116.2, 117.7, 117.8, 121.8, 127.6, 146.7, 150.3. Anal. Calcd for $C_8H_5N_3Se\cdot\frac{1}{3}H_2O$: C, 42.12; H, 2.50; N, 18.42. Found: C, 42.28; H, 2.12; N, 18.04.

4.2.11. *tert*-Butyl 5-chloromethyl-4-ethyl-3-methylpyrrole-2-carboxylate (14b). *N*-Chlorosuccinimide (1.28 g) was added to a solution of *tert*-butyl 4-ethyl-3,5-dimethylpyrrole-2-carboxylate[37] (2.00 g) in carbon tetrachloride (200 mL) and the mixture stirred at room temperature overnight. The solution was washed with water (3× 200 mL) and evaporated under reduced pressure. Recrystallization of the residue from hexanes and subsequent removal of trace solvent in vacuo afforded the title pyrrole (1.60 g, 70%) as a pale brown powder, mp 96 °C, dec. Due to the instability of this compound, it was stored in the freezer. ^{1}H NMR ($CDCl_3$): δ 1.10 (3H, t, J=7.6 Hz), 1.57 (9H, s), 2.24 (3H, s), 2.45 (2H, q, J=7.6 Hz), 4.59 (2H, s), 8.66 (1H, br s); ^{13}C ($CDCl_3$): δ 10.5, 15.7, 17.4, 28.7, 37.0, 81.1, 121.1, 125.7, 126.7, 138.9, 161.2.

4.2.12. *tert*-Butyl 3(5-benzyloxycarbonyl-3-ethyl-4-methyl-2-pyrrolylmethyl)oxadiazolobenzo[4,5-*c*]pyrrole-1-carboxylate (17). A solution of benzyl 5-acetoxymethyl-4-ethyl-3-methylpyrrole-2-carboxylate[38] (185 mg) in acetic acid (15 mL) was added via a syringe pump over a 12 h period to a stirred solution of **9b** (150 mg) and *p*-toluenesulfonic acid (40 mg) in acetic acid under a nitrogen atmosphere. The resulting mixture was stirred for a further 12 h, poured into 200 mL of ice water, and the resulting precipitate suction filtered and dried in vacuo. The crude product was chromatographed on grade III alumina eluting with 5% ethyl acetate–toluene. Once the impurity fractions had been collected, the eluting solvent system was gradually increased in polarity to 10% ethyl acetate–toluene. A bright pink fraction was collected and recrystallized from methanol to afford the title dipyrrylmethane (140 mg, 48%) as pink crystals, mp 161–161.5 °C; ^{1}H NMR (400 MHz, d_6-DMSO): δ 0.65 (3H, t, J=7.2 Hz), 1.59 (9H, s), 2.10 (3H, s), 2.40 (2H, q, J=7.2 Hz), 4.37 (2H, s), 5.27 (2H, s), 7.37 (5H, m), 7.47 (1H, d, J=9.6 Hz), 7.92 (1H, d, J=9.6 Hz), 11.25 (1H, br s), 12.98 (1H, br s); ^{13}C NMR (d_6-DMSO): δ 10.9, 16.1, 17.1, 23.8, 28.7, 65.3, 82.3,

105.7, 112.1, 117.5, 118.5, 124.0, 124.5, 126.5, 128.4, 128.6, 129.1, 129.4, 129.9, 133.3, 137.5, 145.5, 149.6, 160.5, 161.4. Anal. Calcd for $C_{29}H_{30}N_4O_5 \cdot H_2O$: C, 65.40; H, 6.05; N, 10.52. Found: C, 65.26; H, 5.74; N, 10.71.

4.2.13. 8,12,13,17-Tetraethyl-7,18-dimethyloxadiazolobenzo[4,5-*b*]porphyrin (4b). Oxadiazolobenzopyrrole **5b** (100 mg) and acetoxymethylpyrrole **14a**[39] (352 mg) were stirred under reflux with acetic acid (0.75 mL) and toluene (8 mL) under nitrogen for 16 h. The solution was cooled, diluted with dichloromethane, washed with water and evaporated to dryness under reduced pressure to give the crude tripyrrane **15b** as a deep burgundy colored solid that was used without further purification. ^{1}H NMR (400 MHz, d_6-DMSO, downfield region only): δ 4.13 (2H, s), 4.29 (2H, s), 7.00 (1H, d, $J=9.6$ Hz), 7.49 (1H, d, $J=9.6$ Hz), 10.88 (1H, br s), 11.01 (1H, br s), 11.61 (1H, br s). Crude **15b** (100 mg) was dissolved in TFA (2.5 mL) and stirred for 5 min at room temperature under nitrogen. The mixture was diluted with dichloromethane (40 mL), followed by the immediate addition of dialdehyde **16**[23,40] (30 mg) and the mixture stirred under nitrogen for an additional 2 h. The dark solution was poured into a separatory funnel and shaken for 5 min with 0.1% aqueous ferric chloride solution. The organic layer was separated, the aqueous phase back extracted with dichloromethane, and the combined organic phases washed with water, 10% sodium bicarbonate solution, and water. The solvent was evaporated under reduced pressure and the residue chromatographed on grade III alumina eluting with 30% dichloromethane–toluene. A deep green colored fraction was collected, the solvent evaporated, and the residue recrystallized from chloroform–methanol to give porphyrin **4b** (29 mg, 31%) as green crystals, mp >300 °C; UV–vis (1% Et_3N–$CHCl_3$): λ_{max} ($\log_{10}\varepsilon$) 373 (4.83), 393 (4.58), 434 (5.12), 567 (4.42), 592 nm (4.33); UV–vis (1% TFA–$CHCl_3$): λ_{max} ($\log_{10}\varepsilon$) 426 (5.39), 567 (4.06), 591 (3.56), 618 nm (4.30); ^{1}H NMR (400 MHz, TFA–$CDCl_3$): $\delta -3.37$ (4H, br), 1.73–1.88 (12H, m), 3.70 (3H, s), 3.72 (3H, s), 4.17–4.30 (8H, m), 8.74 (1H, d, $J=9.2$ Hz), 9.54 (1H, d, $J=9.6$ Hz), 10.71 (1H, s), 10.73 (1H, s), 11.11 (1H, s), 11.62 (1H, s); ^{13}C NMR (TFA–$CDCl_3$): δ 11.9, 16.3, 16.4, 17.2, 20.1, 20.3, 20.5, 99.0, 99.4, 99.9, 101.0, 119.5, 121.3, 126.5, 134.9, 136.4, 137.1, 138.8, 138.9, 142.3, 143.1, 143.5, 144.1, 144.4, 144.8, 145.1, 145.6, 146.3, 150.6; HRMS (ESI): Calcd for $C_{34}H_{34}N_6O+H$: 543.2872. Found: 543.2859.

4.2.14. Nickel(II) complex 20b. Porphyrin **4b** (12 mg) and nickel(II) acetate tetrahydrate (42 mg) were stirred with DMF (10 mL) in the dark under reflux overnight. The solution was cooled to room temperature, diluted with chloroform, and washed with water (3×200 mL). The organic layer was evaporated under reduced pressure and the residue recrystallized from chloroform–methanol to give the nickel complex (7.5 mg, 63%) as green sheets, mp >300 °C. UV–vis (1% Et_3N–$CHCl_3$): λ_{max} ($\log_{10}\varepsilon$) 379 (4.66), 428 (4.82), 553 (3.92), 601 nm (4.52); HRMS (FAB): Calcd for $C_{34}H_{32}N_6ONi$: 598.1991. Found: 598.1992.

4.2.15. Copper(II) complex 21b. A saturated solution of copper(II) acetate in methanol (10 mL) was added to a solution of porphyrin **4b** (10 mg) in chloroform (10 mL), and the mixture stirred under reflux in the dark overnight. The solution was cooled to room temperature, diluted with chloroform, and washed with water (3×200 mL). The organic layer was evaporated under reduced pressure and the residue recrystallized from chloroform–methanol to give the copper complex (6 mg, 57%) as a deep green powder, mp >300 °C. UV–vis (1% Et_3N–$CHCl_3$): λ_{max} ($\log_{10}\varepsilon$) 380 (4.75), 432 (4.98), 559 (3.93), 608 nm (4.63); HRMS (FAB): Calcd for $C_{34}H_{32}N_6OCu$: 603.1933. Found: 603.1932.

4.2.16. Zinc complex 22b. A saturated solution of zinc(II) acetate in methanol (10 mL) was added to a solution of porphyrin **4b** (10 mg) in chloroform (10 mL), and the mixture stirred under reflux in the dark overnight. The solution was cooled to room temperature, diluted with chloroform, and washed with water (3×200 mL). The organic layer was evaporated under reduced pressure and the residue recrystallized from chloroform–methanol to give the zinc complex (9.5 mg, 87%) as a green powder, mp >300 °C. UV–vis (1% Et_3N–$CHCl_3$): λ_{max} ($\log_{10}\varepsilon$) 384 (4.71), 443 (4.84), 566 (3.86), 617 nm (4.48); HRMS (FAB): Calcd for $C_{34}H_{32}N_6OZn$: 604.1929. Found: 604.1929.

4.2.17. 3^2-Chloro-8,12,13,17-tetraethyl-7,18-dimethyloxadiazolobenzo[4,5-*b*]porphyrin (4c). Pyrrole **5c** (100 mg) and acetoxymethylpyrrole **14a** (288 mg) were stirred under reflux with acetic acid (0.75 mL) and xylene (8 mL) under nitrogen for 16 h. The solution was cooled, diluted with dichloromethane, washed with water and evaporated to dryness under reduced pressure to give the crude tripyrrane **15c** as a reddish brown solid that was used without further purification. ^{1}H NMR (400 MHz, d_6-DMSO, downfield region only): δ 4.16 (2H, s), 4.26 (2H, s), 7.77 (1H, s), 10.75 (1H, br s), 11.03 (1H, br s), 11.72 (1H, br s). Crude **15c** (100 mg) deprotected with TFA (2.5 mL) and reacted with dialdehyde **16** (30 mg) under the conditions described for **4b**. The crude product was chromatographed on grade III alumina eluting with 30% dichloromethane–toluene and recrystallized from chloroform–methanol to give porphyrin **4c** (16 mg, 17%) as dark green crystals, mp >300 °C; UV–vis (1% Et_3N–$CHCl_3$): λ_{max} ($\log_{10}\varepsilon$) 374 (4.86), 436 (5.00), 570 (4.36), 596 nm (4.35); UV–vis (1% TFA–$CHCl_3$): λ_{max} ($\log_{10}\varepsilon$) 427 (5.33), 568 (4.05), 620 nm (4.30); ^{1}H NMR (400 MHz, TFA–$CDCl_3$): $\delta -2.96$ (4H, br), 1.72–1.86 (12H, m), 3.67 (3H, s), 3.68 (3H, s), 4.13–4.26 (8H, m), 9.50 (1H, s), 10.63 (1H, s), 10.65 (1H, s), 10.97 (1H, s), 11.49 (1H, s); ^{13}C NMR (TFA–$CDCl_3$): δ 11.7, 16.2, 16.3, 17.2, 20.1, 20.3, 20.4, 99.1, 99.5, 100.1, 100.7, 119.5, 121.2, 125.6, 135.2, 135.9, 136.0, 136.2, 139.4, 139.4, 142.1, 142.2, 143.0, 143.1, 143.6, 144.2, 144.6, 145.3, 145.6, 150.2; HRMS (ESI): Calcd for $C_{34}H_{33}ClN_6O+H$: 577.2483. Found: 577.2506.

4.2.18. Nickel(II) complex 20c. Porphyrin **4c** (10 mg) and nickel(II) acetate tetrahydrate (40 mg) were reacted in DMF (10 mL) under the conditions described for **20b**. Recrystallization from chloroform–methanol gave the nickel complex (4.8 mg, 32%) as green sheets, mp >300 °C. UV–vis (1% Et_3N–HCl_3): λ_{max} ($\log_{10}\varepsilon$) 381 (4.40), 440 (4.39), 610 nm (4.23); HRMS (FAB): Calcd for $C_{34}H_{31}ClN_6ONi$: 632.1601. Found: 632.1603.

4.2.19. Copper(II) complex 21c. Porphyrin **4c** (8 mg) and copper(II) acetate monohydrate (48 mg) were reacted in DMF (10 mL) under the conditions described for **20b**. Recrystallization from chloroform–methanol gave the copper complex (6.2 mg, 77%) as a blue-green powder, mp > 300 °C. UV–vis (1% Et_3N–$CHCl_3$): λ_{max} ($\log_{10}\varepsilon$) 325 (4.12), 382 (4.61), 442 (4.59), 618 nm (4.39); FD MS: *m/z* (rel. int.) 642 (7), 641 (20), 640 (30), 639 (89), 638 (42), 637 (100) (M^+).

4.2.20. Zinc complex 22c. Porphyrin **4c** (7 mg) and zinc acetate dihydrate (50 mg) were reacted in DMF (10 mL) under the conditions described for **20b**. Recrystallization from chloroform–methanol gave the zinc complex (6.3 mg, 86%) as a green powder, mp > 300 °C. UV–vis (1% Et_3N–$CHCl_3$): λ_{max} ($\log_{10}\varepsilon$) 387 (4.67), 445 (4.65), 573 (3.83), 627 nm (4.32); FD MS: *m/z* (rel. int.) 645 (7), 644 (16), 643 (25), 642 (55), 641 (33), 640 (95), 639 (36), 638 (100) (M^+).

4.2.21. 8,12,13,17-Tetraethyl-7,18-dimethylselenadiazolobenzo[4,5-*b*]porphyrin (4d). Selenadiazolobenzopyrrole **5d** (100 mg) was taken up in ethyl acetate (20 mL) and sonicated for 20 min. Acetic acid (92 mL) and acetoxymethylpyrrole **14a** (254 mg) were added, and the resulting mixture was refluxed with stirring under nitrogen for 16 h. The solution was cooled, diluted with dichloromethane, washed with water and sodium bicarbonate solution, and evaporated to dryness under reduced pressure to give the crude tripyrrane **15d** as a dark red solid that was used without further purification. Crude **15d** (100 mg) deprotected with TFA (2.5 mL) and reacted with dialdehyde **16** (29 mg) under the conditions described for **4b**. The crude product was chromatographed on grade III alumina eluting with 30% dichloromethane–toluene and recrystallized from chloroform–methanol to give porphyrin **4d** (9 mg, 9%) as green crystals, mp > 300 °C; UV–vis (1% Et_3N–$CHCl_3$): λ_{max} ($\log_{10}\varepsilon$) 382 (4.78), 450 (4.69), 574 (4.19), 600 nm (4.27); UV–vis (1% TFA–$CHCl_3$): λ_{max} ($\log_{10}\varepsilon$) 369 (4.33), 404 (4.70), 427 (5.04), 569 (3.94), 597 (3.44), 621 nm (4.27); ^{1}H NMR (400 MHz, TFA–$CDCl_3$): δ −3.62 (2H, br), −2.96 (2H, br), 1.73–1.88 (12H, m), 3.66 (3H, s), 3.69 (3H, s), 4.14–4.29 (8H, m), 8.74 (1H, d, J=9.2 Hz), 9.57 (1H, d, J=9.6 Hz), 10.62 (1H, s), 10.64 (1H, s), 11.04 (1H, s), 12.05 (1H, s); ^{13}C NMR (TFA–$CDCl_3$): δ 12.0, 16.6, 16.7, 17.5, 20.2, 20.3, 20.4, 20.5, 98.1, 99.2, 99.5, 102.1, 124.4, 126.7, 126.8, 134.5, 137.3, 137.6, 138.1, 138.3, 141.6, 142.3, 142.6, 143.4, 143.5, 143.9, 144.1, 144.4, 145.2, 156.3, 161.9; HRMS (ESI): Calcd for $C_{34}H_{34}N_6Se$ + H: 607.2088. Found: 607.2075.

4.2.22. Nickel(II) complex 20d. Porphyrin **4d** (5 mg) and nickel(II) acetate tetrahydrate (40 mg) were reacted in DMF (10 mL) under the conditions described for **20b**. Recrystallization from chloroform–methanol gave the nickel complex (3.2 mg, 58%) as dark green sheets, mp > 300 °C. UV–vis (1% Et_3N–$CHCl_3$): λ_{max} ($\log_{10}\varepsilon$) 350 (4.15), 389 (4.49), 438 (4.33), 445 (4.33), 614 nm (4.24); HRMS (FAB): Calcd for $C_{34}H_{32}N_6SeNi$: 662.1207. Found: 662.1207.

4.2.23. Copper(II) complex 21d. Porphyrin **4d** (8 mg) and copper(II) acetate monohydrate (48 mg) were reacted in DMF (10 mL) for 3 h under the conditions described for **20b**. Recrystallization from chloroform–methanol gave the copper complex (8 mg, 85%) as a green powder, mp > 300 °C. UV–vis (1% Et_3N–$CHCl_3$): λ_{max} ($\log_{10}\varepsilon$) 347 (4.00), 392 (4.51), 441 (4.23), 447 (4.22), 620 nm (4.11); HRMS (FAB): Calcd for $C_{34}H_{32}N_6SeCu$: 667.1150. Found: 667.1151.

4.2.24. Zinc complex 22d. Porphyrin **4d** (7 mg) and zinc acetate dihydrate (50 mg) were reacted in DMF (10 mL) for 3 h under the conditions described for **20b**. Recrystallization from chloroform–methanol gave the zinc complex (5.3 mg, 60%) as a green powder, mp > 300 °C. UV–vis (1% Et_3N–$CHCl_3$): λ_{max} ($\log_{10}\varepsilon$) 348 (4.22), 393 (4.64), 458 (4.40), 627 nm (4.25); HRMS (FAB): Calc for $C_{34}H_{32}N_6SeZn$: 668.1145. Found: 668.1146.

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Porphyrins with exocyclic rings. Part 21: Influence of pyrrolic and carbocyclic ring alkyl substituents on the synthesis of porphyrins bearing six-membered exocyclic rings☆

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Abstract—A series of 5-substituted 4,5,6,7-tetrahydroindoles were prepared by reacting 4-substituted cyclohexanones with phenylhydrazones derived from esters of acetoacetic acid under Knorr-type reaction conditions. Related 6,6-dimethyltetrahydroindoles were also prepared by reacting dimedone with oximes by the Knorr pyrrole syntheses, followed by selective reduction of the remaining ketone moiety with diborane. The substituted tetrahydroindoles were regioselectively oxidized with lead tetraacetate to give the related 7-acetoxy derivatives, and these reacted with 5-unsubstituted pyrrole esters to give pyrrolyltetrahydroindoles. In one case, a bromo substituent was used to protect the β-position of the pyrrole reactant. Cleavage of the benzyl ester protective groups with hydrogen over Pd/C, which also removes the bromo-protective group, gave four dipyrrole carboxylic acids. These were condensed with a dipyrrylmethane dialdehyde using the MacDonald '2+2' condensation to give substituted porphyrins with six-membered exocyclic rings. These structures are useful for comparison to porphyrin samples found in organic-rich sediments such as oil shales and petroleum. The presence of methyl substituents on the six-membered ring for the tetrahydroindole precursors slightly decreases the yields for porphyrin synthesis, and this effect is enhanced when the system becomes more sterically crowded due to the presence of an ethyl group of the adjacent pyrrole ring. 5-Alkyl substituted tetrahydroindoles were also converted to tetrapropanoporphyrins via a cyclotetramerization procedure. The alkyl substituents again decreased the yields, although 5-alkyl substituents were found to have a far less deleterious effect than 6-alkyl groups. In addition to providing samples to help assign the vibrational spectra of geoporphyrin samples, these results demonstrate that highly substituted porphyrin systems can be prepared from tetrahydroindole derivatives.
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1. Introduction

Metalloporphyrins with five-, six- or seven-membered exocyclic rings (**1**–**3**, Chart 1) are commonly found in organic-rich sediments such as oil shales and petroleum, usually in the form of nickel(II) or vanadyl complexes.[1–5] These materials are believed to be derived from the photosynthetic pigments associated with algae or bacteria and can be considered to be molecular fossils of the chlorophylls from which they are derived.[1] The structures of these porphyrins give insights into the environment that existed at that time; for instance, the presence of bacteriopetroporphyrins derived from the *Chlorobiaceae* or brown sulfur bacteria provides evidence for an anoxic environment in the water column.[6] Furthermore, the modifications that these structures undergo can be related to the thermal maturity of the sediments and may provide additional information on the conditions leading to fossil fuel formation.[7] Petroporphyrins also provide a unique fingerprint for petroleum resources that can find application in environmental monitoring, for example, for oil spills.[8] The most abundant of the sedimentary cycloalkanoporphyrins (CAPs) is deoxophylloerythroetioporphyrin (DPEP; **1**, $R=R^1=Et$, $R^2=R^3=H$) but a large number of related DPEP-type petroporphyrins have been identified, including porphyrins with substituted cycloalkano-rings (**1**; R^2 or $R^3=Me$).[1–5] Methyl substituted CAPs **2a**,**b** with six-membered exocyclic rings are also known,[9,10] as well as a related hydroxymethyl-CAPs **2c** and **2d**.[11] In order to provide standards for the analysis of petroporphyrins, we have developed synthetic routes to CAPs starting from cyclic ketones.[12–17] For instance, *meso*,β-propanoporphyrins **4** were synthesized using the MacDonald '2+2' condensation (Scheme 1).[17b,c] Cyclohexanone reacts with oximes or phenylhydrazones **5** in the presence of zinc and acetic acid

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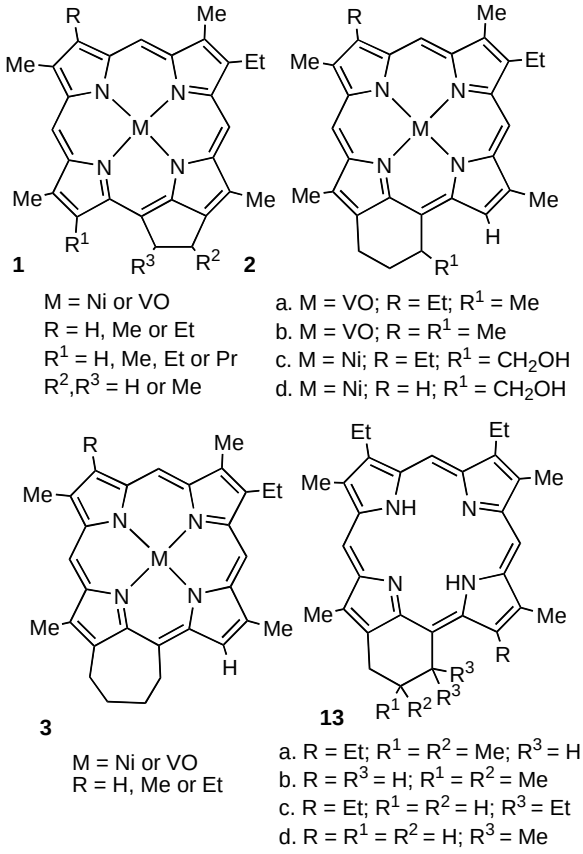
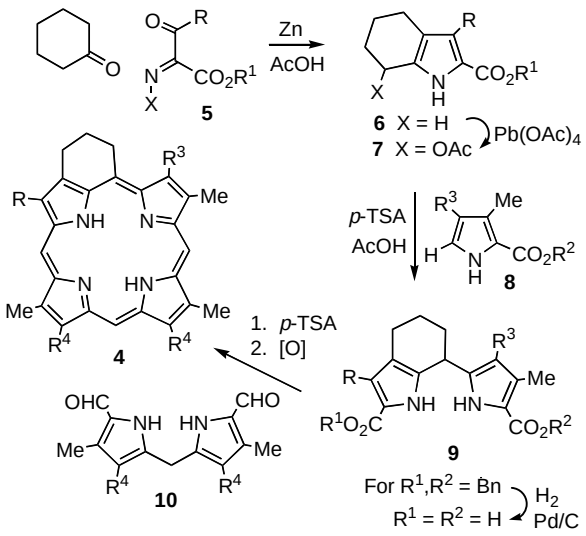
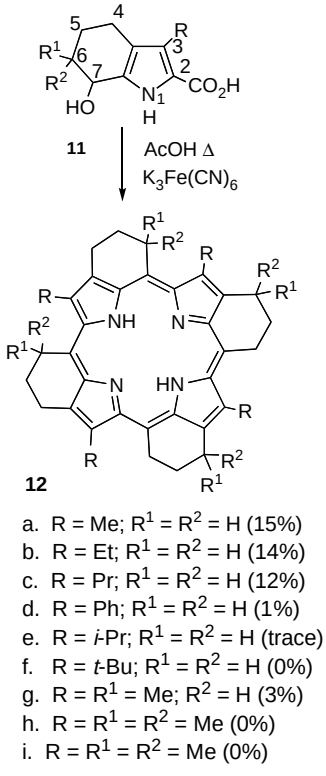


Chart 1.



Scheme 1.

to give 4,5,6,7-tetrahydroindoles **6**[16,17] and these react with lead tetraacetate in acetic acid to regioselectively afford the acetoxy derivatives **7**.[16,17] These undergo acid catalyzed condensations with α-unsubstituted pyrroles **8** to give dipyrroles **9**.[17] Deprotection of the terminal ester groups gives the corresponding dicarboxylic acids and these react with dipyrrylmethane dialdehydes **10** in the presence of *p*-toluenesulfonic acid to give, following the addition of zinc acetate and air oxidation, >20% yield of the propanoporphyrins **4**.[17] This chemistry has been applied to the synthesis of porphyrins with five-, six-, seven-, and eight-membered exocyclic rings.[14,15,17] In addition, the dipyrrolic intermediates have been utilized in stepwise syntheses of naturally occurring petroporphyrins.[14e,f,15a,17a] Furthermore, 7-acetoxy-4,5,6,7-tetrahydroindoles **7** can be hydrolyzed to give hydroxy carboxylic acids **11**, and these undergo cyclotetramerization with potassium ferricyanide in refluxing acetic acid to give the unusual tetrapropanoporphyrins **12** in moderate yields (Scheme 2).[16] Ring size greatly effects the efficiency of these syntheses, probably by altering the conformation of intermediary tetrapyrroles prior to macrocycle formation.[12] Less work has been conducted on the synthesis of porphyrins bearing substituents on the exocyclic rings. For the cyclotetramerization chemistry shown in Scheme 2, the presence of a methyl group at position 6 on the tetrahydroindole intermediate **11g** greatly decreases the yield of tetrapropanoporphyrin, while no product at all is formed for the 6,6-dimethyl substituted structures **11h** and **11i**.[16] In an attempt to more fully explore the versatility of this chemistry, and to provide samples for structurally characterizing geoporphyrins using resonance Raman spectroscopy,[18–26] the dimethylpropanoporphyrins **13a–d** were targeted for synthesis. In addition, the influence of 5-alkyl substitutents on the cyclotetramerization chemistry was also investigated.

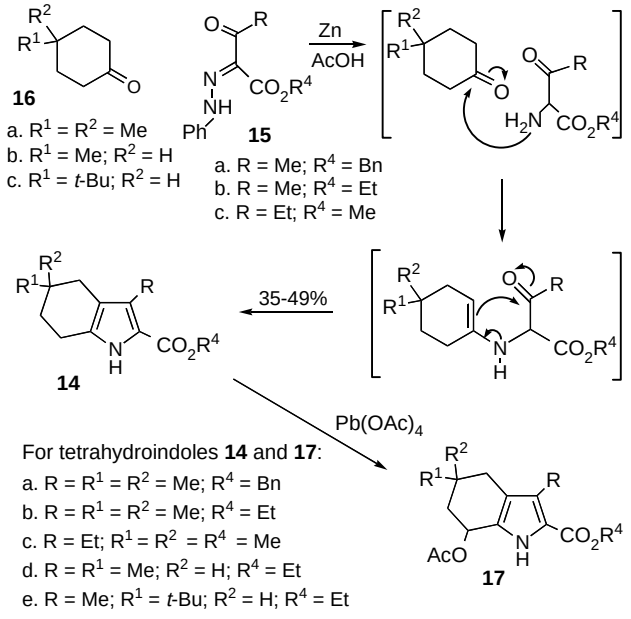


Scheme 2.

2. Results and discussion

In order to synthesize porphyrins with substituted six-membered exocyclic rings, it was first necessary to prepare a series of previously unknown 5- and 6-substituted tetrahydroindole (THI) derivatives. THIs **6** were easily prepared

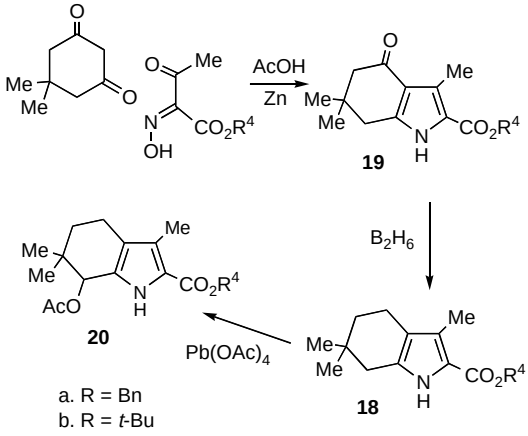
by a modified Knorr-type pyrrole condensation from cyclohexanone (Scheme 1),[16,17] and this approach is well suited for the synthesis of 5-substituted systems (Scheme 3). Reaction of phenylhydrazones **15a–c** with 4,4-dimethylcyclohexanone (**16a**) in the presence of zinc dust and sodium acetate in acetic acid gave the required THIs **14a–c**. The zinc initially reduces the imine unit and cleaves aniline to generate an α-aminoketone, and this condenses and cyclizes onto the cyclic ketone to generate the pyrrolic products. Phenylhydrazone **15b** similarly reacted with 4-methylcyclohexanone (**16b**) or 4-*tert*-butylcyclohexanone (**16c**) to give THIs **14d** and **14e**, respectively. Treatment of **14a–e** with lead tetraacetate in acetic acid gave the 7-acetoxy derivatives **17a–e** in excellent yields, although it is worth noting that **17d** and **17e** were obtained as a mixture of two diastereomers.



Scheme 3.

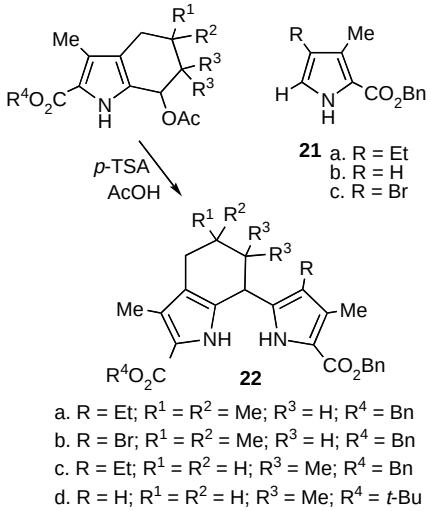
Due to the symmetry of ketones **16a–c**, the required 5-substituted THIs **14** are generated in isomerically pure form. However, 6-alkyl substituted THIs **18** cannot be prepared in this fashion as 3-substituted cyclohexanones would generate mixtures of 4- and 6-substituted products. The problem can be overcome by using substituted 1,3-cyclohexanones to direct the chemistry,[16,17] followed by reduction of the superfluous carbonyl grouping. Dimedone reacts with oximes under conventional Knorr pyrrole condensation conditions to give 4-oxoTHI **19** with the correct substitution pattern (Scheme 4). Hence, benzyl or *tert*-butyl acetoacetate were reacted with nitrous acid to give the corresponding oximes and these in turn were condensed with dimedone in the presence of zinc dust in acetic acid to give **19a** and **19b**, respectively. The ketone moiety was then selectively reduced with diborane to afford the required 6,6-dimethylTHIs in good yields. These were again selectively oxidized with lead tetraacetate to give the acetoxy derivatives **20**.

In order to assess how steric factors affect the yields in porphyrin synthesis, as well as to provide standards suitable

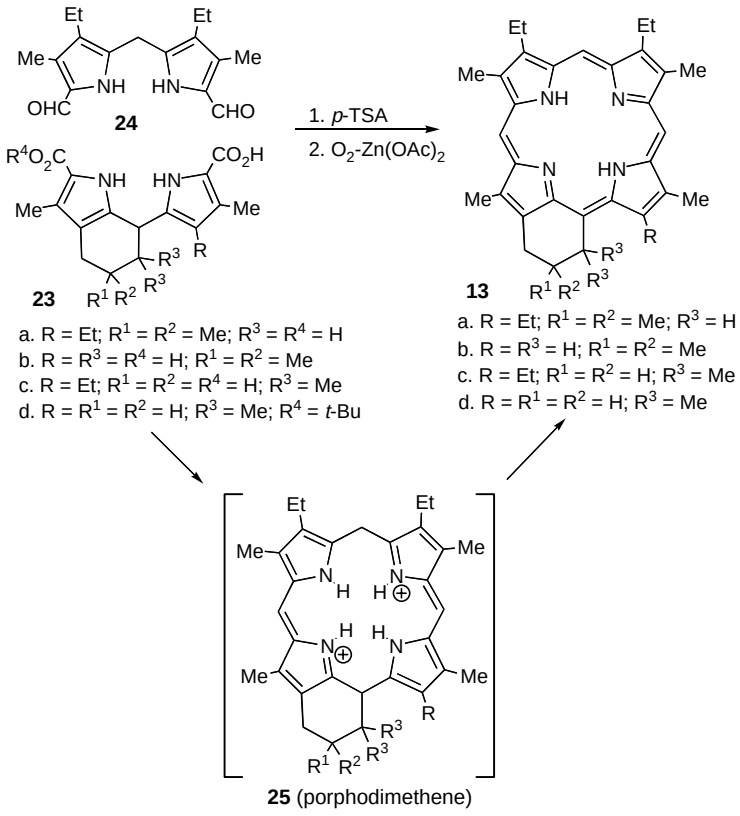


Scheme 4.

for the analysis of vibrational spectra for geochemical samples, porphyrins with an ethyl or a hydrogen next to the exocyclic ring were required. The ethyl substituted dipyrroles **22a** and **22c** were obtained by condensing acetoxyTHIs **14a** and **20a** with 5-unsubstituted pyrrole **21a** in the presence of *p*-toluenesulfonic acid in acetic acid. Similarly, **20b** reacted with the 4,5-unsubstituted pyrrole ester **21b** to give the corresponding pyrrolyltetrahydroindole **22d** in 38% yield (Scheme 5). However, attempts to react acetoxyTHI **14a** with **21b** failed to give more than trace amounts of the required dipyrrolic product. This problem had been encountered previously when two adjacent α and β sites are unsubstituted on the pyrrole nucleus.[17a,27] The absence of an electron-donating β-substituent decreases the reactivity of the pyrrole, and it is also possible that substitution could occur at both positions. These problems were overcome by using a 4-bromo-protected pyrrole **21c** instead of **21b**.[17a] The reaction of **20b** with **21c** did not work well with the usual conditions but when zinc chloride was used as the catalyst in dichloromethane, the bromo-substituted dipyrrole **22d** was isolated in 39% yield. Hydrogenolysis of **22a–d** over 10% palladium–charcoal with trace amounts of triethylamine in acetone or ethanol gave the corresponding carboxylic acids **23a–c** (Scheme 6) in high yields. As expected, treatment of



Scheme 5.



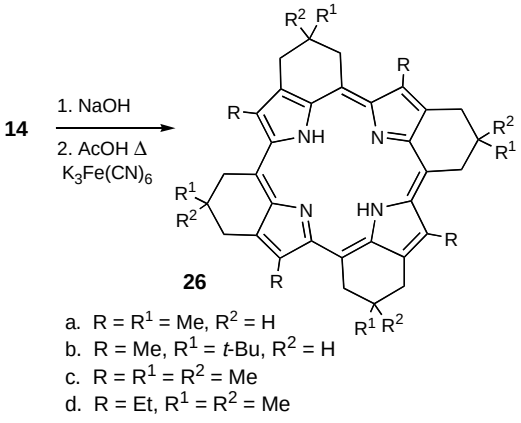
Scheme 6.

the bromo-dipyrrole **22b** with hydrogen and trace triethylamine in methanol led to reductive cleavage of both the bromo-substituent and the benzyl ester moiety to afford the β-unsubstituted dipyrrole **23b**.

Dipyrroles **23a–d** were the final precursors for porphyrin synthesis (Scheme 6). Although several routes for synthesizing porphyrins with exocyclic rings have been explored previously,[12,28] including cyclizations of *a*,*c*-biladienes,[15a,17] the MacDonald condensation[29] had been found to be the most versatile route for preparing cycloalkanoporphyrins. This '2+2' methodology requires that one of the two condensing dipyrrolic reactants is symmetrical or two porphyrin isomers will be formed.[29] As we planned to use the symmetrical dialdehyde **24** in our studies, this methodology was the best suited for our purposes. MacDonald condensations of **23a–c** were conducted under conventional conditions[29b] by reacting the dicarboxylic acids with dipyrrylmethane dialdehyde **24** in the presence of *p*-toluenesulfonic acid. The intermediary porphodimethenes **25** were treated with a solution of zinc acetate in methanol and air oxidized for 1–3 days[17c] to give the dimethylpropanoporphyrins **13a–c**. Dipyrrole *tert*-butyl ester **23d** was first treated with TFA to cleave the protective group and then reacted with **24** under the same conditions. The yields in each case were lower than had been observed for unsubstituted *meso*,β-propanoporphyrins **4** (Scheme 1).[17] However, the yields correlate well with the steric factors involved. The best yield (16%) was obtained for porphyrin **13b** where the *gem*-dimethyl unit is placed at the second carbon of the propano chain and the adjacent β-pyrrolic site is unsubstituted. When the β-substituent is changed to an ethyl, the yield drops to 14%. Placement of the *gem*-dimethyl unit next to the *meso*-carbon increases potential steric interactions and even when the pyrrolic substituent is H, the yield dropped to 10%. Moreover, when the ethyl group is present next to the methyls in **13c**, the yield was only 3.5–7%. The MacDonald condensation takes place via porphodimethene intermediates that still retain an sp^3 carbon at the bridge incorporating the exocyclic ring. This relieves some of the steric congestion that results in the more planar aromatic porphyrin structures **13**. Once the macrocycle has been generated, slow air oxidation gives the porphyrin product. The decreased yields probably result from the dipyrrolic intermediates taking on conformations due to these steric interactions that are not favorable for macrocycle formation, and further condensation would then lead to oligopyrrolic products. Although the yields for **13a**, **13b** and **13d** could be raised to 28–39% by using dilution techniques, this approach must be handled with some caution. The pyrrolic intermediates can undergo acidolysis reactions followed by random recombinations that can give rise to mixtures of porphyrin products,[30] and attempts to improve the yield of **13c** gave material that showed additional resonances in the porphyrin's proton NMR spectrum. Nonetheless, pure porphyrin can be obtained under conventional conditions, and these results provide some insights into the limitations of this methodology.

The UV–vis spectra for **13a**, **13b** and **13d** in chloroform showed strong Soret bands at 405 nm and the usual series of four smaller Q bands through the visible region. The Q bands are generally labeled I–IV, with the longest

wavelength band being designated I and the shortest wavelength absorption labeled IV. These bands showed a phyllo type pattern[31] where the relative intensities of the absorptions were IV > II > III > I. The sterically congested porphyrin **13c** showed a slightly red shifted Soret band at 409 nm, and the Q bands were also slightly shifted to longer wavelengths. In 1% TFA–chloroform, the corresponding dications $\mathbf{13}H_2^{2+}$ for **13a**, **13b** and **13d** gave reddish-pink solutions with intensified Soret bands at 411–412 nm and two relatively weak Q bands at 553–556 and 594–601 nm. However, $\mathbf{13c}H_2^{2+}$ afforded green solutions that gave a bathochromically shifted Soret band at 418 nm and Q bands at 564 and 610 nm. This result suggests that the dication exhibits further distortion due to a combination of the peripheral steric crowding and the presence of 4 internal NHs. The proton NMR spectra for porphyrin **13a**, **13b** and **13d** showed typical shifts due to the presence of a powerful diatropic ring current. Three 1H singlets for the *meso*-protons were observed in each case between 9.88 and 10.07 ppm, while the four methyl substituents gave rise to several singlets between 3.53 and 3.77 ppm. Small differences were noted for the highly crowded propanoporphyrin **13c**, where one of the *meso*-protons was slightly shifted upfield to a value of 9.74 ppm, but most of the remaining resonances fell into the same general range. The CH_2 on the exocyclic ring connected to the β-pyrrolic carbon gave a singlet near 3.7 ppm for **13a** and **13b**, while the corresponding triplet for this unit in **13c** and **13d** was observed at 3.88 and 3.86 ppm, respectively. The exocyclic ring CH_2 connected to the *meso*-carbon in **13a** and **13b** was further deshielded to 4.91 or 4.80 ppm, respectively. The *gem*-dimethyl groups on porphyrins **13a** and **13b** gave rise to a 6H singlet near 1.5 ppm, while the corresponding resonances in **13c** and **13d** were further deshielded and gave the 6H singlet at 2.5 ppm, as expected given the relative proximity of the methyl groups to the porphyrin macrocycle. It is also notable that for **13c**, one of the ethyl triplets was shifted upfield to 1.24 ppm compared to typical values of 1.7–1.9 ppm. In the presence of TFA, the corresponding dications for **13a**, **13b** and **13d** showed the presence of four internal NH resonances that fell into the range of −2.8 to −4.0 ppm. The relatively large separation between the individual NH resonances indicates a large degree of asymmetry within the porphyrin cavity. The proton NMR spectrum of $\mathbf{13c}H_2^{2+}$ in TFA–$CDCl_3$ also showed four NH peaks but these were shifted downfield to a range of −1.9 to −3.4 ppm. The external *meso*-protons were all shifted to values above 10 ppm, although these shifts were slightly smaller for $\mathbf{13c}H_2^{2+}$. The decreased ring current was also evident from the chemical shifts for the methyl resonances of $\mathbf{13c}H_2^{2+}$, which gave values of 3.14, 3.35, 3.45 and 3.47 ppm compared to chemical shifts of 3.54–3.74 ppm for the other three porphyrin dications. The exocyclic ring for $\mathbf{13c}H_2^{2+}$ also showed evidence for crowding where the resonances for the two CH_2 units appeared as broadened triplets at 2.20 and 3.77 ppm; at 25 °C the latter resonance was very broad but this resolved into a broadened triplet at 50 °C. Finally, the ethyl unit adjacent to the exocyclic ring in $\mathbf{13c}H_2^{2+}$, gave a triplet that was abnormally shifted upfield to 0.53 ppm. This unit appears slightly upfield in the free base **13c** but the effect is far more apparent in the dication. Presumably this is due to the highly distorted



Scheme 7.

macrocycle of $\mathbf{13c}H_2^{2+}$, and to a lesser extent for free base **13c**, placing this ethyl unit over the porphyrin π-system.

Porphyrins with four six-membered exocyclic rings have been prepared previously, but the presence of alkyl substitutents at position 6 of the THI precursors (Scheme 2) had a strongly deleterious effect on this chemistry. Given the results that we had obtained for the MacDonald condensation, it seemed likely that tetrapropanoporphyrins could be obtained more readily from 5-substituted THIs. AcetoxyTHIs **17b–e** were hydrolysed with sodium hydroxide and the resulting hydroxy carboxylic acids were heated under reflux in acetic acid with potassium ferricyanide. Following purification by column chromatography and recrystallization from chloroform–methanol, the new tetrapropanoporphyrins **26** were isolated in moderate yield (Scheme 7). The 5-methyl substituted THI **17d** gave the porphyrin product **26a** in 10.4% yield, although the 5-*tert*-butyl substituted THI **17e** gave porphyrin **26b** in only 2.5% yield. The *gem*-dimethyl THIs **17b** and **17c** gave intermediary results affording porphyrins **26c** and **26d**, respectively, in 6–8% yields. Again, the presence of 5-alkyl substituents decreases the yields but to a far lesser extent than for 6-alkyl substituted THIs. In addition, the presence of two substituents exerts a larger detrimental influence that is further exacerbated for the *tert*-butyl THI **14e**. Porphyrins **26a** and **26b** gave complex NMR spectra that were consistent with the presence of diastereomers. The *gem*-dimethyl porphyrins **26c** and **26d** have no chiral centers and are therefore generated as single isomers. However, they are virtually insoluble in chloroform and only dissolved sufficiently well in TFA–$CDCl_3$ to give NMR data. Even then the signals were somewhat broadened. This broadening was temperature dependent and indicates that the *gem*-dimethyl units introduce constraints to the mobility of the exocyclic rings. The *gem*-dimethyl units for **26c** gave a very broad peak at 1.1 ppm, while the CH_2 units of the exocyclic rings afforded broad singlets at 3.5 and 4.8 ppm. The methyl substituents were noted at 3.3 ppm, while the NH resonance was observed at −2.5 ppm, confirming that most of the diatropic character for the crowded porphyrin system has been retained.[32] The carbon-13 NMR spectrum of **26c** in TFA–$CDCl_3$ showed five resonances in the aliphatic region between 14 and 45 ppm, and five aromatic resonances between 115 and 142 nm for the 44 carbon structure, confirming the expected high degree of symmetry for this porphyrin. Similar results were obtained for the tetraethylporphyrin **26d**. The UV–vis spectra for these

compounds also suggest that **26c** and **26d** are more conformationally distorted. The UV–vis spectra for **26a** and **26b** are very similar, showing Soret bands at 418 or 419 nm and a series of Q bands at 518, 550, 591 and 647 nm (phyllo-type spectrum; IV > II > III > I). However, the Soret band shifts to 422 nm for **26c**, while all of the bands are further red shifted to values of 425, 523, 558, 597 and 654 nm for **26d**. Bathochromic shifts of this type are commonly associated with distorted nonplanar porphyrin systems.[33]

3. Conclusions

Porphyrins with six-membered exocyclic rings have previously been synthesized from tetrahydroindoles, and the new results demonstrate that this approach can also be applied to the synthesis of porphyrins with substituted exocyclic rings. Tetrahydroindoles bearing two 5- or 6-alkyl substituents were prepared and used to generate dipyrrolic precursors to dimethylpropanoporphyrins needed in the assignment of vibrational spectra for petroporphyrin samples. The alkyl groups had a deleterious effect on the yields, which increased with the degree of steric crowding, but the methodology still allows the synthesis of diverse porphyrin structures. Cyclotetramerization of 5-alkyl or 5,5-dimethyl tetrahydroindoles gave four examples of substituted tetrapropanoporphyrins. Although this chemistry can only be applied to porphyrins with four six-membered exocyclic rings, these new examples extend this class of crowded porphyrin systems.

4. Experimental

4.1. General

Ethyl acetoacetate, *tert*-butyl acetoacetate, dimedone, 4-methylcyclohexanone, 4-*tert*-butylcyclohexanone, 4,4-dimethyl-2-cyclohexenone, lead tetraacetate, 10% wt % palladium on activated carbon, triethylamine, and *p*-toluenesulfonic acid were purchased from Aldrich or Acros, and were used without further purification. Benzyl acetoacetate was purchased from TCI America. Chromatography was performed using grade III neutral alumina or 70–230 mesh silica gel. Melting points were determined in open capillary tubes on a Mel-Temp apparatus and are uncorrected. UV–vis absorption spectra were run on a Beckmann DU-40 spectrophotometer or a Varian Cary Spectrophotometer. Proton and carbon-13 NMR data were obtained on a Varian Gemini 300 or 400 MHz FT NMR spectrometer. Mass spectral determinations were conducted at the Mass Spectral Laboratory, School of Chemical Sciences, University of Illinois at Urbana-Champaign, and elemental analyses were obtained from Micro-Analysis, Inc., Wilmington, DE 19808 or the School of Chemical Sciences Microanalysis Laboratory at the University of Illinois.

4.2. Synthetic procedures

4.2.1. 4,4-Dimethylcyclohexanone. A solution of 4,4-dimethyl-2-cyclohexenone (25.0 g) in acetone (75 mL) was shaken with 10% palladium–charcoal under a hydrogen atmosphere at room temperature and 30 psi for 90 min. The catalyst was removed by suction filtration and the solvent evaporated under reduced pressure to give the crude cyclic ketone (25.0 g; quantitative) as colorless glacial crystals. ^{1}H NMR ($CDCl_3$): δ 1.09 (6H, s), 1.66 (4H, t, J=6.8 Hz), 2.34 (4H, t, J=6.8 Hz); ^{13}C NMR ($CDCl_3$): δ 27.6, 29.9, 38.1, 39.2, 212.6.

4.2.2. Benzyl 3,5,5-trimethyl-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (14a). A mixture of 4,4-dimethylcyclohexanone (6.35 g), sodium acetate (71.19 g) and glacial acetic acid (475 mL) were placed in a 1 L Erlenmeyer flask and the stirred mixture was heated on a oil bath to 120 °C. A mixture of phenylhydrazone **15a**[17c] (14.92 g) and zinc dust (30 g) was added in small portions to the flask, while maintaining the temperature of reaction between 125–130 °C. After the addition was complete, the reaction mixture was stirred at 110 °C for 1 h. The mixture was then cooled to 70 °C and the solution decanted into ice/water. The resulting precipitate was filtered, washed thoroughly with water and recrystallized from ethanol to give white crystals (5.33 g; 35%), mp 134–135 °C. IR (Nujol mull): ν 3287 (NH str.), 1670 cm^{-1} (C=O str.); ^{1}H NMR ($CDCl_3$): δ 0.97 (6H, s), 1.53 (2H, t, J=6.5 Hz), 2.17 (2H, s), 2.23 (3H, s), 2.52 (2H, t, J=6.5 Hz), 5.29 (2H, s), 7.25–7.43 (5H, m), 8.78 (1H, br s); ^{13}C NMR ($CDCl_3$): δ 10.4, 20.1, 28.1, 30.1, 35.1, 35.7, 65.3, 117.2, 119.4, 126.7, 128.0, 128.5, 131.5, 136.8, 161.6. Anal. Calcd for $C_{19}H_{23}NO_2$: C, 76.73; H, 7.79; N, 4.71. Found: C, 76.62; H, 7.63; N, 4.67.

4.2.3. Ethyl 3,5-dimethyl-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (14d). A mixture of 4-methylcyclohexanone (5.60 g), sodium acetate (12.50 g) and acetic acid (75 mL) were placed in a 500 mL Erlenmeyer flask and heated to 70 °C on a water bath. A solution of phenylhydrazone **15b**[16b] (12.00 g) in acetic acid (75 mL) was added to the mixture while simultaneously adding zinc dust (35 g) in small portions and maintaining the temperature of the reaction between 90–100 °C. Once the addition was complete, the mixture was stirred for 1 h on a boiling water bath. The mixture was cooled to 70 °C and poured into ice/water (2 L). The precipitate was filtered, washed with water and recrystallized from ethanol–water to give the title compound (5.46 g; 49%) as off-white crystals, mp 94.5–95.5 °C. IR (Nujol mull): ν 3310 (NH str.), 1656 cm^{-1} (C=O str.); ^{1}H NMR ($CDCl_3$): δ 1.07 (3H, d, J=6.5 Hz), 1.34 (3H, t, J=7.1 Hz), 1.4 (1H, m), 1.67–2.02 (3H, m), 2.22 (3H, s), 2.45–2.65 (3H, m), 4.29 (2H, q, J=7.1 Hz), 8.59 (1H, br s); ^{13}C NMR ($CDCl_3$): δ 10.2, 14.6, 21.7, 22.5, 29.6, 31.2, 59.5, 117.5, 119.8, 125.7, 132.0, 162.0. Anal. Calcd for $C_{13}H_{19}NO_2$: C, 70.54; H, 8.67; N, 6.33. Found: C, 70.70; H, 8.45; N, 6.14.

4.2.4. Ethyl 5-*tert*-butyl-3-methyl-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (14e). Prepared from 4-*tert*-butylcyclohexanone (7.70 g) and phenylhydrazone **15b**[16b] (11.70 g) by the previous procedure, except that the temperature during the initial addition was maintained at 130 °C. Recrystallization from methanol gave the title pyrrole (5.95 g; 45%) as small white flakes, mp 173–173.5 °C. IR (Nujol mull): ν 3293 (NH str.), 1666 cm^{-1} (C=O str.); ^{1}H NMR ($CDCl_3$): δ 0.96 (9H, s), 1.33 (3H, t), 1.3–1.4 (1H, m), 1.99–2.18 (2H, m), 2.25

(3H, s), 2.48–2.71 (4H, m), 4.31 (2H, q), 8.88 (1H, br s); ^{13}C NMR ($CDCl_3$): δ 10.4, 14.7, 22.5, 23.7, 24.6, 27.6, 32.7, 45.6, 59.7, 117.7, 120.3, 126.3, 132.8, 162.4. Anal. Calcd for $C_{16}H_{25}NO_2$: C, 72.96; H, 9.57; N, 5.32. Found: C, 72.60; H, 9.57; N, 5.16.

4.2.5. Ethyl 3,5,5-trimethyl-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (14b). Prepared by the previous procedure from 4,4-dimethylcyclohexanone (6.30 g) and phenylhydrazone **15b**[16b] (11.70 g). Recrystallization from ethanol gave the tetrahydroindole (5.76 g; 49%) as small white needles, mp 117.5–119.5 °C. Further crystallization from ethanol gave an analytical sample as white crystals, mp 119.5–120 °C. IR (Nujol mull): ν 3303 (NH str.), 1671 cm^{-1} (C=O str.); ^{1}H NMR ($CDCl_3$): δ 0.98 (6H, s), 1.34 (3H, t, J=7.2 Hz), 1.55 (2H, t, J=6.5 Hz), 2.18 (2H, br s), 2.21 (3H, s), 2.56 (2H, t, J=6.5 Hz), 4.29 (2H, q, J=7.2 Hz), 8.70 (1H, br s); ^{13}C NMR ($CDCl_3$): δ 10.3, 14.7, 20.2, 28.2, 30.2, 35.2, 35.9, 59.7, 117.9, 119.6, 126.4, 131.3, 162.3. Anal. Calcd for $C_{14}H_{21}NO_2$: C, 71.45; H, 8.99; N, 5.95. Found: C, 71.43; H, 9.12; N, 5.87.

4.2.6. Methyl 3-ethyl-5,5-dimethyl-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (14c). Prepared by the previous procedure from 4,4-dimethylcyclohexanone (6.30 g) and phenylhydrazone **15c**[16b] (11.70 g). Recrystallization from ethanol gave the title pyrrole (4.21 g; 36%) as small white chunky needles, mp 153–154 °C. Further crystallization from ethanol gave an analytical sample as white crystals, mp 154–154.5 °C. IR (Nujol mull): ν 3310 (NH str.), 1662 cm^{-1} (C=O str.); ^{1}H NMR ($CDCl_3$): δ 0.98 (6H, s), 1.10 (3H, t, J=7.5 Hz), 1.56 (2H, t, J=6.3 Hz), 2.21 (2H, br s), 2.56 (2H, t, J=6.6 Hz), 2.69 (2H, q, J=7.5 Hz), 3.82 (3H, s), 8.60 (1H, br s); ^{13}C NMR ($CDCl_3$): δ 15.1, 18.4, 20.2, 28.1, 30.3, 35.2, 35.8, 51.0, 116.9, 118.9, 131.5, 133.4, 162.4. Anal. Calcd for $C_{14}H_{21}NO_2$: C, 71.45; H, 8.99; N, 5.95. Found: C, 71.42; H, 9.26; N, 5.79.

4.2.7. Benzyl 3,6,6-trimethyl-4-oxo-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (19a). Benzyl acetoacetate (38.44 g) was dissolved in acetic acid (62 mL) and cooled to 10 °C in a ice-salt bath. A solution of sodium nitrite (22.00 g) in water (62 mL) was added dropwise to the stirred mixture, maintaining the reaction temperature below 15 °C throughout. After stirring for 1 h at room temperature, the mixture was extracted with dichloromethane (3×50 mL) and the combined organic solutions washed with water, 10% $NaHCO_3$ solution and water. The solution was dried over Na_2SO_4, filtered and the solvent evaporated. The resulting oxime was obtained as a yellow oil (43.03 g; 97%) and was used without further purification.

Anhydrous sodium acetate (10.0 g) and dimedone (11.20 g) were added to glacial acetic acid (100 mL) and heated on a water bath to 70 °C. A solution of the foregoing oxime (19.20 g) in acetic acid (50 mL) was added dropwise to the stirred mixture, while simultaneously adding zinc dust (30 g) and maintaining the reaction temperature at 85–90 °C. After the addition was complete, the mixture was heated on a boiling water bath for 1 h, cooled to 70 °C and poured into ice/water. The resulting precipitate was filtered, washed thoroughly with water to remove traces of acetic acid and recrystallized from chloroform–hexanes to give the title compound (15.43 g; 54%) as an off-white powder, mp 162.5–162.5 °C; IR (Nujol mull): ν 3206 (NH str.), 1712, 1689 cm^{-1} (2×C=O str); ^{1}H NMR ($CDCl_3$): δ 1.07 (6H, s), 2.32 (2H, s), 2.61 (3H, s), 2.62 (2H, s), 5.32 (2H, s), 7.27–7.47 (5H, m), 9.28 (1H, br s); ^{13}C NMR ($CDCl_3$): δ 11.6, 28.5, 35.2, 37.0, 53.0, 68.2, 119.4, 119.6, 128.1, 128.7, 129.0, 136.0, 144.4, 161.7, 194.6. Anal. Calcd for $C_{19}H_{21}NO_3$: C, 73.28; H, 6.81; N, 4.50. Found: C, 73.13; H, 6.70; N, 4.60.

4.2.8. *tert*-Butyl 3,6,6-trimethyl-4-oxo-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (19b). *tert*-Butyl acetoacetate (31.64 g) was dissolved in acetic acid (62 mL) and cooled to 10 °C in a ice-salt bath. A solution of sodium nitrite (22.0 g) in water (62 mL) was added dropwise to the stirred mixture, while maintaining the temperature below 15 °C. After the addition was complete, the resulting oxime solution was allowed to stir at room temperature for 1 h.

A solution of sodium acetate (20.00 g) and dimedone (28.04 g) were added to glacial acetic acid (200 mL) and heated on a water bath to 60 °C. The crude oxime solution was added dropwise to the stirred mixture, while simultaneously adding zinc dust (40 g) and maintaining the reaction at 80 °C. After the addition was complete, the mixture was heated on a boiling water bath for 1 h, cooled to 70 °C and poured into ice/water. The resulting precipitate was filtered, washed thoroughly with water to remove traces of acetic acid and recrystallized from methanol to give the required tetrahydroindole (10.35 g; 19%) as a white powder, mp 207–209 °C. IR (Nujol mull): ν 3292 (NH str.), 1655 cm^{-1} (C=O str.); ^{1}H NMR ($CDCl_3$): δ 1.10 (6H, s), 1.59 (9H, s), 2.34 (2H, s), 2.58 (3H, s), 2.67 (2H, s), 9.51 (1H, br s); ^{13}C NMR ($CDCl_3$): δ 11.5, 28.5, 35.2, 37.1, 53.0, 81.5, 118.9, 120.8, 127.4, 143.9, 161.7, 194.8. Anal. Calcd for $C_{16}H_{23}NO_3$: C, 69.27; H, 8.37; N, 5.05. Found: C, 68.82; H, 8.09; N, 4.98.

4.2.9. Benzyl 3,6,6-trimethyl-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (18a). Sodium borohydride (5.72 g) was dissolved in anhydrous THF (130 mL) and placed in a 500 mL 3-neck flask equipped with a nitrogen flow inlet, addition funnel, a condenser and a thermometer. 4-Oxotetrahydroindole **19a** (11.85 g) in anhydrous THF (230 mL) was added and the mixture was cooled to −5 °C in an ice/salt bath. The system was purged with nitrogen and the slow addition of boron trifluoride etherate (26.7 mL) was initiated, while maintaining a temperature below 0 °C. After the addition was complete, the mixture was allowed to stir at room temperature for 2 h. The mixture was poured into ice/water and the resulting precipitate filtered off, washed well with warm water and recrystallized from dichloromethane–hexane to give the tetrahydroindole (7.63 g; 67%) as a white powder, mp 142.5–143 °C. IR (Nujol mull): ν 3295 (NH str.), 1664 cm^{-1} (C=O str.); ^{1}H NMR ($CDCl_3$): δ 0.97 (6H, s), 1.52 (2H, t, J=6.5 Hz), 2.17 (2H, s), 2.23 (3H, s), 2.50 (2H, t, J=6.5 Hz), 5.29 (2H, s), 7.23–7.45 (5H, m), 8.90 (1H, br s); ^{13}C NMR ($CDCl_3$): δ 10.4, 20.1, 28.1, 30.1, 35.1, 35.7, 65.3, 117.3, 119.4, 126.7, 128.0, 128.5, 131.7, 136.8, 161.7. Anal. Calcd for $C_{19}H_{23}NO_2$: C, 76.72; H, 7.81; N, 4.71. Found: C, 76.46; H, 7.65; N, 4.76.

4.2.10. *tert*-Butyl 3,6,6-trimethyl-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (18b). Using the procedure described above, **19b** (7.45 g) afforded the title compound (4.95 g; 70%) after recrystallization from methanol as white crystals, mp 150.5–151.5 °C. IR (Nujol mull): ν 3304 (NH str.), 1654 cm^{-1} (C=O str.); ^1H NMR ($CDCl_3$): δ 0.98 (6H, s), 1.51 (2H, t, $J=6.4$ Hz), 1.56 (9H, s), 2.21 (3H, s), 2.33 (2H, s), 2.38 (2H, t, $J=6.4$ Hz), 8.67 (1H, br s); ^{13}C NMR ($CDCl_3$): δ 10.5, 18.5, 28.0, 28.6, 30.5, 36.3, 36.8, 79.9, 117.9, 118.7, 124.8, 131.5, 161.6. Anal. Calcd for $C_{16}H_{25}NO_2$: C, 72.95; H, 9.59; N, 5.32. Found: C, 72.82; H, 9.29; N, 5.34.

4.2.11. *tert*-Butyl 7-acetoxy-3,6,6,-trimethyl-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (20b). Lead tetraacetate (5.31 g) was added in one portion to a stirred solution of **18b** (5.31 g) in acetic acid (57 mL) and acetic anhydride (3 mL). The mixture was stirred for 2.5 h at room temperature, extracted with dichloromethane and washed with water, 5% sodium bicarbonate solution and water. The organic layer was dried over sodium sulfate and evaporated under reduced pressure to give the desired acetoxy compound as a yellow oil (quantitative). Crystallization from hexane gave the 7-acetoxytetrahydroindole (2.36 g; 64.5%) as white crystals, mp 137.5–138.5 °C. IR (Nujol mull): ν 3300 (NH str.), 1715 (acetoxy C=O str.), 1699 cm^{-1} (pyrrole C=O str.); ^1H NMR ($CDCl_3$): δ 0.93 (3H, s), 1.06 (3H, s), 1.55 (9H, s), 1.43–1.62 (1H, m) 1.83 (1H, m), 2.06 (3H, s), 2.20 (3H, s), 2.25–2.53 (2H, m), 5.21 (1H, s), 8.91 (1H, br s); ^{13}C NMR ($CDCl_3$): δ 10.2, 18.2, 21.1, 23.9, 25.6, 28.5, 31.9, 33.9, 72.5, 80.4, 120.6, 120.7, 123.6, 128.6, 161.1, 172.3. Anal. Calcd for $C_{18}H_{27}NO_4$: C, 67.26; H, 8.47; N, 4.36. Found: C, 67.85; H, 8.70; N, 4.37.

4.2.12. Benzyl 7-acetoxy-3,5,5-trimethyl-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (17a). Prepared by the previous procedure from **14a** (3.00 g). The 7-acetoxytetrahydroindole was recrystallized from hexane to give off-white crystals (2.83 g; 79%), mp 88.5–90.5 °C. IR (Nujol mull): ν 3302 (NH str.), 1729 (acetoxy C=O str.), 1686 cm^{-1} (pyrrole C=O str.); ^1H NMR ($CDCl_3$): δ 1.01 (3H, s), 1.09 (3H, s), 1.71–1.95 (2H, m), 2.08 (3H, s), 2.12–2.35 (2H, AB quartet), 2.22 (3H, s), 5.24–5.38 (2H, AB quartet), 5.69 (1H, t, $J=5.9$ Hz), 7.26–7.46 (5H, m), 9.10 (1H, br s); ^{13}C NMR ($CDCl_3$): δ 10.3, 21.3, 28.4, 29.0, 31.6, 35.1, 41.8, 65.6, 66.2, 119.4, 122.1, 125.4, 128.0, 128.5, 136.5, 161.3, 172.4. Anal. Calcd for $C_{21}H_{25}NO_4$: C, 70.96; H, 7.09; N, 3.94. Found: C, 71.15; H, 7.25; N, 3.97.

4.2.13. Benzyl 7-acetoxy-3,6,6-trimethyl-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (20a). Prepared from **19a** (1.00 g) by the procedure detailed for **20b**. The 7-acetoxytetrahydroindole was obtained as a pale yellow oil (1.16 g; 97%) and was used without further purification. An analytical sample was obtained by recrystallization from hexanes as off-white crystals, mp 75.5–76.5 °C. IR (Nujol mull): ν 3452 (NH str.), 1710 (acetoxy C=O str.), 1667 cm^{-1} (pyrrole C=O str.); ^1H NMR ($CDCl_3$): δ 0.92 (3H, s), 1.06 (3H, s), 1.44–1.56 (1H, m), 1.77–1.91 (1H, m), 2.05 (3H, s), 2.22 (3H, s), 2.38–2.65 (2H, m), 5.20 (1H, s), 5.23–5.38 (2H, AB quartet), 7.28–7.46 (5H, m), 8.89 (1H, br s); ^{13}C NMR ($CDCl_3$): δ 10.3, 18.2, 21.1, 23.9, 25.6, 26.0, 31.8, 33.9, 65.5, 72.4, 119.1, 120.8, 125.0, 128.0, 128.1, 128.5, 129.6, 136.6, 161.3, 172.4. Anal. Calcd for $C_{21}H_{25}NO_4$: C, 70.96; H, 7.09; N, 3.94. Found: C, 70.99; H, 7.02; N, 4.01.

4.2.14. Ethyl 7-acetoxy-3,5-dimethyl-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (17d). Lead tetraacetate (4.21 g) was added in one portion to a stirred solution of ethyl 3,5-dimethyl-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (**14d**; 2.00 g) in acetic acid (18 mL) and acetic anhydride (2 mL). The mixture was stirred for 3 h at room temperature, extracted with dichloromethane and washed with water, 5% sodium bicarbonate solution and water. The acetoxy derivative was obtained as a pale brown oil (2.42 g; 96%) and used without further purification. Crystallization from hexanes gave an analytical sample as white crystals, mp 120–121 °C; IR (Nujol mull): ν 3313 (NH str.), 1729 (acetoxy C=O str.), 1662 cm^{-1} (pyrrole C=O str.); ^1H NMR ($CDCl_3$): δ 1.12 (3H, d, $J=6.7$ Hz) 1.34 (3H, t, $J=7.2$ Hz), 1.61 (1H, m), 1.81–2.17 (3H, m), 2.05 (3H, s), 2.23 (3H, s), 2.60 (1H, dd), 4.30 (2H, m), 5.65 (1H, m) 9.06 (1H, br s); ^{13}C NMR ($CDCl_3$): δ 10.0, 14.5, 21.2, 21.4, 21.6, 25.6, 29.4, 29.6, 37.3, 59.7, 65.4, 67.7, 119.4, 119.5, 122.5, 122.8, 124.2, 128.5, 129.5, 161.4, 172.3. Anal. Calcd for $C_{13}H_{19}NO_4$: C, 64.50; H, 7.56; N, 5.01. Found: C, 64.46; H, 7.56; N, 5.03.

4.2.15. Ethyl 7-acetoxy-5-*tert*-butyl-3-methyl-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (17e). Prepared from **14e** (1.00 g) by the procedure detailed above. The acetoxy derivative was obtained as a yellow oil (1.08 g; 88%), which was used without further purification. An analytical sample was obtained as white crystals by recrystallization from hexane, mp 121–123 °C. IR (Nujol mull): ν 3279 (NH str.), 1734 (acetoxy C=O str.), 1674 cm^{-1} (pyrrole C=O str.); ^1H NMR ($CDCl_3$): δ 0.98 (9H, s), 1.35 (3H, t, $J=7.1$ Hz), 1.62 (1H, m), 1.78 (1H, m), 1.92–2.21 (2H, m), 2.05 (3H, s), 2.24 (3H, s), 2.57 (1H, dd), 4.30 (2H, m), 5.67 (1H, m), 9.06 (1H, br s); ^{13}C NMR ($CDCl_3$): δ 10.0, 14.5, 21.2, 22.3, 27.4, 30.5, 33.4, 40.3, 59.8, 65.5, 119.6, 123.0, 124.7, 128.5, 161.5, 172.4. Anal. Calcd for $C_{18}H_{27}NO_4$: C, 67.25; H, 8.48; N, 4.36. Found: C, 66.96; H, 8.18; N, 4.36.

4.2.16. Ethyl 7-acetoxy-3,5,5-trimethyl-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (17b). Following the foregoing procedure, **14b** (2.00 g) and lead tetraacetate (4.00 g) gave the title compound as a pale yellow oil (2.48 g, quantitative). An analytical sample was obtained by slow crystallization from hexane as white crystals, mp 81–82 °C. IR (Nujol mull): ν 3310 (NH str.), 1734 (acetoxy C=O str.), 1662 cm^{-1} (pyrrole-C=O str.); ^1H NMR ($CDCl_3$): δ 1.01 (3H, s), 1.09 (3H, s), 1.34 (3H, t, $J=6.9$ Hz), 1.73–1.80 (1H, dd, $J=13.8$, 5.7 Hz), 1.88–1.94 (1H, dd, $J=13.6$, 6.1 Hz), 2.09 (3H, s), 2.16–2.33 (2H, AB quartet, $J=15.3$ Hz), 2.21 (3H, s), 4.22–4.37 (2H, m), 5.68 (1H, t, $J=5.8$ Hz), 9.01 (1H, br s); ^{13}C NMR ($CDCl_3$): δ 10.2, 14.6, 21.4, 28.6, 29.0, 31.7, 35.2, 41.9, 60.0, 66.4, 120.0, 122.2, 125.3, 128.4, 161.9, 172.9. Anal. Calcd for $C_{16}H_{23}NO_4$: C, 65.51; H, 7.90; N, 4.77. Found: C, 65.58; H, 7.85; N, 4.56.

4.2.17. Methyl 7-acetoxy-3-ethyl-5,5-dimethyl-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (17c). Prepared by

the foregoing procedure from **14c** (2.00 g) and lead tetraacetate (4.00 g). Recrystallization from hexane gave the acetoxy derivative (1.93 g; 77%) as white crystals, mp 93–94 °C. IR (Nujol mull): ν 3300 (NH str.), 1731 (acetoxy C=O str.), 1676 cm^{-1} (pyrrole-C=O str.); ^1H NMR ($CDCl_3$): δ 1.01 (3H, s), 1.09 (6H, overlapping triplet and singlet), 1.74–1.81 (1H, dd, $J=13.9$, 5.5 Hz), 1.88–1.95 (1H, dd, $J=13.8$, 6.3 Hz), 2.09 (3H, s), 2.19–2.36 (2H, AB quartet, $J=15.4$ Hz), 2.66–2.74 (2H, m), 3.82 (3H, s), 5.68 (1H, t, $J=5.8$ Hz), 9.03 (1H, br s); ^{13}C NMR ($CDCl_3$): δ 15.1, 18.2, 21.4, 28.5, 29.0, 31.7, 35.2, 41.8, 51.1, 66.5, 119.0, 121.6, 128.5, 132.2, 161.9, 173.0. Anal. Calcd for $C_{16}H_{23}NO_4$: C, 65.51; H, 7.90; N, 4.77. Found: C, 65.41; H, 7.99; N, 4.70.

4.2.18. Benzyl 7-(5-benzyloxycarbonyl-3-ethyl-4-methyl-2-pyrrolyl)-3,6,6-trimethyl-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (22c). Benzyl 4-ethyl-3-methylpyrrole-2-carboxylate[34] (**21a**) (0.81 g) and *p*-toluenesulfonic acid (36 mg) were dissolved in acetic acid (14 mL) and placed in a 50 mL Erlenmeyer flask. A solution of benzyl 7-acetoxy-3,6,6-trimethyl-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (**20a**) (1.18 g) in acetic acid (20 mL) was slowly added over 30 min to the stirred reactants and the mixture was then allowed to stir for a further 3 h at room temperature. The solution was extracted with dichloromethane and washed with water, saturated sodium bicarbonate solution and water, and dried over sodium sulfate. The solvent was removed under reduced pressure and the residue chromatographed on silica, eluting with dichloromethane. Recrystallization with ethanol gave the title dipyrrole (0.71 g; 40%) as a white powder, mp 136.5–137.5 °C. IR (Nujol mull): ν 3282 (NH str.), 1678 cm^{-1} (C=O str.) cm; ^1H NMR ($CDCl_3$): δ 0.90 (3H, s), 1.03 (3H, s), 1.14 (3H, t, $J=7$ Hz), 1.69 (2H, m), 2.25 (3H, s), 2.31 (3H, s), 2.48 (4H, m), 3.85 (1H, s), 5.11–5.29 (4H, two overlapping AB quartets), 7.25–7.45 (10H, m), 8.75 (2H, br); ^{13}C NMR ($CDCl_3$): δ 10.7, 11.1, 15.4, 17.7, 18.4, 22.7, 29.1, 35.4, 37.1, 42.8, 65.5, 65.6, 118.3, 119.4, 125.6, 126.4, 126.6, 127.9, 128.5, 130.7, 132.7, 136.6, 136.6, 161.6. Anal. Calcd for $C_{34}H_{38}N_2O_4 \cdot {}^1/_2H_2O$: C, 74.56; N, 7.18; N, 5.11. Found: C, 74.39; H, 7.04; N, 5.02.

4.2.19. Benzyl 7-(5-benzyloxycarbonyl-3-ethyl-4-methyl-2-pyrrolyl)-3,5,5-trimethyl-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (22a). Prepared by the procedure detailed above from **17a** (0.80 g) and **21a** (0.52 g). The residue was chromatographed on silica eluting with dichloromethane, to give the title dipyrrole as a yellow oil (0.44 g; 38%) that solidified on standing. Attempts to recrystallize this compound were unsuccessful. IR (Nujol mull): ν 3264 (NH str.), 1674 cm^{-1} (C=O str.); ^1H NMR ($CDCl_3$): δ 1.00 (3H, s), 1.10 (3H, s), 1.11 (3H, t), 1.70 (2H, m), 2.21 (3H, s), 2.27 (3H, s), 2.22–2.34 (2H, m), 2.46 (2H, m), 4.06 (1H, t), 5.01–5.34 (4H, two overlapping AB quartets), 7.24–7.45 (10H, m), 9.59 (2H, br s); ^{13}C NMR ($CDCl_3$): δ 10.8, 16.3, 17.4, 24.8, 30.4, 31.2, 31.8, 35.3, 45.5, 65.5, 117.7, 118.4, 120.3, 124.3, 125.9, 126.5, 127.8, 127.9, 128.1, 128.5, 131.6, 134.5, 136.5, 161.8, 162.0.

4.2.20. *tert*-Butyl 7-(5-benzyloxycarbonyl-4-methyl-2-pyrrolyl)-3,6,6-trimethyl-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (22d). Prepared from **20b** (2.00 g) and benzyl 3-methylpyrrole-2-carboxylate[35] (**21b**; 1.34 g) by the method described previously for **22c**. The residue was chromatographed on silica eluting with 10% ethyl acetate/dichloromethane and recrystallized from ethanol to give the title dipyrrole as a light yellow solid (1.36 g; 46%), mp 157.5–159.5 °C. IR (Nujol mull): ν 3283 (NH str.), 1679, 1655 cm^{-1} (C=O str.); ^1H NMR ($CDCl_3$): δ 0.79 (3H, s), 1.02 (3H, s), 1.48–1.75 (2H, m), 1.52 (9H, s), 2.22 (3H, s), 2.29 (3H, s), 2.45 (2H, m), 3.65 (1H, s), 5.23 (2H, AB quartet), 5.80 (1H, m), 7.26–7.44 (5H, m), 8.87 (1H, br s), 9.09 (1H, br s); ^{13}C NMR ($CDCl_3$): δ 10.5, 13.2, 18.4, 29.3, 28.5, 28.6, 34.7, 36.2, 45.4, 65.7, 80.2, 113.5, 118.2, 119.1, 120.0, 124.1, 128.0, 128.4, 128.5, 131.0, 135.3, 136.5, 161.6. Anal. Calcd for $C_{29}H_{36}N_2O_4$: C, 73.08; H, 7.61; N, 5.88. Found: C, 72.70; H, 7.45; N, 5.68.

4.2.21. Benzyl 7-(5-benzyloxycarbonyl-3-bromo-4-methyl-2-pyrrolyl)-3,5,5-trimethyl-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (22b). Benzyl 4-bromo-3-methylpyrrole-2-carboxylate[17a] (**21c**; 0.42 g) and zinc chloride (0.103 g) were dissolved in dichloromethane (20 mL) and cooled to below 0 °C. Benzyl 7-acetoxy-3,5,5 trimethyl-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (**17a**; 0.51 g) was dissolved in dichloromethane (20 mL) and placed in an addition funnel. The acetoxy mixture was slowly added over 40 min while maintaining the temperature below 0 °C. The mixture was allowed to stir at 0 °C for a further 2 h and then at room temperature overnight. The solution was washed successively with 3 M hydrochloric acid, water, 5% sodium bicarbonate solution and water, and dried over sodium sulfate. The solvent was removed under reduced pressure. The residue was then purified by flash chromatography, eluting with 8.5% ethyl acetate/hexane and recrystallized from ethanol to give the title dipyrrole as a light pink solid (0.325 g; 39%), mp 115–118 °C. IR (Nujol mull): ν 3256 (NH str.), 1676 cm^{-1} (C=O str.); ^1H NMR ($CDCl_3$): δ 1.04 (3H, s), 1.13 (3H, s), 1.59 (1H, t, $J=$ 12.4 Hz), 1.88 (1H, dd), 2.18 (3H, s), 2.25 (3H, s), 2.31 (2H, m), 4.26 (1H, dd), 4.86–5.39 (4H, two overlapping AB quartets), 7.27–7.48 (10H, m), 10.63 (2H, br); ^{13}C NMR ($CDCl_3$): δ 10.9, 12.2, 24.7, 31.1, 31.4, 31.8, 35.6, 43.6, 65.9, 66.3, 100.5, 117.7, 118.5, 120.3, 125.3, 126.5, 127.7, 127.8, 128.1, 128.4, 128.5, 131.2, 135.9, 136.5, 136.8, 161.6, 162.6. Anal. Calcd for $C_{32}H_{33}N_2O_4Br \cdot {}^1/_4H_2O$: C, 64.70; H, 5.68; N, 4.72. Found: C, 64.70; H, 5.56; N, 4.72.

4.2.22. 7-(5-Carboxy-3-ethyl-4-methyl-2-pyrrolyl)-3,6,6-trimethyl-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylic acid (23c). Dipyrrole **22c** (1.00 g) was dissolved in acetone (150 mL) and placed in a hydrogenation vessel. Triethylamine (20 drops) was added to the solution, the vessel purged with nitrogen and 10% palladium–charcoal (200 mg) was added. The mixture was shaken under an atmosphere of hydrogen (40 psi) at room temperature overnight. The solution was filtered to remove the catalyst and the solvent removed under reduced pressure. The residue was taken up in a 5% aqueous ammonia solution and cooled to 5 °C. Glacial acetic acid was added to neutralize the solution, while maintaining temperature at 0–5 °C. The resulting precipitate was filtered, washed with water to remove all traces of acid and dried in vacuo overnight. The dicarboxylic acid (548 mg; 82%) was obtained as a pale purple powder, mp 173 °C dec. IR (Nujol mull): ν 3306 (NH str.), 1655 cm^{-1} (C=O str.); ^1H NMR ($CDCl_3$): δ 0.86

(3H, s), 1.02 (3H, s), 1.11 (3H, t, $J=6.8$ Hz), 1.68 (2H, m), 2.26 (3H, s), 2.38 (3H, s), 2.48 (4H, m), 3.85 (1H, s), 8.24 (2H, br). Anal. Calcd for $C_{20}H_{26}N_2O_4$: C, 67.02; H, 7.31; N, 7.81. Found: C, 67.35; H, 7.64; N, 7.78.

4.2.23. 7-(5-Carboxy-3-ethyl-4-methyl-2-pyrrolyl)-3,5,5-trimethyl-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylic acid (23a). Prepared from **22a** (435 mg) by the procedure detailed above. The dicarboxylic acid powder (256 mg; 88%) was obtained as a pale greyish-purple powder, mp 208–212 °C dec. IR (Nujol mull): ν 3307 (NH str.), 1645 cm^{-1} (C=O str.); ^1H NMR (d_6-DMSO): δ 0.88 (6H, s), 1.04 (3H, t), 1.64 (2H, m), 2.09 (2H, m), 2.15 (3H, s), 2.18 (3H, s), 2.23 (2H, m), 4.05 (1H, m), 10.38 (1H, br s), 10.74 (1H, br s), 11.77 (2H, br). Anal. Calcd for $C_{20}H_{26}N_2O_4$: C, 67.02; H, 7.31; N, 7.81. Found: C, 67.41; H, 7.40; N, 7.31.

4.2.24. *tert*-Butyl 7-(5-carboxy-4-methyl-2-pyrrolyl)-3,6,6-trimethyl-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (23d). Prepared from **22d** (500 mg) by the procedure described for **23c** but using ethanol as the solvent. The carboxylic acid was obtained as a white solid (344 mg; 85%), mp 158–163 °C. IR (Nujol mull): ν 3218 (NH str.), 1672 cm^{-1} (C=O str.); ^1H NMR (d_6-DMSO): δ 0.72 (3H, s), 0.92 (3H, s), 1.09–1.17 (1H, m), 1.58–1.75 (1H, m), 1.48 (9H, s), 2.14 (3H, s), 2.15 (3H, s), 2.20–2.48 (2H, m), 3.67 (1H, s), 10.61 (1H, br s), 11.10 (1H, br s), 11.85 (1H, br). Anal. Calcd for $C_{22}H_{30}N_2O_4$: C, 68.37; H, 7.82; N, 7.25. Found: C, 68.39; H, 7.80; N, 7.26.

4.2.25. 7-(5-Carboxy-4-methyl-2-pyrrolyl)-3,5,5-trimethyl-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylic acid (23b). Prepared from bromo-dipyrrole **22b** (500 mg) by the procedure detailed for **23c**, except that methanol was used as the solvent. The title carboxylic acid (227 mg; 81%) was obtained as a light tan solid, mp 198–202 °C dec. IR (Nujol mull): ν 3305 (NH str.), 1646 cm^{-1} (C=O str.); ^1H NMR (d_6-DMSO): δ 0.86 (3H, s), 1.05 (3H, s), 1.71 (2H, m), 2.12 (3H, s), 2.19 (3H, s), 2.15 (2H, m), 3.91 (1H, m), 5.67 (1H, s), 10.36 (1H, br s), 11.26 (1H, br s). Anal. Calcd for $C_{18}H_{22}N_2O_4$: C, 65.44; H, 6.71; N, 8.48. Found: C, 65.60; H, 6.95; N, 8.06.

4.2.26. 7,13,17-Triethyl-2,8,12,18-tetramethyl-3,5-(2,2-dimethylpropano)porphyrin (13a). *Method A*. A solution of *p*-toluenesulfonic acid (80 mg) in methanol (1.4 mL) was added in one portion to a stirred mixture of **24**[17c] (80 mg) and **23a** (100 mg) in dichloromethane (10 mL). The mixture was allowed to stir in the dark overnight. A saturated solution of zinc acetate in methanol (1.6 mL) was added and the mixture was again allowed to stir in the dark overnight. The solution was washed with water, 5% HCl, 5% aqueous ammonia and water, and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure and the residue chromatographed on alumina eluting with dichloromethane. The red fractions were combined, evaporated and rechromatographed on alumina eluting with toluene. The fractions containing porphyrin were evaporated and recrystallized from dichloromethane–methanol to give dark maroon crystals (20 mg; 14%), mp >300 °C; UV–vis (1% Et_3N–$CHCl_3$): λ_{max} ($\log_{10}\varepsilon$) 405 (5.22), 504 (4.20), 538 (3.83), 571 (3.88), 625 nm (3.61); UV–vis (1% TFA–$CHCl_3$): λ_{max} ($\log_{10}\varepsilon$) 391 (sh, 4.85), 412 (5.53), 556 (4.28), 599 nm (3.95); ^1H NMR ($CDCl_3$): δ −3.28 (2H, br s), 1.45 (6H, s), 1.79–1.88 (9H, three overlapping triplets), 3.53 (3H, s), 3.58 (3H, s), 3.64 (3H, s), 3.66 (5H, s), 4.01 (2H, q), 4.05–4.12 (4H, two overlapping quartets), 4.91 (2H, s), 9.88 (1H, s), 10.02 (1H, s), 10.07 (1H, s); ^1H NMR (TFA–$CDCl_3$): δ −3.76 (1H, br s), −3.56 (1H, s), −3.54 (1H, br s), −2.96 (1H, br s), 1.29 (6H, s), 1.69 (3H, t, $J=7.8$ Hz), 1.74 (6H, t, $J=7.6$ Hz), 3.48 (3H, s), 3.52 (3H, s), 3.59 (3H, s), 3.60 (3H, s), 3.75 (2H, s), 3.89 (2H, q, $J=7.6$ Hz), 4.02–4.11 (4H, two overlapping quartets), 5.05 (2H, s), 10.35 (1H, s), 10.43 (1H, s), 10.51 (1H, s); ^{13}C NMR (TFA–$CDCl_3$): δ 11.7, 11.9, 15.9, 16.4, 16.5, 20.2 (2), 21.6, 28.6, 37.3, 37.8, 44.8, 96.2, 97.6, 99.4, 118.7, 135.5, 137.7, 138.2, 138.7, 140.3, 140.4, 140.9, 141.0, 141.2, 142.5, 142.9, 143.3, 143.4, 144.5; EIMS: *m/z* (rel int.) 518 (100, M^+), 503 (8, $[M-CH_3]^+$); HRMS: Calcd for $C_{35}H_{42}N_4$: 518.3412. Found: 518.3404. Anal. Calcd for $C_{35}H_{42}N_4\cdot{}^1/_4H_2O$: C, 80.34; H, 8.19; N, 10.71. Found: C, 80.14; H, 8.26; N, 10.48. *Method B*. A solution of **24** (80 mg) and **23a** (100 mg) in methanol (2 mL) and dichloromethane (40 mL) was added dropwise to a stirred solution of *p*-toluenesulfonic acid (80 mg) in methanol (2 mL) and dichloromethane (40 mL) under nitrogen over a period of 1 h. The resulting mixture was allowed to stir in the dark open to the air overnight, and then further reacted under the conditions described for method A. Recrystallization from chloroform–methanol gave the title porphyrin (53 mg, 37%) as dark maroon crystals, mp > 300 °C.

4.2.27. 7,13,17-Triethyl-2,8,12,18-tetramethyl-3,5-(3,3-dimethylpropano)porphyrin (13c). Prepared from **24** (120 mg) and **23c** (150 mg) in 30 mL of CH_2Cl_2 and 3 mL of methanol using method A as detailed above. Recrystallization from dichloromethane–methanol gave the porphyrin (16 mg; 7%) as small lustrous purple needles, mp 256–257 °C; UV–vis (1% Et_3N–$CHCl_3$): λ_{max} ($\log_{10}\varepsilon$) 409 (5.17), 509 (4.15), 543 (3.84), 578 (3.85), 628 nm (3.66); UV–vis (1% TFA–$CHCl_3$): λ_{max} ($\log_{10}\varepsilon$) 397 (sh, 4.81), 418 (5.35), 564 (4.18), 610 nm (4.13); ^1H NMR ($CDCl_3$): δ −2.74 (2H, br s), 1.24 (3H, t, $J=7.6$ Hz), 1.80–1.86 (6H, two overlapping triplets), 2.45 (2H, t, $J=6.4$ Hz), 2.51 (6H, s), 3.54 (3H, s), 3.57 (3H, s), 3.58 (3H, s), 3.59 (3H, s), 3.88 (2H, t, $J=6$ Hz), 3.94–4.04 (4H, two overlapping quartets), 4.30 (2H, q, $J=7.6$ Hz), 9.74 (1H, s), 9.91 (1H, s), 10.06 (1H, s); ^1H NMR (TFA–$CDCl_3$): δ −3.39 (1H, s), −2.99 (1H, s), −2.43 (1H, s), −1.90 (1H, s), 0.53 (3H, t, $J=7.6$ Hz), 1.66 (3H, t, $J=7.8$ Hz), 1.70 (3H, t, $J=7.8$ Hz), 2.20 (2H, br t), 2.54 (6H, s), 3.14 (3H, s), 3.35 (3H, s), 3.45 (3H, s), 3.47 (3H, s), 3.77 (2H, br), 3.87–3.99 (6H, m), 10.01 (1H, s), 10.06 (1H, s), 10.29 (1H, s); ^{13}C NMR (TFA–$CDCl_3$): δ 11.5, 11.6, 11.8 (2), 14.8, 16.2, 20.0, 20.1, 20.8, 22.8, 31.5, 39.1, 46.1, 95.4, 95.7, 102.3, 128.5, 132.6, 133.7, 135.2, 137.8, 138.9, 139.5, 140.6, 140.8, 141.3, 142.6, 142.8, 143.6, 143.8, 145.1; EIMS: *m/z* (rel int.) 518 (100, M^+), 503 (64, $[M-CH_3]^+$); HRMS: Calcd for $C_{35}H_{42}N_4$: 518.3412. Found: 518.3393. Anal. Calcd for $C_{35}H_{42}N_4\cdot{}^1/_2H_2O$: C, 79.65; H, 8.21; N, 10.62. Found: C, 79.64; H, 8.28; N, 10.47.

4.2.28. 13,17-Diethyl-2,8,12,18-tetramethyl-3,5-(2,2-dimethylpropano)porphyrin (13b). The title porphyrin was prepared using method A, as detailed for porphyrin **13a**, from **24** (87 mg) and **23b** (100 mg). The residue was

chromatographed on silica eluting with dichloromethane. The red fractions were combined, evaporated and crystallized from dichloromethane–methanol to give the cycloalkanoporphyrin (24 mg; 16%) as deep purple crystals, mp >300 °C. Under the conditions described in method B for **13a**, the yield was raised to 39%. UV–vis (1% Et_3N–$CHCl_3$): λ_{max} ($\log_{10}\varepsilon$) 405 (5.25), 502 (4.19), 537 (3.80), 571 (3.86), 625 nm (3.53); UV–vis (1% TFA–$CHCl_3$): λ_{max} ($\log_{10}\varepsilon$) 390 (sh, 4.86), 411 (5.56), 553 (4.32), 594 nm (3.72); ^{1}H NMR ($CDCl_3$): δ −3.37 (2H, br s), 1.52 (6H, s), 1.85–1.89 (6H, two overlapping triplets), 3.55 (3H, s), 3.60 (3H, s), 3.62 (3H, s), 3.69 (2H, s), 3.77 (3H, s), 4.02–4.11 (4H, two overlapping quartets), 4.80 (2H, s), 9.26 (1H, s), 9.94 (1H, s), 9.99 (1H, s), 10.06 (1H, s); ^{1}H NMR (TFA–$CDCl_3$): δ −3.98 (1H, br s), −3.92 (1H, br s), −3.88 (1H, br s), −3.70 (1H, br s), 1.55 (6H, s), 1.70–1.77 (6H, two overlapping triplets), 3.61 (3H, s), 3.65 (3H, s), 3.66 (3H, s), 3.74 (3H, s), 3.81 (2H, s), 4.10–4.16 (4H, two overlapping quartets), 4.94 (2H, s), 9.46 (1H, s), 10.54 (1H, s), 10.58 (1H, s), 10.68 (1H, s); ^{13}C NMR (TFA–$CDCl_3$): δ 11.7, 11.8, 14.0, 16.4, 20.2, 20.3, 28.6, 36.3, 38.0, 44.7, 97.2, 98.2, 100.1, 118.1, 125.7, 136.2, 138.8, 139.4, 140.2, 140.9, 141.2, 141.8, 141.9, 142.1, 142.6, 142.9, 143.0, 143.6, 144.4, 145.1; EIMS: *m*/*z* (rel int.) 490 (100, M^+), 475 (13, $[M-CH_3]^+$); HRMS: Calcd for $C_{33}H_{38}N_4$: 490.3099. Found: 490.3108. Anal. Calcd for $C_{33}H_{38}N_4\cdot{}^1/_8CHCl_3$: C, 78.69; H, 7.60; N, 11.08. Found: C, 78.67; H, 7.78; N, 10.92.

4.2.29. 13,17-Diethyl-2,8,12,18-tetramethyl-3,5-(3,3-dimethylpropano)porphyrin (13d). Dipyrrole *tert*-butyl ester **23d** (250 mg) was dissolved in trifluoroacetic acid (9.5 mL) and allowed to stir for 15 min. The mixture was diluted with dichloromethane (40 mL) and washed with water (95 mL), 5% sodium bicarbonate solution (95 mL) and water (95 mL), and dried over anhydrous potassium carbonate. The solvent was removed under reduced pressure keeping the temperature below 40 °C. The residue was dissolved in dichloromethane (25 mL) and diformyldipyrrylmethane **24** (185 mg) was added. A solution of *p*-toluenesulfonic acid (185 mg) in methanol (3.5 mL) was added in one portion and the mixture allowed to stir in the dark overnight. A saturated solution of zinc acetate in methanol (3.6 mL) was added and the mixture was again allowed to stir in the dark overnight. The solution was washed with water, 5% HCl, 5% aqueous ammonia and water, and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure and the residue was chromatographed on alumina eluting with dichloromethane. The fractions containing porphyrin were evaporated and recrystallized from dichloromethane–methanol to give deep purple crystals (32 mg; 10%), mp 281–282 °C. Alternatively, following deprotection of the *tert*-butyl ester **23d**, the conditions described in method B for **13a** gave porphyrin **13d** in 28% yield. UV–vis (1% Et_3N–$CHCl_3$): λ_{max} ($\log_{10}\varepsilon$) 405 (5.05), 504 (4.02), 539 (3.69), 573 (3.72), 625 nm (3.48); UV–vis (1% TFA–$CHCl_3$): λ_{max} ($\log_{10}\varepsilon$) 392 (sh, 4.70), 412 (5.33), 555 (4.13), 601 nm (3.90); ^{1}H NMR ($CDCl_3$): δ −3.28 (1H, br s), −3.15 (1H, br s), 1.85 (3H, t, J=7.8 Hz), 1.86 (3H, t, J=7.6 Hz), 2.50 (6H, s), 2.72 (2H, t, J=6.4 Hz), 3.56 (3H, s), 3.60 (3H, s), 3.61 (3H, s), 3.74 (3H, s), 3.86 (2H, t, J=6.2 Hz), 4.00–4.08 (4H, two overlapping quartets), 9.47 (1H, s), 9.88 (1H, s), 10.00 (1H, s), 10.07 (1H, s); ^{1}H NMR (TFA–$CDCl_3$): δ −3.42 (1H, br s), −3.35 (1H, br s), −3.03 (1H, br s), −2.83 (1H, br s), 1.69–1.76 (6H, two overlapping triplets), 2.54 (6H, s), 2.74 (2H, t, J=6.4 Hz), 3.54 (3H, s), 3.55 (3H, s), 3.58 (3H, s), 3.60 (3H, s), 3.94 (2H, t, J=6.2 Hz), 4.01–4.10 (4H, two overlapping quartets), 9.26 (1H, s), 10.33 (1H, s), 10.38 (1H, s), 10.50 (1H, s); ^{13}C NMR (TFA–$CDCl_3$): δ 11.7, 11.8, 14.0, 16.4, 20.2, 20.3, 28.6, 36.3, 38.0, 44.7, 97.2, 98.2, 100.1, 118.1, 125.7, 136.2, 138.8, 139.4, 140.2, 140.9, 141.2, 141.8, 141.9, 142.1, 142.6, 142.9, 143.0, 143.6, 144.4, 145.1; EIMS: *m*/*z* (rel int.) 490 (100, M^+), 475 (55, $[M-CH_3]^+$); HRMS: Calcd for $C_{33}H_{38}N_4$: 490.3099. Found: 490.3118. Anal. Calcd for $C_{33}H_{38}N_4\cdot{}^1/_8CHCl_3$: C, 78.69; H, 7.60; N, 11.08. Found: C, 78.73; H, 7.66; N, 11.00.

4.2.30. 2,7,12,17-Tetramethyl-3,5:8,10:13,15:18,20-tetrakis(2-methylpropano)porphyrin (26a). Pyrrole **17d** (2.40 g) in methanol (4.5 mL) was added to a solution of KOH (3.27 g) in water (10.9 mL) and methanol (10.9 mL), and the mixture refluxed for 2 h on a hot water bath. The mixture was cooled to 0 °C in an ice-salt bath and carefully neutralized with 6 M HCl, while maintaining a temperature below 5 °C. The mixture was extracted with chloroform, dried over magnesium sulfate and the solvent evaporated under reduced pressure while maintaining the temperature below 30 °C. Acetic acid (7.6 mL) and potassium ferricyanide (0.185 g) were added to the residual solid, and the mixture was heated on a boiling water bath for 1 h. The mixture was partitioned between dichloromethane and water, and the organic phase washed with water, 5% aqueous ammonia solution and water, and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure and the residue chromatographed on grade III alumina eluting with dichloromethane. The red fractions were combined, evaporated under reduced pressure and rechromatographed on grade III alumina eluting with toluene. The fractions containing porphyrin were evaporated and recrystallized from dichloromethane–methanol to give deep purple crystals (131 mg; 10.4%), mp >300 °C; UV–vis ($CHCl_3$): λ_{max} ($\log_{10}\varepsilon$) 419 (5.29), 518 (4.12), 550 (3.61), 591 (3.62), 647 nm (3.43); ^{1}H NMR ($CDCl_3$): δ −2.78 (2H, br s), 1.38–1.72 (12H, m), 2.14–2.97 (4H, m), 3.43 (12H, s), 3.13–3.98 (8H, m), 4.25–5.29 (8H, m); FABMS: *m*/*z* (rel int.) 583 (100) $[M+H]^+$.

4.2.31. 2,7,12,17-Tetramethyl-3,5:8,10:13,15:18,20-tetrakis(2-*tert*-butylpropano)porphyrin (26b). Prepared by the previous procedure from **17e** (2.40 g). The title porphyrin (35 mg; 2.5%) was obtained as deep purple crystals, mp >300 °C; UV–vis ($CHCl_3$): λ_{max} ($\log_{10}\varepsilon$) 419 (5.29), 518 (4.12), 550 (3.61), 591 (3.62), 647 nm (3.43); λ_{max} ($\log_{10}\varepsilon$) 418 (5.36), 518 (4.16), 552 (3.66), 591 (3.66), 647 nm (3.60); ^{1}H NMR ($CDCl_3$): δ −2.94 (1H, br s), −2.82 (1H, br s), 1.34–1.48 (36H, m), 2.14–2.96 (4H, m), 3.16–4.21 (20H, m), 4.35–4.66 (4H, m), 5.09–5.51 (4H, m); FABMS: *m*/*z* (rel int.) 751 (100) $[M+H]^+$, 693 (27).

4.2.32. 2,7,12,17-Tetramethyl-3,5:8,10:13,15:18,20-tetrakis(2,2-dimethylpropano)-porphyrin (26c). The title porphyrin was obtained from **17b** in 8% yield as deep purple crystals, mp >300 °C; UV–vis ($CHCl_3$): λ_{max} ($\log_{10}\varepsilon$) 422 (5.33), 520 (4.20), 552 (3.83), 592 (3.88), 646 nm (3.79); UV–vis (1% TFA–$CHCl_3$): λ_{max} ($\log_{10}\varepsilon$) 430 (5.43), 584 (4.00), 634 nm (4.19); ^{1}H NMR

(TFA–$CDCl_3$; 17 °C): δ −2.47 (4H, s), 1.12 (24H, br s), 3.34 (12H, s), 3.54 (8H, br s), 4.77 (8H, s); ^{13}C NMR (TFA–$CDCl_3$): δ 14.6, 28.6 (br), 36.1, 37.5, 44.7, 115.3, 133.3, 138.2, 139.2, 141.6; EIMS: *m/z* (rel int.) 638 (100), 637 (23), 636 (38), 621 (25); HRMS: Calcd for $C_{44}H_{54}N_4$: 638.4322. Found: 638.4325.

4.2.33. 2,7,12,17-Tetraethyl-3,5:8,10:13,15:18,20-tetrakis(2,2-dimethylpropano)porphyrin (26d). The title porphyrin was obtained from **17c** in 6% yield as deep purple crystals, mp >300 °C; UV–vis (1% Et_3N–$CHCl_3$): λ_{max} ($\log_{10}\varepsilon$) 425 (5.34), 523 (4.21), 558 (3.81), 597 (3.80), 654 nm (3.64); UV–vis (1% TFA–$CHCl_3$): λ_{max} ($\log_{10}\varepsilon$) 432 (5.46), 588 (4.08), 638 nm (4.34); ^1H NMR (TFA–$CDCl_3$; 45 °C): δ −2.14 (4H, s), 1.10 (24H, br s), 1.63 (12H, t, J=7.5 Hz), 3.51 (8H, br s), 3.73 (8H, q, J=7.5 Hz), 4.78 (8H, br s); ^{13}C NMR (TFA–$CDCl_3$): δ 15.6, 21.2, 28.5 (v br), 36.1, 37.3, 44.2, 115.4, 138.6, 139.3, 139.7, 140.9; EIMS: *m/z* (rel int.) 694 (100), 693 (8), 692 (10), 677 (3); HRMS: Calcd for $C_{48}H_{62}N_4$: 694.4974. Found: 694.4975.

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Vinylsulfones versus alkylsulfones in the addition to chiral imines. Synthesis of *N*-(*tert*-butoxycarbonyl)-L-homophenylalanine

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Abstract—A study of the addition of vinylsulfones versus alkylsufones has been done, and applied to the synthesis of *N*-(*tert*-butoxycarbonyl)-L-homophenylalanine.
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1. Introduction

The addition of organometallics to imines derived from (*R*)-2,3-diprotected-D-glyceraldehyde, has been the subject of numerous papers, in particular related to the synthesis of natural and unnatural aminoacids.[1] Recently we have published the addition of a cyclopropylvinylsulfone to the benzylimine of (*R*)-2,3-*O*-isopropylidene-D-glyceraldehyde **1**, for the synthesis of unnatural aminoacids.[2] (Fig. 1).

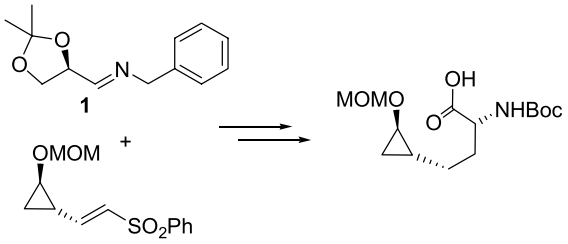

Figure 1. Addition of cyclopropylvinylsulfone to imine **1**, and transformation into an unnatural protected aminoacid.

In order to extend this approach to other systems we decided to study the addition of phenylsulfones to the chiral imine **1**. In this manner the synthesis of one of the more interesting unnatural aminoacids, L-homophenylalanine, could be achieved. This amino acid is found in several pharmaceuticals such as the angiotensin converting enzyme (ACE) inhibitors, for example, Cilazapril[3] and Enalapril[4] among others (Fig. 2). To the best of our knowledge there is no report of a synthesis applying this approach to L-homophenylalanine, although this molecule has been synthesized several times in both enantiomeric forms.[5] Initially, we decided to study the addition of sulfone **2** to the chiral imine **1** (Scheme 1).

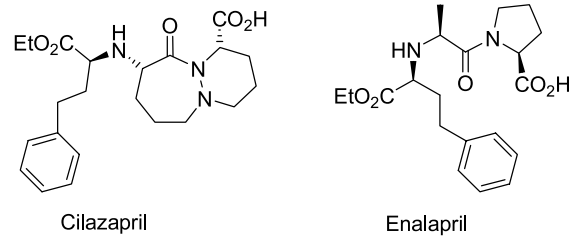

Figure 2. Some ACE inhibitors containing the L-HPA moiety.

2. Results and discussion

When sulfone **2** was treated with *n*-BuLi in THF and made to react with imine **1**, several compounds **3** (45%) **4**–**6** (21%) and **7** (30%) were obtained, which were isolated by column chromatography. Compound **3** was hydrogenated in presence of Boc_2O, giving a 48% yield of derivative **8**, which was then transformed into D-homophenylalanine using Pericàs methodology.[6] Thus, deprotection of the acetonide and oxidation with $RuCl_3$ and sodium periodate led to *N*-Boc-D-homophenylalanine[7] **10** in 59% yield from **8**, hence establishing the stereochemistry for **8**.

The other three compounds **4**, **5** and **6** were debenzylated

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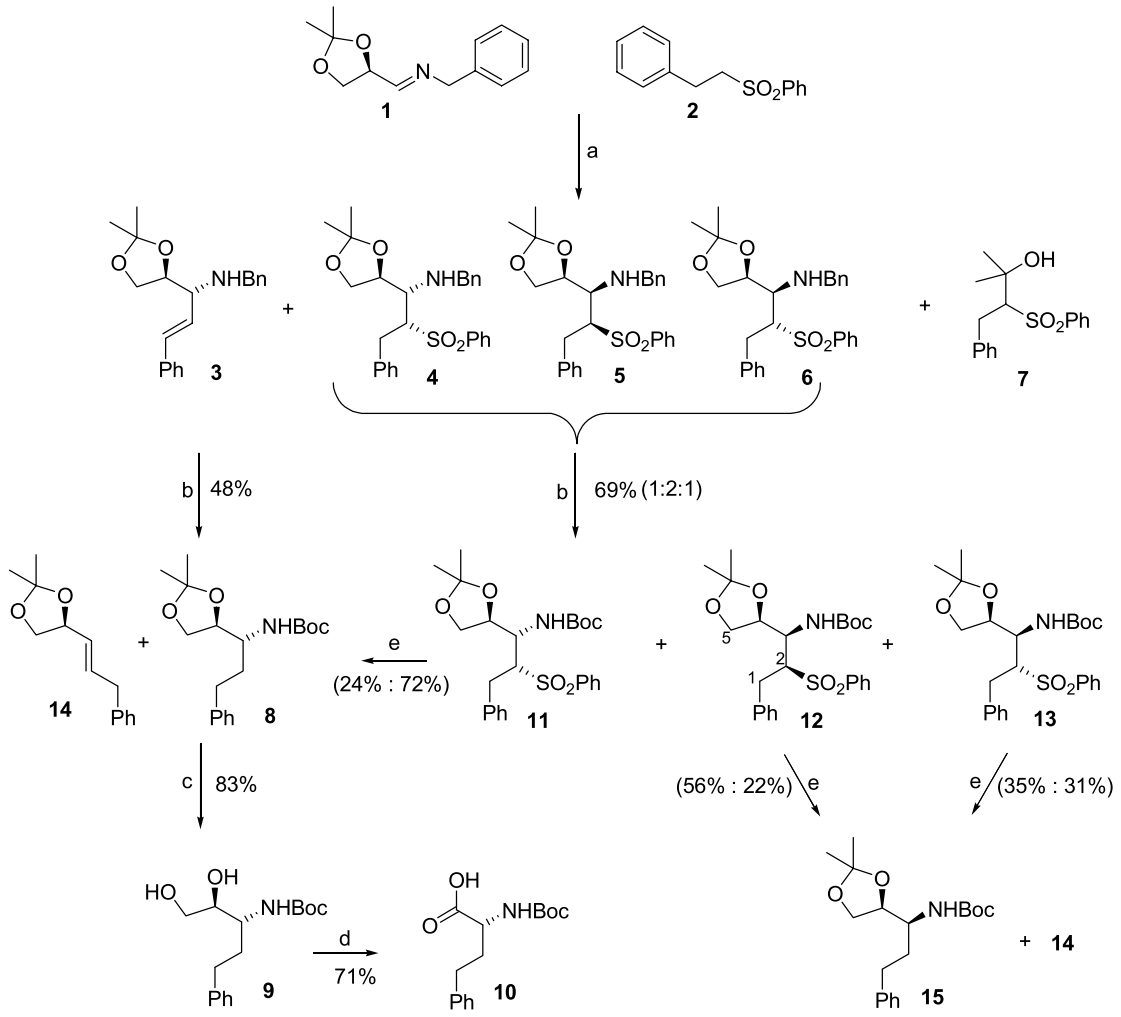


Scheme 1. Reagents: (a) *n*-BuLi, THF, −78→0 °C, 5 h (**3**:45%, **4**–**6**:21%, **7**:30%); (b) H_2, Boc_2O, $Pd(OH)_2$, EtOAc, rt, 24 h; (c) CF_3COOH, $MeOH/H_2O$ 3:1, rt, 7 h; (d) $RuCl_3$–H_2O, $NaIO_4$, $MeCN/CCl_4/H_2O$ 2:2:3, rt, 1 h; (e) Na–Hg, MeOH, 1–2 h.

under the same conditions as **3**, and *N*-Boc derivatives **11**, **12**, and **13** were obtained in 69% yield in a 1:2:1 ratio. In order to establish the stereochemistry of these compounds, they were desulfonylated with sodium amalgam. Derivative **11** gave a 3:7 mixture of the olefin **14**, via a Julia type reaction,[8] and the *N*-Boc compound **8**, in nearly quantitative yield. Compounds **12** and **13**, when similarly treated, separately gave the same desulfonylated compound **15** in quantitative yield, along with **14**. This compound **15** has already been described and the spectroscopic properties and rotation were coincident with those taken from the literature.[9] The stereochemistry of the sulfone carbon C-2 was tentatively determined for **11**, **12** and **13** by extensive NOE and comparative NMR studies. We felt sufficiently confident of this assignment for the purposes of this study, particularly since this stereocentre was in any event going to be removed.

Thus, in this case, the addition of an anion stabilised by an alkyl sulfone to the imine proceeded with moderate stereoselectivity (3:1 ratio), following a course similar to that found by Díaz-de-Villegas and Galvez et al for the addition of benzylmagnesium chloride to imine **1**,[10] and in contrast to the addition of other organometalics to the same imine observed by these authors.[11]

In order to further study the stereoselectivity of the sulfone addition, we decided to examine the behaviour of the more rigid phenylstyrylsulfone **16** in a similar manner, in the expectation that we might find changes in the stereoselectivity that would help determine the causes of that selectivity. The reaction of the anion of **16** (generated by treatment with *n*-BuLi in THF) with imine **1** gave compounds **17**, **18**, **19** and **20** in an excellent 93% yield and with complete stereoselectivity, as will be demonstrated below (Scheme 2).

The stereoselectivity obtained has been explained for analogous systems in terms of α-chelate controlled addition[1b,12] but, while the Cram, Cram-chelate, and Felkin-Anh models have all been applied to the addition of nucleophiles to imines,[1c] the substrate **1** provides a particularly complex case. As mentioned above, the stereochemical outcome of additions to **1** is known to depend upon the nucleophile used, with a complete reversal of the (nearly complete) stereoselectivity on changing, for example, from PhMgBr to $PhCH_2MgCl$[11]—this has been postulated to be due to changes in the preferred chelate formed (with the α or β oxygen of the acetonide unit). In any event, this means that the classical models provide no guidance in this context as to possible outcomes prior to

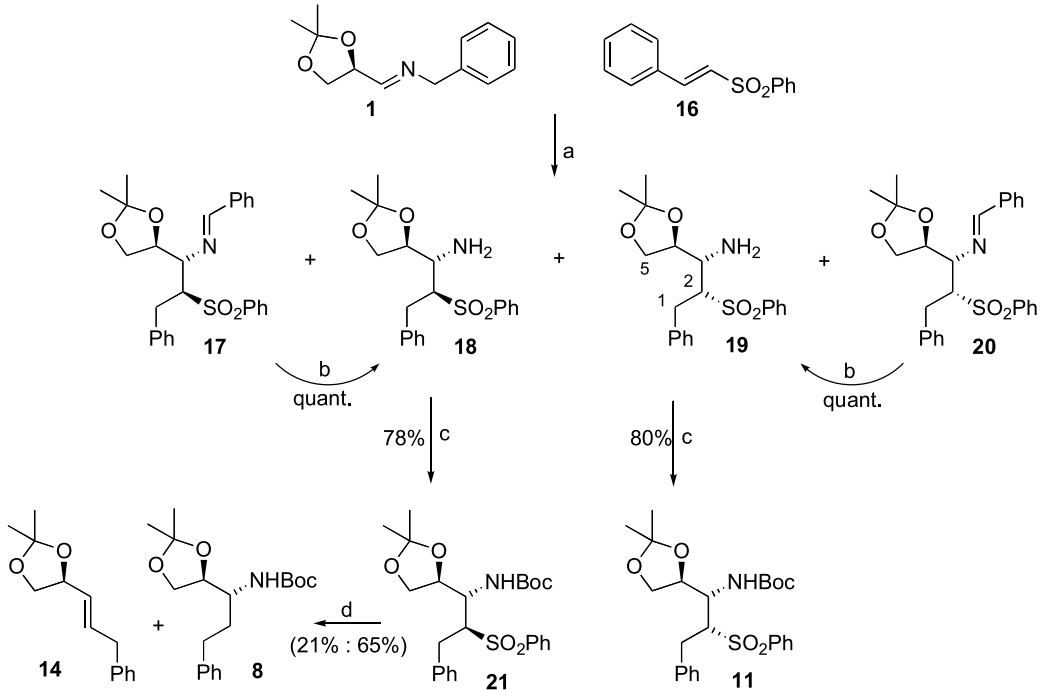
synthesis, since there is no a priori way to decide, which of the chelated forms will predominate for a particular nucleophile. Therefore, we undertook a molecular modeling study to determine what might be controlling the stereoselective addition of our anions to imine **1**. Our objective in undertaking such a study was to provide an approximate model that could be generated in a practical amount of time, rather than to do a full investigation of the many chelated and/or solvated forms of every possible conformation of each possible intermediate or transition state. While the result is necessarily approximate, this is a characteristic of many useful models, such as are indeed the Cram or Felkin–Anh models; our hope was to find a model that was consistent with the observed results, and which might serve as a guide to predict the outcome of related reactions, with a minimal increase in complexity over the classical Cram chelate model.

Given the dependence of the stereochemical outcome of these additions on the nucleophile, we considered the possibility that the addition might proceed through a late transition state, or that the addition might be reversible; under either of these circumstances, the relative stability of the possible diastereoisomeric products might serve as a model to explain the stereoselectivity of the reaction. Therefore, the addition product of **16** to **1**, with a hydrogen atom on the amine nitrogen, was sketched in Maestro (v. 7.0.113)[13] and subjected to Monte Carlo multiple minimum/low mode conformational search with Macromodel (v. 9.0.016)[13] using up to 5000 iterations of TNCG minimization of each conformer with the MMFFs forcefield, with all other parameters selected using the automatic setup options within Maestro. The result of a conformational search with 1000 attempts to find new conformers suggested that the more stable product should be the *S*,*S* stereoisomer

Figure 3. Top panel: lowest-energy conformation of the *S*,*S* addition product of **1** and **16**. Bottom panel: lowest-energy conformation of the *S*,*R* addition product.

(MMFF94s energy 429.88 kJ/mol versus *S*,*R* isomer 434.64 kJ/mol; the *S* stereocentre is that in the dioxolane ring, the other is the new stereocentre, with nitrogen, created by the reaction). This difference is in the right direction and probably would be sufficient to explain the modest stereoselectivity of the related reaction of **1** with the anion of **2**, and hence this simple model may serve as a rough guide to the stereochemical outcome of these reactions.

However, given the small energetic difference, it seems that



Scheme 2. Reagents: (a) *n*-BuLi, THF, −78→0 °C, 7 h (**17** and **20**:42%, **18**:33%, **19**:18%); (b) Silica gel (quantitative); (c) Boc_2O, THF, rt, overnight; (d) Na–Hg, MeOH, 1–2 h.

the hypothesis that the stability of the intermediates (or nearby transition states) corresponding to the structures in Figure 3 might only be a partial explanation for the total stereoselectivity observed for the reaction of **1** with the anion of **16**, even allowing for the approximations—such as the use of the amine rather than the lithium amide-used.

Compounds **17** and **20** are the result of addition to the imine followed by hydride transfer to the resulting vinylsulfone. This hydride transfer has been observed previously by us with the cyclopropylvinylsulfone of Figure 1.[2] Hydride transfer may further enhance the stereoselectivity of the reaction in the vinylsulfone case, as the conformation shown in the top panel of Figure 3 (*S*,*S* stereochemistry) is also ideally set up for the this process to take place via a chair like transition state, and rapid, irreversible hydride transfer (rapid transfer is supported by the absence of any of the immediate product of addition in the product mixture) would hence result in a trapping of this intermediate and greater stereoselectivity.

Amines **18** and **19** are the result of the hydrolysis of derivatives **17** and **20**, respectively (Scheme 2). Compound **19** has the same stereochemistry at C-3 as **18** but the opposite at C-2. The stereochemistry of these four compounds was established as follows. Imine **17** was hydrolyzed on silica gel to give compound **18** quantitatively. Compounds **18** and **19** were transformed separately into their *N*-Boc-protected derivatives **21** and **11**. The stereochemistry of **11** has been determined previously. When compound **21** was submitted to desulfonylation, compounds **8** and **14** were obtained in a similar ratio as was the case with **11**, so the stereochemistry of compound **21**, and hence of **18**, is the same at C-3 and different at C-2.

3. Conclusions

The addition of the phenylstyrylsulfone to the imine **1** was thus completely stereoselective, an improvement upon the addition of the alkylsulfone. The hydride transfer previously reported is also confirmed as a somewhat general phenomenon by this work, and its synthetic utility is being investigated. This methodology constitutes an alternative procedure for the synthesis of L-homophenylalanine, and opens a new methodology for the synthesis of unnatural aminoacids.

4. Experimental

4.1. General

Unless otherwise stated, all chemicals were purchased as the highest purity commercially available and were used without further purification. IR spectra were recorded on a BOMEM 100 FT-IR or an AVATAR 370 FT-IR Thermo Nicolet spectrophotometers. ^{1}H and ^{13}C NMR spectra were performed in $CDCl_3$ and referenced to the residual peak of $CHCl_3$ at δ 7.26 and 77.0 ppm, for ^{1}H and ^{13}C, respectively, using Varian 200 VX and Bruker DRX 400 instruments. Chemical shifts are reported in δ ppm and coupling constants (*J*) are given in Hz. MS were performed at a VG-TS 250 spectrometer at 70 eV ionising voltage. Mass spectra are presented as *m*/*z* (% rel int.). HRMS were recorded on a VG Platform (Fisons) spectrometer using chemical ionization (ammonia as gas) or Fast Atom Bombardment (FAB) technique. For some of the samples, QSTAR XL spectrometer was employed for electrospray ionization (ESI). Optical rotations were determined on a Perkin-Elmer 241 polarimeter in 1 dm cells. Diethyl ether and THF were distilled from sodium, and dichloromethane was distilled from calcium hydride under Argon atmosphere.

Imine **1** was prepared from (*R*)-2,3-*O*-isopropylidene-D-glyceraldehyde and benzylamine in the presence of anhydrous magnesium sulphate according to the literature procedure.[14]

Numbering of compounds in the experimental part corresponds with Schemes in the discussion and not with the name, for spectral interpretation and comparison purposes.

4.1.1. Reaction of 2 with imine 1. *n*-BuLi 1.6 M (0.80 mL, 1.29 mmol) was added to a solution of **2** (264 mg, 1.07 mmol) in THF (5 mL) at −78 °C under Ar atmosphere. After 15 min, imine **1** (350 mg, 1.60 mmol) was added to the reaction flask via cannula as a solution in THF (5 mL). The reaction mixture was left to stir for 5 h at −78→0 °C under Ar before addition of saturated ammonium chloride solution (3 mL). The product was extracted into EtOAc (3×). The organic extracts were combined, washed with brine, dried over anhydrous Na_2SO_4, filtered and removed the solvent in vacuo. The resultant oil was submitted to flash silica column chromatography (hexane/EtOAc, 95:5) to yield 156 mg (0.48 mmol, 45%) of **3**, 104 mg (0.22 mmol, 21%) of the mixture **4**–**6** and 98 mg (0.32 mmol, 30%) of **7**. *Compound* **3**. $[\alpha]_D^{22}$ −50.0 (*c* 1.2, $CHCl_3$). IR: 3330, 2985, 2934, 1495, 1454, 1371, 1213, 1146, 1067, 852, 748, 697 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.35 and 1.36 (3H each, 2s, Me_2C), 3.17–3.33 (1H, m, H-3), 3.68 and 3.95 (1H each, 2d, J=13.6 Hz, $NHCH_2Ph$), 3.86 (1H, dd, J=5.4, 8.4 Hz, H_A-5), 3.91–3.94 (1H, m, H_B-5), 4.10–4.20 (1H, m, H-4), 6.03 (1H, dd, J=8.8, 16.0 Hz, H-2), 6.59 (1H, d, J=16.0 Hz, H-1), 7.26–7.43 (10H, m, Ar). ^{13}C NMR (50 MHz, $CDCl_3$) δ 25.7 and 27.2 (Me_2C), 51.0 ($NHCH_2Ph$), 64.0 (C-3), 67.0 (C-5), 78.3 (C-4), 110.0 (Me_2C), 126.8 (C_{meta} Ph), 127.3 (C_{para} $NHCH_2Ph$), 128.0 (C-2), 128.2 (C_{para} Ph), 128.5 (C_{meta} $NHCH_2Ph$), 128.7 (C_{ortho} $NHCH_2Ph$), 128.9 (C_{ortho} Ph), 134.8 (C-1), 136.6 (C_{ipso} $NHCH_2Ph$), 140.0 (C_{ipso} Ph). MS: 324 $[M+H]^+$, 279, 217, 159, 149, 117. HRMS: calcd for $C_{21}H_{26}NO_2$ $[M+H]^+$: 324.1964, found: 324.1928.

Compounds **4**–**6**. IR: 3525, 3355, 2985, 2933, 1447, 1371, 1305, 1214, 1149, 1084, 1029, 743, 699, 609 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.21 and 1.34 (3H each, 2s, Me_2C), 3.01–3.42, 3.59–3.86, 3.93–4.21, 4.45–4.55 and 4.76–4.84 (9H, series of 5m, H-1, H-2, H-3, H-4, H-5 and $NHCH_2Ph$), 6.79–7.97 (15H, series of m, Ar). ^{13}C NMR (50 MHz, $CDCl_3$) δ 25.6 and 26.9 (Me_2C), 28.9 and 30.9 (C-1), 52.8 and 57.7 ($NHCH_2Ph$), 58.5 (C-3), 66.2 (C-2), 69.5 (C-5), 76.9 (C-4), 109.5 (Me_2C), 126.9–129.6 (14C Ar), 133.7 and 134.1 (C_{para} SO_2Ph), 137.5, 137.7, 139.2,

139.5 and 140.3 ($3C_{ipso}$ Ar). MS: 466 $[M+H]^+$, 408, 279. HRMS: calcd for $C_{27}H_{32}NO_4S$ $[M+H]^+$: 466.2052, found: 466.2018.

Compound **7**. IR: 3600–3300, 3063, 2980, 2936, 1447, 1381, 1292, 1197, 1140, 1081, 746, 689, 610 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 1.33 and 1.67 (3H each, 2s, Me_2C), 3.04 (1H, dd, $J=5.4$, 15.8 Hz, H_A-1), 3.19 (1H, dd, $J=7.0$, 15.8 Hz, H_B-1), 3.72 (1H, dd, $J=5.4$, 7.0 Hz, H-2), 4.63 (1H, br s, OH), 6.76–6.81 (2H, m, H_{ortho} Ph), 7.03–7.07 (3H, m, H_{meta} and H_{para} Ph), 7.33–7.41 (2H, m, H_{meta} SO_2Ph), 7.48–7.55 (1H, m, H_{para} SO_2Ph), 7.62–7.66 (2H, m, H_{ortho} SO_2Ph). ^{13}C NMR (50 MHz, $CDCl_3$) δ 26.5 and 30.3 (Me_2C), 33.5 (C-1), 74.2 (C-3), 75.5 (C-2), 126.8 (C_{para} Ph), 128.1 (C_{meta} Ph), 128.6 (C_{ortho} SO_2Ph and C_{ortho} Ph), 129.2 (C_{meta} SO_2Ph), 133.6 (C_{para} SO_2Ph), 137.9 (C_{ipso} Ph), 140.4 (C_{ipso} SO_2Ph).

4.1.2. (1*R*,4′*S*)-*N*-*tert*-butoxycarbonyl-1-2′2′-dimethyl [1′,3′]-dioxolan-4′-yl-3-phenylpropylamine, 8. To a solution of **3** (67 mg, 0.21 mmol) and di-*tert*-butyl dicarbonate (68 mg, 0.31 mmol) in dry EtOAc (2.5 mL) was added 55 mg (0.10 mmol) of 20% wt $Pd(OH)_2$, and the mixture was hydrogenated with H_2 at atmospheric pressure by stirring at room temperature for 24 h. After completion the reaction mixture was filtered and concentrated in vacuo to afford a crude product, which was purified by flash chromatography (hexane/EtOAc, 9:1) to provide 34 mg (0.10 mmol, 48%) of **8** as a white solid. $[\alpha]_D^{22}$ +23.2 (*c* 1.10, $CHCl_3$). IR: 3450, 3358, 2981, 2932, 1715, 1498, 1367, 1169, 1057, 700 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 1.33 and 1.43 (3H each, 2s, Me_2C), 1.46 (9H, s, $CO_2C(CH_3)_3$), 1.78–1.91 (2H, m, H-2), 2.58–2.79 (2H, m, H-1), 3.66 (1H, dd, $J=7.4$, 7.8 Hz, H_A-5), 3.67–3.77 (1H, m, H-3), 3.98 (1H, dd, $J=6.8$, 7.8 Hz, H_B-5), 4.11–4.18 (1H, m, H-4), 4.71 (1H, d, $J=9.8$ Hz, NH), 7.18–7.32 (5H, m, Ph). ^{13}C NMR (50 MHz, $CDCl_3$) δ 25.3 and 26.5 (Me_2C), 28.6 ($CO_2C(CH_3)_3$), 32.7 (C-2), 35.8 (C-1), 50.5 (C-3), 66.6 (C-5), 77.7 (C-4), 79.7 ($CO_2C(CH_3)_3$), 109.3 (Me_2C), 126.1 (C_{para} Ph), 128.6 (C_{ortho} and C_{meta} Ph), 141.9 (C_{ipso} Ph), 156.4 ($CO_2C(CH_3)_3$). MS: *m*/*z* (%) 336 (10) $[M+H]^+$, 280 (15), 222 (65), 117 (25), 91 (55). HRMS: calcd for $C_{19}H_{30}NO_4$ $[M+H]^+$: 336.2175, found: 336.2153.

4.1.3. (2*S*,3*R*)-3-(*tert*-butoxycarbonylamino)-5-phenyl-1, 2-pentanediol, 9. To a solution of **8** (93 mg, 0.28 mmol) in MeOH/H_2O 3:1 (1.5 mL), CF_3COOH (12 μL, 0.15 mmol) was added and the mixture was left to stir for 7 h. The solution was then poured into a separting funnel, extracted with CH_2Cl_2 (3×), washed with brine (1×), dried over anhydrous Na_2SO_4, filtered and removed the solvent. The product was purified by flash silica column chromatography (hexane/EtOAc 6:4) to yield 68 mg (0.23 mmol, 83%) of **9** as a white solid. $[\alpha]_D^{22}$ +15.9 (*c* 1.1, $CHCl_3$). IR: 3600–3100, 2977, 2932, 1682, 1513, 1455, 1392, 1366, 1250, 1169, 1053, 872, 751, 700 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 1.45 (9H, s, $CO_2C(CH_3)_3$), 1.76–1.93 (2H, m, H-2), 2.64–2.74 (2H, m, H-1), 3.10 (2H, br s, 2OH), 3.48–3.76 (4H, m, H-3, H-4 and H-5), 4.89 (1H, d, $J=9.2$ Hz, NH), 7.15–7.31 (5H, m, Ar). ^{13}C NMR (50 MHz, $CDCl_3$) δ 28.6 ($CO_2C(CH_3)_3$), 32.8 (C-2), 34.1 (C-1), 51.2 (C-3), 63.9 (C-5), 73.6 (C-4), 80.2 ($CO_2C(CH_3)_3$), 126.2 (C_{para} Ph), 128.7 (C_{ortho} and C_{meta} Ph), 141.7 (C_{ipso} Ph), 157.4 ($CO_2C(CH_3)_3$). MS: 318 $[M+Na]^+$, 296 $[M+H]^+$, 240, 196. HRMS: calcd for $C_{16}H_{26}NO_4$ $[M+H]^+$: 296.1862, found: 296.1843.

4.1.4. (2*R*)-(*tert*-butoxycarbonylamino)-4-phenyl butanoic acid 10. To a solution of **9** (61 mg, 0.21 mmol) in MeCN/CCl_4/H_2O 2:2:3 (4 mL) was slowly added $NaIO_4$ (178 mg, 0.83 mmol). The mixture was vigorously stirred for 5 min before addition of $RuCl_3$–H_2O (2 mg, 0.01 mmol). It was then left to stir at room temperature for 1 h. CH_2Cl_2 was then added and the product extracted with $NaHCO_3$ 1 M aqueous solution (3×). The aqueous extracts were combined, washed with Et_2O, and slowly acidulated with solid $KHSO_4$ until pH=3. Product was then extracted with Et_2O, dried over anhydrous Na_2SO_4, filtered and removed the solvent in vacuo. It was purified by flash chromatography (hexane/EtOAc, 1:1) to provide 41 mg (0.15 mmol, 71%) of **10**. $[\alpha]_D^{22}$ −5.2 (*c* 1.1, EtOH). Lit.[7] value for (2*S*)-(*tert*-butoxycarbonylamino)-4-phenylbutanoic acid: $[\alpha]_D^{22}$ +5.8 (*c* 1, EtOH). IR: 3450–3100, 2978, 1717, 1498, 1406, 1368, 1248, 1166, 700 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 1.46 (9H, s, $CO_2C(CH_3)_3$), 1.94–2.30 (2H, m, H-2), 2.73 (2H, t, $J=7.8$ Hz, H-1), 4.28–4.43 (1H, m, H-3), 5.10 (1H, d, $J=7.4$ Hz, NH), 7.18–7.32 (5H, m, Ar), 10.6 (1H, br s, COOH). ^{13}C NMR (50 MHz, $CDCl_3$) δ 28.5 ($CO_2C(CH_3)_3$), 31.8 (C-2), 34.3 (C-1), 53.4 (C-3), 80.5 ($CO_2C(CH_3)_3$), 126.4 (C_{para} Ph), 128.7 (C_{ortho} and C_{meta} Ph), 140.9 (C_{ipso}, Ph), 155.8 ($CO_2C(CH_3)_3$), 177.6 (COOH). MS: 302 $[M+Na]^+$, 280 $[M+H]^+$, 224, 180, 134, 117. HRMS: calcd for $C_{15}H_{22}NO_4$ $[M+H]^+$: 280.1549, found: 280.1540.

4.1.5. *N*-*tert*-butoxycarbonyl-1-2′2′-dimethyl-[1′,3′]-dioxolan-4′-yl-3-phenyl-2-(phenylsulfonyl) propyl amines 11, 12 and 13. To a solution of **4**–**6** (94 mg, 0.20 mmol) and di-*tert*-butyl dicarbonate (68 mg, 0.31 mmol) in dry EtOAc (2.5 mL) was added 53 mg (0.10 mmol) of 20% wt $Pd(OH)_2$, and the mixture was hydrogenated with H_2 at atmospheric pressure by stirring at room temperature for 24 h. After completion the reaction mixture was filtered and concentrated in vacuo to afford a crude product, which was purified by flash chromatography (hexane/EtOAc, 95:5) to provide 15 mg (0.03 mmol, 16%) of **11**, 32 mg (0.07 mmol, 34%) of **12** and 18 mg (0.04 mmol, 19%) of **13**. *Compound* **11**. $[\alpha]_D^{22}$ −17.0 (*c* 0.9, $CHCl_3$). IR: 3442, 2981, 2935, 1713, 1495, 1448, 1369, 1305, 1233, 1147, 1082, 918, 862, 734, 690, 666 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 1.34 (9H, s, $CO_2C(CH_3)_3$), 1.39 and 1.43 (3H each, 2s, Me_2C), 3.13 (1H, dd, $J=5.6$, 14.8 Hz, H_A-1), 3.25 (1H, dd, $J=7.6$, 14.8 Hz, H_B-1), 3.58 (1H, dd, $J=7.0$, 8.0 Hz, H_A-5), 3.74–3.82 (1H, m, H-2), 4.08 (1H, dd, $J=7.0$, 8.0 Hz, H_B-5), 4.42 (1H, dt, $J=2.6$, 2.6, 9.0 Hz, H-3), 4.78–4.87 (2H, m, H-4 and NH), 6.93–6.98 (2H, m, H_{ortho} Ph), 7.08–7.16 (3H, m, H_{meta} and H_{para} Ph), 7.42–7.61 (3H, m, H_{meta} and H_{para} SO_2Ph), 7.80–7.83 (2H, m, H_{ortho} SO_2Ph). ^{13}C NMR (50 MHz, $CDCl_3$) δ 25.4 and 26.5 (Me_2C), 28.4 ($CO_2C(CH_3)_3$), 31.5 (C-1), 50.1 (C-3), 67.4 (C-2 and C-5), 74.5 (C-4), 80.0 ($CO_2C(CH_3)_3$), 110.2 (Me_2C), 126.8 (C_{para} Ph), 128.6 (C_{ortho} SO_2Ph), 128.7 (C_{meta} and C_{ortho} Ph), 129.4 (C_{meta} SO_2Ph), 133.9 (C_{para} SO_2Ph), 138.1 (C_{ipso} Ph), 139.3 (C_{ipso} SO_2Ph), 155.2 ($CO_2C(CH_3)_3$). MS: 498 $[M+Na]^+$, 476 $[M+H]^+$, 420,

376, 362. HRMS: calcd for $C_{25}H_{34}NO_6S$ $[M+H]^+$: 476.2107, found: 476.2112.

Compound **12**. $[\alpha]_D^{22}$ +7.5 (*c* 1.2, $CHCl_3$). IR: 3368, 2982, 2934, 1716, 1498, 1448, 1370, 1307, 1243, 1150, 1078, 1058, 734, 691 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 1.21 (6H, s, Me_2C), 1.45 (9H, s, $CO_2C(CH_3)_3$), 3.12 (1H, dd, J= 8.8, 14.6 Hz, H_A-1), 3.38 (1H, dd, J=5.2, 14.6 Hz, H_B-1), 3.70–3.91 (4H, m, H-2, H-3 and H-5), 4.16–4.27 (1H, m, H-4), 5.04 (1H, d, J=9.8 Hz, NH), 7.12–7.25 (5H, m, Ph), 7.48–7.62 (3H, m, H_{meta} and H_{para} SO_2Ph), 7.88–7.92 (2H, m, H_{ortho} SO_2Ph). ^{13}C NMR (50 MHz, $CDCl_3$) δ 25.6 and 26.6 (Me_2C), 28.5 ($CO_2C(CH_3)_3$), 30.8 (C-1), 52.2 (C-3), 66.3 (C-2), 68.0 (C-5), 75.8 (C-4), 80.4 ($CO_2C(CH_3)_3$), 110.2 (Me_2C), 127.3 (C_{para} Ph), 129.0 (C_{ortho} SO_2Ph), 129.2 (C_{ortho} and C_{meta} Ph), 129.4 (C_{meta} SO_2Ph), 134.0 (C_{para} SO_2Ph), 137.0 (C_{ipso} Ph), 138.4 (C_{ipso} SO_2Ph), 155.4 ($CO_2C(CH_3)_3$). MS: 476 $[M+H]^+$, 420, 376, 362. HRMS: calcd for $C_{25}H_{34}NO_6S$ $[M+H]^+$: 476.2107, found: 476.2094.

Compound **13**. $[\alpha]_D^{22}$ +6.2 (*c* 1.0, $CHCl_3$). IR: 3392, 2982, 2929, 1716, 1506, 1448, 1370, 1306, 1237, 1153, 1074, 666 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 0.76 and 1.17 (3H each, 2s, Me_2C), 1.50 (9H, s, $CO_2C(CH_3)_3$), 2.89 (1H, dd, J=10.6, 13.8 Hz, H_A-1), 3.03 (1H, dd, J=3.2, 13.8 Hz, H_B-1), 3.79 (1H, dd, J=3.8, 8.8 Hz, H_A-5), 3.86–4.05 (3H, m, H-2, H-3 and H_B-5), 4.45–4.54 (1H, m, H-4), 5.61 (1H, d, J=10.6 Hz, NH), 7.00–7.04 (2H, m, H_{ortho} Ph), 7.16–7.22 (3H, m, H_{meta} and H_{para} Ph), 7.56–7.70 (3H, m, H_{meta} and H_{para} SO_2Ph), 7.90–7.98 (2H, m, H_{ortho} SO_2Ph). ^{13}C NMR (50 MHz, $CDCl_3$) δ 25.6 and 26.4 (Me_2C), 28.5 ($CO_2C(CH_3)_3$), 33.4 (C-1), 52.9 (C-3), 65.6 (C-2), 67.6 (C-5), 76.1 (C-4), 80.4 ($CO_2C(CH_3)_3$), 110.0 (Me_2C), 127.2 (C_{para} Ph), 128.5 (C_{ortho} SO_2Ph), 128.9 (C_{meta} Ph), 129.6 (C_{meta} SO_2Ph and C_{ortho} Ph), 134.2 (C_{para} SO_2Ph), 136.7 (C_{ipso} Ph), 139.7 (C_{ipso} SO_2Ph), 156.1 ($CO_2C(CH_3)_3$). MS:476 $[M+H]^+$, 420, 376, 362. HRMS: calcd for $C_{25}H_{34}NO_6S$ $[M+H]^+$: 476.2107, found: 476.2076.

4.1.6. Reaction of 11 with Na amalgam: synthesis of (1*R*, 4′*S*)-*N*-*tert*-butoxycarbonyl-1-2′2′-dimethyl [1′,3′]dioxolan-4′-yl-3-phenylpropylamine 8. A solution of **11** (53 mg, 0.11 mmol) in dry MeOH (1.5 mL) was added to Na–Hg 5% amalgam (250 mg) via cannula. The reaction mixture was left to stir for 1 h at room temperature under Ar. The residue was filtered and diluted with EtOAc. The mixture was washed with H_2O and brine, dried over anhydrous Na_2SO_4, filtered and removed the solvent. The crude product was submitted to flash silica column chromatography (hexane/EtOAc 9:1) to yield 6 mg (0.03 mmol, 24%) of **14** and 27 mg (0.08 mmol, 72%) of **8**. *Compound* **14**. $[\alpha]_D^{22}$ +31.6 (*c* 0.2, $CHCl_3$). IR: 2986, 2928, 2856, 1451, 1371, 1156, 1061, 968, 862, 665 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.38 and 1.42 (3H each, s, Me_2C), 3.40 (2H, d, J=6.8 Hz, H-1), 3.58 (1H, dd, J=7.6, 8.4 Hz, H_A-5), 4.07 (1H, dd, J= 6.4, 8.4 Hz, H_B-5), 4.48–4.53 (1H, m, H-4), 5.51 (1H, dd, J=7.6, 15.2 Hz, H-3), 5.95 (1H, dt, J=6.8, 6.8, 15.2 Hz, H-2), 7.16–7.22 (2H, m, H_{ortho} Ph), 7.26–7.31 (3H, m, H_{meta} and H_{para} Ph). ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.8 and 26.7 (Me_2C), 38.6 (C-1), 69.4 (C-5), 77.0 (C-4), 109.1 (Me_2C), 126.1 (C_{para} Ph), 128.4 (C_{meta} Ph), 128.5 (C_{ortho} Ph), 128.7 (C-2), 134.0 (C-3), 139.5 (C_{ipso} Ph). MS: 241 $[M+Na]^+$, 159, 143, 117. HRMS: calcd for $C_{14}H_{18}O_2Na$ $[M+Na]^+$: 241.1204, found: 241.1193.

4.1.7. Reaction of 12 with Na amalgam: synthesis of (1*S*, 4′*S*)-*N*-*tert*-butoxycarbonyl-1-2′2′-dimethyl [1′,3′]dioxolan-4′-yl-3-phenylpropylamine 15. A solution of **12** (43 mg, 0.09 mmol) in dry MeOH (1.5 mL) was added to Na–Hg 5% amalgam (200 mg) via cannula. The reaction mixture was left to stir for 1 h at room temperature under Ar. The residue was filtered and diluted with EtOAc. The mixture was washed with H_2O and brine, dried over anhydrous Na_2SO_4, filtered and removed the solvent. The crude product was submitted to flash silica column chromatography (hexane/EtOAc 95:5) to yield 4 mg (0.02 mmol, 22%) of **14** and 17 mg (0.05 mmol, 56%) of **15**. *Compound* **15**. $[\alpha]_D^{22}$ −21.3 (*c* 1.0, $CHCl_3$). Lit.[9] $[\alpha]_D$ −22.3 (*c* 1, $CHCl_3$). ^1H NMR (200 MHz, $CDCl_3$) δ 1.32 and 1.38 (3H each, 2s, Me_2C), 1.46 (9H, s, $CO_2C(CH_3)_3$), 1.54–1.73 and 1.87–2.08 (1H each, 2m, H-2), 2.55–2.85 (2H, m, H-1), 3.61–3.80 (2H, m, H-3 and H_A-5), 3.96–4.05 (2H, m, H-4 and H_B-5), 4.51 (1H, d, J=10.0 Hz, NH). 7.14–7.32 (5H, m, Ph). This compound **15** has already been described and the spectroscopic properties and rotation were coincident with those taken from literature.[9]

4.1.8. Reaction of 13 with Na amalgam: synthesis of (1*S*, 4′*S*)-*N*-*tert*-butoxycarbonyl-1-2′2′-dimethyl [1′,3′]dioxolan-4′-yl-3-phenylpropylamine 15. A solution of **13** (23 mg, 0.05 mmol) in dry MeOH (1.5 mL) was added to Na–Hg 5% amalgam (150 mg) via cannula. The reaction mixture was left to stir for 1 h at room temperature under Ar. The residue was filtered and diluted with EtOAc. The mixture was washed with H_2O and brine, dried over anhydrous Na_2SO_4, filtered and removed the solvent. The crude product was submitted to flash silica column chromatography (hexane/EtOAc 95:5) to yield 3.3 mg (0.015 mmol, 31%) of **14** and 6 mg (0.02 mmol, 35%) of **15**.

4.1.9. Reaction of 16 with imine 1. *n*-BuLi 1.6 M (1.4 mL, 2.24 mmol) was added to a solution of **16** (500 mg, 2.05 mmol) in THF (10 mL) at −78 °C under Ar atmosphere. After 15 min, imine **1** (685 mg, 3.12 mmol) was added to the reaction flask via cannula as a solution in THF (15 mL). The reaction mixture was left to stir for 7 h at −78→0 °C under Ar before addition of saturated ammonium chloride solution (4 mL). The product was extracted into EtOAc (3×). The organic extracts were combined, washed with brine, dried over anhydrous Na_2SO_4, filtered and removed the solvent in vacuo. The resultant oil was submitted to flash silica column chromatography (hexane/EtOAc, 95:5) to yield 255 mg (0.55 mmol, 27%) of **17**, 142 mg (0.31 mmol, 15%) of the mixture **17** and **20**, 254 mg (0.68 mmol, 33%) of **18** and 139 mg (0.37 mmol, 18%) of **19**. *Compound* **17**. $[\alpha]_D^{22}$ −85.1 (*c* 1.0, $CHCl_3$). IR: 3063, 2986, 2936, 2876, 1643, 1447, 1381, 1306, 1217, 1148, 1071, 841, 746, 692 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 1.13 and 1.32 (3H each, 2s, Me_2C), 3.27–3.60 (5H, m, H-1, H-2 and H-5), 3.68–3.78 (1H, m, H-4), 4.08 (1H, d, J=8.2 Hz, H-3), 7.23–7.26, 7.37–7.49, 7.68–7.72 and 7.79–7.83 (15H, 4m, Ar), 8.22 (1H, s, N=C*H*Ph). ^{13}C NMR (50 MHz, $CDCl_3$) δ 25.6 and 26.8 (Me_2C), 31.0 (C-1), 66.4 (C-5), 69.4 (C-2), 70.3 (C-3), 76.4 (C-4), 110.0 (Me_2C), 127.1 (C_{para} Ph), 128.6 (C_{ortho}

SO_2Ph), 128.9 (C_{ortho} and C_{meta} Ph), 129.2 (C_{meta} SO_2Ph), 129.4 (C_{ortho} and C_{meta} N=CHPh), 131.2 (C_{para} N=CHPh), 133.8 (C_{para} SO_2Ph), 136.2 (C_{ipso} N=CHPh), 138.3 (C_{ipso} Ph), 138.5 (C_{ipso} SO_2Ph), 164.6 (N=*C*HPh). MS: *m*/*z* (%) 464 (5) $[M+H]^+$, 89 (30). HRMS: calcd for $C_{27}H_{30}NO_4S$ $[M+H]^+$: 464.1896, found: 464.1902.

Compound **18**. $[\alpha]_D^{22}$ −3.7 (*c* 1.4, $CHCl_3$). IR: 3395, 2986, 2936, 2880, 1674, 1497, 1447, 1371, 1304, 1215, 1148, 1067, 914, 845, 743, 691 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.22 and 1.31 (3H each, 2s, Me_2C), 2.11 (2H, br s, NH_2), 3.15 (1H, dd, $J=5.2$, 14.8 Hz, H_A-1), 3.23–3.27 (1H, m, H-2), 3.41 (1H, dd, $J=6.8$, 14.8 Hz, H_B-1), 3.49 (1H, dd, $J=6.8$, 7.6 Hz, H_A-5), 3.60–3.63 (2H, m, H_B-5 and H-3), 3.79 (1H, q, $J=6.8$ Hz, H-4), 7.02–7.04 (2H, m, H_{ortho} Ph), 7.13–7.20 (3H, m, H_{meta} and H_{para} Ph), 7.49–7.55 (2H, m, H_{meta} SO_2Ph), 7.60–7.64 (1H, m, H_{para} SO_2Ph), 7.84–7.86 (2H, m, H_{ortho} SO_2Ph). ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.3 and 26.4 (Me_2C), 28.6 (C-1), 51.9 (C-3), 66.5 (C-5), 68.9 (C-2), 77.9 (C-4), 109.7 (Me_2C), 126.7 (C_{para} Ph), 128.6 (C_{ortho} SO_2Ph and C_{meta} Ph), 128.7 (C_{ortho} Ph), 129.3 (C_{meta} SO_2Ph), 133.8 (C_{para} SO_2Ph), 138.0 (C_{ipso} SO_2Ph), 138.2 (C_{ipso} Ph). MS: *m*/*z* 376 (75) $[M+H]^+$, 318 (15), 274 (20), 154 (30), 91 (100). HRMS: calcd for $C_{20}H_{26}NO_4$ $[M+H]^+$: 376.1583, found: 376.1560.

Compound **19**. $[\alpha]_D^{22}$ −8.9 (*c* 1.2, $CHCl_3$). IR: 3399, 2986, 2936, 2886, 1497, 1449, 1371, 1304, 1254, 1215, 1146, 1067, 849, 731, 691 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 1.32 (6H, s, Me_2C), 2.17 (2H, br s, NH_2), 3.00 (1H, dd, $J=$ 4.8, 14.4 Hz, H_A-1), 3.28 (1H, dd, $J=8.8$, 14.4 Hz, H_B-1), 3.28–3.31 (2H, m, H-2 and H-3), 3.50 (1H, dd, $J=7.2$, 8.0 Hz, H_A-5), 4.09 (1H, dd, $J=6.4$, 8.0 Hz, H_B-5), 4.64–4.73 (1H, m, H-4), 6.95-7.02 (2H, m, H_{ortho} Ph), 7.12–7.19 (3H, m, H_{meta} and H_{para} Ph), 7.46–7.66 (3H, m, H_{meta} and H_{para} SO_2Ph), 7.82–7.87 (2H, m, H_{ortho} SO_2Ph). ^{13}C NMR (50 MHz, $CDCl_3$) δ 25.4 and 26.7 (Me_2C), 32.5 (C-1), 53.3 (C-3), 67.0 (C-5), 71.5 (C-2), 76.5 (C-4), 109.6 (Me_2C), 127.1 (C_{para} Ph), 128.7 (C_{ortho} SO_2Ph), 128.8 (C_{meta} Ph), 129.0 (C_{ortho} Ph), 129.4 (C_{meta} SO_2Ph), 134.0 (C_{para} SO_2Ph), 137.3 (C_{ipso} Ph), 139.3 (C_{ipso} SO_2Ph). MS: *m*/*z* (%) 376 (30) $[M+H]^+$, 274 (10), 154 (100), 91 (45). HRMS: calcd for $C_{20}H_{26}NO_4S$ $[M+H]^+$: 376.1583, found: 376.1578.

4.1.10. *N-tert*-butoxycarbonyl-1-2′2′-dimethyl-[1′,3′]-dioxolan-4′-yl-3-phenyl-2-(phenylsulfonyl)-propyl-amines 21 and 11. To a solution of **18** (250 mg, 0.67 mmol) in THF (2.5 mL) was added Boc_2O (230 mg, 1.05 mmol) as a solution in THF (2 mL). The mixture was left to stir for 20 h before addition of 5% $NaHCO_3$ solution (2 mL). The product was extracted into EtOAc (3×). The organic extracts were combined, washed with brine, dried over anhydrous Na_2SO_4, filtered and removed the solvent in vacuo. The resultant oil was purified by flash silica column chromatography (hexane/EtOAc, 9:1) to yield 249 mg (0.52 mmol, 78%) of **21**. $[\alpha]_D^{22}$ −16.9 (*c* 1.3, $CHCl_3$). IR: 3443, 3375, 2981, 2932, 1716, 1497, 1369, 1306, 1247, 1150, 1072, 748, 691, 666 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.26 and 1.28 (3H each, 2s, Me_2C), 1.43 (9H, s, $CO_2C(CH_3)_3$), 2.94 (1H, dd, $J=7.6$, 14.8 Hz, H_A-1), 3.21 (1H, dd, $J=4.0$, 14.8 Hz, H_B-1), 3.60 (1H, dd, $J=6.8$, 7.6 Hz, H_A-5), 3.71–3.74 (1H, m, H-2), 3.99 (1H, t, $J=$ 7.6 Hz, H_B-5), 4.42–4.48 (2H, m, H-3 and H-4), 4.73 (1H, d, $J=10.0$ Hz, NH), 7.04–7.07 (2H, m, H_{ortho} Ph), 7.12–7.22 (3H, m, H_{meta} and H_{para} Ph), 7.49–7.53 (2H, m, H_{meta} SO_2Ph), 7.59–7.63 (1H, m, H_{para} SO_2Ph), 7.89–7.91 (2H, m, H_{ortho} SO_2Ph). ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.8 and 25.9 (Me_2C), 28.2 ($CO_2C(CH_3)_3$), 32.1 (C-1), 49.9 (C-3), 66.4 (C-5), 67.7 (C-2) 76.9 (C-4), 79.9 ($CO_2C(CH_3)_3$), 109.4 (Me_2C), 126.6 (C_{para} Ph), 128.5 (C_{ortho} SO_2Ph, C_{meta} Ph and C_{ortho} Ph), 129.1 (C_{meta} SO_2Ph), 133.7 (C_{para} SO_2Ph), 137.7 (C_{ipso} Ph), 138.2 (C_{ipso} SO_2Ph), 155.5 ($CO_2C(CH_3)_3$). MS: *m*/*z* (%) 498 (20) $[M+Na]^+$, 476 (5) $[M+H]^+$, 420 (10), 362 (70), 154 (30), 91 (50). HRMS: calcd for $C_{25}H_{34}NO_6S$ $[M+H]^+$: 476.2107, found: 476.2117.

To a solution of **19** (114 mg, 0.30 mmol) in THF (1.5 mL) was added Boc_2O (110 mg, 0.50 mmol) as a solution in THF (1.5 mL). The mixture was left to stir for 15 h before addition of 5% $NaHCO_3$ solution (1 mL). The product was extracted into EtOAc (3×). The organic extracts were combined, washed with brine, dried over anhydrous Na_2SO_4, filtered and removed the solvent in vacuo. The resultant oil was purified by flash silica column chromatography (hexane/EtOAc, 9:1) to yield 114 mg (0.24 mmol, 80%) of **11**.

4.1.11. Reaction of 21 with Na amalgam: synthesis of 8. A solution of **21** (205 mg, 0.43 mmol) in dry MeOH (3 mL) was added to Na–Hg 5% amalgam (600 mg) via cannula. The reaction mixture was left to stir for 30 min at room temperature under Ar. The residue was filtered and diluted with EtOAc. The mixture was washed with H_2O and brine, dried over anhydrous Na_2SO_4, filtered and removed the solvent. The crude product was submitted to flash silica column chromatography (hexane/EtOAc 95:5) to yield 20 mg (0.09 mmol, 21%) of **14** and 94 mg (0.28 mmol, 65%) of **8**.

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Biomimetic approach to *Galbulimina* type I alkaloids

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Abstract—On treatment with trifluoroacetic acid the tetraene precursor **23** underwent Boc deprotection, condensation and an iminium ion accelerated intramolecular Diels–Alder cycloaddition resulting in an iminium species **12**, which was further converted into himbacine **1**, himbeline **3** and himandravine **4**, three out of four *Galbulimina* type I alkaloids thus providing strong evidence for the proposed biogenesis of this important family of alkaloids.
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1. Introduction

As early as 1948 Webb discovered that the bark of relic trees belonging to the *Galbulimina baccata* genus and found in New Guinea and North Queensland (Australia) reacted strongly to alkaloid tests and this was subsequently verified by Ritchie 7 years later when he isolated nine novel alkaloids from the same bark.[1] So far 28 *Galbulimina* alkaloids have been isolated and they appear to fall into four classes based upon their structures.[2] Class I consists of four tetracyclic lactones as shown in Figure 1.

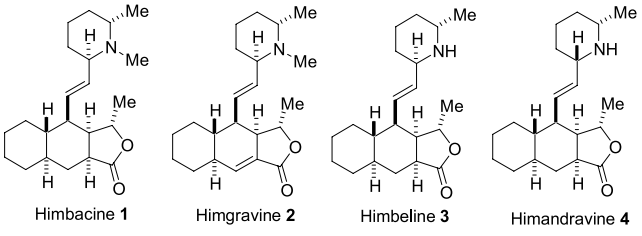
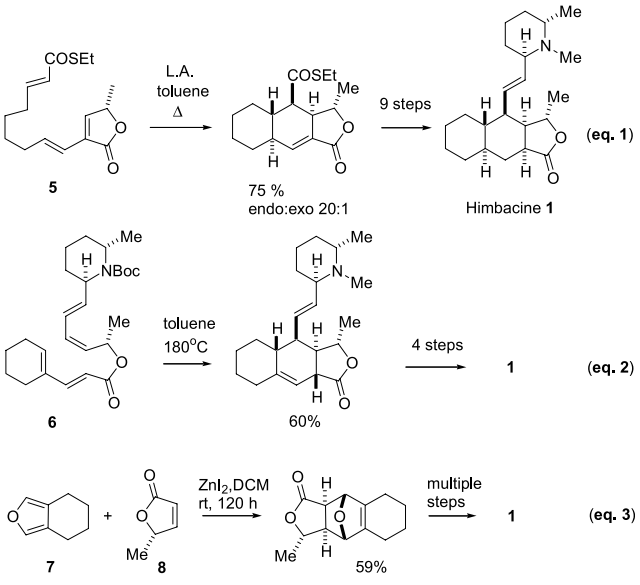


Figure 1. Class I *Galbulimina* alkaloids.

Himbacine **1**, being the major representative of the family, was the first to have it's structure identified.[3] It was originally shown to exhibit anti-spasmodic activity with low toxicity and few side effects.[4] In the 1990s though, himbacine has been shown to be a selective muscarinic antagonist and thus a potential new lead in the treatment of Alzheimer's disease.[5] Mostly due to the latter discovery himbacine has attracted significant synthetic attention.[6–9]

Three successful total syntheses of himbacine have been reported, each having similar features in common. A Diels–Alder cycloaddition was employed to construct the decalin fragment and all of the authors approached himbacine **1** via methylation of the piperidine nitrogen of himbeline **3**. Hart has synthesised the tricyclic fragment via a highly stereoselective Lewis acid catalysed cycloaddition of thioester **5** (Scheme 1, Eq. 1). Completion of the tetracycle



Scheme 1. Previous successful syntheses of himbacine **1**.

Keywords: Biomimetic synthesis; *Galbulimina* alkaloids; Himbacine; Himandravune; Himbeline.
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doi:10.1016/j.tet.2005.09.050

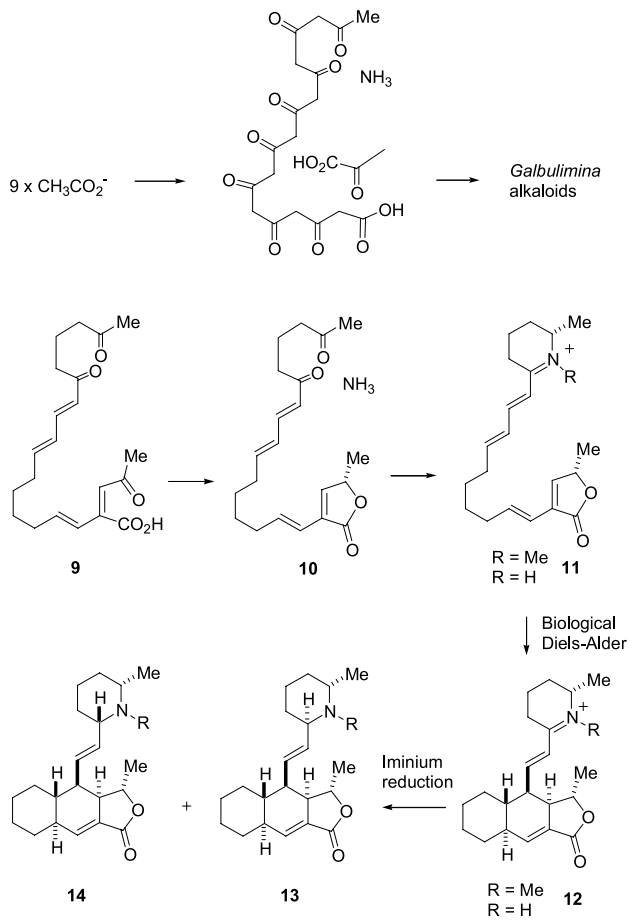
required nine extra synthetic steps[7] including a forced reduction, protection and further reoxidation of the lactone.

In what is considered as the most efficient published total synthesis of himbacine **1**, Chackalamannil performed a reverse electron demand Diels–Alder of teraene **6** (Scheme 1 Eq. 2). The *exo*-selective cycloaddition resulted in a compound with opposite stereochemistry at the centre α to the lactone to the one required for the total synthesis. Epimerization was achieved on treatment of the tetracycle with DBU in toluene and the total synthesis was accomplished in four further synthetic steps.[8] Terashima's synthesis has employed an intermolecular Diels–Alder of furan derivative **7** and butenolide **8** (Scheme 1 Eq. 3). Completion of the total synthesis required over 15 synthetic steps, although a more modular approach allowed synthesis of multiple unnatural analogues.[9] Remarkably, only Chackalamannil has synthesized himandravine **4** in a later synthetic effort.[10]

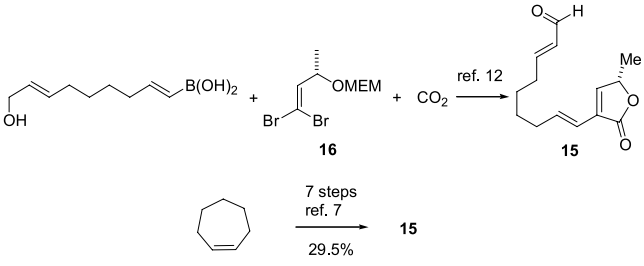
Our interest in the *Galbulimina* alkaloids dates back to the late 1980s when we decided to establish a general biomimetic approach to all members of the class I alkaloids.[11] The proposed approach should also be applicable, with modification, to the synthesis of more structurally complex class II and class III alkaloids.

2. Biomimetic approach

Ritchie speculated that nine C_2 acetate units, one C_3 pyruvate unit and a molecule of NH_3 were used to construct the alkaloids (Scheme 2),[12] without being specific on the particular biotransformations involved. Our biosynthetic pathway for the *Galbulimina* class I alkaloids[13] postulates ketide **9** formation from the same nine acetates and a pyruvate via standard polyketide biosynthesis. Reductive lactonisation would result in butenolide **10**, which on reductive amination followed by iminium ion formation via *N*-methylation or N-protonation would provide the Diels–Alder precursor **11**. Intramolecular Diels–Alder cycloaddition via an *endo* transition state would afford tetracycle **12**. Finally, hydride reduction of the iminium from either the α or β face would furnish either the himbacine (*trans*-piperidine ring) precursor **13** or the himandravine (*cis*-piperidine ring) precursor **14** (Scheme 2). Our primary goal was to establish the possibility of a biological iminium catalysed Diels–Alder reaction leading to the tricyclic lactone core of these alkaloids.



Scheme 2. Postulated biogenesis of class I alkaloids.



Scheme 3. Approaches to aldehyde **15**.

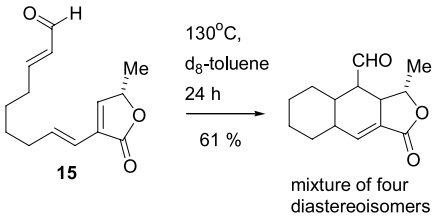
Our initial approach to aldehyde **15** is outlined in Scheme 3 and was based on a Suzuki reaction of the *trans*-bromide followed by carboxylation of the cis-position of the dibromo-alkene **16**.[13] Later, we adopted a more elegant approach by Hart[7] and accessed **15** from cycloheptene in seven synthetic steps and 29.5% overall yield.

3. Results and discussion

3.1. Gassman Diels–Alder

It was our expectation, that the Diels–Alder type reaction of butenolides like **10** would require strong activation of the dienophile. Aldehydes are known to be among the best substituents for such activation, as their LUMO energy lowering effect can be dramatically enhanced via formation of oxacarbenium species on treatment with Lewis Acids. Thus, we chose the aldehyde **15** (Scheme 3) as our initial model to study the possibility of a biological Diels–Alder.

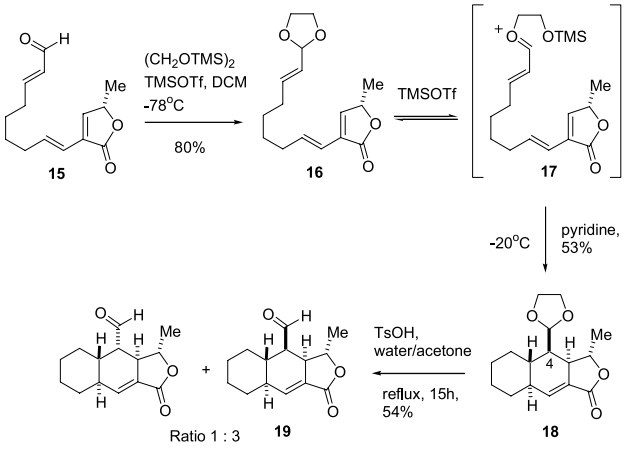
First, we attempted a thermal cycloaddition of aldehyde **15** (Scheme 4). A solution of the enal **15** in d_8-toluene was heated in the presence of 0.2 equiv of the radical inhibitor, 3-*tert*-butyl-4-hydroxy-5-methylphenyl sulphide, at 130 °C in a sealed tube for 24 h. Pleasingly an IMDA process occurred and the cycloaddition product appeared to consist of a 2.5:2.3:1.0:0.25 mixture of diastereoisomers. The isolated yield after column chromatography was a reasonable 61%, although the diastereoisomers could not be separated.



Scheme 4. Thermal cycloaddition of enal **15**.

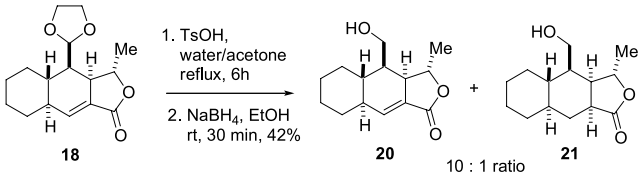
Attempts to accelerate the Diels–Alder by use of Me_2AlCl or $SnCl_4$ were unsuccessful. This was attributed to the more likely coordination of the Lewis Acids to the more basic carbonyl of the butenolide, rather then the enal.

Next, our attention was drawn to Gassman's findings indicating that acrolein acetals in the presence of Lewis or protic acids form oxonium ions, which undergo accelerated IMDA cycloadditions with a range of dienes at low temperatures.[14] At first we attempted to form a cyclic acetal of the enal **15**. The Noyori procedure involves low temperature conditions using 1,2-bis(trimethylsilyloxy)-ethane as the acetal source and TMSOTf as catalyst.[15] Application of these conditions to the enal **15** at −78 °C afforded the acetal **16**, which could be isolated if the reaction was quenched by addition of pyridine. However, if the reaction was allowed to warm to −20 °C, the presence of TMSOTf, acting as a Lewis Acid, promoted a Gassman intramolecular Diels–Alder reaction presumably via the formation of an oxonium ion intermediate **17**. Pleasingly, tricyclic acetal **18** was isolated in a 53% yield with a 40:1 ratio of diastereomers (Scheme 5).



Scheme 5. Gassman Diels–Alder.

At first we expected the minor cycloadduct to be an *exo*-pathway product, but our first attempt to hydrolyse the acetal **18** led to isolation of the aldehyde **19** as a 3:1 mixture of epimers. Epimerisation under the hydrolysis conditions could only have occurred at C4 and this suggested that the Gassman Diels–Alder produced only *endo*-cycloaddition product with some minor epimerization at C4 position occurring under the reaction conditions. Our next aim was to obtain unambiguous proof of the stereochemistry of the cycloadduct. A briefer aqueous hydrolysis and an in situ $NaBH_4$ reduction of the aldehyde **19** avoided epimerization at C4 position in the alcohol **20**. Quite unexpectedly 1,4-addition of the hydride was competitive under the same conditions resulting in formation of a minor saturated tricycle **21** (Scheme 6).



Scheme 6. Synthesis of alcohol **20**.

The alcohol **20** is a white crystalline solid and we expected to obtain crystals of the major component on attempted crystallization of the mixture of **20** and **21**. To our surprise the single crystal that was chosen for X-ray analysis contained the minor component **21**. Gratifyingly, the diffraction analysis data[16] (Fig. 2) confirmed that the stereochemistry of the tricyclic core of **21** is the same as that of himbacine **1**.

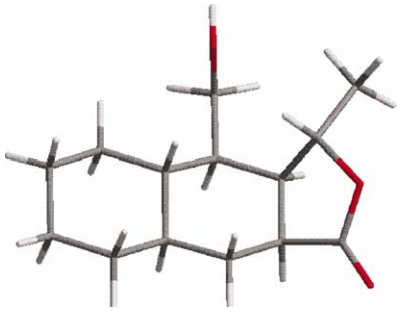


Figure 2. Chem 3D plot of the X-ray of **21**.

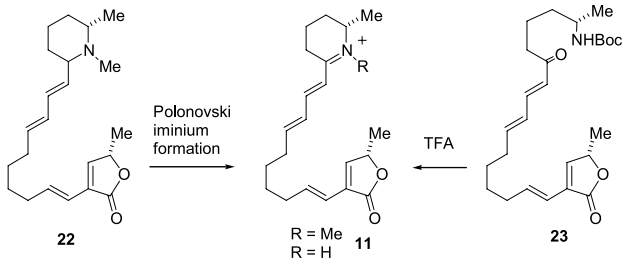
Having obtained proof that with sufficient dienophile activation, the intramolecular Diels–Alder reaction of a butenolide like **15** gives *endo*-cyclisation products with high level of stereocontrol, we moved on to investigate the proposed biomimetic cycloaddition of iminium species like **11**.

3.2. Biomimetic Diels–Alder reaction

Two possible synthetic pathways towards the iminium **11** were investigated. The first one centered on a Polonovski[17] generation of **11** (R=Me) from the piperidine species **22**. The second envisaged formation of the iminium species **11** (R=H) on acid catalysed cleavage of the *N*-Boc protecting group and condensation of the tethered amine in an open chain precursor **23** (Scheme 7).

Our multiple attempts to synthesise himgravine **2** type adducts in Polonovski-type oxidations of the piperidine compound **21** were unsuccessful[18] and will not be discussed further in this publication. On the other hand, positive results were obtained in the investigation of the alternative condensation pathway.

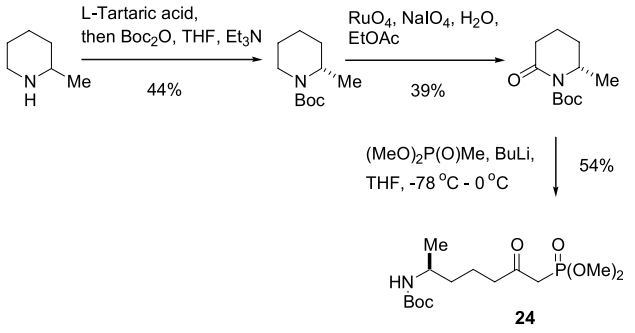
It was expected that the cyclisation precursor **23** would be accessed via a Wittig type olefination of aldehyde **15** and phosphonate **24**.



Scheme 7. Approaches to iminium species **11**.

The synthesis of the chiral phosphonate **24** was accomplished in three steps. 2-Methyl piperidine was resolved via crystallization with L-tartaric acid,[19] which was followed by Boc protection and oxidation.[20] Treatment of the piperidinone with a small excess of the lithiated dimethyl methylphosphonate produced the desired Horner–Emmons reagent **24** in 54% yield after column chromatography (Scheme 8).

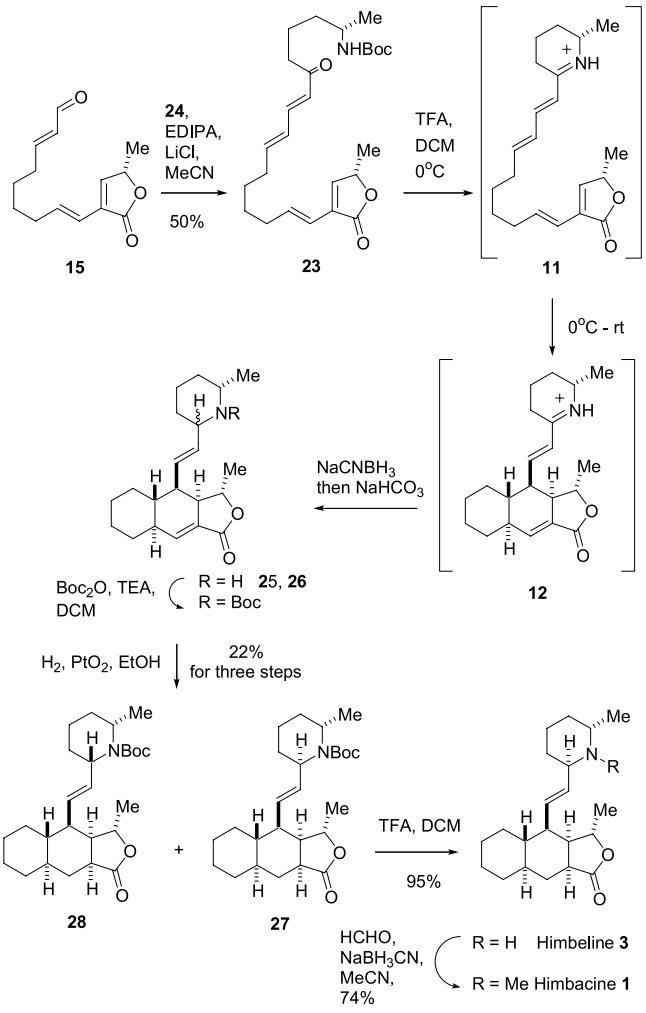
Reaction of the phosphonate **24** with the aldehyde **15**



Scheme 8. Synthesis of phosphonate **23**.

employing the modified Masamune–Roush conditions[21] produced the tetraene **23**, which was treated with trifluoroacetic acid at 0 °C in dichloromethane effecting Boc cleavage and condensation to the desired iminium species **11**. The reaction mixture was then allowed to warm up slowly to room temperature and stirred for an additional hour then quenched by addition of an excess of sodium cyanoborohydride almost immediately followed by addition of saturated aqueous sodium bicarbonate. At this point ^{1}H NMR analysis of the crude reaction mixture showed complete disappearance of resonances corresponding to the starting material along with the appearance of two characteristic peaks at δ 6.62 and 6.69 ppm, corresponding to the resonances of the protons of the α,β unsaturated double bond of two epimeric products **25** and **26** derived from consecutive *N*-Boc deprotection, condensation, IMDA cycloaddition and iminium ion reduction (Scheme 9). The reduction proved to be non-facial selective and both β-hydrogen peaks had identical integration in the 500 MHz ^{1}H NMR.

We found that direct separation of the diastereomeric mixture of **25** and **26** from the complex crude reaction mixture was impossible and required *N*-Boc protection

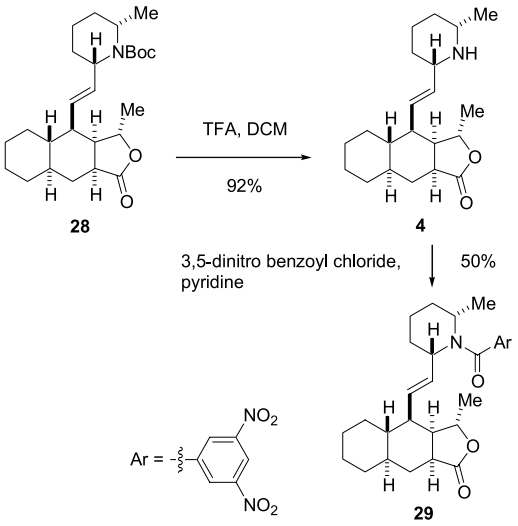


Scheme 9. Completeion of synthesis.

followed by a highly selective hydrogeneation of the trisubstituted double bond over Adam's catalyst[3] in order to separate the *N*-Boc protected himbeline **27** and himandravine **28** derivatives. Boc deprotection of **27** yielded synthetic himbeline **3**, which was *N*-methylated following literature method[7] to give synthetic himbacine **1**, whose structure was confirmed via ^{1}H NMR in a doping experiment with an authentic sample of the natural product.[22]

N-Boc deprotection of **28** led to isolation of synthetic himandravine **4** (Scheme 10). In absence of a natural product sample to run a doping experiment, it was decided to establish the structure via X-ray analysis. Thus, **4** was converted into a dinitro-benzoate derivative **29**. The results of the single crystal X-ray analysis of **29** are presented in Figure 3.

In summary, we have demonstrated a single step biomimetic transformation of **23** into a tetracyclic iminium species **12**, which was further transformed into himbeline **3**, himbacine **1** and himandravine **4**. We believe this proceeds via a consecutive *N*-Boc deprotection, condensation and iminium ion activated intramolecular Diels–Alder cycloaddition process. This provides strong support for our proposed biogenesis of these class 1 *Galbulimina* alkaloids.



Scheme 10. Synthesis of himandravine derivative **29**.

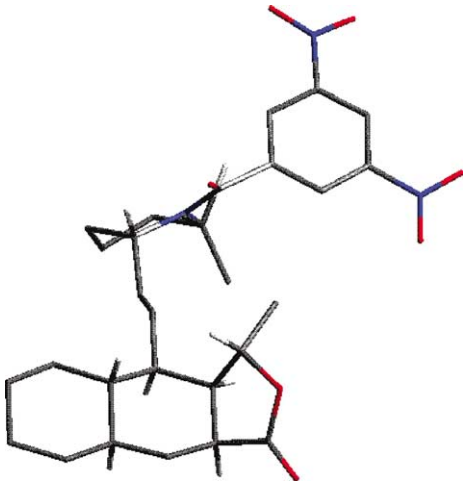


Figure 3. Chem 3D plot of the X-ray structure of **29**.

3.3. A new hypothesis

It was our original intent to propose a more complete biogenetic pathway analysis, which would be applicable not only to the class I but to other classes of *Galbulimina* alkaloids as well. Himbosine **30** is a representative of the highly oxygenated hexacyclic class II alkaloids. Similarities between alkaloids of the two classes is indicated by numbering in Figure 4.

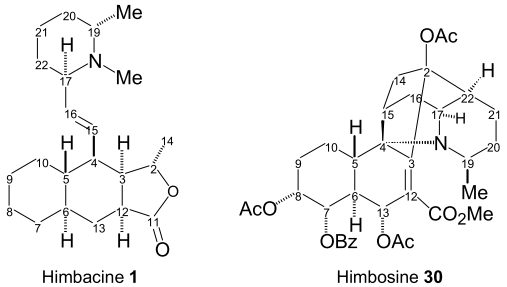
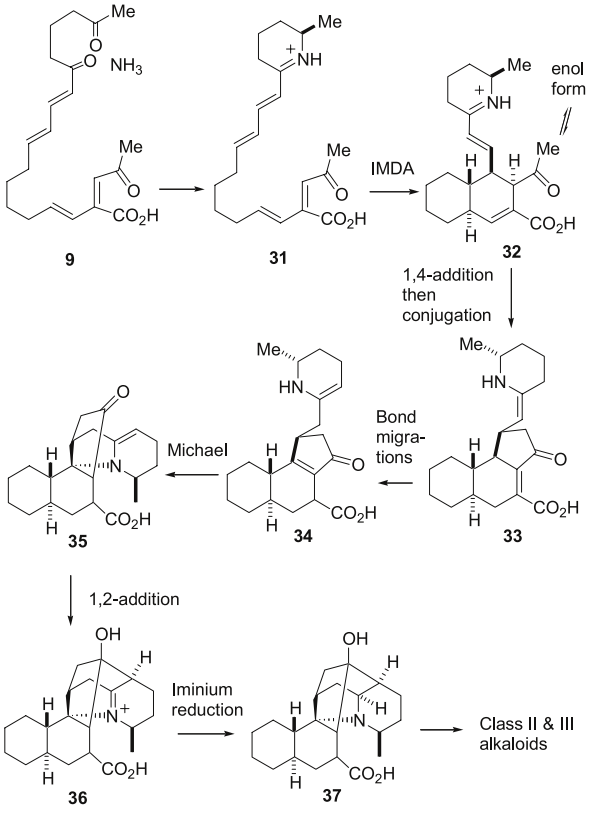


Figure 4. Class I and II *Galbulimina* alkaloids.

Analysis of both structures shows that the class II alkaloids have extra carbon–carbon bonds between C22 and C2, C14 and C15 and that the piperidine nitrogen becomes connected to C4. Extra oxygenation of C8, C7, and C13 of the decalin unit could be attributed to later metabolic oxidation steps.

Our successful biomimetic synthesis of the class I alkaloids (notably both major alkaloids himbacine **1** and himandravine **2** were synthesized in the same synthetic route) employing an iminium ion activated intramolecular Diels–Alder reaction allows us to postulate that similar chemistry would be involved in the biosynthesis of the class II and III alkaloids. Our proposed biosynthetic pathway is presented in Scheme 11. The scheme starts with the same polyketide **9**, which would not undergo a reductive lactonisation, but would directly give the iminium **31** on condensation with ammonia. **31** is set for an iminium ion activated intramolecular Diels–Alder reaction producing the tricyclic **32**. The iminium activates the exocyclic double bond as an acceptor and the tautomerised enolic form of the ketone would add in a conjugate fashion to give the C14–C15 carbon–carbon bond. Further tautomerisation of the enamine **33** accompanied by double bond migration into **34** would be followed by Michael addition to give the *N*–C4 connectivity in **35**. 1,2-Addition of the enamine would accomplish the C2–C22 bond in polycycle **36**, which on iminium reduction would give **37**, a possible intermediate in the biosynthesis of class II and III *Galbulimina* alkaloids.



Scheme 11. Postulated biogenesis of class II and III alkaloids.

4. Experimental

4.1. General

4.1.1. Preparation of ketal 18. To a solution of enal[7] **15** (88.9 mg, 0.38 mmol) in dichloromethane (6 ml) cooled to −78 °C under an atmosphere of argon was added 1,2-bis-(trimethylsiloxy)-ethane (235 mg, 1.14 mmol). This solution was stirred at −78 °C for 15 min at which stage trimethylsilyltrifluoromethylsulphonate (84.4 mg, 0.38 mmol) was added dropwise. The resultant bright yellow solution was stirred at −78 °C for a further 3 h, at which stage the temperature of the reaction mixture was rapidly increased to −20 °C. The reaction mixture was stirred for 2 h between −30 and −20 °C during which time the colour of the reaction mixture darkened considerably. Excess pyridine (0.5 ml) was added discharging the dark colour. The reaction mixture was then warmed to room temperature, diluted with dichloromethane (20 ml) and poured into saturated sodium hydrogen carbonate (30 ml). The layers were separated and the aqueous layer extracted with dichloromethane (2×20 ml). The organics were combined, dried over magnesium sulphate, filtered and volatiles removed in vacuo. The obtained yellow oil was purified by flash chromatography (diethyl ether/pet. ether 4:7) to yield the product **18** as a colourless oil (56 mg, 53%); $[\alpha]_D^{22}$ +54.3 (*c* 1, $CHCl_3$); *m/z* (CI^+) found 279.1596, $C_{16}H_{22}O_4+H^+$ requires 279.1596; ν_{max}/cm^{-1} (film) 2854 (m), 1757 (s), 1683 (m), 1408 (m), 1226 (m), 979 (m); δ_H (500 MHz, $CDCl_3$) 1.12 (1H, m), 1.16 (1H, m), 1.26–1.46 (3H, m), 1.47 (3H, d, *J*=6.0 Hz), 1.72 (1H, m), 1.75–1.80 (2H, m), 2.05–2.11 (2H, m), 2.15 (1H, ddd, *J*=9.5, 7.5, 4.0 Hz), 2.61 (1H, ddd, *J*=9.5, 7.5, 3.0 Hz), 3.81–3.83 (2H, m), 3.94–3.96 (2H, m), 4.78 (1H, d, *J*=4.0 Hz), 4.94 (1H, dq, *J*=7.5, 6.0 Hz), 6.71 (1H, dd, *J*=3.5, 3.0 Hz); δ_c (125 MHz, $CDCl_3$) 21.84, 26.12, 26.58, 32.24, 33.86, 41.28, 41.35, 43.51, 44.31, 64.37, 64.62, 77.47, 105.1, 131.4, 141.2, 169.3.

4.1.2. Preparation of alcohol 20. To a solution of ketal **18** (56 mg, 0.2 mmol) in acetone–water (2/1, 9 ml) was added *para*-toluenesulfonic acid (114 mg, 0.6 mmol). The solution was brought to a gentle reflux which was maintained for 6 h. The reaction mixture was then cooled, diluted with dichloromethane (50 ml) and washed with water (25 ml). The aqueous portion was extracted with dichloromethane (25 ml), the organics combined, dried over magnesium sulphate, filtered and the volatiles removed in vacuo. The crude mixture was dissolved in ethanol (7 ml) and the solution was cooled to 0 °C. To this rapidly stirred solution was added sodium borohydride (15 mg, 0.4 mmol). The reaction mixture was then stirred at room temperature for 20 min at which stage excess sodium borohydride (30 mg, 0.8 mmol) was added and the reaction mixture was stirred for a further 10 min. The reaction was quenched by careful addition of acetone (6 ml) over a period of 2 min and then poured into water (20 ml) and diluted with dichloromethane (20 ml). Dilute hydrochloric acid (1 M, 3 ml) was added to the biphasic mixture, the layers were separated and the aqueous portion was extracted with dichloromethane (20 ml). The organics were combined, dried over magnesium sulphate, filtered and the volatiles removed in vacuo. The obtained yellow oil was purified by flash chromatography (diethyl ether/pet. ether 2:3) giving the product **20** as a white crystalline solid (20 mg, 42%); mp 149–151.5 °C; $[\alpha]_D^{22}$ +52.5 (*c* 0.4, $CHCl_3$); *m/z* (CI^+) found 254.1756, $C_{14}H_{20}O_3+NH_4^+$ requires 254.1756; ν_{max}/cm^{-1} (KBr) 3435 (br s), 2839 (s), 1735 (s), 1630 (m), 1442 (m), 1380 (m); δ_H (500 MHz, $CDCl_3$) 0.87 (1H, m), 1.13 (1H, m), 1.25–1.39 (3H, m), 1.56 (3H, d, *J*=6.0 Hz), 1.76–1.84 (3H, m), 1.90 (1H, ddt, *J*=9.5, 7.0, 6.5 Hz), 1.97 (1H, m), 2.07 (1H, m), 2.70 (1H, ddd, *J*=12.5, 7.0, 3.5 Hz), 3.73 (2H, d, *J*=6.5 Hz) 4.80 (1H, dq, *J*=12.5, 6.0 Hz), 6.65 (1H, dd, *J*=3.5, 3.0 Hz); δ_c (125 MHz, $CDCl_3$) 21.62, 26.04, 26.44, 31.79, 32.43, 40.56, 40.83, 42.99, 45.76, 61.68, 78.28, 131.4, 140.9, 169.5.

4.1.3. Preparation of phosphonate 24. To a stirred solution of dimethyl methylphosphonate (1.86 g, 15 mmol) in THF (50 ml) was added *n*-butyl lithium (1.6 M solution in hexanes, 10 ml) dropwise at −78 °C. The reaction mixture was stirred for an hour and (2*S*)-methyl-*N*-Boc-piperidinone[20] (2.13 g, 10 mmol) was added. Reaction mixture was allowed to warm to room temperature and stiring was continued for an additional hour. Water (20 ml) was added and the resulting mixture was poured into a separating funnel containing ethyl acetate (100 ml) and water (100 ml). Layers were separated and the aqueous layer was extracted with ethyl acetate (2×25 ml). Combined organics were dried ($MgSO_4$), filtered and volatiles removed in vacuo. Flash chromatography (short column, EtOAc with 2.5% MeOH) yielded the title compound as a colourless oil (1.86 g, 54%); $[\alpha]_D^{22}$ −2.9 (*c* 1.0, $CHCl_3$); *m/z* (CI^+) found 337.1661, $C_{14}H_{28}NO_6P+H^+$ requires 337.1654; ν_{max}/cm^{-1} (film) 3318 (br m), 2972 (s), 1710 (s), 1690 (s), 1253 (m), 1174 (m), 1033 (s); δ_P (125 MHz, $CDCl_3$) 23.91; δ_H (500 MHz, $CDCl_3$) 1.09 (3H, d, *J*=6.5 Hz), 1.31–1.46 (2H, m), 1.39 (9H, s), 1.59 (2H, m), 2.62 (2H, m), 3.07 (2H, d, *J*=22.5 Hz), 3.59 (1H, m), 3.73 (3H, s), 3.79 (3H, s), 4.39 (1H, br s); δ_c (125 MHz, $CDCl_3$) 19.63, 21.08, 26.28, 36.11, 40.63, 41.66, 43.59, 45.98, 52.85, 78.85, 155.29, 201.48.

4.1.4. Preparation of tetraene 23. A mixture of aldehyde **15** (270 mg, 1.15 mmol), phosphonate **24** (656 mg, 1.94 mmol), lithium chloride (210 mg, 5 mmol) and diisopropyl ethyl amine (246 mg, 2 mmol) in acetonitrile (10 ml) was stirred for 12 h. Water (10 ml) and diethyl ether (10 ml) were added and layers were separated. Aqueous layer was extracted with diethyl ether (2×10 ml), combined organics dried ($MgSO_4$), filtered and concentrated in vacuo. Flash chromatography (petrol ether/ethyl acetate 6:1) gave the title compound **23** as a colourless oil (256 mg, 50%); $[\alpha]_D^{22}$ +48.1 (*c* 1.0, $CHCl_3$); *m/z* (CI^+) found 446.2898, $C_{26}H_{39}NO_5+H^+$ requires 446.2906; ν_{max}/cm^{-1} (film) 3320 (br m), 2971 (s), 1735 (s), 1710 (s), 1650 (s), 1630 (m), 1525 (m), 1253 (s), 1033 (m); δ_H (500 MHz, $CDCl_3$) 1.10 (3H, d, *J*=5.5 Hz), 1.36–158 (8H, m), 1.40 (9H, s), 1.43 (3H, d, *J*=6.0 Hz), 1.62 (2H, m), 2.12–2.30 (2H, m), 2.54 (2H, m), 3.62 (1H, m), 4.40 (1H, br s), 5.10 (1H, dq, *J*=2.5, 6.0 Hz), 5.84 (1H, m), 5.90 (1H, m), 6.06–6.11 (2H, m), 6.81 (1H, dt, *J*=16.0, 5.5 Hz), 7.07 (1H, m), 7.16 (1H, d, *J*=2.5 Hz); δ_c (125 MHz, $CDCl_3$) 17.9, 19.1, 20.3, 21.1, 27.6, 28.3, 28.5, 29.3, 32.0, 36.5, 39.5, 46.0, 77.4, 116.8, 122.2, 128.4, 130.3, 138.9, 146.7, 146.9, 148.9, 171.0, 172.9, 200.3.

4.1.5. Preparation of *N*-Boc-himbeline 27 and *N*-Boc-himandravine 28. To a stirred solution of tetraene **23** (120 mg, 0.27 mmol) in DCM (5 ml) at 0 °C was added trifluoroacetic acid (100 μl, 1.34 mmol), the reaction mixture was stirred for 30 min and allowed to warm to room temperature. Stirring was continued for an additional hour, when ethanol (5 ml) and sodium cyanoborohydride (100 mg, 1.6 mmol) were added. The reaction was stirred for 30 s and quenched by addition of saturated aqueous sodium bicarbonate solution (10 ml). The resulting mixture was poured into a separating funnel and diluted with ethyl acetate (20 ml). Layers were separated and the aqueous phase was extracted with ethyl acetate (2×10 ml). Combined organics were dried (Na_2SO_4), filtered and concentrated to give crude cycloaddition products as a yellow oil, which was diluted with dichloromethane (10 ml). Triethylamine (30 mg, 0.3 mmol) and di-*tert*-butyl-dicarbonate (65 mg, 0.3 mmol) were added and the resulting solution was stirred for 14 h. The volatiles were removed in vacuo and the residue was dissolved in ethanol (20 ml). To the solution was added platinum (IV) oxide (10 mg, cat.) and the resulting mixture was stirred under atmosphere of hydrogen for 12 h. Filtration through a plug of Celite, which was washed with ethyl acetate (10 ml), and concentration in vacuo afforded the crude products as a brown oil, which was purified by flash chromatography (petrol ether/ethyl acetate 6:1).

N-Boc-himbeline **27**. A colourless oil (12.4 mg, 11%); $[\alpha]_D^{22}$ +58.2 (*c* 0.5, $CHCl_3$); lit.[7] +60.6 (*c* 0.55, $CHCl_3$); *m/z* (CI^+) found 432.3111, $C_{26}H_{41}NO_4+H^+$ requires 432.3114; ν_{max}/cm^{-1} (film) 3055 (s), 1765 (s), 1670 (m); δ_H (500 MHz, $CDCl_3$) 0.69 (1H, m), 0.97 (3H, m), 1.08–1.36 (3H, m), 1.22 (3H, d, *J*=8.0 Hz), 1.40 (3H, d, *J*=6.0 Hz), 1.43 (9H, s), 1.45–2.10 (12H, m), 2.23 (1H, m), 2.61 (1H, dt, *J*=6.5, 12.5 Hz), 4.00 (1H, m), 4.42 (1H, m), 4.62 (1H, dq, *J*=10.0, 6.0 Hz), 5.21 (1H, dd, *J*=15.0, 10.0 Hz), 5.52 (1H, dd, *J*=15.0, 6.0 Hz); δ_c (125 MHz, $CDCl_3$) 13.05, 20.66, 21.92, 25.19, 25.86, 25.95, 26.10, 28.22, 30.95, 31.75, 33.40, 39.78, 41.30, 42.0, 45.41, 46.76, 48.47, 51.95, 78.85, 131.05, 133.89, 154.74, 178.21.

N-Boc-himandravine **28**. A colourless oil (12.2 mg, 11%); $[\alpha]_D^{22}$ +62.1 (*c* 0.6, $CHCl_3$); *m/z* (CI^+) found 432.3118, $C_{26}H_{41}NO_4+H^+$ requires 432.3114; ν_{max}/cm^{-1} (film) 3055 (s), 1765 (s), 1670 (m); δ_H (500 MHz, $CDCl_3$) 0.72 (1H, m), 0.99 (2H, m), 1.11 (3H, d, *J*=6.0 Hz), 1.11–1.29 (4H, m), 1.29 (3H, d, *J*=6.5 Hz), 1.43 (9H, s), 1.45–1.90 (11H, m), 2.08 (1H, m), 2.21 (1H, m), 2.60 (1H, dt, *J*=6.0, 13.0 Hz), 4.30 (1H, m), 4.61 (1H, dq, *J*=10.0, 6.0 Hz), 4.73 (1H, m), 5.31 (1H, dd, *J*=15.0, 10.5 Hz), 5.53 (1H, dd, *J*=15.0, 7.5 Hz); δ_c (125 MHz, $CDCl_3$) 14.48, 20.48, 22.25, 26.14, 26.45, 28.28, 28.50, 30.10, 31.39, 32.04, 33.60, 40.04, 41.76, 42.32, 45.65, 46.08, 48.98, 50.22, 77.05, 79.28, 131.73, 134.32, 154.82, 178.34.

4.1.6. Preparation of himbeline 3. To a solution of *N*-Boc-himbeline **27** (12.4 mg, 0.027 mmol) in dichloromethane (3 ml) was added TFA (0.3 ml) and the reaction mixture was stirred for 1 h. The volatiles were removed in vacuo and the residual oil dissolved in dichloromethane (10 ml). The solution was washed with saturated aqueous sodium bicarbonate solution (2×5 ml). The aqueous phase was extracted with dichloromethane (2×5 ml) and the combined organic phase was dried (K_2CO_3), filtered and evaporated to give the title compound **3** as an oil (9.3 mg, 95%) which required no further purification. $[\alpha]_D^{22}$ +17.5 (*c* 0.95, $CHCl_3$); lit.[3] +19 (2.4% in $CHCl_3$); *m/z* (FAB) found 332.2595, $C_{21}H_{33}NO_2+H^+$ requires 332.2590; ν_{max}/cm^{-1} (film) 3055 (s), 1765 (s), 1670 (m); δ_H (500 MHz, $CDCl_3$) 0.72 (1H, m), 1.00 (3H, m), 1.08 (3H, d, *J*=6.5 Hz), 1.10–1.30 (4H, m), 1.40 (3H, d, *J*=6.0 Hz), 1.42 (1H, m), 1.45–1.80 (10H, m), 2.09 (1H, m), 2.23 (1H, dt, *J*=10.0, 6.5 Hz), 2.62 (1H, dt, *J*=13.0, 6.0 Hz), 3.09 (1H, m), 3.53 (1H, m), 4.64 (1H, dq, *J*=10.5, 6.0 Hz), 5.24 (1H, dd, *J*=15.5, 10.5 Hz), 5.70 (1H, dd, *J*=15.5, 6.5 Hz); δ_c (125 MHz, $CDCl_3$) 19.62, 21.31, 22.22, 26.07, 26.37, 30.96, 31.27, 31.93, 32.51, 33.58, 39.88, 41.42, 42.22, 45.50, 46.28, 48.96, 53.01, 76.80, 131.46, 135.00, 178.32.

4.1.7. Preparation of himbacine 1.[7] Synthetic himbeline **3** (6.5 mg, 0.02 mmol) in acetonitrile (4 ml) was added sodium cyanoborohydride (6.5 mg, 0.1 mmol) and 37% aqueous formaldehyde solution (25 mg, 0.03 mmol). The reaction mixture was stirred at room temperature for 1 h and neutralized (pH 7) by dropwise addition of glacial acetic acid and allowed to stir for an additional 2 h. The solvents were removed in vacuo and the residue was dissolved in dichloromethane (10 ml). The solution was washed with saturated aqueous sodium bicarbonate solution (10 ml), the aqueous phase was extracted with dichloromethane (4×5 ml) and the combined organics were dried over potassium carbonate, filtered and evaporated. The crude product was purified by flash chromatography on basic alumina (petrol ether/ethyl acetate 5:1) to give the title compound **1** as an oil[23] (5.0 mg, 74%); $[\alpha]_D^{22}$ +47.5 (*c* 0.25, $CHCl_3$); lit.[7] +51.4 (*c* 1.01, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 0.74 (1H, m), 1.00 (3H, d, *J*=6.5 Hz), 0.91–1.08 (3H, m), 1.10–1.30 (3H, m), 1.40 (3H, d, *J*=6.0 Hz), 1.37–1.48 (2H, m), 1.50–1.58 (2H, m), 1.63–1.80 (6H, m), 1.87 (1H, m), 2.10 (1H, m), 2.20–2.27 (1H, m), 2.22 (3H, s), 2.62 (1H, dt, *J*=12.5, 6.5 Hz), 2.84 (1H, m), 3.02 (1H, m), 4.63 (1H, dq, *J*=10.5, 6.0 Hz), 5.26 (1H, dd, *J*=15.0, 10.0 Hz), 5.57 (1H, dd, *J*=15.0, 9.0 Hz); δ_c (125 MHz, $CDCl_3$) 13.95, 18.91, 22.19, 26.06, 26.43, 31.41, 31.98, 32.57, 33.21, 33.54, 39.83, 41.15, 41.49, 42.18, 45.67, 49.09, 53.35, 61.29, 76.77, 133.30, 133.48, 178.32.

4.1.8. Preparation of himandravine 4. To a solutiom of *N*-Boc-himandravine **28** (11.2 mg, 0.026 mmol) in dichloromethane (3 ml) was added TFA (0.3 ml) and the reaction mixture was stirred for 1 h. The volatiles were removed in vacuo and the residual oil dissolved in dichloromethane (10 ml). The solution was washed with saturated aqueous sodium bicarbonate solution (2×5 ml). The aqueous phase was extracted with dichloromethane (2×5 ml) and the combined organic phase was dried (K_2CO_3), filtered and evaporated to give the title compound **4** as an oil (7.9 mg, 92%) which required no further purification. $[\alpha]_D^{22}$ +20.5 (*c* 0.25, $CHCl_3$); lit.[3] +23 (1.9% in $CHCl_3$); *m/z* (FAB) found 332.2595, $C_{21}H_{33}NO_2+H^+$ requires 332.2590; ν_{max}/cm^{-1} (film) 3055 (s), 1765 (s), 1670 (m); δ_H (500 MHz, $CDCl_3$) 0.69 (1H, m), 0.92–1.43 (8H, m), 1.07 (3H, d, *J*=6.5 Hz), 1.42 (3H, d, *J*=6.0 Hz), 1.53–1.89 (9H, m), 2.03 (1H, m), 2.21 (1H, m), 2.59 (1H, dt, *J*=13.0, 6.5 Hz), 2.69 (1H, m), 3.11 (1H, m), 4.61 (1H, dq,

$J=10.0$, 6.0 Hz), 5.28 (1H, dd, $J=15.0$, 10.5 Hz), 5.51 (1H, dd, $J=15.0$, 6.5 Hz); δ_c (125 MHz, $CDCl_3$) 19.79, 22.52, 23.11, 24.67, 26.25, 26.55, 31.32, 32.13, 32.46, 33.79, 40.02, 41.53, 42.36, 45.40, 48.83, 52.45, 59.46, 77.50, 131.40, 136.04, 178.47.

4.1.9. Preparation of benzoate 29. To a stirred solution of 3,5-dinitro benzoyl chloride (30 mg, 0.13 mmol) in dichloromethane (5 ml) was added solution of himandravine **4** (22.3 mg, 0.067 mmol) in pyridine (5 ml) dropwise over 10 min. The resulting mixture was stirred at room temperature for 6 h and poured into water (20 ml). Organics were extracted with ethyl acetate (3×10 ml), dried ($MgSO_4$), filtered and concentrated in vacuo to give a brown oil. Flash chromatography (petrol ether/ethyl acetate 2:1) gave the product as an oil (19.3 mg, 50%). The product was obtained as white prisms on slow evaporation of a diethyl ether solution. $[\alpha]_D^{22}$ +29.9 (*c* 0.83, $CHCl_3$); mp 166–167 °C; ν_{max}/cm^{-1} (film) 2930 (m), 1772 (s), 1756 (s), 1629 (s), 1544 (s), 1418 (m), 1344 (s); δ_H (500 MHz, $CDCl_3$) 0.76 (1H, m), 0.98–1.12 (2H, m), 1.06 (3H, d, $J=$ 7.0 Hz), 1.15–1.30 (3H, m), 1.28 (3H, d, $J=6.0$ Hz), 1.34–1.47 (2H, m), 1.58–1.94 (11H, m), 2.02 (1H, m), 2.20 (1H, m), 2.27 (1H, m), 2.66 (1H, dt, $J=13.0$, 6.5 Hz), 4.66 (1H, dq, $J=10.0$, 6.0 Hz), 5.50 (1H, dd, $J=15.5$, 10.0 Hz), 5.78 (1H, dd, $J=15.5$, 5.5 Hz), 8.52 (2H, d, $J=2.0$ Hz), 9.08 (1H, t, $J=2.0$ Hz); δ_c (125 MHz, $CDCl_3$) (one carbon not identified due to peak broadening) 14.40, 20.90, 21.93, 25.89, 26.26, 27.66, 30.22, 31.42, 31.80, 33.37, 39.84, 41.56, 42.08, 45.81, 48.87, 76.36, 77.09, 119.05, 126.33, 132.31, 133.59, 140.30, 148.47, 166.04, 177.77.

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22. It was observed that on standing in chloroform *Galbulimina* alkaloids were undergoing protonation resulting in significant broadening of peaks in NMR spectra. All characterisations were carried out on samples, which were freshly filtered through a short plug of basic alumina.
23. No attempts to obtain crystalline form of the product were undertaken due to low availability of the synthetic material. The identity of the synthetic and natural compounds was established via NMR experiment of a sample premixed with reference 1 mg sample obtained from Fisher Scientific UK (Acros cat. no. 32912 0010).

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Mild and efficient direct aromatic iodination

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Abstract—Aryl iodides are important synthetic intermediates that can be transformed into tritium labelled compounds by metal-mediated hydrodehalogenation and also react in a number of important synthetic transformations. We present ICl/In(OTf)$_3$ as a new reagent combination for mild iodination, suitable for acid-sensitive substrates such as carbohydrates.
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1. Introduction

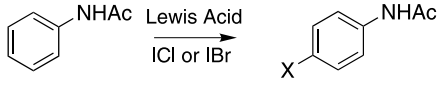
Aryl iodides can easily be transformed into tritium labelled compounds by metal-mediated hydrodehalogenation and are thus, important intermediates in medicinal chemistry.[1–3] The number of functional transformations, for example, Heck reactions as well as Stille and Negishi cross couplings, originating from aryl iodides also make these compounds valuable synthetic intermediates.[4] However, the low electrophilicity of molecular iodine, compared to that of molecular bromine and chlorine, renders direct iodination difficult, even in combination with activating Lewis acids. The direct iodination is also hampered by the formation of HI, which can cause proteolytic cleavage of sensitive compounds. To overcome these problems, iodinations are often performed under oxidative conditions where iodide ions, formed in the reaction, are reoxidized to molecular iodine. However, the oxidizing reagents can degrade sensitive groups, and are, therefore, not always feasible.

There has been a number of reports on direct aromatic iodination (i.e., by direct formation of a carbon–iodine bond from an iodonium species),[4] but few Lewis acids have been evaluated. The most common Lewis acids are silver and mercuric salts in combination with I_2. Recently a super active iodinating reagent, capable of iodinating even deactivated aromatic compounds, using ICl was reported. However, the use of strong acids (i.e., H_2SO_4) renders this and other similar methods less suitable for sensitive compounds such as carbohydrates.[5–8] In order to perform iodination under mild, yet effective, conditions we present the ICl/In(OTf)$_3$ system.

2. Optimization of aromatic iodination

The activating properties of various Lewis acids, together with either ICl or IBr, were examined using the halogenation of acetanilide as a model reaction. Acetanilide was treated with interhalogen in the presence of different Lewis acids for 15 min at room temperature. The reaction mixtures were then extracted and the crude mixtures were analyzed by NMR in order to estimate the reaction conversion of acetanilide into halogenated products. The results are summarized in Table 1.

Table 1. The halogenating properties of ICl and IBr in combination with various Lewis acids



1 **2a** X=I **2b** X=Br

Entry	Lewis acid	ICl (%) X=I[a]	IBr (%) X=I[a]	X=Br
1	HOTf	0	0	3
2	Sc(OTf)$_3$	0	0	8
3	Sn(OTf)$_2$	0	0	0
4	Al(OTf)$_3$	14	0	14
5	Yb(OTf)$_2$	16	0	14
6	—[b]	21	0	21
7	Mg(OTf)$_2$	24	0	26
8	Mn(OTf)$_2$	25	0	17
9	LiOTf	25	0	18
10	Cu(OTf)$_2$	30	0	36
11	AgOTf	43	13	32
12	Zn(OTf)$_2$	46	0	19
13	In(OTf)$_3$	79	27	11
14	Hg(OTf)$_2$	100	100	0

[a] Conversion into halogenated product. The reaction conditions are chosen for best comparison of the Lewis acids, and are by no means optimized.
[b] No Lewis acid added.

Keywords: Iodination; Lewis acids; Interhalogens; Carbohydrates.
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As expected, the most potent activator was $Hg(OTf)_2$ (entry 14, Table 1), a well known activator of iodinations,[4] but to our surprise we found that $In(OTf)_3$ (entry 13) was an even better activator than AgOTf (entry 11) in the ICl mediated iodination. In a preparative reaction using 1.5 equiv of ICl and 1.0 equiv of $In(OTf)_3$, 4-iodoacetanilide was isolated in 99% yield after 30 min reaction time.

In order to optimize the amount of $In(OTf)_3$ used, acetanilide was dissolved in CD_3CN in an NMR tube together with various equivalents of $In(OTf)_3$ (0, 0.1, 0.5, 1.0). The reactions were started by the addition of 1.0 equiv ICl and the conversion into 4-iodoacetanilide was estimated using NMR. The results, shown in Figure 1, indicate that only 0.5 equiv of $In(OTf)_3$ are necessary for efficient conversion into iodinated product.

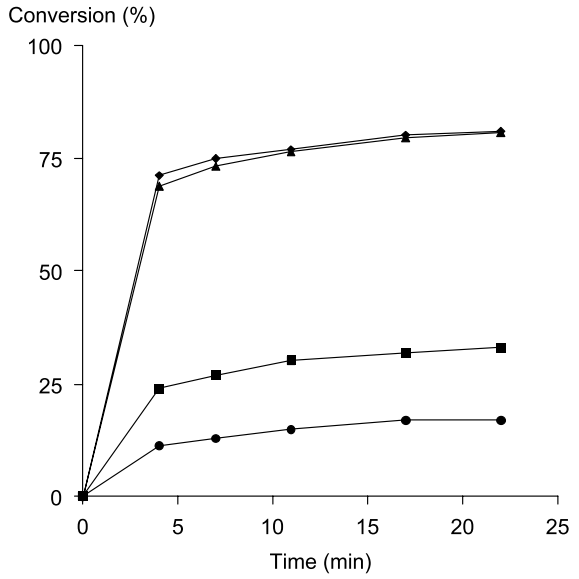
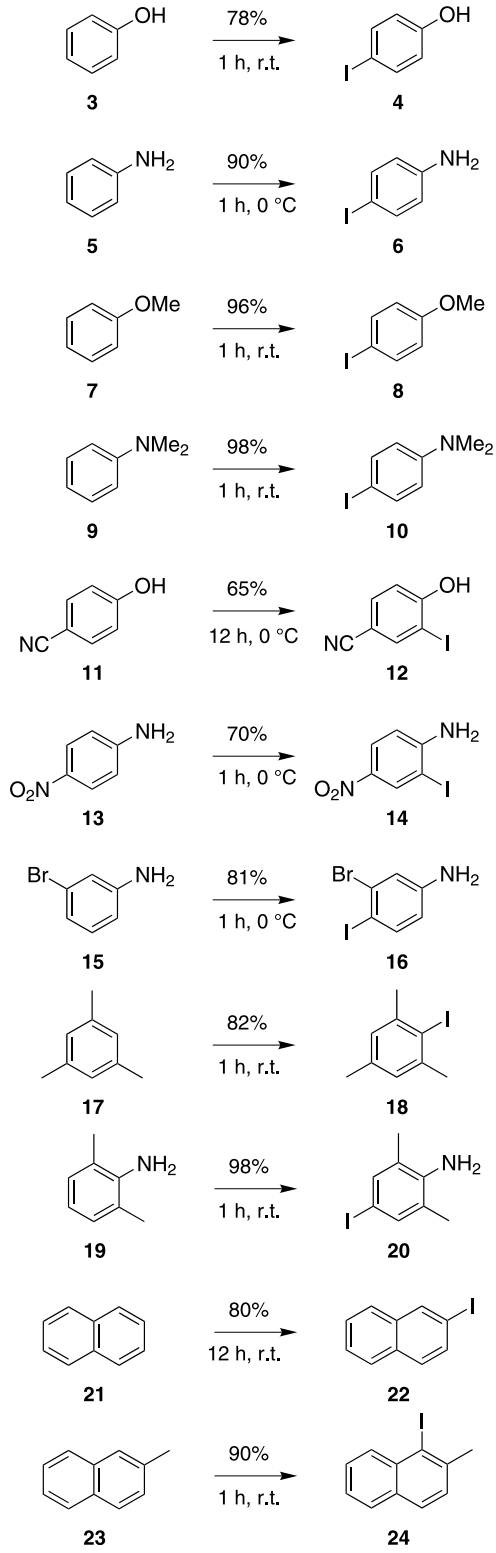


Figure 1. Iodination of acetanilide using various amounts of $In(OTf)_3$. ICl/acetanilide 1:1. ♦, 1 equiv $In(OTf)_3$; ▲, 0.5 equiv $In(OTf)_3$; ■, 0.1 equiv $In(OTf)_3$; ●, 0 equiv $In(OTf)_3$.

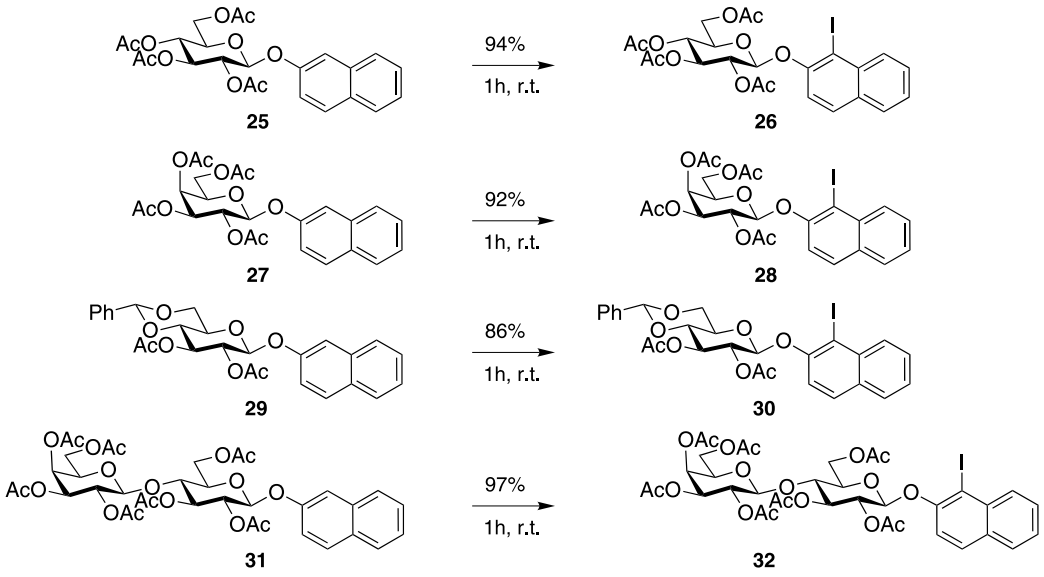
3. Application of aromatic iodination

The novel iodination reagent $ICl/In(OTf)_3$ was applied to a wide range of aromatic compounds (Scheme 1). Deactivated and weakly activated compounds, that is, nitrobenzene, acetophenone, bromobenzene, benzene and toluene were not iodinated, whereas more activated compounds were cleanly converted into the *para*-iodinated product with no *ortho*-product observed. However, by using $Hg(OTf)_2$ it was possible to iodinate toluene to give a mixture of *para*-iodinated and *ortho*- and *para*-diiodinated compounds in 30 and 23% yield. All reactions were performed at room temperature unless otherwise stated. The highly activated aniline (**5**) was *para*-iodinated (66%) as well as *ortho*- and *para*-diiodinated (13%) at room temperature but the reaction gave 90% *para*-iodinated product and only 6% diiodinated product when the temperature was lowered to 0 °C. 4-Hydroxy-benzonitrile (**11**), an easily reducible compound[9] was iodinated in 16% yield in room temperature, but prolonged reaction time at 0 °C gave 65% product. Iodination of 4-nitroaniline (**13**) at room temperature gave



Scheme 1. The halogenating properties of $ICl/In(OTf)_3$. Aryl/$In(OTf)_3$/ICl 1:0.5:1.1, MeCN.

63% yield. By adding molecular sieves, which can function as a neutral acid scavenger,[10] the yield increased to 67% while reaction with molecular sieves at 0 °C gave 70% product. Iodination of 3-bromo-aniline (**15**) gave 73% at room temperature and the yield increased to 81% at 0 °C. Naphthalene (**21**) was converted into 2-iodonaphthalene



Scheme 2. The halogenating properties of ICl/In(OTf)$_3$. Aryl/In(OTf)$_3$/ICl 1:0.5:1.1, MeCN.

(**22**) in 42% yield after 60 min, whereas prolonged reaction time increased the yield to 80%.

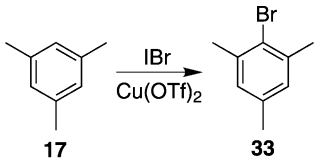
To verify the mildness of the reaction conditions, aromatic carbohydrate aglycons were iodinated in excellent yields (Scheme 2) without decomposition of the acid sensitive carbohydrate moiety. The more acid sensitive 2-naphthyl 4,6-*O*-benzylidene-2,3-di-*O*-acetyl-β-D-glucopyranoside (**29**) was iodinated in the presence of molecular sieves as a neutral acid scavenger.[10] All attempts to iodinate unprotected carbohydrates resulted in complete decomposition.

4. Aromatic bromination

The halogenations using IBr gave either bromination (through the in situ formation of molecular bromine from IBr)[11,12] or iodination, or a mixture of both, compared with ICl that gave pure iodinated product (Table 1). Apparently IBr is less suitable for aromatic iodination due to the competing bromination reaction. However, of the Lewis acids capable of activating IBr (i.e., compared to no Lewis acid added) both Cu(OTf)$_2$ (entry 10, Table 1) and Hg(OTf)$_2$ (entry 14) gave clean conversion into either brominated or iodinated product, that is, the choice of Lewis acid control whether an electrophilic bromonium or iodonium species is reacting in the aromatic halogenations using IBr.

The brominating capabilities of IBr/Cu(OTf)$_2$ was investigated using mesitylene as a model system (Fig. 2). To CD_3CN, 1.0 equiv of mesitylene and various equivalents of Cu(OTf)$_2$ were added. The reaction was initiated by addition of 1.0 equiv of IBr. The reactions were run in NMR tubes and the conversion of mesitylene into bromomesitylene was monitored. The results (Fig. 2) indicate that the bromination does not follow the stoichiometry of the reported reaction pathway, where 2 equiv of IBr are required for brominating 1 equiv of an aromatic compound.[11,12] Instead, it is shown that IBr is needed only in stoichiometric amount when in combination with Cu(OTf)$_2$.

It is known that copper(II) ions are capable of oxidizing iodide ions to molecular iodine, and this has been used in iodinations of aromatic compounds.[13] Apparently the brominating properties of IBr/Cu(OTf)$_2$ are enhanced due



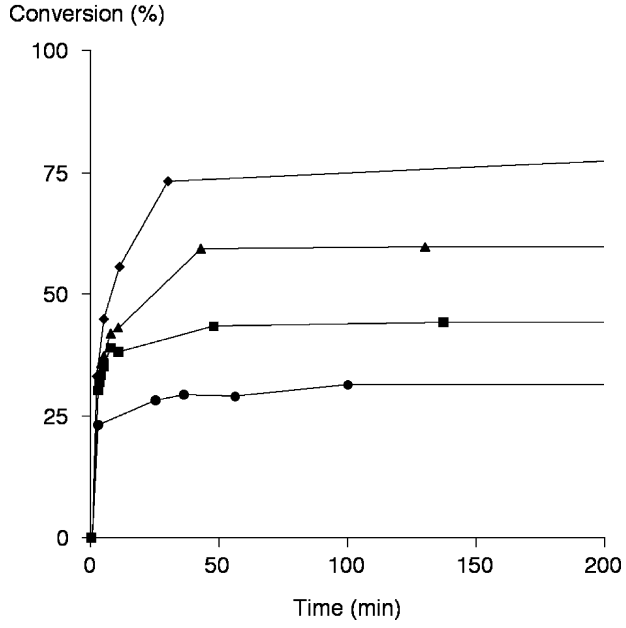


Figure 2. Bromination of mesitylene using various equivalents of Cu(OTf)$_2$. The time interval is minimized for clarity; after 8000 min the reaction promoted by 1.0 equiv Cu(OTf)$_2$ had reached 92% conversion, while the others had reached their maximum after 200 min. IBr/mesitylene 1:1 ♦, 1.0 equiv Cu(OTf)$_2$; ▲, 0.50 equiv Cu(OTf)$_2$; ■, 0.25 equiv Cu(OTf)$_2$; ●, 0 equiv Cu(OTf)$_2$.

to the regeneration of molecular bromine from bromide ions formed in the bromination reaction by the oxidation of copper(II) ions.

5. Conclusion

We have introduced ICl/$In(OTf)_3$ as a mild and effective reagent combination for aromatic iodination, suitable even for acid sensitive compounds, such as carbohydrates. We have also shown that the halogenating (i.e., bromination or iodination) properties of IBr can be controlled by the appropriate choice of Lewis acid. These results can hopefully be correlated to other reactions involving iodonium ions, for example, glycosylations.[14,15]

6. Experimental

6.1. General

General experimental conditions: NMR spectra were recorded at 300 or 400 MHz. ^1H NMR spectra were assigned using 2D-methods (COSY). Chemical shifts are given in ppm downfield from the signal for Me_4Si, with reference to residual $CHCl_3$. Reactions were monitored by TLC using alumina plates coated with silica gel and visualized using either UV light or by charring with *para*-anisaldehyde. Preparative chromatography was performed with silica gel (35–70 μm, 60 Å). MeCN used in iodination reactions was distilled from CaH_2 before use. ICl and IBr are commercially available as 1 M solutions in CH_2Cl_2. Compounds **1**–**9**, **11**, **13**–**15**, **17**–**19**, **21**, **23**, **27** and **33** are commercially available. Known and commercially available compounds were in agreement with previously published data (e.g., NMR).

Table 1. General procedure for evaluating Lewis acid mediated halogenation of acetanilide. Solution A: acetanilide (135 mg, 1 mmol) was dissolved in MeCN (10 mL). Solution B: Lewis Acid (0.2 mmol) was dissolved in MeCN (4 mL). Solution C: ICl (0.95 mL, 1.0 M in CH_2Cl_2) was dissolved in MeCN (9.05 mL). Solution B (0.74 mL) and solution A (0.37 mL) were added to an argon flushed test tube. To the stirred mixture was then added solution C (0.39 mL). After 15 min diisopropyl amine (0.10 mL, excess) and CH_2Cl_2 (5 mL) were added and the mixture was extracted twice with acidified water (2 mL, H_2SO_4, pH 2) and once with water (2 mL). The mixture was then concentrated before analysis using NMR.

Figure 1. General procedure for NMR reactions with acetanilide. To CD_3CN (0.5 mL) were added acetanilide (5.0 mg, 0.037 mmol) and $In(OTf)_3$ (0.1–1.0 equiv). The reaction was initiated by the addition of ICl (0.037 mL, 1.0 M in CH_2Cl_2).

6.1.1. 4-Iodoacetanilide (2a). Acetanilide (**1**) (68 mg, 0.50 mmol) and $In(OTf)_3$ (281 mg, 0.50 mmol) were dissolved in MeCN (5 mL). ICl (1 M in CH_2Cl_2, 0.750 mL, 0.75 mmol) was added and the mixture was stirred for 30 min at room temperature. The solution was then diluted with CH_2Cl_2, washed with satd aq $NaHCO_3$ and $Na_2S_2O_3$, dried (Na_2SO_4), concentrated and chromatographed (SiO_2, 1:1 heptane/EtOAc) to give 4-iodoacetanilide (130 mg, 99%) as an amorphous off-white solid.

6.1.2. 4-Iodophenol (4). Phenol (**3**) (48 mg, 0.51 mmol) and $In(OTf)_3$ (140 mg, 0.25 mmol) were dissolved in MeCN (5 mL). ICl (1 M in CH_2Cl_2, 0.550 mL, 0.55 mmol) was added dropwise and the mixture was stirred for 60 min at room temperature. Triethylamine (1.5 mL) followed by $Na_2S_2O_3$ (1.5 g) were added and the mixture was concentrated and chromatographed (SiO_2, 2:1 heptane/EtOAc) to give 4-iodophenol (88 mg, 78%) as an amorphous off-white solid.

6.1.3. 4-Iodoaniline (6). Aniline (**5**) (0.046 mL, 0.50 mmol), and $In(OTf)_3$ (141 mg, 0.25 mmol) were dissolved in MeCN (5 mL). ICl (1 M in CH_2Cl_2, 0.550 mL, 0.55 mmol) was added dropwise and the mixture was stirred for 60 min at 0 °C. Triethylamine (1.5 mL) followed by $Na_2S_2O_3$ (1.5 g) were added and the mixture was concentrated and chromatographed (SiO_2, 3:1 heptane/EtOAc) to give 4-iodoaniline (99 mg, 90%) as an amorphous off-white solid.

6.1.4. 4-Iodoanisole (8). Anisole (**7**) (0.055 mL, 0.51 mmol), and $In(OTf)_3$ (142 mg, 0.25 mmol) were dissolved in MeCN (5 mL). ICl (1 M in CH_2Cl_2, 0.550 mL, 0.55 mmol) was added dropwise and the mixture was stirred for 60 min at room temperature. Triethylamine (1.5 mL) followed by $Na_2S_2O_3$ (1.5 g) were added and the mixture was concentrated and chromatographed (SiO_2, heptane) to give 4-iodoanisole (113 mg, 96%) as an amorphous off-white solid.

6.1.5. 4-Iodo *N*,*N*-dimethylaniline (10). *N*,*N*-dimethylaniline (**9**) (0.064 mL, 0.51 mmol) and $In(OTf)_3$ (140 mg, 0.25 mmol) were dissolved in MeCN (5 mL). ICl (1 M in CH_2Cl_2, 0.550 mL, 0.55 mmol) was added dropwise and the mixture was stirred for 60 min at room temperature. Triethylamine (1.5 mL) followed by $Na_2S_2O_3$ (1.5 g) were added and the mixture was concentrated and chromatographed (SiO_2, 10:1 heptane/EtOAc) to give 4-iodo *N*,*N*-dimethylaniline[16,17] (122 mg, 98%) as an amorphous off-white solid.

6.1.6. 4-Hydroxy-3-iodo-benzonitrile (12). 4-Hydroxybenzonitrile (**11**) (60 mg, 0.50 mmol) and $In(OTf)_3$ (141 mg, 0.25 mmol) were dissolved in MeCN (5 mL). ICl (1 M in CH_2Cl_2, 0.550 mL, 0.55 mmol) was added dropwise and the mixture was stirred for 12 h at 0 °C. Triethylamine (1.5 mL) followed by $Na_2S_2O_3$ (1.5 g) were added and the mixture was concentrated and chromatographed (SiO_2, 1:0→1:1 CH_2Cl_2/acetone) to give 4-hydroxy-3-iodo-benzonitrile[18,19] (80 mg, 65%) as an amorphous white solid.

6.1.7. 2-Iodo-4-nitroaniline (14). 4-Nitroaniline (**13**) (71 mg, 0.52 mmol) $In(OTf)_3$ (141 mg, 0.25 mmol) and molecular sieves 3 Å (1 g) were dissolved in MeCN (5 mL). ICl (1 M in CH_2Cl_2, 0.550 mL, 0.55 mmol) was added dropwise and the mixture was stirred for 60 min at 0 °C. Triethylamine (1.5 mL) followed by $Na_2S_2O_3$ (1.5 g) were added and the mixture was concentrated and

chromatographed (SiO_2, 3:2 heptane/EtOAc + 1% triethylamine) to give 2-iodo-4-nitroaniline (95 mg, 70%) as an amorphous yellow solid.

6.1.8. 3-Bromo-4-iodoaniline (16). 3-Bromoaniline (**15**) (0.054 mL, 0.50 mmol) and $In(OTf)_3$ (140 mg, 0.25 mmol) were dissolved in MeCN (5 mL). ICl (1 M in CH_2Cl_2, 0.550 mL, 0.55 mmol) was added dropwise and the mixture was stirred for 60 min at 0 °C. Triethylamine (1.5 mL) followed by $Na_2S_2O_3$ (1.5 g) were added and the mixture was concentrated and chromatographed (SiO_2, 3:1 heptane/EtOAc) to give 3-bromo-4-iodoaniline[20] (119 mg, 81%) as an amorphous off-white solid.

6.1.9. Iodomesitylene (18). Mesitylene (**17**) (0.070 mL, 0.50 mmol) and $In(OTf)_3$ (141 mg, 0.25 mmol) were dissolved in MeCN (5 mL). ICl (1 M in CH_2Cl_2, 0.550 mL, 0.55 mmol) was added dropwise and the mixture was stirred for 60 min at room temperature. Triethylamine (1.5 mL) followed by $Na_2S_2O_3$ (1.5 g) were added and the mixture was concentrated and chromatographed (SiO_2, heptane) to give iodomesitylene (102 mg, 82%) as an amorphous off-white solid.

6.1.10. 4-Iodo-2,6-dimethylaniline (20). 2,6-Dimethylaniline (**19**) (0.062 mL, 0.50 mmol) and $In(OTf)_3$ (139 mg, 0.25 mmol) were dissolved in MeCN (5 mL). ICl (1 M in CH_2Cl_2, 0.550 mL, 0.55 mmol) was added dropwise and the mixture was stirred for 60 min at room temperature. Triethylamine (1.5 mL) followed by $Na_2S_2O_3$ (1.5 g) were added and the mixture was concentrated and chromatographed (SiO_2, 3:1 heptane/EtOAc) to give 4-iodo-2,6-dimethylaniline[21,22] (122 mg, 98%) as an amorphous off-white solid.

6.1.11. 2-Iodonaphthalene (22). Naphthalene (**21**) (64 mg, 0.50 mmol) and $In(OTf)_3$ (141 mg, 0.25 mmol) were dissolved in MeCN (5 mL). ICl (1 M in CH_2Cl_2, 0.550 mL, 0.55 mmol) was added dropwise and the mixture was stirred for 12 h at room temperature. Triethylamine (1.5 mL) followed by $Na_2S_2O_3$ (1.5 g) were added and the mixture was concentrated and chromatographed (SiO_2, heptane) to give 2-iodonaphthalene[23,24] (102 mg, 80%) as an amorphous off-white solid.

6.1.12. 1-Iodo-2-methylnaphthalene (24). 2-Methylnaphthalene (**23**) (72 mg, 0.47 mmol) and $In(OTf)_3$ (141 mg, 0.25 mmol) were dissolved in MeCN (5 mL). ICl (1 M in CH_2Cl_2, 0.550 mL, 0.55 mmol) was added dropwise and the mixture was stirred for 60 min at room temperature. Triethylamine (1.5 mL) followed by $Na_2S_2O_3$ (1.5 g) were added and the mixture was concentrated and chromatographed (SiO_2, heptane) to give 1-iodo-2-methylnaphthalene[25,26] (114 mg, 90%) as a yellow oil.

6.1.13. 2-(1-Iodonaphthyl) 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (26). 2-Naphthyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside[27] (**25**) (83 mg, 0.175 mmol) and $In(OTf)_3$ (51 mg, 0.09 mmol) were dissolved in MeCN (5 mL). ICl (1 M in CH_2Cl_2, 0.275 mL, 0.28 mmol) was added dropwise and the mixture was stirred for 60 min at room temperature. Triethylamine (1.5 mL) followed by $Na_2S_2O_3$ (1.5 g) were added and the mixture was concentrated and chromatographed (SiO_2, 1:2 heptane/EtOAc) to give 2-(1-iodonaphthyl) 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (99 mg, 94%). Recrystallization from EtOAc/heptane gave an analytical sample of white crystals; mp 180.0–181.0 °C; $[\alpha]_D^{22}$ −62.6 (*c* 0.9, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 8.16 (d, 1H, J=8.4 Hz), 7.80 (d, 1H, J=8.9 Hz), 7.76 (d, 1H, J=8.1 Hz), 7.57 (dt, 1H, J=8.5, 1.2 Hz), 7.45 (dt, 1H, J=7.9, 0.9 Hz), 7.32 (d, 1H, J=8.9 Hz), 5.48 (dd, 1H, J=9.3, 7.9 Hz), 5.33 (dd, 1H, J=9.4, 9.3 Hz), 5.24 (dd, 1H, J=9.7, 9.5 Hz), 5.18 (d, 1H, J=7.8 Hz), 4.32 (dABq, 1H, J=12.3, 5.3 Hz), 4.23 (dABq, 1H, J=12.3, 2.5 Hz) 3.93–3.89 (m, 1H), 2.13, 2.10 (s, 3H each), 2.06 (s, 6H). ^{13}C NMR ($CDCl_3$): δ 170.7, 170.5, 169.53, 169.47, 154.5, 135.5, 132.0, 131.1, 130.5, 128.5, 128.3, 125.7, 116.9, 100.4, 90.2, 72.9, 72.4, 71.1, 68.4, 62.1, 21.5, 20.9, 20.81, 20.77. HRMS calcd for $C_{24}H_{25}IO_{10}Na$ (M+Na) 623.0390, found 623.0356.

6.1.14. 2-(1-Iodonaphthyl) 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (28). 2-Naphthyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (**27**) (118 mg, 0.25 mmol) and $In(OTf)_3$ (73 mg, 0.13 mmol) were dissolved in MeCN (5 mL). ICl (1 M in CH_2Cl_2, 0.275 mL, 0.28 mmol) was added dropwise and the mixture was stirred for 60 min at room temperature. Triethylamine (1.5 mL) followed by $Na_2S_2O_3$ (1.5 g) were added and the mixture was concentrated and chromatographed (SiO_2, 1:1 heptane/EtOAc) to give 2-(1-iodonaphthyl) 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (138 mg, 92%). Recrystallization from ether gave an analytical sample of white crystals; mp 147.0–148.0 °C; $[\alpha]_D^{21}$ −39.0 (*c* 1.0, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 8.17 (d, 1H, J=8.5 Hz), 7.80 (d, 1H, J=9.0 Hz), 7.76 (d, 1H, J=8.1 Hz), 7.57 (t, 1H, J=7.8 Hz), 7.45 (t, 1H, J=7.7 Hz), 7.35 (d, 1H, J=8.9 Hz), 5.70 (dd, 1H, J=10.4, 8.0 Hz), 5.50 (d, 1H, J=3.3 Hz), 5.15 (d, 1H, J=7.8 Hz), 5.14 (dd, 1H, J=10.5, 3.4 Hz), 4.30 (dABq, 1H, J=11.2, 7.0 Hz), 4.20 (dABq, 1H, J=11.2, 6.2 Hz), 4.12 (t, 1H, J=6.4 Hz), 2.22, 2.14, 2.09, 2.04 (s, 3H each). ^{13}C NMR ($CDCl_3$): δ 170.5, 170.44, 170.35, 169.5, 154.7, 135.5, 132.0, 131.0, 130.5, 128.5, 128.3, 125.6, 116.7, 100.9, 89.8, 71.4, 71.1, 68.4, 67.0, 61.6, 21.6, 20.86, 20.85, 20.8. HRMS calcd for $C_{24}H_{25}IO_{10}Na$ (M+Na) 623.0390, found 623.0375.

6.1.15. 2-Naphthyl 4,6-*O*-benzylidene-2,3-di-*O*-acetyl-β-D-glucopyranoside (29). 2-Naphthyl β-D-glucopyranoside (363 mg, 1.19 mmol) and benzaldehyde dimethyl acetal (0.325 mL, 2.17 mmol) were suspended in MeCN (5.5 mL). *p*TSA (5 mg, 0.03 mmol) was added and the mixture was stirred for 50 min at room temperature. Triethylamine (0.5 mL) was added and the mixture was co-concentrated with toluene. The crude product was dissolved in pyridine (5 mL) and acetic anhydride (4 mL, 42.4 mmol) was added and the mixture was stirred for 3.5 h at room temperature. Co-concentration with toluene and chromatography (SiO_2, 2:1→0:1 heptane/EtOAc) gave 2-naphthyl 4,6-*O*-benzylidene-2,3-di-*O*-acetyl-β-D-glucopyranoside (523 mg, 92%). Recrystallization from EtOAc/heptane gave an analytical sample of white crystals; mp 194.0–196.0 °C; $[\alpha]_D^{21}$ −19.3 (*c* 1.1, $CHCl_3$). ^1H NMR (C_6D_6): δ 7.64–7.09 (m, 12H), 5.68–5.61 (m, 2H), 5.16 (s, 1H), 5.07 (d, 1H, J=7.5 Hz), 4.00 (dd, 1H, J=10.4, 5.0 Hz), 3.48 (t, 1H, J=9.6 Hz), 3.36 (t, 1H, J=10.3 Hz), 3.13 (m, 1H), 1.78, 1.74 (s,

3H each). ^{13}C NMR (C_6D_6): δ 169.8, 169.3, 155.3, 137.8, 134.8, 130.7, 130.1, 129.3, 128.7, 128.4, 127.6, 127.4, 127.0, 126.7, 124.9, 119.1, 112.0, 101.8, 99.7, 78.3, 72.8, 72.2, 68.5, 66.6, 20.4, 20.3. HRMS calcd for $C_{27}H_{27}O_8$ (M+H) 479.1706, found 479.1682.

6.1.16. 2-(1-Iodonaphthyl) 4,6-*O*-benzylidene-2,3-di-*O*-acetyl-β-D-glucopyranoside (30). 2-Naphthyl 4,6-*O*-benzylidene-2,3-di-*O*-acetyl-β-D-glucopyranoside (**29**) (121 mg, 0.25 mmol), $In(OTf)_3$ (70 mg, 0.12 mmol) and molecular sieves 3 Å (1 g) were dissolved in MeCN (5 mL). ICl (1 M in CH_2Cl_2, 0.275 mL, 0.28 mmol) was added dropwise and the mixture was stirred for 60 min at room temperature. Triethylamine (1.5 mL) followed by $Na_2S_2O_3$ (1.5 g) were added and the mixture was concentrated and chromatographed (SiO_2, 2:1 heptane/EtOAc) to give 2-(1-iodonaphthyl) 4,6-*O*-benzylidene-2,3-di-*O*-acetyl-β-D-glucopyranoside (131 mg, 86%). Recrystallization from EtOAc/heptane gave an analytical sample of white crystals; mp 223.0–225.0 °C; $[\alpha]_D^{22}$ −100.1 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 8.17 (d, 1H, *J*=8.6 Hz), 7.82 (d, 1H, *J*=9.0 Hz), 7.77 (d, 1H, *J*=8.0 Hz), 7.57 (dt, 1H, *J*=8.5, 1.3 Hz), 7.48–7.43 (m, 3H), 7.39–7.37 (m, 3H), 7.31 (d, 1H, *J*=8.9 Hz), 5.57 (s, 1H), 5.50–5.40 (m, 2H), 5.31 (d, 1H, *J*=7.4 Hz), 4.45 (dd, 1H, *J*=10.5, 5.0 Hz), 3.92 (t, 1H, *J*=9.6 Hz), 3.89 (t, 1H, *J*=10.4 Hz), 3.74 (dt, 1H, *J*=9.7, 4.9 Hz), 2.12, 2.10 (s, 3H each). ^{13}C NMR ($CDCl_3$): δ 170.5, 169.8, 154.6, 136.9, 132.0, 131.1, 130.7, 129.5, 128.6, 128.5, 128.4, 126.4, 125.7, 116.5, 101.9, 100.8, 90.0, 78.2, 72.2, 72.1, 68.8, 67.0, 21.5, 21.1. HRMS calcd for $C_{27}H_{25}IO_8Na$ (M+Na) 627.0492, found 627.0530.

6.1.17. 2-Naphthyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (31). Peracetylated lactose (683 mg, 1.00 mmol) and 2-naphthol (231 mg, 1.6 mmol) were dissolved in CH_2Cl_2 (10 mL, filtered through Al_2O_3). $BF_3{\cdot}OEt_2$ (0.190 mL, 1.5 mmol) was added and the mixture was stirred for 60 min at room temperature under argon. Triethylamine (1.5 mL) was added and the mixture was concentrated and chromatographed (SiO_2, 1:2 heptane/EtOAc) to give 2-naphthyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (609 mg, 79%). Recrystallization from EtOAc/heptane gave an analytical sample of white crystals; mp 169.5–170.5 °C; $[\alpha]_D^{21}$ −19.1 (*c* 0.9, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 7.80–7.71 (m, 3H), 7.46 (dt, 1H, *J*=7.1, 1.0 Hz), 7.39 (dt, 1H, *J*=7.0, 1.0 Hz), 7.32 (d, 1H, *J*=2.3 Hz), 7.16 (dd, 1H, *J*=8.9, 2.4 Hz), 5.37 (d, 1H, *J*=3.0 Hz), 5.32 (t, 1H, *J*=8.3 Hz), 5.26–5.19 (m, 2H), 5.14 (dd, 1H, *J*=10.4, 7.9 Hz), 4.98 (dd, 1H, *J*=10.4, 3.4 Hz), 4.55–4.49 (m, 1H), 4.53 (d, 1H, *J*=8.0 Hz), 4.19–4.07 (m, 3H), 3.92 (t, 1H, *J*=9.9 Hz), 3.92–3.88 (m, 2H), 2.16, 2.09 (s, 3H each), 2.07 (s, 12H), 1.97 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 170.52, 170.48, 170.3, 170.2, 169.9, 169.8, 169.3, 154.7, 134.2, 130.2, 129.8, 127.9, 127.2, 126.8, 124.8, 118.9, 111.5, 101.3, 98.9, 76.5, 73.00, 72.97, 71.7, 71.1, 70.9, 69.2, 66.8, 62.3, 61.0, 21.0, 20.92, 20.85, 20.8, 20.7. HRMS calcd for $C_{36}H_{42}O_{18}Na$ (M+Na) 785.2269, found 785.2239.

6.1.18. 2-(1-Iodonaphthyl) 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (32). 2-Naphthyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**31**) (188 mg, 0.25 mmol) and $In(OTf)_3$ (69 mg, 0.12 mmol) were dissolved in MeCN (5 mL). ICl (1 M in CH_2Cl_2, 0.275 mL, 0.28 mmol) was added dropwise and the mixture was stirred for 60 min at room temperature. Triethylamine (1.5 mL) followed by $Na_2S_2O_3$ (1.5 g) were added and the mixture was concentrated and chromatographed (SiO_2, 2:3 heptane/EtOAc) to give 2-(1-iodonaphthyl) 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (212 mg, 97%). Recrystallization from ether gave an analytical sample of white crystals; mp 195.0–196.0 °C; $[\alpha]_D^{21}$ −50.0 (*c* 1.2, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 8.15 (d, 1H, *J*=8.4 Hz), 7.78 (d, 1H, *J*=9.0 Hz), 7.75 (d, 1H, *J*=8.2 Hz), 7.56 (dt, 1H, *J*=6.9, 1.0 Hz), 7.44 (dt, 1H, *J*=7.9, 0.7 Hz), 7.29 (d, 1H, *J*=9.0 Hz), 5.37 (t, 1H, *J*=9.0 Hz), 5.36 (d, 1H, *J*=3.5 Hz), 5.31 (t, 1H, *J*=8.8 Hz), 5.17 (d, 1H, *J*=7.2 Hz), 5.14 (dd, 1H, *J*=10.4, 7.9 Hz), 4.98 (dd, 1H, *J*=10.4, 3.4 Hz), 4.56 (dd, 1H, *J*=11.8, 2.1 Hz), 4.55 (d, 1H, *J*=7.9 Hz), 4.18 (t, 1H, *J*=5.5 Hz), 4.15–4.08 (m, 2H), 4.01 (t, 1H, *J*=8.6 Hz), 3.91 (t, 1H, *J*=6.9 Hz), 3.86–3.83 (m, 1H), 2.16, 2.12, 2.11, 2.08, 2.07, 2.06, 1.97 (s, 3H each). ^{13}C NMR ($CDCl_3$): δ 170.5, 170.4, 170.3, 170.2, 169.9, 169.7, 169.2, 154.5, 135.5, 131.9, 131.0, 130.5, 128.5, 128.3, 125.6, 116.6, 101.3, 99.8, 90.0, 76.2, 73.1, 71.4, 71.1, 70.9, 69.2, 66.8, 62.0, 61.0, 21.4, 21.0, 20.9, 20.81, 20.80, 20.78, 20.7. HRMS calcd for $C_{36}H_{41}IO_{18}Na$ (M+Na) 911.1235, found 911.1212.

Figure 2. General procedure for NMR reactions with mesitylene. To CD_3CN (0.4 mL) were added mesitylene (**17**) (0.0025 mL, 0.018 mmol) and $Cu(OTf)_2$ (0.25–1.0 equiv). The reaction was initiated by the addition of IBr (0.018 mL, 1.0 M in CH_2Cl_2). The NMR spectrum of bromomesitylene was in agreement with published data.

Acknowledgements

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Modular chiral selenium-containing oxazolines: synthesis and application in the palladium-catalyzed asymmetric allylic alkylation

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Abstract—A new series of modular chiral selenium-containing oxazolines has been synthesized from inexpensive and commercially available L-serine and L-aspartic acid. These new compounds were evaluated as chiral ligands in the palladium-catalyzed asymmetric allylic alkylation reaction, furnishing the product in high enantiomeric excess, using Cs_2CO_3/CH_2Cl_2 as the base/solvent system.
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1. Introduction

The preparation of new and efficient enantiopure ligands providing a chiral environment to metals for asymmetric catalysis is currently one of the major challenges in synthetic organic chemistry. Among the transition metal-catalyzed reactions known to form carbon–carbon and carbon-heteroatom bonds, the palladium-catalyzed allylic substitution stands out as one of the most valuable synthetic tools available.[1] The development of effective chiral ligands for this process has grown steadily over the past few years since several new catalysts have been successfully employed for asymmetric induction in this reaction. Among the wide variety of catalysts designed, hetero-bidentate ligands play an important role once they capitalize upon the difference in electronic character of the two donor atoms to exert a stereoelectronic bias upon intermediate π-allyl complexes. Stereoelectronically, the palladium-allyl terminus opposite to the more powerful acceptor atom will be longer; hence, more susceptible to cleavage as a result of nucleophilic attack.[2]

In this context, chiral bidentate ligands, equipped with an oxazoline framework and a soft donor heteroatom, have been the subject of research of many groups. The pioneering chiral phosphine-oxazolines developed by Pfaltz,[3] Helmchen,[4] and Williams[5] are by far the most widely studied[6] and have served as inspiration for the design of many other heterobidentate *P*,*N* ligands with an oxazoline backbone.[7,8] Some of these ligands are shown in Figure 1 and all of them have been successfully employed in the enantioselective allylation reaction.

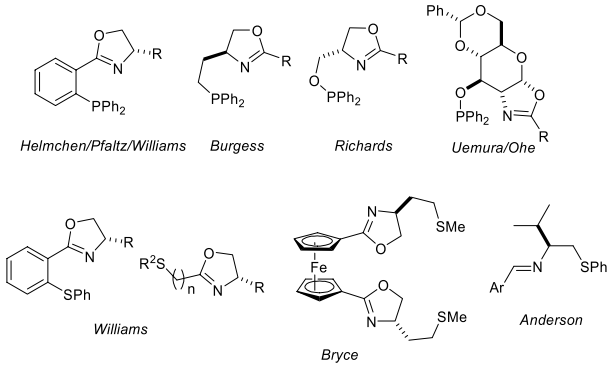

Figure 1. Oxazoline ligands for palladium-catalyzed allylic alkylations.

Mixed *S*,*N*-ligands have also been extensively studied in palladium-catalyzed allylic substitutions. Various sulfur-containing oxazolines were reported to act as chiral catalysts in this reaction.[9] Ferrocenyl-oxazolines incorporating a thioether moiety were applied to such a process as well.[10] A series of sulfur-imine mixed donors, derived from amino acids has also been described to induct high degrees of enantioselectivity in the allylation reaction.[11] Our group has recently published the synthesis of cysteine and methionine-derived *N*,*S*-ligands and its application to asymmetric allylations.[12]

On the other hand, chiral organoselenium compounds have

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attracted much attention of organic chemists over the last decade, and are now a very important tool for stereoselective transformations.[13] These compounds have found use in the stereoselective ring opening of epoxides,[14] anti-stereoselectivity and Markownikoff regioselectivity in the electrophilic selenenylation of alkenes.[15] Most importantly, chiral diselenides have been employed as useful ligands in various asymmetric transformations such as asymmetric hydrosilylation of acetophenone,[16] enantioselective diethylzinc addition to aldehydes,[17,18] and 1,4 addition of Grignard reagents to enones.[19]

In the course of our current interest in chiral organoselenium mediated asymmetric transformations,[18,19] and as only few examples of chiral ligands containing a selenium atom coordinated to palladium have been described,[20] we decided to prepare a new class of chiral oxazoline ligands **1** and **2** (Fig. 2) with an organoselenium moiety as a soft donor, starting from an easily available and inexpensive chiral pool. These chiral selenium-containing oxazolines had their efficiency examined as catalysts in the enantioselective palladium-catalyzed allylic alkylation reaction.

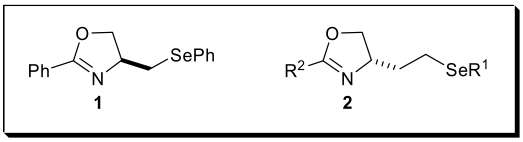
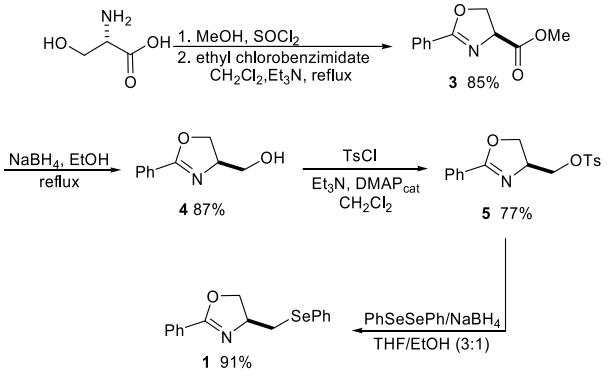


Figure 2. Selenium-containing oxazolines **1** and **2**.

2. Results and discussion

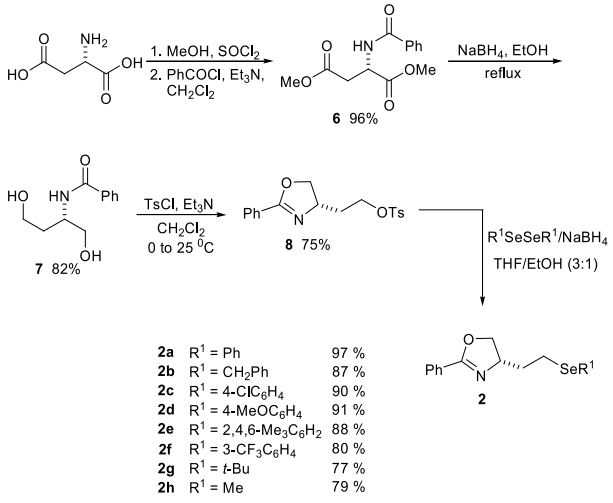
Ligands **1** and **2** were synthesized starting from natural amino acids L-serine and L-aspartic acid, respectively. Oxazolinyl selenide **1** was prepared by the sequence shown in Scheme 1. First, esterification of L-serine with $SOCl_2$/MeOH, followed by cyclization of the resulting ester with ethyl chlorobenzimidate resulted in the corresponding oxazolinyl ester **3** in 85% yield for the two steps.[21] The ester was further reduced to the alcohol **4**, which was treated with TsCl in dichloromethane affording the tosylate **5** in good yield.[22] Thus the desired oxazolinyl selenide was obtained by nucleophilic displacement with PhSeSePh/$NaBH_4$ in a 3:1 mixture of THF/ethanol as solvents.



Scheme 1. Synthesis of ligand **1**.

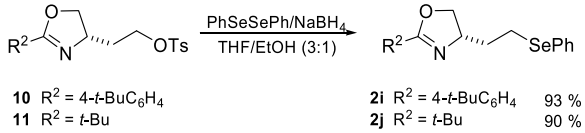
Ligand **2a** was obtained by esterification of both carboxyl groups of aspartic acid, followed by acylation at nitrogen with benzoyl chloride. The diester **6** was cleanly reduced to the diol **7**[23] which was treated, without further purification, with TsCl in dichloromethane using triethylamine as base. The ditosylated intermediate immediately cyclizes to the entropically favored oxazoline **8**.[7c] The organoselenium functionalization took place again by nucleophilic displacement of the tosylate leaving group by a phenyl selenide anion generated by reduction of PhSeSePh with $NaBH_4$ in a 3:1 mixture of THF and ethanol.

Ligands **2b–h** were prepared in a similar way as **2a** by nucleophilic displacement of tosylate leaving group with the selenide anion generated by reaction of the corresponding diorganoyl diselenide with $NaBH_4$ in THF/EtOH. The desired oxazolinyl selenides were obtained with yields ranging from 77 to 97% (Scheme 2).



Scheme 2. Synthesis of ligands **2a–h**.

Structural variations at R^2 positions were also introduced. Thus, we prepared tosylates **10** and **11** in a similar way to **8**, and they were further submitted to substitution with phenylselenolate anion to afford oxazolinyl selenide **2i** and **2j** (Scheme 3).



Scheme 3. Synthesis of ligands **2i–j**.

One of the major advantages of the strategy developed is its modular construction and, thus, modifications in the structure of the catalysts can be easily introduced. This characteristic is important for the fine-tuning of the catalytic activity and for a deeper understanding of the steric and electronic effects in the reaction outcome (Fig. 3). Moreover, it is interesting to point out that, for the first time, an organoselenium group is attached to a sp^3 carbon in a ligand designed for palladium-catalyzed allylic alkylations. This structural feature is highly interesting, since it facilitates the

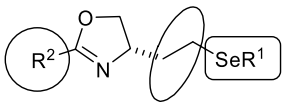


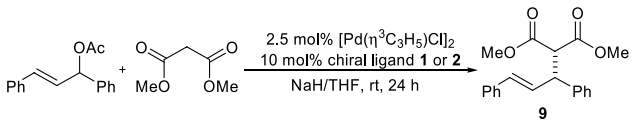
Figure 3. Modular construction of chiral selenium-containing oxazolines.

introduction of the selenium atom into the framework of the molecule thus permiting a highly flexible strategy for steric and electronic modifications at R^1 position.

With this set of ligands in hand, we turned our attention to investigate their potential in the palladium-catalyzed asymmetric allylic alkylation.

We studied the alkylation reaction of racemic 1,3-diphenyl-2-propenyl acetate with sodium dimethyl malonate, using the chiral selenium-containing oxazolines **1** and **2** as chiral ligands (10 mol%) in the presence of $[Pd(\eta^3\text{-}C_3H_5)Cl]_2$ (2.5 mol%) in THF as solvent. The results of these studies are summarized in Table 1.

Table 1.



#	Ligand	R^1	R^2	Yield (%)[a]	ee (%)[b]
1	**1**	—	—	71	23
2	**2a**	Ph	Ph	99	85
3	**2b**	CH_2Ph	Ph	93	79
4	**2c**	4-ClPh	Ph	85	63
5	**2d**	4-MeOPh	Ph	81	75
6	**2e**	2,4,6-Me_3Ph	Ph	67	6[c]
7	**2f**	3-CF_3Ph	Ph	83	54
8	**2g**	*t*-Bu	Ph	91	37
9	**2h**	Me	Ph	89	70
10	**2i**	Ph	4-*t*-BuPh	68	58
11	**2j**	Ph	*t*-Bu	63	5

[a] Isolated yields.
[b] Determined by HPLC with a Daicel Chiralcel OD column, hexane/isopropanol 99:1; 0.5 mL/min; 254 nm.
[c] The opposite enantiomer was obtained.

Ligand **1** furnished quite disappointing results, once the alkylation product was obtained with poor enantioselectivity. On the other hand, ligands of type **2** performed much more successfully.

Ligand **2a** has proven to be more efficient than **1**, giving the alkylated product in 85% ee (Table 1, entry 2). We could observe that the nature of the group attached to the selenium atom plays an important role in the enantioselection event. The catalyst **2b** with a benzyl substituent at selenium was tested, but no increase in the ee could be achieved (entry 3). Since the best result was obtained using catalyst **2a**, which has R^1=Ph, we attempted to test ligands with several different substituents at the aromatic ring of the organoselenium moiety. Ligands with electron-withdrawing groups like chlorine (**2c**) and trifluormethyl (**2f**) at the selenium donor were also evaluated. Unfortunately, a decrease in the enantioselectivity was observed together with diminished yields. A plausible explanation for this account is that the presence of the electron-withdrawing group reduces the ability of the selenium to coordinate to palladium, which would account for the lowering in the yields of **9** (see entries 4 and 7). When catalyst **2d** bearing an electron-donating group was employed, an ee of 75% was obtained (entry 5).

Steric effects seem to be crucial in our catalytic system. The increase of the bulk around selenium causes a negative effect in the enantioselectivity of the reaction. This can be easily evidenced by a dramatic decrease in the ee by using ligand **2g**, which has an *t*-Bu substituent directly attached to selenium (entry 6). A more pronounced effect can be seen when the highly encumbered ligand **2e** was employed. A poor yield of **9** was achieved and interestingly the opposite enantiomer was formed with a very low enantioselectivity of only 6% (entry 6).

The influence of the sterics at the oxazoline ring was also evaluated. When the phenyl group in **2a** was replaced by bulkier ones such as in the case of **2i** and **2j**, a decrease in the ee was detected (entries 10 and 11). **2i** gave an ee of 58% and in the case of ligand **2j** an essentially racemic product was obtained (5% ee, entry 11).

All ligands furnished the alkylated product with the (*R*) configuration as the major product, except for ligand **2e**, which furnished a slight excess of the opposite enantiomer. The stereochemistry of **9** was assigned by comparison of the sign of optical rotation with literature data.[7b]

A plausible explanation for such a high difference in the level of enantioselection showed between **1** and **2a** can be found in the difference of the bite angle of the chiral ligand. It is known that as the length of the tether in bidentate ligands is increased, the bite angle can be increased as well.[9b,24] This increase places the chiral environment of the ligand closer to the allyl system and it may result in greater asymmetric induction. So, as ligand **2a** has a longer side chain, it coordinates to palladium in a greater bite angle. This behavior results in closer proximity of the oxazoline moiety to the allyl unit and, hence, greater enantioselectivity should be observed in comparison with ligand **1** (Fig. 4).

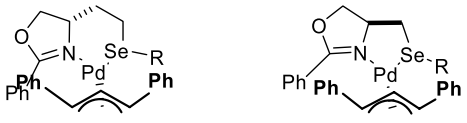


Figure 4. Effect of the bite angle.

Attempts to study the loading of the catalyst used were also performed. As depicted in the Figure 5, quantities of 15, 10, 5, and 2.5 mol% of chiral ligand **2a** were employed in the asymmetric allylic alkylation. The best results were obtained by using 10 mol% of **2a** and 2.5 mol% of the palladium source. This amount corresponds to a 2:1 ratio of ligand to palladium. Changing this ratio to 1:1, by lowering the amount of **2a** to 5 mol%, a decrease of the ee to 78% was observed. Moreover, a dramatic decrease in the ee was verified when 2.5 mol% of the ligand was used.

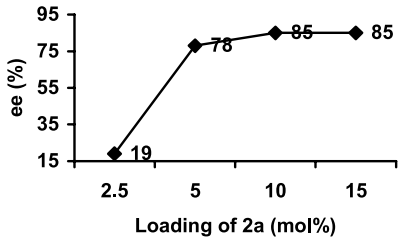
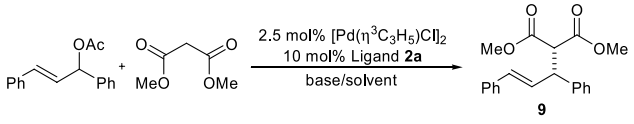


Figure 5. Variation in the amount of **2a** in the presence of 2.5 mol% of $[Pd(\eta^3\text{-}C_3H_5)Cl]_2$.

The results obtained with ligand **2a** encouraged us to continue our search to find out some improvements in the reaction conditions using this catalyst. The results of these studies are summarized in Table 2.

Table 2. Effect of the base/solvent system in the asymmetric allylic alkylation



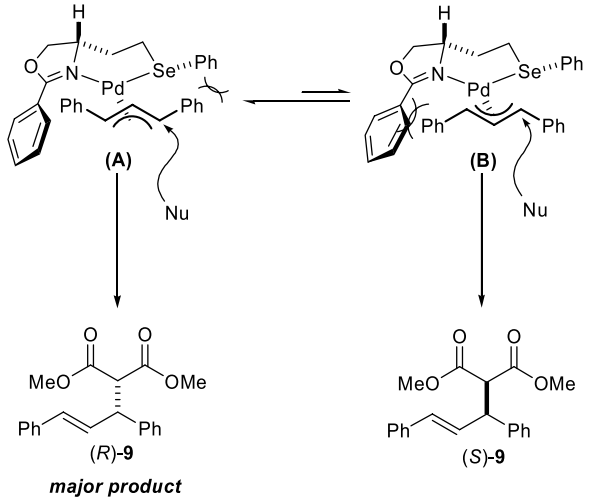
Entry	Base	Solvent	Time (h)	Yield (%)[a]	ee (%)[b]
1[c]	BSA/KOAc	CH_2Cl_2	24	71	23
2	BSA/KOAc	CH_2Cl_2	24	97	81
3[d]	BSA/KOAc	CH_2Cl_2	24	93	79
4	BSA/KOAc	CH_3CN	24	95	79
5	BSA/KOAc	Toluene	24	63	66
6	NaH	THF	24	99	85
7[d]	NaH	THF	24	96	85
8	Cs_2CO_3	CH_2Cl_2	2	99	85
9[d]	Cs_2CO_3	CH_2Cl_2	10	99	91
10	Cs_2CO_3	Toluene	24	49	32

[a] Isolated yields.
[b] Determined by HPLC with a Daicel Chiralcel OD column, hexane/isopropanol 99:1; 0.5 mL/min; 254 nm.
[c] Reaction carried out using **1** as ligand.
[d] Reaction was carried out at 0 °C.

We examined the effect of another base/solvent system and results are depicted in Table 2. Changing the base from NaH to *N*,*O*-bis(trimethylsilyl)acetamide (BSA) and the solvent from THF to dichloromethane, a decrease in the ee was observed (entries 2 and 3). Another change to a more polar solvent, acetonitrile, and to the apolar toluene, did not result in any improvement (see entries 4 and 5). When using acetonitrile the ee of the product did not change significantly. With toluene, however, the enantioselectivity decreased to 66% ee.

On the other hand, employing cesium carbonate as base and performing the reaction in dichloromethane, the reaction proceeded smoothly and the product was isolated in 85% of enantiomeric excess and full conversion after only 2 h at room temperature (entry 8). The use of these conditions gave the same ee of product **9** when compared to the NaH/THF system; however, the reaction goes to completion in much reduced reaction time. On decreasing the temperature to 0 °C, the reaction took 10 h to completion and, to our delight the ee of **9** increased to 91% (entry 9). Again, reducing the polarity of the solvent proved to negatively influence the reaction outcome, since a dramatic drop in the ee was observed when toluene was used instead of dichloromethane (compare entries 9 and 10).

In order to propose a plausible explanation of the stereoselectivity observed, a schematic reaction pathway is shown in Scheme 4.[25]



Scheme 4. Plausible reaction pathways for the asymmetric allylic alkylation.

Taking into analogy the previous work of Anderson where he proposes that the attack of the nucleophile occurs trans to a nitrogen donor,[11b] which is the contrary of that expected for a nitrogen–sulfur chelate complex, we assumed that our system behaves in a similar way, that is, the nucleophile attacks preferentially at the allylic position trans to the Pd–N bond in the π-allylpalladium complex.

Since (*R*)-**9** is the major product of the alkylation reaction of racemic 1,3-diphenyl-2-propenyl acetate with dimethyl malonate, the reaction appears to proceed preferentially via the intermediate (**A**) in the equilibrium depicted in Scheme 4.

The steric repulsion in the intermediate (**A**) between a phenyl terminus of the allylic substrate arranged in a 'W' orientation and the selenophenyl group seems to be smaller than the repulsion of the phenyl ring attached to the 2-position of the oxazoline ring and the phenyl moiety in structure (**B**), which is disposed in a 'M' orientation.

These disfavoring steric interactions, which are present in intermediate (**B**) would lead to a predominance of the intermediate (**A**) in the equilibrium and explain the stereoselectivity observed in favor of (*R*)-**9** (Scheme 4).

3. Conclusions

In summary, we have described herein a new class of chiral selenium-containing oxazoline chiral ligands, which were prepared in a concise and flexible synthetic route in good yields. This flexibility permits the preparation of a wide range of compounds with varied steric and electronic combination of substituents. This feature confers to this

class of compounds a modular character allowing the synthesis of small libraries of new chiral selenium compounds.

Additionally, these compounds were systematically screened as chiral ligands in the asymmetric palladium-catalyzed allylic alkylation of racemic 1,3-diphenyl-2-propenyl acetate with dimethyl malonate. We were able to identify among the set of ligands prepared, a catalyst that can effectively promote the allylic alkylation reaction in high ee. This approach demonstrates the importance of the easy access to modification in the structure of the chiral ligand in order to readily identify an efficient ligand for a given catalytic setup through a correct combination of structural and electronic properties of the catalyst and refinement of reaction conditions.

We also believe that this modular approach, which permits ready access of a range of new chiral selenium compounds, may have significant importance in the design of new Se, N-ligand systems for application in asymmetric catalysis.

4. Experimental

4.1. General procedures

Melting Points are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively, with tetramethylsilane as internal standard. High-resolution mass spectra were recorded on a Bruker BioApex 70e FT-ICR (Bruker Daltonics, Billerica, USA) instrument in ESI-mode. Column chromatography was performed using Merck Silica Gel (230–400 mesh) following the methods described by Still.[26] Thin-layer chromatography (TLC) was performed using Merck Silica Gel GF_{254}, 0.25 mm thickness. For visualization, TLC plates were either placed under ultraviolet light, or stained with iodine vapor, or acidic vanillin. THF was dried over sodium benzophenone ketyl and distilled prior to use. Dichloromethane and acetonitrile were distilled from phosphorus pentoxide. All other solvents were used as purchased unless otherwise noted. Racemic 1,3-diphenyl-3-acetoxyprop-1-ene was prepared according to literature procedure.[27] Compounds **3**,[21] **6** and **7**[7c] were prepared by the procedures described in the cited references.

4.1.1. (*R*)-(2-phenyl-4,5-dihydrooxazol-4-yl)methanol 4.[28] Under an argon atmosphere, sodium borohydride (0.760 g, 20 mmol) was added, at 0 °C, to a solution of oxazolinyl ester **3** (1.025 g, 5 mmol) in dry ethanol (20 mL). The reaction was then stirred for 12 h under reflux and after this time, cooled to room temperature, diluted with CH_2Cl_2 (30 mL) and washed with saturated $NaCl_{(aq)}$ (20 mL). The organic layer was dried with $MgSO_4$, filtered and the solvent removed under vacuum. Alcohol **4** was used without further purification. Yield 87%; $[\alpha]_D^{20}$ +35 (*c* 0.42, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ=7.81–7.79 (m, 2H), 7.43–7.26 (m, 3H), 4.44–4.30 (m, 3H), 3.93 (dd, J^1=11.6 Hz, J^2=4.0 Hz, 1H), 3.63 (dd, J^1=11.6 Hz, J^2=4 Hz, 1H), 2.89 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ=165.45, 131.38, 128.24, 128.14, 126.95, 69.17, 68.00, 63.60; HRMS *m/z* calcd for $C_{10}H_{11}O_2N+Na^+$ 200.0682, found 200.0683.

4.1.2. (*S*)-(2-phenyl-4,5-dihydrooxazol-4-yl)methyl 4-methylbenzenesulfonate 5.[29] Under an argon atmosphere, TsCl (0.420 g, 2.2 mmol) was added in one portion, at 0 °C, to a solution of alcohol **4** (0.354 g, 2 mmol) in dichloromethane (5 mL) and Et_3N (0.6 mL, 4 mmol) in the presence of a catalytic amount of DMAP (25 mg, 10 mol%). The mixture was then stirred for 24 h at room temperature diluted with CH_2Cl_2 (30 mL) and washed with saturated $NaCl_{(aq)}$ (20 mL). The organic layer was dried with $MgSO_4$, filtered and the solvent evaporated. The crude product was purified by flash chromatography eluting with a mixture of hexanes–ethyl acetate (80/20). Yield: 77%; $[\alpha]_D^{24}$ +56.6 (*c* 1.0, EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz): δ=7.86–7.79 (m, 2H), 7.77–7.69 (m, 2H), 7.49–7.20 (m, 5H), 4.55–4.38 (m, 2H), 4.33–4.19 (m, 2H), 4.05–3.96 (m, 1H), 2.39 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ=166.0, 145.0, 132.5, 131.8, 129.9, 128.4, 128.3, 128.0, 127.0, 70.8, 69.8, 65.1, 21.7.

4.1.3. (*S*)-2-(2-phenyl-4,5-dihydrooxazol-4-yl)ethyl 4-methylbenzenesulfonate 8.[7c] Under an argon atmosphere, TsCl (2.88 g, 15 mmol) was added in one portion to a solution of diol **7** (1.045 g, 5 mmol) in dichloromethane (50 mL) and Et_3N (4.2 mL, 30 mmol) at 0 °C. The mixture was then stirred for 24 h and slowly warmed to 25 °C. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed with 1 M $HCl_{(aq)}$ (20 mL), saturated $NaHCO_{3(aq)}$ (20 mL) and saturated $NaCl_{(aq)}$ (20 mL). The organic layer was dried with $MgSO_4$, filtered, and concentrated. The crude product was purified by flash chromatography eluting with a mixture of hexanes–ethyl acetate (70/30). Yield: 75%; $[\alpha]_D^{24}$ −59 (*c* 2.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ=7.86 (d, J=8.4 Hz, 2H), 7.79 (d, J=8.4 Hz, 2H), 7.46–7.30 (m, 5H), 4.48–4.44 (m, 1H), 4.35–4.23 (m, 3H), 4.04–4.00 (m, 1H), 2.42 (s, 3H), 2.03–1.97 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ=164.01, 144.75, 132.76, 131.37, 129.78, 128.29, 128.22, 128.15, 127.83, 72.28, 67.88, 63.34, 34.98, 21.54.

4.1.4. (*S*)-2-(2-*tert*-butyl-phenyl-4,5-dihydrooxazol-4-yl) ethyl 4-methylbenzenesulfonate 10. This compound was prepared in the same method used for **8**, starting from (*S*)-2-*tert*-butyl-benzoylamino-1,4-butanediol (0.795 g, 3 mmol). Yield 55%; $[\alpha]_D^{20}$ −56 (*c* 0.6, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz): δ=7.80 (d, J=8.4 Hz, 4H), 7.40 (d, J=8.4 Hz, 2H), 7.31 (d, J=8.4 Hz, 2H), 4.47–4.42 (m, 1H), 4.33–4.23 (m, 3H), 4.03–3.99 (m, 1H), 2.42 (s, 3H), 2.01–1.96 (m, 2H), 1.30 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ=164.11, 154.91, 144.67, 133.02, 129.78, 128.05, 127.85, 125.19, 124.60, 72.22, 67.91, 63.36, 35.07, 34.87, 31.08, 21.52; HRMS *m/z* calcd for $C_{22}H_{27}O_4NS+H^+$ 402.1729, found 402.1733.

4.1.5. (*S*)-2-(2-*tert*-butyl-4,5-dihydrooxazol-4-yl)ethyl 4-methylbenzenesulfonate 11.[30] This compound was prepared in the same method used for **8**, starting from (*S*)-2-trimethyl-acetylamino-1,4-butanediol (0.567 g, 3 mmol). Yield 62%; $[\alpha]_D^{20}$ −54 (*c* 0.6, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz): δ=7.78 (d, J=8.4 Hz, 2H), 7.34 (d, J=8.4 Hz, 2H), 4.26–4.07 (m, 4H), 3.85–3.81 (m, 1H), 2.44 (s, 3H), 1.89–1.86 (m, 2H), 1.16 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ=174.32, 144.62, 132.44, 129.75, 128.74,

71.94, 67.72, 62.69, 34.89, 32.93, 27.17, 21.39; HRMS *m/z* calcd for $C_{16}H_{24}O_4NS+H^+$ 326.1416, found 326.1420.

4.2. General procedure for the synthesis of ligands 1 and 2

Under an argon atmosphere, sodium borohydride was added to a solution of the diorganoil diselenide (0.55 mmol) in THF (4 mL). Ethanol (2 mL) was then dropwise added and the clear solution formed was stirred at room temperature for 10 min. After this time a THF (1 mL) solution of the appropriate oxazolinyl tosylate (1 mmol) was added dropwise. After stirring for 24 h at room temperature, the reaction mixture was quenched with aqueous saturated NH_4Cl (10 mL) and extracted with CH_2Cl_2 (3×15 mL). The combined organic layers were dried with $MgSO_4$, filtered, and concentrated. The crude product was purified by flash chromatography first eluting with hexanes and then with a mixture of hexanes–ethyl acetate (80/20).

4.2.1. (*S*)-2-phenyl-4-(phenylselanylmethyl)-4,5-dihydrooxazole 1. Yield: 91%; $[\alpha]_D^{20}$ −15 (*c* 0.5, CH_2Cl_2); ^1H NMR ($CDCl_3$, 400 MHz): δ=7.90 (d, *J*=7.2 Hz, 2H), 7.56–7.53 (m, 8H), 4.53–4.46 (m, 2H), 4.25–4.22 (m, 1H), 3.42–3.38 (m, 1H), 2.95–2.89 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ=164.66, 133.02, 131.47, 129.15, 128.29, 128.29, 128.27, 127.48, 127.28, 72.53, 66.58, 32.73; HRMS *m/z* calcd for $C_{16}H_{15}ONSe+H^+$ 318.0392, found 318.0391.

4.2.2. (*S*)-2-phenyl-4-(2-(phenylselanyl)ethyl)-4,5-dihydrooxazole 2a. Yield: 97%; $[\alpha]_D^{20}$ −57 (*c* 0.55, CH_2Cl_2); ^1H NMR ($CDCl_3$, 400 MHz): δ=7.93 (d, *J*=7.16 Hz, 2H), 7.52–7.37 (m, 5H), 7.26–7.20 (m, 3H), 4.48–4.37 (m, 2H), 4.02–3.98 (m, 1H), 3.14–2.99 (m, 2H), 2.10–2.01 (m, 1H), 2.00–1.94 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ=165.88, 132.48, 131.24, 130.04, 129.14, 128.21, 128.17, 127.99, 126.74, 72.12, 66.46, 36.45, 24.04; HRMS *m/z* calcd for $C_{17}H_{17}ONSe+Na^+$ 354.0366, found 354.0367.

4.2.3. (*S*)-4-(2-(benzylselanyl)ethyl)-2-phenyl-4,5-dihydrooxazole 2b. Yield: 87%; $[\alpha]_D^{20}$ −58 (*c* 0.5, CH_2Cl_2); ^1H NMR ($CDCl_3$, 400 MHz): δ=7.93 (d, *J*=7.2 Hz, 2H), 7.45–7.36 (m, 3H), 7.29–7.17 (m, 5H), 4.42–4.38 (m, 1H), 4.33–4.29 (m, 1H), 3.96–3.92 (m, 1H), 3.78 (s, 2H), 2.62–2.59 (m, 2H), 2.04–1.95 (m, 1H), 1.90–1.81 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ=163.33, 139.07, 131.02, 128.59, 128.19, 128.01, 127.91, 127.50, 126.39, 71.87, 66.30, 36.38, 26.79, 19.56; HRMS *m/z* calcd for $C_{18}H_{19}ONSe+Na^+$ 368.0563, found 368.0526.

4.2.4. (S)-4-(2-(4-chlorophenylselanyl)ethyl)-2-phenyl-4,5-dihydrooxazole 2c. Yield: 90%; $[\alpha]_D^{20}$ −48 (*c* 0.55, CH_2Cl_2); ^1H NMR ($CDCl_3$, 400 MHz): δ=7.93 (d, *J*=7.2 Hz, 2H), 7.47–7.40 (m, 5H), 7.22 (d, *J*=8.4 Hz, 2H), 4.50–4.45 (m, 1H), 4.40–3.37 (m, 1H), 4.02–3.98 (m, 1H), 3.11–3.01 (m, 2H), 2.05–1.95 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ=163.73, 133.76, 132.93, 131.27, 129.11, 128.23, 128.21, 128.15, 127.56, 72.06, 66.36, 36.36, 24.08; HRMS *m/z* calcd for $C_{17}H_{16}ONSeCl+H^+$ 366.0154, found 366.0163.

4.2.5. (S)-4-(2-(4-methoxyphenylselanyl)ethyl)-2-phenyl-4,5-dihydrooxazole 2d. Yield: 91%; $[\alpha]_D^{20}$ −48 (*c* 0.55, CH_2Cl_2); ^1H NMR ($CDCl_3$, 400 MHz): δ=7.92 (d, *J*=7.2 Hz, 2H), 7.49–7.39 (m, 5H), 6.81 (d, *J*=8.8 Hz, 2H), 4.47–4.35 (m, 2H), 4.00–3.96 (m, 1H), 3.78 (s, 3H), 3.02–2.91 (m, 2H), 2.07–1.99 (m, 1H), 1.96–1.89 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ=163.60, 159.21, 135.47, 131.19, 128.17, 128.13, 127.64, 119.60, 114.71, 72.09, 66.44, 55.12, 36.47, 25.10; HRMS *m/z* calcd for $C_{18}H_{19}O_2NSe+H^+$ 362.0648, found 362.0653.

4.2.6. (S)-4-(2-(mesitylselanyl)ethyl)-2-phenyl-4,5-dihydrooxazole 2e. Yield: 88%; $[\alpha]_D^{20}$ −35 (*c* 0.5, CH_2Cl_2); ^1H NMR ($CDCl_3$, 400 MHz): δ=7.91 (d, *J*=8.0 Hz, 2H), 7.47–7.36 (m, 3H), 6.91 (s, 2H), 4.46–4.44 (m, 1H), 4.36–4.32 (m, 1H), 3.99–3.95 (m, 1H), 2.82–2.77 (m, 2H), 2.53 (s, 6H), 2.25 (s, 3H), 1.98–1.86 (s, 1H), 1.84–1.83 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ=163.54, 142.95, 137.91, 131.15, 128.40, 128.37, 128.34, 127.65, 127.31, 72.04, 66.69, 36.65, 24.42, 24.62, 20.80; HRMS *m/z* calcd for $C_{20}H_{23}ONSe+H^+$ 374.1011, found 374.1017.

4.2.7. (*S*)-2-phenyl-4-(2-(3-(trifluoromethyl)phenylselanyl)-ethyl)-4,5-dihydrooxazole 2f. Yield: 80%; $[\alpha]_D^{20}$ −59 (*c* 0.5, CH_2Cl_2); ^1H NMR ($CDCl_3$, 400 MHz): δ=7.93 (d, *J*=7.2 Hz, 2H), 7.74 (s, 1H), 7.46 (d, *J*=7.2 Hz, 1H), 7.42–7.35 (m, 5H), 4.51–4.49 (m, 1H), 4.47–4.41 (m, 1H), 4.03–4.00 (m, 1H), 3.19–3.16 (m, 1H), 3.13–3.11 (m, 1H), 2.05–1.99 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ=163.89, 135.17, 131.59, 131.34, 131.32 (q, *J*=32.1 Hz), 129.26, 128.55, 128.51, 128.26, 128.22, 127.58, 123.43 (q, *J*=3.8 Hz), 72.11, 66.36, 36.37, 23.92; HRMS *m/z* calcd for $C_{18}H_{16}ONSe+H^+$ 400.0442, found 400.0421.

4.2.8. (*S*)-4-(2-(*tert*-butylselanyl)ethyl)-2-phenyl-4,5-dihydrooxazole 2g. Yield: 77%; $[\alpha]_D^{20}$ −75 (*c* 0.5, CH_2Cl_2); ^1H NMR ($CDCl_3$, 400 MHz): δ=7.94 (d, *J*=7.12 Hz, 2H), 7.47–7.38 (m, 3H), 4.53–4.49 (m, 1H), 4.39–4.36 (m, 1H), 4.07–4.03 (m, 1H), 2.79–2.71 (m, 2H), 2.08–2.04 (m, 1H), 1.97–1.94 (m, 1H), 1.46 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ=163.63, 131.19, 128.20, 128.19, 127.74, 72.25, 66.95, 38.86, 37.09, 32.46, 17.91; HRMS *m/z* calcd for $C_{15}H_{21}ONSe+Na^+$ 334.0672, found 334.0680.

4.2.9. (*S*)-4-(2-(methylselanyl)ethyl)-2-phenyl-4,5-dihydrooxazole 2h. Yield: 79%; $[\alpha]_D^{20}$ −82 (*c* 0.55, CH_2Cl_2); ^1H NMR ($CDCl_3$, 400 MHz): δ=7.94 (d, *J*=7.2 Hz, 2H), 7.47–7.38 (m, 3H), 4.53–4.49 (m, 1H), 4.42–4.38 (m, 1H), 4.07–4.03 (m, 1H), 2.72–2.67 (m, 2H), 2.07–1.95 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ=163.68, 131.24, 128.22, 128.18, 127.69, 72.18, 66.52, 36.42, 21.20, 4.05; HRMS *m/z* calcd for $C_{12}H_{15}ONSe+H^+$ 270.0386, found 270.0397.

4.2.10. (S)-2-(4-*tert*-butylphenyl)-4-(2-(phenylselanyl) ethyl)-4,5-dihydrooxazole 2i. Yield: 93%; $[\alpha]_D^{20}$ −51 (*c* 0.55, CH_2Cl_2); ^1H NMR ($CDCl_3$, 400 MHz): δ=7.85 (d, *J*=8.4 Hz, 2H), 7.50 (d, *J*=7.2 Hz, 2H), 7.41 (d, *J*=8.4 Hz, 2H), 7.26–7.23 (m, 3H), 4.47–4.39 (m, 2H), 4.01–3.98 (m, 1H), 3.10–3.01 (m, 2H), 2.06–2.04 (m, 1H), 1.98–1.96 (m, 1H), 1.32 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ=163.69, 154.68, 132.42, 130.08, 128.98, 127.98, 126.71, 125.17, 124.81, 71.97, 66.42, 36.48, 34.83, 31.08, 23.68; HRMS *m/z* calcd for $C_{21}H_{25}ONSe+H^+$ 388.1168, found 388.1179.

4.2.11. (S)-2-*tert*-butyl-4-(2-(phenylselanyl)ethyl)-4,5-dihydrooxazole 2j. Yield: 90%; $[\alpha]_D^{20}$ −26 (*c* 0.55, CH_2Cl_2); ^1H NMR ($CDCl_3$, 400 MHz): δ=7.48 (d, *J*= 7.6 Hz, 2H), 7.27–7.21 (m, 3H), 4.26–4.21 (m, 1H), 4.16–4.13 (m, 1H), 3.84–3.80 (m, 1H), 3.01–2.97 (m, 1H), 2.95–2.91 (m, 1H), 1.94–1.88 (m, 2H), 1.20 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ=174.11, 132.29, 130.16, 129.00, 126.70, 71.90, 65.85, 36.53, 33.13, 27.78, 23.33; HRMS *m/z* calcd for $C_{15}H_{21}ONSe+H^+$312.0869, found 312.0866.

4.3. General procedure for the asymmetric allylic alkylation with NaH/THF

A THF (1 mL) solution of $[Pd(\eta^3\text{-}C_3H_5)Cl]_2$ (10 mg, 2.5 mol%), catalyst (10 mol%) was stirred for 30 min under an argon atmosphere and then 1,3-diphenyl-2-propenyl acetate (252 mg, 1.0 mmol) was added. The mixture was stirred for 10 min and a solution of sodium dimethyl malonate, prepared from dimethyl malonate (264 mg, 2.0 mmol) and sodium hydride (36 mg, 1.5 mmol) in THF (3 mL), was added at room temperature. The reaction mixture was then stirred for 24 h at room temperature. After this time, saturated $NH_4Cl_{(aq)}$ was added and the aqueous solution was extracted with CH_2Cl_2 (3× 15 mL). The combined organic layers were dried with $MgSO_4$, the solvent was evaporated and the crude product was purified by flash chromatography eluting with hexane–ethyl acetate (98/2). Enantiomeric excess was determined by chiral HPLC (Chiralcel OD column, 0.5 mL/min, hexane/2-propanol 99:1, 254 nm). The optical rotation of the product was compared with literature data to assign the absolute configuration (*R*).

4.4. General procedure for the asymmetric allylic alkylation with Cs_2CO_3/CH_2Cl_2

A solution of $[Pd(\eta^3\text{-}C_3H_5)Cl]_2$ (10 mg, 2.5 mol%), catalyst (10 mol%) in dichloromethane (2.5 mL) was stirred for 1 h under an argon atmosphere, at room temperature, and then cooled to 0 °C, when 1,3-diphenyl-2-propenyl acetate (126 mg, 0.5 mmol) was added. The mixture was stirred for 10 min at this temperature and dimethyl malonate (173 mg, 1.5 mmol), and cesium carbonate (489 mg, 1.5 mmol) were sequentially added. The reaction mixture was then stirred for 10 h at 0 °C. After this time, saturated $NH_4Cl_{(aq)}$ was added and the aqueous solution was extracted with CH_2Cl_2 (3×15 mL). The combined organic layers were dried with $MgSO_4$, the solvent was evaporated and the crude product was purified by flash chromatography eluting with hexane–ethyl acetate (98/2). Enantiomeric excess was determined by chiral HPLC (Chiralcel OD column, 0.5 mL/min, hexane/2-propanol 99:1, 254 nm). The optical rotation of the product was compared with literature data to assign the absolute configuration (*R*).

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Synthesis of (+)-agelasine C. A structural revision

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Abstract—An efficient synthesis of (+)-agelasine C has been achieved from *ent*-halimic acid. The structure and absolute configuration of the natural product (−)-agelasine C was established and a structure for *epi*-agelasine C, is proposed.
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1. Introduction

Agelasines are a diterpene-alkaloid group, 7,9-dialkylpurine salts, isolated from marine sponges of the genus *Agelas sp.*[1] Until now only 11 natural products have been described, wearing all of them a diterpene side chain in position 7 of the adenine. Until now only (−)-agelasine A,[2] (−)-agelasine B,[3] (±)-agelasine F[4] and (+)-agelasine D[5] have been synthesized.

Agelasines are associated with biological activities with antimicrobial and cytotoxic effects, as well as contractive response of smooth muscles and inhibition of Na,K-ATPase, strong activity against *Mycobacterium tuberculosis* and antifouling agent for macroalgae.[6] Agelasine C is one of the first four known agelasines isolated by Nakamura et al. in 1984,[7] from Okinawan sea sponge *Agelas sp.* Agelasine C showed powerful inhibitory effects on Na,K-ATPase and antimicrobial activities (Fig. 1).

The union of the diterpenic part with the halimane skeleton (rearranged labdane skeleton) to the nitrogen atom at 7′ position of the 9′-methyladeninium group, was fixed spectroscopically. The configuration at C-8 and C-9 was established by chemical correlation as it was done with agelasine A, being perfectly established the relative *cis* configuration for the methyls at C-8 and C-9. The configuration at C-5 and so the absolute configuration was tentatively determined by circular dichroism and spectroscopical studies of a ketone. Nakamura et al. have presented the structural formula **1** for agelasine C.

Epi-agelasine C[8] was isolated in 1997 for Hattori et al. from the marine sponge *Agelas mauritiana* as an antifouling substance active against macroalgae. The structure of this substance was elucidated by spectral means. The stereoestructure of *epi*-agelasine C was established by its NOE difference spectrum. The absolute configuration still is not known, Hattori et al. presented the structural formula **2** for *epi*-agelasine C (Fig. 1).

Due to the interest of *epi*-agelasine C as antifouling agent[9] and having the possibility of establish the absolute configuration of this compound the synthesis of **19**, has been carried out in this work.

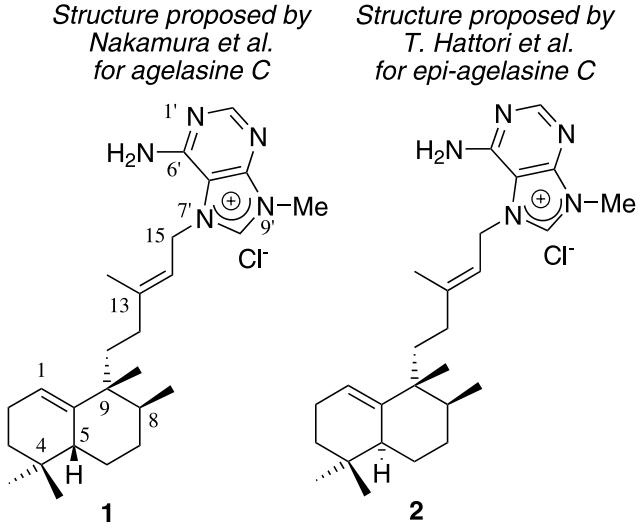

Figure 1.

Keywords: Agelasine C; *Epi*-agelasine C; Diterpenes alkaloids; *Ent*-halimic acid.
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2. Results and discussion

The synthesis of **19** was planned following an analogue scheme to the one for other agelasines, this is coupling of the terpenic fragment with the corresponding purine as **4** (Scheme 1).

As starting material for the terpenic part, *ent*-halimic acid methyl ester **3**, duly transformed its functional groups into the bromoderivative **5**, will contribute the necessary stereogenic centres. *Ent*-halimic acid is a known natural product used already for the synthesis of chettaphanin I and II,[10] *ent*-halimanolides[11] and sesterterpenolides.[12]

The synthesis of **19** is carried out in a convergent way, synthesis of the purine fragment and the diterpenic part.

The purinic fragment **4** was done following the methodology of Fujii[13] from adenine (Scheme 2).

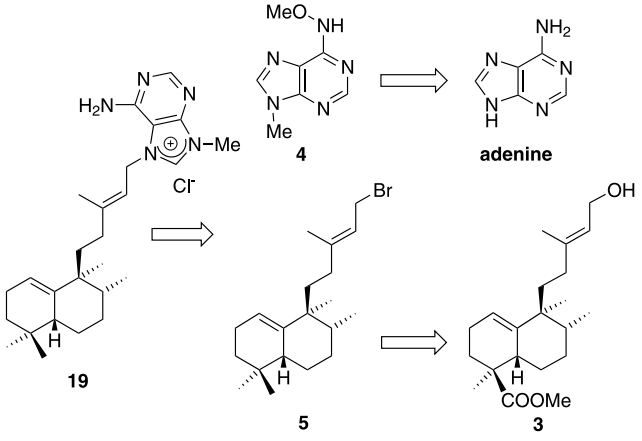
Oxidation of adenine with peracetic acid[14] gives oxide **6** that by methylation produce the iodohydrate **7** that lead to **8** when pass through an amberlite column. The *N*-methyl oxide **8** was selectively methylated in 9 position by treatment with MeI in DMA (dimethyl acetamide) giving compound **9** from which, imine **10** is obtained when passed through an amberlite column. When compound **10** is heated in refluxing water the required compound **4** is obtained.

The second part of the synthesis is the transformation of *ent*-halimic acid methyl ester **3** into the bromoderivative **5**. This transformation required the reduction of the methoxycarbonyl at C-18 to a methyl group and substitution of the alcohol in C-15 for bromine (Scheme 3).

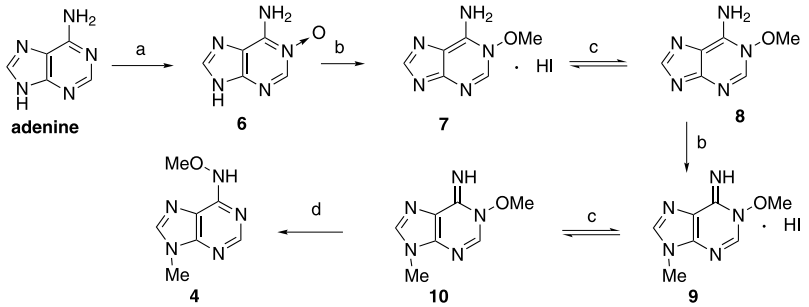
In order to reduce C-18 (Scheme 3) is necessary to protect previously C-15 as tetrahydropyrane derivative. The reaction of **3** with DHP[15] in *p*-TsOH gives **11** that by reduction with LAH lead to the hydroxyderivative **12**. The reaction of **12** with CS_2 followed by treatment with MeI in alkaline media led to the xantogenate **13**, that by reduction with Bu_3SnH in presence of AIBN gave the reduction product **15** in a modest yield.[16] This yield was increased when aldehyde **14**, obtained by oxidation of **12** with TPAP, was reduced using Huang–Minlon methodology.[17] Deprotection of the primary hydroxy group followed by the treatment of the hydroxyderivative **16** with CBr_4[18] in presence of PPh_3 gave the required bromoderivative **5**.

Once the two fragments were obtained the synthesis was continued. Alkylation of methoxyadenine **4** with the bromoderivative **5** (Scheme 4) gives a mixture of **17** and **18**.[19] Compound **18** is the required one and **17** is the isomer corresponding to the alkylation of the N in C-6 (Scheme 4). The separation of these compounds is very difficult and was achieved using polar solvents as CH_2Cl_2/MeOH/NH_4OH in silica gel column chromatography.

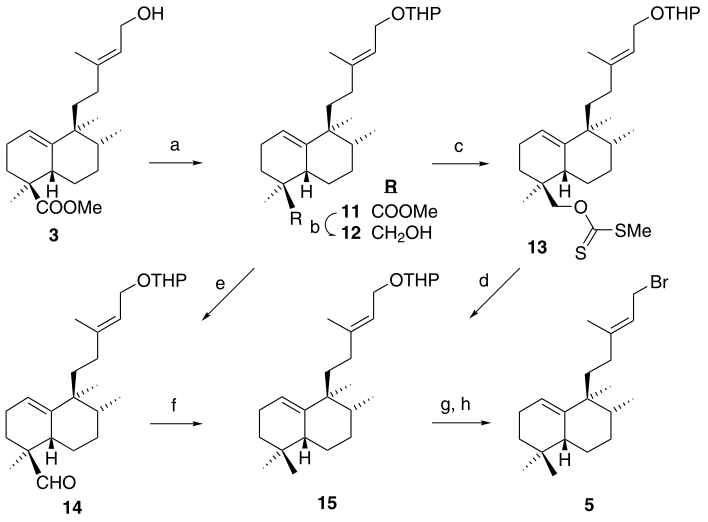
Finally reduction of **18** with Zn/AcOH gives compound **19**. The physical properties of **19** are very different to the natural product *epi*-agelasine C, whose proposed structure **2**, (Fig. 1) should be revised. By contrary when compared the



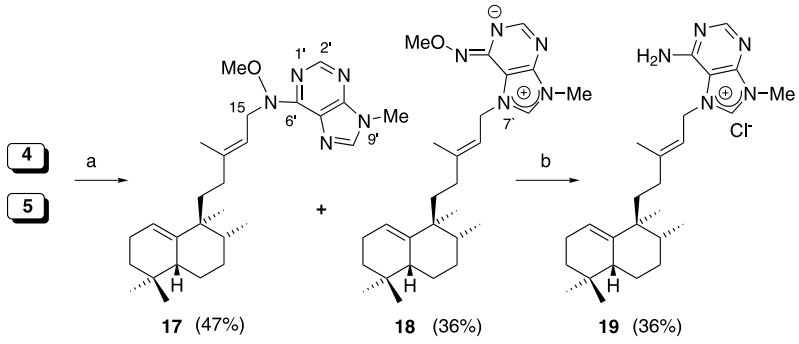
Scheme 1.



Scheme 2. (a) AcOOH, AcOH, 10%, 24 h (31%); (b) MeI, DMA, 20 h; (c) Amberlite IRA-402 (HCO_3^-), H_2O (44%); (d) H_2O, reflux, 90 min, (77%).



Scheme 3. (a) DHP, *p*-TsOH, C_6H_6, 15 min, rt, (98%); (b) LAH, Et_2O, 0 °C and then rt, 4 h, (99%); (c) *n*BuLi, THF, −78 °C→0 °C, 30 min then CS_2, 2 h, rt then MeI, 1 h, rt, (98%); (d) nBu_3SnH, AIBN, toluene, 120 °C, 20 min, (31%); (e) TPAP, NMO, sieves, DCM, Ar, 45 min, (94%); (f) diethylene glycol, NH_2–$NH_2 \cdot H_2O$, KOH, 175 °C→230 °C, 18 h, (81%); (g) *p*-TsOH, MeOH, 30 min, (81%); (h) CBr_4, PPh_3, DCM, 3 h, (76%).



Scheme 4. (a) DMA, 50 °C, 16 h, (**17**: 47%; **18**: 36%); (b) Zn, MeOH, H_2O, AcOH, argon, 60 °C, 2 days, (**18**: 47%; **19**: 36%).

^{13}C NMR spectra of **19** with agelasine C, structure proposed **1**, showed to be identical, as their ^{1}H NMR, although the rotation for **19** ($[\alpha]_D^{22}+36.7$) and agelasine C ($[\alpha]_D^{25}-55.1$) have different sign. So it should be concluded that the structure for (−)-agelasine C should be corrected to structure **20**, enantiomer of the synthesis product **19** (+)-agelasine C (Fig. 2).

Considering the spectroscopical properties of *epi*-agelasine C (C-5 and C-9 configurations were unequivocally established by NOE experiments)[8] and by comparison with the ones of some derivatives with the same decaline as the sesterterpenes with cladocorane skeleton,[20] can be concluded that when methyls Me-17 and Me-20 (*ent*-halimane skeleton) or Me-24 and Me-25 (cladocorane skeleton) are in disposition *cis*, Me-20 or its equivalent Me-25 is always more shielded than in compounds with a *trans* disposition of these methyls. This is the case for **19** and *epi*-agelasine C, in **19**, the signal for Me-20 in the ^{1}H NMR spectrum appears at 0.96 ppm while in *epi*-agelasine C the signal for Me-20 appears at 1.06 ppm and consequently in the last compound Me-20 and Me-17 should be *trans*, as in **21**.

Rotation for *epi*-agelasine C ($[\alpha]_D^{25}+33.9$) and **19** ($[\alpha]_D^{22}+25.0$) allow us to propose for the natural compound the absolute configuration of **21** (Fig. 2), considering the sign and the contribution to the α_D value of the stereogenic centre C-8, that can be deduced by comparison of the epimers compounds of cladocorane skeleton, already mentioned.

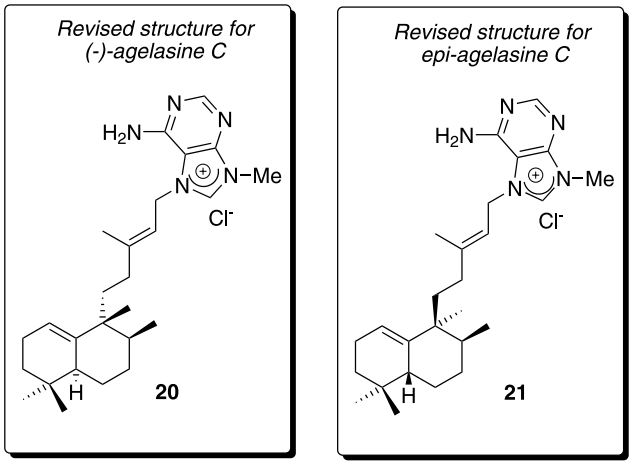


Figure 2. Structure revised for (−)-agelasine C and proposed for *epi*-agelasine C.

3. Conclusions

From the present results, the structure of agelasine C is clearly indicated in **20** and for the natural product *epi*-agelasine C is proposed as **21**.

4. Experimental

Unless otherwise stated, all chemicals were purchased as the highest purity commercially available and were used without further purification. IR spectra were recorded on a MATTSON-GENESIS II FT-IR spectrophotometer. ^{1}H and ^{13}C NMR spectra were performed in deuterochloroform and referenced to the residual peak of $CHCl_3$ at δ 7.26 ppm and δ 77.0 ppm, for ^{1}H and ^{13}C, respectively, at a Bruker WP-200 SY and a Bruker DRX 400 MHz. Chemical shifts are reported in δ ppm and coupling constants (J) are given in Hz. MS were performed at a VG-TS 250 spectrometer at 70 eV ionizing voltage. Mass spectra are presented as *m/z* (% rel int). HRMS were recorded on a VG Platform (Fisons) spectrometer using chemical ionization (ammonia as gas). Optical rotations were determined on a digital ADP 220 polarimeter in 1 dm cells. Diethyl ether, THF and benzene were distilled from sodium, and pyridine and dichloromethane were distilled from calcium hydride under an Ar atmosphere.

4.1. Reaction of adenine with AcOOH: 6

Adenine (10.0 g, 74.07 mmol) was solved in softly hot AcOH (69 mL). To this solution, AcOOH 35% in AcOH (28 mL) was added at room temperature. As result, a solution of AcOOH in AcOH 10% was formed. After 24 h stirring a substancial proportion of precipitated product was formed, filtered off and washed with a little acetic acid. Crystallization of adenine oxide from boiling water yielded filamentous crystals **6** (3.5 g, 31%).

4.1.1. Adenine *N*-oxide (6). Mp 300–301 °C; UV (H_2O): 231, 262 nm; ^{1}H RMN (D_2O) δ: 8.52 and 8.23 (1H, s each, H-2, H-8); ESIMS: 152 ($M^+ + 1$, 100), 135 (20); ESIHRMS: calcd for $C_5H_6N_5O$ $(M)^+$ 152.0567; found $(M)^+$ 152.0577.

4.2. Reaction of 6 with MeI: 8

A mixture of **6** (3.5 g, 20.83 mmol), MeI (3.23 mL, 52.07 mmol) and $AcNMe_2$ (28 mL) was stirred at room temperature for 20 h. The resultant precipitates were filtered off, washed with a small amount of EtOH and evaporated in vacuo. The residue was recrystallized from 70% EtOH/H_2O and filtered off in vacuo obtaining **7** (Mp 222 °C). The hydriodide **7** was dissolved in H_2O (97 mL) and the solution was passed trough a column (Ø: 1 cm) of Amberlite IRA-402 (29 mL) in which previously, a saturated aqueous solution of $NaHCO_3$ and then H_2O till pH: 7, was passed. Slowly elution with water (200 mL) and evaporation of the eluate in vacuo left **8** (1.3 g, 44%).

4.2.1. 1-Methoxyadenine (8). Mp 255–257 °C; UV (EtOH): 201, 227, 273 nm; ^{1}H RMN (D_2O) δ: 8.54 and 8.03 (1H, s each, H-2, H-8), 4.18 (3H, s, MeO–); ^{13}C RMN (D_2O) δ: 140.6 (C-2), 156.3 (C-4), 118.9 (C-5), 146.9 (C-6), 155.7 (C-8), 68.1 (MeO–); ESIMS: 166 ($M^+ + 1$, 100), 152 (20), 135 (85); ESIHRMS: calcd for $C_6H_8N_5O$ $(M)^+$ 166.0723; found $(M)^+$ 166.0721.

4.3. Reaction of 8 with MeI: 10

A mixture of **8** (7.0 g, 41.42 mmol), MeI (6.51 mL, 103.48 mmol) and $AcNMe_2$ (56 mL) was stirred at room temperature for 23 h. The resultant precipitates were filtered off, washed with EtOH to give a white solid **9**. A solution of **9** in H_2O (250 mL) was passed through a column (Ø: 1 cm) of amberlite IRA-402 (100 mL) in which previously, a saturated aqueous solution of $NaHCO_3$ and then H_2O until pH: 7, was passed. Slowly elution of the solution with water (300 mL) and evaporation of the eluent in vacuo left **10** (5.0 g, 68%).

4.3.1. 1-Methoxy-9-methyladenine (10). Mp 172 °C.

4.4. Reaction of 10 with H_2O/110 °C: 4

Compound **10** (900 mg, 5.06 mmol) was treated with boiling water for 1.5 h. The mixture was cooled, evaporated in vacuo and crystallized in H_2O obtaining **4** (634 mg, 77%).

4.4.1. 6-Methoxyamine-9-methylpurine. (*N*-methoxy-9-methyl-9*H*-purin-6-amine) (4). Mp 236 °C; UV (EtOH 95% aq): 268 nm; ^{1}H RMN (D_2O) δ: 7.65 and 7.61 (1H, s each, H-2, H-8), 3.79 (3H, s, MeO–), 3.56 (3H, s, N–Me); ^{13}C RMN (D_2O) δ: 146.1 (C-2), 145.8 (C-4), 116.6 (C-5), 145.9 (C-6), 140.9 (C-8), 62.0 (MeO–), 23.9 (N–Me); ESIMS: 180 ($M^+ + 1$, 100), 150 (30), 149 (95), 145 (20), 132 (30), 127 (10), 113 (70); ESIHRMS: calcd for $C_7H_{10}N_5O$ $(M)^+$ 180.0879; found $(M)^+$ 180.0880.

4.5. Reaction of 3 with DHP: 11

To a solution of **3** (2.1 g, 6.23 mmol) in C_6H_6 (25 mL), *p*-TsOH (237 mg, 1.25 mmol) and DHP (1.75 mL, 18.68 mmol) was added. After stirring 15 min at room temperature, K_2CO_3 (200 mg) was added. The reaction mixture was stirred for an additional 30 min, filtered off and extracted with AcOEt. The combined organic extracts were washed with Na_2CO_3 6%, brine and H_2O. After drying over Na_2SO_4, the solvent was evaporated in vacuo and the residue was chromatographed over silica gel to give **11** (2.5 g, 98%).

4.5.1. Methyl-15-tetrahydropyranyloxy-*ent*-halima-1(10),13*E*-dien-18-oate (11). $[\alpha]_D^{22} + 24.5$ (c 0.4, $CHCl_3$); IR (film): 2940, 1732, 1456, 1256, 1200, 1117, 1024 cm^{-1}; ^{1}H NMR δ: 5.35–5.31 (2H, m, H-1, H-14), 4.98–4.93 and 4.66–4.60 (1H, m each, H-1′), 4.23 (1H, dd, J=12.1, 6.4 Hz, H-15_A), 4.00 (1H, dd, J=12.1, 7.5 Hz, H-15_B), 3.93–3.88 (1H, m, H-5′), 3.66 (3H, s, –COOMe), 3.58–3.53 (1H, m, H-5′), 2.72–2.55 (1H, m, H-5), 2.15–1.00 (19H, m), 1.69 (3H, s, Me-16), 1.11 (3H, s, Me-19), 0.91 (3H, s, Me-20), 0.80 (3H, d, J=7.0 Hz, Me-17); ^{13}C NMR δ: 119.6 (C-1), 22.9 (C-2), 30.7 (C-3), 44.9 (C-4), 38.4 (C-5), 22.9 (C-6), 28.3 (C-7), 38.4 (C-8), 42.8 (C-9), 141.3 (C-10), 37.7 (C-11), 34.0 (C-12), 141.3 (C-13), 120.1 (C-14), 63.8

(C-15), 16.7 (C-16), 15.5 (C-17), 178.4 (C-18), 19.6 (C-19), 22.4 (C-20), 51.6 (–COO*Me*), 97.9 (C-1′), 30.7 (C-2′), 25.5 (C-3′), 19.7 (C-4′), 62.2 (C-5′); EIMS: 418 (M^+, 1), 235 (35), 175 (35), 137 (15), 85 (100); EIHRMS: calcd for $C_{26}H_{42}O_4$ (M^+) 418.3083; found (M^+) 418.3090.

4.6. Reaction of 11 with $LiAlH_4$: 12

A solution of methoxy ether **11** (3.5 g, 8.37 mmol) in ether (80 mL) was reduced with $LiAlH_4$ (477 mg, 12.50 mmol) at 0 °C for 4 h. Then aqueous AcOEt was added and after filtration, the solvent was evaporated to yield alcohol **12** (3.2 g, 99%).

4.6.1. 15-Tetrahydropyranyloxy-*ent*-halima-1(10),13*E*-dien-18-ol (12). $[\alpha]_D^{22}+36.2$ (*c* 0.8, $CHCl_3$); IR (film): 3468, 2940, 1454, 1379, 1117, 1022 cm^{-1}; ^1H RMN δ: 5.35–5.31 (2H, m, H-1, H-14), 4.63–4.58 (1H, m, H-1′), 4.23 (1H, dd, $J=11.8$, 6.5 Hz, H-15_A), 3.97 (1H, dd, $J=11.8$, 7.5 Hz, H-15_B), 3.90–3.85 (1H, m, H-5′), 3.53–3.47 (1H, m, H-5′), 3.47 (1H, d, $J=10.8$ Hz, H-18_A), 3.24 (1H, d, $J=10.8$ Hz, H-18_B), 2.10–1.10 (20H, m), 1.64 (3H, s, Me-16), 0.88 (3H, s, Me-19), 0.83 (3H, s, Me-20), 0.79 (3H, d, $J=7.0$ Hz, Me-17); ^{13}C RMN δ: 120.0 (C-1), 22.6 (C-2), 29.0 (C-3), 36.5 (C-4), 37.8 (C-5), 23.3 (C-6), 28.6 (C-7), 39.0 (C-8), 43.0 (C-9), 141.5 (C-10), 37.5 (C-11), 34.4 (C-12), 141.2 (C-13), 120.2 (C-14), 63.8 (C-15), 16.6 (C-16), 15.6 (C-17), 69.6 (C-18), 20.6 (C-19), 22.3 (C-20), 97.9 (C-1′), 30.7 (C-2′), 25.5 (C-3′), 19.6 (C-4′), 62.2 (C-5′); EIMS: 390 (M^+, 1), 257 (25), 207 (40), 177 (25), 149 (10), 85 (100); EIHRMS: calcd for $C_{25}H_{42}O_3$ $(M)^+$ 390.3133; found $(M)^+$ 390.3140.

4.7. Reaction of 12 with CS_2/MeI: 13

To a solution of **12** (33 mg, 0.09 mmol) in THF (2 mL) cooled at −78 °C under argon atmosphere, a solution of *n*BuLi 1.6 M in hexane (0.06 mL, 0.10 mmol) was added. The mixture was allowed to stir for 30 min at 0 °C and then CS_2 (0.05 mL, 0.85 mmol) was added. The mixture was stirred for 2 h more at room temperature and then MeI (0.02 mL, 0.25 mmol) was added. After 1 h, ice was added. The mixture was extracted with AcOEt and the combined organic extracts were washed with HCl 0.5 M, H_2O and brine. After drying over Na_2SO_4, the solvent was evaporated in vacuo to yield **13** (40 mg, 98%).

4.7.1. Methyl-15-tetrahydropyranyloxy-*ent*-halima-1(10),13*E*-dien-18-xantogenate (13). $[\alpha]_D^{22}+38.0$ (*c* 0.3, $CHCl_3$); IR (film): 1464, 1380, 1215, 1069, 1023, 666 cm^{-1}; ^1H RMN δ: 5.37–5.30 (2H, m, H-1, H-14), 4.63–4.58 (1H, m, H-1′), 4.42 (1H, d, $J=10.6$ Hz, H-18_A), 4.26 (1H, d, $J=10.6$ Hz, H-18_B), 4.18 (1H, dd, $J=11.8$, 7.2 Hz, H-15_A), 3.98 (1H, dd, $J=11.8$, 7.4 Hz, H-15_B), 3.94–3.83 (1H, m, H-5′), 3.56–3.45 (1H, m, H-5′), 2.54 (3H, s, MeS–), 2.26–1.11 (20H, m), 1.66 (3H, s, Me-16), 0.95 and 0.91 (3H, s each, Me-19, Me-20), 0.81 (3H, d, $J=7.0$ Hz, Me-17); ^{13}C RMN δ: 120.1 (C-1), 23.5 (C-2), 29.0 (C-3), 35.9 (C-4), 38.4 (C-5), 22.7 (C-6), 29.1 (C-7), 39.4 (C-8), 43.2 (C-9), 141.3 (C-10), 37.2 (C-11), 34.3 (C-12), 141.1 (C-13), 120.2 (C-14), 63.9 (C-15), 17.0 (C-16), 15.8 (C-17), 80.5 (C-18), 21.6 (C-19), 22.5 (C-20), 98.0 (C-1′), 30.9 (C-2′), 25.7 (C-3′), 19.8 (C-4′), 62.3 (C-5′), 215.9 (–CS_2), 19.0 (MeS–); EIMS: 447 (M^+-33, 3), 331 (15), 270 (15), 189 (70), 85 (100).

4.8. Reaction of 13 with nBu$_3$SnH: 15

To a solution of **13** (373 mg, 0.78 mmol) in toluene (10 mL), AIBN (13 mg, 0.08 mmol) and nBu$_3$SnH (0.52 mL, 1.94 mmol) were added and the mixture was stirred at 120 °C for 20 min. The reaction was filtered through a column of silica and eluted with hexane/AcOEt 98:2 to yield **15** (91 mg, 31%).

4.8.1. 15-Tetrahydropyranyloxy-*ent*-halima-1(10),13*E*-diene (15). IR (film): 2940, 2870, 1653, 1454, 1379, 1200, 1132, 1117, 1042, 907, 870 cm^{-1}; ^1H RMN δ: 5.34–5.30 (2H, m, H-1, H-14), 4.63–4.58 (1H, m, H-1′), 4.20 (1H, dd, $J=11.8$, 6.5 Hz, H-15_A), 3.97 (1H, dd, $J=11.8$, 7.5 Hz, H-15_B), 3.90–3.85 (1H, m, H-5′), 3.53–3.47 (1H, m, H-5′), 2.15–1.00 (20H, m), 1.66 (3H, s, Me-16), 0.89 (3H, s, Me-20), 0.87 (3H, s, Me-19), 0.82 (3H, s, Me-18), 0.80 (3H, d, $J=7.0$ Hz, Me-17); ^{13}C RMN δ: 119.8 (C-1), 23.4 (C-2), 33.5 (C-3), 31.6 (C-4), 43.6 (C-5), 23.9 (C-6), 29.4 (C-7), 39.4 (C-8), 43.1 (C-9), 141.7 (C-10), 37.7 (C-11), 34.5 (C-12), 141.4 (C-13), 120.2 (C-14), 63.9 (C-15), 16.9 (C-16), 15.9 (C-17), 26.3 (C-18), 28.4 (C-19), 22.6 (C-20), 97.9 (C-1′), 31.0 (C-2′), 25.8 (C-3′), 19.9 (C-4′), 62.3 (C-5′); EIMS: 374 (M^+, 8), 272 (15), 191 (90), 121 (14), 85 (100); EIHRMS: calcd for $C_{25}H_{42}O_2$ $(M)^+$ 374.3185; found $(M)^+$ 374.3178.

4.9. Reaction of 12 with TPAP: 14

To a mixture of **12** (3.3 g, 8.46 mmol), *N*-methylmorpholine *N*-oxide (1.7 g, 12.69 mmol) and molecular sieves (4.2 g) in anhydrous DCM (40 mL), under argon and at room temperature, TPAP (148 mg, 0.42 mmol) was added. The reaction mixture was stirred for 45 min and then filtered off on silica gel and celite (EtOAc), the organic layer was evaporated to yield the expected compound **14** (3.1 g, 94%).

4.9.1. 15-Tetrahydropyranyloxy-*ent*-halima-1(10),13*E*-dien-18-al (14). IR (film): 2940, 2872, 1724, 1672, 1456, 1381, 1123, 1024 cm^{-1}; ^1H RMN δ: 9.43 (1H, s, H-18), 5.45–5.25 (2H, m, H-1, H-14), 4.65–4.55 (1H, m, H-1′), 4.20 (1H, dd, $J=11.8$, 6.5 Hz, H-15_A), 3.97 (1H, dd, $J=11.8$, 7.5 Hz, H-15_B), 3.90–3.80 (1H, m, H-5′), 3.55–3.40 (1H, m, H-5′), 2.50–2.35 (1H, m, H-5), 2.20–1.20 (19H, m), 1.66 (3H, s, Me-16), 0.95 (3H, s, Me-19), 0.90 (3H, s, Me-20), 0.79 (3H, d, $J=7.0$ Hz, Me-17); ^{13}C RMN δ: 120.4 (C-1), 22.6 (C-2), 28.7 (C-3), 48.0 (C-4), 36.2 (C-5), 23.2 (C-6), 27.9 (C-7), 38.9 (C-8), 43.2 (C-9), 141.2 (C-10), 37.6 (C-11), 34.4 (C-12), 141.1 (C-13), 120.3 (C-14), 63.9 (C-15), 16.9 (C-16), 15.8 (C-17), 205.9 (C-18), 17.6 (C-19), 22.5 (C-20), 97.9 (C-1′), 30.9 (C-2′), 25.8 (C-3′), 19.9 (C-4′), 62.3 (C-5′).

4.10. Reaction Huang–Minlon of 14: 15

To a solution of **14** (1.76 g, 4.53 mmol) in diethylene glycol (40 mL), KOH (1.6 g, 26.6 mmol) and hydrazine hydrate 25% (6 mL, 120.0 mmol) were added. The reaction was heated at 175 °C for 18 h, the condenser was removed for 15 min and after putting back the condenser, the heat raised

to 230 °C. After 4 h stirring at that temperature, the reaction was cooled and H_2O (25 mL) and HCl 6 M (25 mL) were added. Extraction with Et_2O followed by washing with H_2O, dried and evaporation of the organic layer, yield a crude, which was chromatographed over silica (63 g) and eluted with hexane/AcOEt 98:2 to yield **15** (1.37 g, 81%).

4.10.1. Reaction of 15 with *p*-TsOH and CBr_4/PPh_3: 5. To a solution of **15** (8 mg, 0.02 mmol) in MeOH (0.5 mL), *p*-TsOH (2 mg, 0.01 mmol) was added. After 30 min stirring, the reaction mixture was diluted with AcOEt and washed successively with a 6% aqueous solution of $NaHCO_3$ and water. The organic layer was dried and evaporated to give a crude, which was chromatographed on silica gel (hexane/AcOEt 95:5→9:1) to yield (5 mg, 81%) of *ent*-halima-1(10),13*E*-dien-15-ol. $[\alpha]_D^{22}+83.0$ (*c* 0.9, $CHCl_3$); IR (film): 3327, 3048, 2918, 2870, 1669, 1456, 1379, 1364, 1001, 851 cm^{-1}; ^1H RMN δ: 5.38 (1H, t, J= 7.0 Hz, H-14), 5.31 (1H, t, J=3.2 Hz, H-1), 4.13 (2H, d, J= 7.0 Hz, H-15), 2.05–1.98 (2H, m, H-6), 2.00–1.95 (1H, m, H-11), 1.98–1.95 (1H, m, H-7), 1.84 (1H, dt, J=14.8, 5.2 Hz, H-12), 1.70 (1H, dt, J=14.8, 4.8 Hz, H-12), 1.69–1.63 (1H, m, H-5), 1.66 (3H, s, Me-16), 1.54–1.50 (1H, m, H-8), 1.35–1.30 (3H, m), 1.25–1.21 (1H, m, H-11), 1.15–1.08 (2H, m, H-3), 0.90 (3H, s, Me-20), 0.88 (3H, s, Me-19), 0.83 (3H, s, Me-18), 0.81 (3H, d, J=7.0 Hz, Me-17); ^{13}C RMN δ: 119.8 (C-1), 23.6 (C-2), 33.4 (C-3), 31.4 (C-4), 43.4 (C-5), 23.1 (C-6), 29.1 (C-7), 39.1 (C-8), 42.9 (C-9), 141.7 (C-10), 37.7 (C-11), 34.2 (C-12), 141.2 (C-13), 122.8 (C-14), 59.4 (C-15), 16.4 (C-16), 15.6 (C-17), 25.9 (C-18), 28.2 (C-19), 22.3 (C-20); EIMS: 290 (M^+, 1), 192 (100), 129 (17), 107 (15), 91 (16), 77 (22); EIHRMS: calcd for $C_{20}H_{34}O$ $(M)^+$ 290.2609; found $(M)^+$ 290.2607.

To a solution of *ent*-halima-1(10),13*E*-dien-15-ol (160 mg, 0.55 mmol) in DCM (6 mL), PPh_3 (307 mg, 1.17 mmol) and CBr_4 (387 mg, 1.16 mmol) were added. The reaction was allowed to stir under argon atmosphere for 3 h and then the solvent was removed. The different solubility in hexane allowed to separate **5** (148 mg, 76%).

4.10.2. 15-Bromide of *ent*-halima-1(10),13*E*-dienyl (5). IR (film): 2924, 1653, 1456, 1379, 1200, 1119, 851 cm^{-1}; ^1H RMN δ: 5.49 (1H, t, J=7.5 Hz, H-14), 5.30 (1H, t, J= 3.8 Hz, H-1), 4.01 (2H, d, J=7.5 Hz, H-15), 2.10–1.05 (14H, m), 1.71 (3H, s, Me-16), 0.89 (3H, s, Me-20), 0.88 (3H, s, Me-19), 0.83 (3H, s, Me-18), 0.81 (3H, d, J=7.0 Hz, Me-17); ^{13}C RMN δ: 120.0 (C-1), 23.1 (C-2), 33.4 (C-3), 31.4 (C-4), 43.5 (C-5), 23.6 (C-6), 29.1 (C-7), 39.1 (C-8), 42.9 (C-9), 141.5 (C-10), 37.3 (C-11), 34.4 (C-12), 145.0 (C-13), 120.0 (C-14), 29.7 (C-15), 16.1 (C-16), 15.6 (C-17), 25.8 (C-18), 28.3 (C-19), 22.2 (C-20); EIMS: 273 (M^+ − Br, 10), 191 (100), 135 (12), 95 (12), 81 (13).

4.11. Reaction of 5 with 6-methoxyamine-9-methyl-purine 4: 17, 18

To a mixture of **5** (43 mg, 0.12 mmol) and 6-methoxy-amine-9-methyl-purine **4** (26 mg, 0.16 mmol) under argon atmosphere, DMA (2 mL) was added and the reaction was stirred and heated at 50 °C for 16 h. After evaporation of DMA the crude was chromatographed over flash silica eluting first with AcOEt/EtOH/NH_3 160:5:2→40:10:1) and then with CH_2Cl_2/MeOH/NH_3 35:5:1 to obtain **17** (26 mg, 47%) and **18** (20 mg, 36%).

4.11.1. *N*-[15-*ent*-halima-1(10),13*E*-dienyl]-*N*-methoxy-9-methyl-9*H*-purin-6-amine (17). $[\alpha]_D^{22}+44.3$ (*c* 0.8, $CHCl_3$); IR (film): 1578, 1453, 1378, 1327, 1296, 644 cm^{-1}; ^1H RMN δ: 8.48 (1H, s, H-2′), 7.81 (1H, s, H-8′), 5.40 (1H, t, J=7.1 Hz, H-14), 5.27 (1H, t, J=3.3 Hz, H-1), 4.73 (2H, d, J=6.8 Hz, H-15), 3.93 (3H, s, MeO–), 3.83 (3H, s, MeN–), 2.10–1.95 (2H, m, H-6), 2.05–1.87 (1H, m, H-7), 2.00–1.90 (1H, m, H-11), 1.90–1.80 (1H, m, H-12), 1.77–1.63 (1H, m, H-12), 1.76 (3H, s, Me-16), 1.75–1.65 (2H, m, H-2), 1.71–1.60 (1H, m, H-5), 1.59–1.50 (1H, m, H-8), 1.41–1.34 (1H, m, H-3), 1.40–1.25 (1H, m, H-7), 1.30–1.18 (1H, m, H-11), 1.21–1.05 (1H, m, H-3), 0.87 (3H, s, Me-20), 0.84 (3H, s, Me-19), 0.81 (3H, s, Me-18), 0.79 (3H, d, J=7.0 Hz, Me-17); ^{13}C RMN δ: 119.6 (C-1), 23.5 (C-2), 33.3 (C-3), 31.3 (C-4), 43.3 (C-5), 23.0 (C-6), 29.1 (C-7), 39.0 (C-8), 42.8 (C-9), 141.7 (C-10), 37.4 (C-11), 34.3 (C-12), 141.6 (C-13), 117.7 (C-14), 48.2 (C-15), 16.7 (C-16), 15.5 (C-17), 25.9 (C-18), 28.1 (C-19), 22.2 (C-20), 152.3 (C-2′), 151.7 (C-4′), 119.2 (C-5′), 155.9 (C-6′), 140.8 (C-8′), 62.5 (MeO–), 29.7 (MeN–); EIMS: 451 (M^+, 3), 420 (20), 277 (25), 205 (50), 149 (45); EIHRMS: calcd for $C_{27}H_{41}ON_5$ $(M)^+$ 451.3311; found $(M)^+$ 451.3308.

4.11.2. Compound (18). $[\alpha]_D^{22}+22.8$ (*c* 0.5, $CHCl_3$); IR (film): 3402, 1655, 1578, 1458, 1057, 644 cm^{-1}; ^1H RMN δ: 9.73 (1H, s, H-8′), 7.96 (1H, s, H-2′), 5.42 (1H, br s, H-14), 5.29 (1H, br s, H-1), 5.08 (2H, d, J=7.2 Hz, H-15), 3.99 (3H, s, MeN–), 3.89 (3H, s, MeO–), 2.11–1.93 (2H, m, H-6), 2.04–1.93 (1H, m, H-11), 2.02–1.90 (1H, m, H-12), 1.95–1.90 (1H, m, H-7), 1.86 (3H, s, Me-16), 1.79–1.65 (1H, m, H-12), 1.75–1.65 (2H, m, H-2), 1.68–1.56 (1H, m, H-5), 1.58–1.46 (1H, m, H-8), 1.39–1.25 (1H, m, H-7), 1.30–1.20 (1H, m, H-11), 1.18–1.02 (2H, m, H-3), 0.89 (3H, s, Me-20), 0.86 (3H, s, Me-19), 0.82 (3H, s, Me-18), 0.79 (3H, d, J=7.0 Hz, Me-17); ^{13}C RMN δ: 120.0 (C-1), 23.5 (C-2), 33.2 (C-3), 31.4 (C-4), 43.4 (C-5), 23.0 (C-6), 29.1 (C-7), 39.0 (C-8), 42.9 (C-9), 141.3 (C-10), 37.1 (C-11), 34.4 (C-12), 147.6 (C-13), 114.9 (C-14), 48.4 (C-15), 17.4 (C-16), 15.6 (C-17), 25.9 (C-18), 28.3 (C-19), 22.3 (C-20), 148.7 (C-2′), 141.2 (C-4′), 110.4 (C-5′), 135.9 (C-6′), 137.2 (C-8′), 62.4 (MeO–), 32.2 (MeN–); EIMS: 451 (M^+, 1), 386 (10), 256 (20), 191 (10), 149 (30); EIHRMS: calcd for $C_{27}H_{41}ON_5$ $(M)^+$ 451.3311; found $(M)^+$ 451.3318.

4.12. Reaction of 18 with AcOH/Zn: 19

A mixture of the betaine **18** (36 mg, 0.08 mmol) and Zn powder (48 mg, 0.73 mmol) in MeOH (1.3 mL), water (0.26 mL), and acetic acid (0.05 mL) was stirred under argon atmosphere at 60 °C for 2 days. After cooling, the mixture was filtered and the solid washed with a big amount of MeOH. The filtrate was evaporated and mixed with MeOH (8.5 mL), saturated aqueous NaCl (0.34 mL), and water (2.5 mL) and stirred for 1 h before evaporation. The residue was dissolved in saturated aqueous NaCl (1.27 mL) and water (1.27 mL), extracted several times with CH_2Cl_2, dried and evaporated. The product (32 mg) was purified by flash chromatography on silica gel (2.0 g) eluting with CH_2Cl_2/MeOH 16:1, to obtain **18** (17 mg, 47%) and **19** (12 mg, 36%).

4.12.1. Chloride of 7-[*ent*-halima-1(10),13*E*-dien-15-yl]-9-methyl-adenonium (19). $[\alpha]_D^{22}+36.7$ (*c* 0.1, $CHCl_3$); $[\alpha]_D^{22}+25.0$ (*c* 0.2, MeOH); IR (film): 3308, 3070, 1646, 1609, 1455, 1362, 1295, 1175 666 cm^{-1}; ^1H RMN ($CDCl_3$) δ: 11.1 (1H, s, H-8′), 8.53 (1H, s, H-2′), 6.47 (2H, br s, $-NH_2$), 5.63 (2H, br s, H-15), 5.41 (1H, br s, H-14), 5.31 (1H, t, $J=3.2$ Hz, H-1), 4.10 (3H, br s, MeN–), 2.00–1.00 (14H, m), 1.86 (3H, s, Me-16), 0.88 (3H, s, Me-20), 0.85 (3H, s, Me-18), 0.83 (3H, s, Me-19), 0.80 (3H, d, $J=6.8$ Hz, Me-17); ^1H RMN (CD_3OD) δ: 8.46 (1H, s, H-2′), 5.19 (2H, d, $J=7.1$ Hz, H-15), 5.52 (1H, tq, $J=7.1$, 1.2 Hz H-14), 5.39 (1H, t, $J=3.2$ Hz, H-1), 3.97 (3H, s, MeN–), 2.18–1.85 (5H, m), 1.86 (3H, d, $J=1.2$ Hz, Me-16), 1.75–1.55 (3H, m), 1.40–1.05 (6H, m), 0.96 (3H, s, Me-20), 0.89 (3H, s, Me-18), 0.84 (3H, s, Me-19), 0.85 (3H, d, $J=6.7$ Hz, Me-17); ^{13}C RMN (CD_3OD) δ: 121.4 (C-1), 24.8 (C-2), 34.3 (C-3), 32.4 (C-4), 44.9 (C-5), 24.1 (C-6), 30.2 (C-7), 40.6 (C-8), 44.1 (C-9), 142.7 (C-10), 38.4 (C-11), 35.7 (C-12), 150.1 (C-13), 115.2 (C-14), 48.9 (C-15), 17.1 (C-16), 16.0 (C-17), 26.4 (C-18), 28.8 (C-19), 22.8 (C-20), 157.2 (C-2′), 150.9 (C-4′), 109.4 (C-5′), 153.7 (C-6′), 142.2 (C-8′), 32.0 (MeN–); ESIHRMS: calcd for $C_{26}H_{40}N_5$ $(M+H)^+$ 422.3278; found $(M+H)^+$ 422.3283.

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$BiBr_3$ initiated cyclization–addition reactions: effect of π-nucleophile on oxocarbenium ion addition and total syntheses of (+)-(*S*,*S*)-(cis-6-methyltetrahydropyran-2-yl)acetic acid and its trans-diastereomer

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Abstract—For $BiBr_3$ initiated tandem cyclization–additions of very reactive silyl ketene acetal nucleophiles with δ-silyloxy aldehydes to afford 2,6-disubstituted THP products, diastereoselectivities range from 5–6:1 (trans-/cis-). The selectivity for axial attack on the intermediate oxocarbenium ion is inversely proportional to π-nucleophilicity. We have utilized this chemistry to convert a common starting material to both cis- and trans-diastereomers of an acid first isolated from the secretions of *Viverra civetta*.
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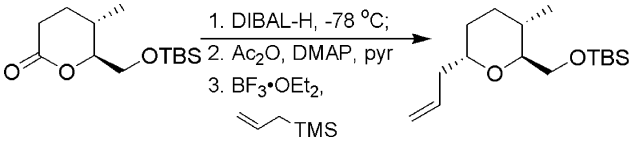
1. Introduction

2,6-Disubstituted tetrahydropyran systems are present in a large number of biologically active natural products such as leucascandrolide,[1] phorboxazole,[2] and spirastrellolide.[3] Control of the relative and absolute stereochemistry can be accomplished in a number of ways, although arguably the most common approach involves three steps from a δ-substituted δ-lactone (Eq. 1): (a) partial reduction of the lactone; (b) acylation or methylation of the intermediate lactol; and (c) addition of a nucleophile to an oxocarbenium ion formed by Lewis acid activation. Typically,[1d,e] 1.2–1.5 equiv of $BF_3 \cdot OEt_2$ or other Lewis acid is used.



(1)

Recently, bismuth compounds have become intensively studied as effective catalysts or initiators in organic synthesis.[4,5] Although many of the reactions that Bi(III) derivatives initiate are simple protection and deprotection sequences, Evans[6,7] and others[8] have described etherification reactions yielding simple ethers and tetrahydropyran derivatives using silyl-protected alcohols and aldehydes and/or ketones.

Based on the chemistry involving intramolecular tandem cyclization–addition[6] and reductive etherification[7,8] reactions initiated by Bi(III), we envisioned that diastereomers of the natural product (6-methyltetrahydropyran-2-yl)acetic acid (cis-**1** and trans-**1**) could be synthesized in short order from a common intermediate (**4**). This acid was originally isolated from the glandular secretions of the civet cat[9,10] and has since been used as a model compound for developing methodology toward 2,6-disubsituted tetrahydropyrans.[11]

Herein, we report the synthesis of both the trans- and cis-diastereomers of (6-methyltetrahydropyran-2-yl)acetic acid (**1**) using $BiBr_3$ intitiated cyclization reactions with δ-triethylsilyloxyhexanal as a common intermediate. We also report the effects that different nucleophiles have on the direction of addition to the putative six-membered oxocarbenium ion intermediate.

2. Result and discussion

2.1. Retrosynthetic analyses

The retrosynthetic analyses of both the cis- and trans-diastereomers of the target (**1**) are shown in Scheme 1.

Keywords: Bismuth; Bi(III); Oxocarbenium; Allylation; Etherification; Tetrahydropyran.
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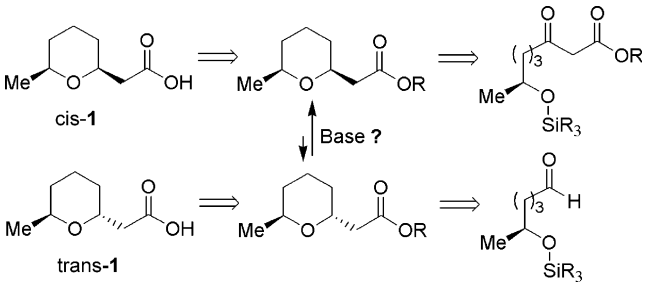


Scheme 1. Retrosynthetic analyses of the cis- and trans-diastereomers of (6-methyltetrahydropyran-2-yl)acetic acid (**1**).

Cyclizations of two different δ-silyloxy carbonyl compounds under either BiX_3 initiated reductive etherification[7] (leading to the cis-isomer) or tandem cyclization–addition[6] (trans-isomer) etherification protocols from the corresponding β-ketoester or aldehyde, respectively, would efficiently provide the target molecules.

In the case of the tandem cyclization–addition reaction toward trans-**1**, we expected that use of the more reactive silyl ketene acetal nucleophile instead of allyltrimethylsilane[6] would still occur with high selectivity via the well-established axial approach of nucleophiles on a cyclic oxocarbenium ion (Fig. 1).[12]

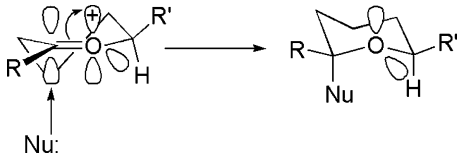
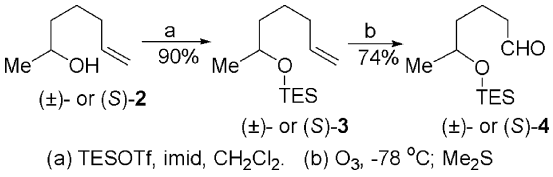


Figure 1. Preferred axial attack of nucleophile on cyclic oxocarbenium ion.

2.2. Synthesis of δ-silyloxy aldehyde, 4

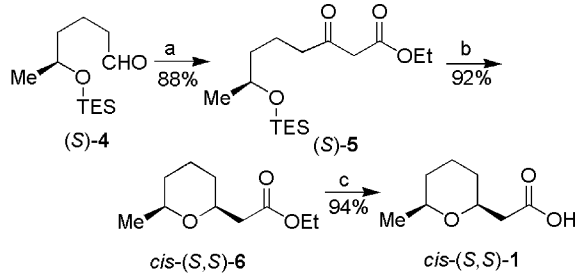
We began our synthesis toward the common intermediate **4** by Grignard addition to propylene oxide to afford the known alcohol, **2**[13] (Scheme 2). The enantiomerically enriched versions of the desired δ-silyloxy carbonyl compounds could be obtained from commercially available[14] (*S*)-propylene oxide (sold as 99% ee and the Mosher's ester of (*S*)-**2** was measured as 96.6 de). Subsequent protection of the hydroxyl moiety as the triethylsilylether (**3**) and ozonolysis of this protected alkenol then provided a good yield of the desired common intermediate, δ-silyloxy aldehyde **4**.



Scheme 2. Synthesis of aldehyde intermediate **4** in the construction of an acid component of civet cat secretion, **1**.

2.3. Synthesis of cis-1

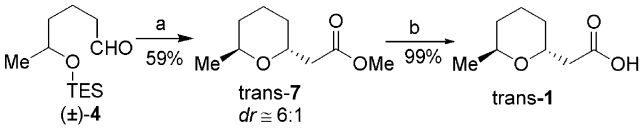
A short and convenient Bi(III) initiated approach to the desired cis-natural product is shown in Scheme 3. Aldehyde (*S*)-**4** was reacted with ethyl diazoacetate using the mild Roskamp protocol[15] to produce β-keto ester (*S*)-**5** in excellent isolated yield as a 6:1 mixture of the dicarbonyl compound and enol tautomer. Reductive etherification[7] initiated by $BiBr_3$ then provided the known desired cyclic ester, cis-(*S*,*S*)-**6**[16] with excellent diastereoselectivity (≥99:1 by GC). Finally, basic hydrolysis under the conditions reported by Nussbaumer and Fráter[10h] gave the title compound cis-(*S*,*S*)-**1** as a white solid in 51% isolated overall yield from (*S*)-**2**. The relative stereochemistry cis-**1** was established by qualitative NOE experiments that clearly show interaction between the two axial hydrogens on either side of the oxygen in the THP ring. No such interaction was observed in trans-**1** (vide infra).



Scheme 3. Stereoselective conversion of aldehyde **4** to cis-(*S*,*S*)-**1**.

2.4. Synthesis of trans-1

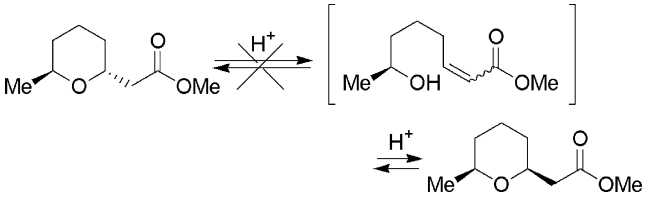
We initially embarked on the synthesis of the trans-stereoisomer of the title compound via a $BiBr_3$ initiated tandem cyclization–addition reaction (Scheme 4). This approach would provide a very short entry to the non-natural isomer of the 2,6-disubstituted tetrahydropyran. The expected ester product was indeed obtained by addition of $CH_2{=}C(OTMS)OMe$ to the putative intermediate oxocarbenium ion. This extension of the allylation methodology[6] to include the more reactive silyl ketene acetal nucleophile resulted in the desired cyclization–addition product, **7**, in 59% yield, albeit with dr≅6:1 (trans-/cis-). Separation of the isomers and conversion of trans-**7** to trans-**1** was then accomplished in quantitative yield by the method of Nussbaumer and Fráter.[10h] Thus, we synthesized trans-**1** in four steps from known alcohol **2**.



Scheme 4. Conversion of aldehyde **4** to intermediates to (trans-6-methyltetrahydropyran-2-yl)acetic acid, trans-**1**.

Many attempts were made to improve the selectivity for axial approach of $CH_2{=}C(OTMS)OMe$ via changes in concentration and variations in the amount of $BiBr_3$ initiator (10–30%). One possibility for attenuation of diastereoselectivity is that

adventitious water hydrolyzes the $BiBr_3$ to HBr[17] and that the Brønsted acid catalyzes equilibration of the product(s) to the more thermodynamically stable, diequatorial cis-diastereomer via an elimination–addition reaction (Scheme 5).

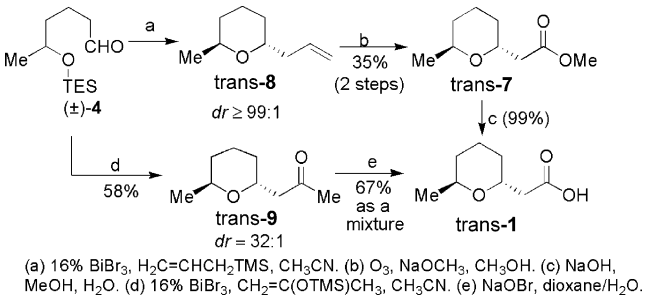


Scheme 5. Possible trans- to cis-equilibration pathway.

Conducting the reactions in NMR tubes containing 10–15% $BiBr_3$ and an excess of π-nucleophile showed that the initial ratio of trans-/cis-tetrahydropyran remained constant even after 16 h at rt. Furthermore, addition of imidazole (up to three times the amount of $BiBr_3$ initiator) immediately before workup to consume any possible HBr does not change the diastereomeric ratio. These observations lead us to believe that the reduced diastereoselectivity is a kinetic effect rather than a thermodynamic equilibration.

2.5. Alternative approaches to trans-diastereomer

Due to the modest selectivity of CH_2=C(OTMS)OMe addition to the intermediate oxocarbenium ion, we turned our attention toward an approach involving conversion of the allylated THP (trans-**8**) to trans-**1** (Scheme 6). Allylation of **4** under standard conditions (3.0 equiv CH_2=CH–CH_2–TMS, CH_3CN, rt)[6] provided trans-**8** with high selectivity, but the volatility of this product made isolation somewhat challenging. Although we did carry this intermediate to trans-**1**, the modest isolated yield after basic ozonlysis (35% for two steps) prompted us to attempt a third route toward the unnatural trans-diastereomer.



Scheme 6. Alternative approaches to (trans-6-methyltetrahydropyran-2-yl) acetic acid, cis-**1**.

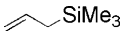
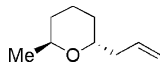
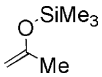
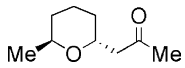
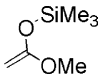
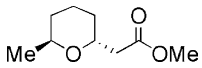
The final attempted route involved addition of the commercially available silyl enol ether of acetone (CH_2=C(OTMS)CH_3) to **4** under $BiBr_3$ initiated conditions in CH_3CN. This tandem cyclization–addition reaction directly afforded trans-**9** with dr=32:1 (GC) and in reasonable yield. We then converted the methyl ketone to the corresponding acid under the bromoform conditions described by Dixon et al.[10a] for the cis-isomer. Unfortunately, epimerization under these conditions led to mixtures of cis-**1** and trans-**1**. Although we were able to synthesize trans-**1** in as few as four steps from alcohol **2**, the most selective approach, involved allylation and basic ozonolysis in five steps from **2**.

2.6. Discussion of diastereoselectivities of different nucleophiles

The moderate preference for axial addition of the silyl ketene acetal nucleophile to the putative oxocarbenium ion is in significant contrast to the high trans-selectivity generally observed when using allyltrimethylsilane (≥99:1).[6] The reactivity appears to be inversely proportional to the π-nucleophilicity parameters (*N*) elegantly described by Mayr and co-workers.[18] The π-nucleophilicity parameter decreases from approximately 8.5[19] to 5.41 and to 1.79, for CH_2=C(OTMS)OMe, CH_2=C(OTMS)CH_3, CH_2=$CHCH_2$–TMS, respectively. The selectivity in this series, however, increases from 5–6:1 to 32:1 and to ≥99:1. Mayr's parameters indicate that the silyl ketene acetal reacts approximately seven orders of magnitude faster than allyltrimethylsilane. Our data show that the greater the nucleophilicity, the lower the stereoelectronic preference for axial attack. The observed trend between π-nucleophilicity and the observed diastereoselectivity is displayed in Table 1 and strongly indicates that the selectivity is likely a kinetic effect of nucleophilic addition. The diastereomeric ratio for CH_2=$CHCH_2$TMS addition is represented by a ratio of 99:1 given the commonly accepted limit for GC analysis included in Ref. 6.

It should be noted that if the elimination–addition sequence (Scheme 5) were operative, equilibration of either **7** or **9**

Table 1. Diastereoselectivity of cyclization–addition reactions of π-nucleophiles and aldehyde **4**

Nucleophile	Product	trans-/cis-[a]	π-Nucleophilicity parameter, *N*[b]
		99:1	1.79
		32:1	5.41
		5–6:1	~8.5[c]

[a] The ratio was determined by capillary GC of crude reaction mixtures before chromatography.
[b] Nucleophilicity parameters obtained from Ref. 18 and Refs therein.
[c] This parameter is an estimate.[19]

should occur to give similar ratios of the diequatorial cis-isomers. Furthermore, equilibration of **9** should occur more readily than equilibration of **7** due to the lower pK_a of hydrogens α- to the ketone in **9** versus α- to the ester in **7**.

3. Conclusions

We have established routes from common aldehyde **4** to both diastereomers of the 2,6-disubstituted tetrahydropyran compound, **1**, in good yields and moderate to high diastereoselectivities. The key step of each route is initiated by Bi(III) and the resulting stereochemistry is consistent with the recognized preference for axial addition to a six-membered cyclic oxocarbenium ion. Relative to both allyltrimethysilane or triethylsilane (Et_3SiH), however, the more reactive silyl enol ether and silyl ketene acetal nucleophiles result in diminished selectivity for the trans-product. Our data in conjuction with the nucleophilicity parameters (*N*) described by Mayr et al.[18] for $CH_2{=}CHCH_2{-}TMS$, $CH_2{=}C(OTMS)CH_3$, and $CH_2{=}C(OTMS)OMe$,[19] indicate that the axial approach of a π-nucleophile is likely dictated by the relative voracity of the nucleophile: the greater the nucleophilicity, the more rapid the addition and less discriminating the approach to the oxocarbenium ion. We are in the process of further evaluating this selectivity effect with other π-systems of varying nucleophilicity.

The applications of Bi(III) methodology to trans-**1** and cis-**1** show the flexibility afforded by tandem cyclization–addition reactions of δ-silyloxy carbonyl compounds. The generally low toxicity of most Bi(III) compounds and their use in catalytic quantities are particularly noteworthy benefits of this methodology. Finally, the bismuth(III) initiated reactions toward oxocarbenium ion intermediates afford extremely convenient alternatives to reactions via prototypical Lewis acids such as $BF_3{\cdot}OEt_2$ or TMSOTf.

4. Experimental

4.1. General methods

All reagents were used as received unless otherwise noted. Dichloromethane was distilled from CaH_2 and THF was purified via a SolvTek® solvent purification system. 2-(Trimethylsilyloxy)propene was purchased from Acros. Ethyl diazoacetate, 3-butenylmagnesium bromide as well as racemic and (*S*)-propylene oxide were purchased from Aldrich Chemical Company, Inc. Methyl trimethylsilyl dimethylketene acetal was synthesized from methyl acetate by the method of Shibasaki.[20] (*S*)-6-Hepten-2-ol,[13] was synthesized by the method of Grubbs.[21] ^{13}C NMR spectra were recorded with the aid of an APT sequence in which methylene and quaternary carbons=e (even) and methyl and methine carbons=o (odd). Coupling constants were determined by the method of Hoye.[22] Thin-layer chromatography was performed on Sigma-Aldrich general-purpose silica gel on glass and flash chromatography was performed using Sorbent Technologies chromatographic silica gel (200–475 mesh). Melting points were recorded by a Mel-Temp® apparatus and are uncorrected. All compounds were judged to be >95% homogeneous by ^1H NMR spectroscopy.

4.1.1. Preparation of cis-2-(6-methyl-tetrahydro-2*H*-pyran-2-yl)acetic acid, (±)-cis-1 and/or (+)-(*S*,*S*)-cis-1. Ester **6** (0.128 g, 0.68 mmol) was weighed into a 5 mL round-bottom flask, methanol (1.0 mL) and 10% aqueous NaOH (2.0 mL) solutions were added, the reaction was refluxed for 4 h, then washed with ethyl ether. The aqueous layer was acidified with concd HCl, extracted with Et_2O (3×5 mL) and the organic layers combined. The solution was dried ($MgSO_4$) and concentrated in vacuo, leaving 0.101 g (94%) of (±)-cis-**1** as a white solid; mp 51–53 °C; for (+)-(*S*,*S*)-cis-**1** as a colorless, viscous oil; spectral data were in accord with those published by Dixon and co-workers for this compound.[10a] Anal. Calcd for $C_8H_{14}O_3$ (158.19): C, 60.81; H, 9.01. Found: C, 60.74; H, 8.92; for (+)-(*S*,*S*)-cis-**1**: $[\alpha]_D^{25}+20.2$ (*c* 1.25, $CHCl_3$), lit: $[\alpha]_D^{25}+18.60$ (*c* 2.77, $CHCl_3$),[10i] $[\alpha]_D^{31}+20.5$ (*c* 1.23, $CHCl_3$)[10a]

4.1.2. Preparation of (±) trans-2-(6-methyl-tetrahydro-2*H*-pyran-2-yl)acetic acid, (±)-trans-1. trans-ester **7** (0.086 g, 0.50 mmol) was weighed into a 5 mL round bottom flask. Methanol (0.70 mL) and 10% aqueous NaOH (1.50 mL) solution were then added. This solution was refluxed for 3 h, then washed with Et_2O. The aqueous layer was then acidified with concd HCl, extracted with Et_2O (3× 5 mL), the combined ether layers dried ($MgSO_4$) and concentrated in vacuo leaving 0.079 g (99%) of trans-**1** as a colorless, viscous oil; spectral data were in agreement with those published by Dixon and co-workers for this compound.[10i]

4.1.3. Preparation of (±)-triethyl(hept-6-en-2-yloxy)-silane, (±)-3 or (*S*)-3. A solution of 6-hepten-2-ol (**2**) (0.610 g, 5.34 mmol) in freshly distilled CH_2Cl_2 (11 mL) was placed in a flame-dried round bottom flask under argon. Imidazole (0.52 g, 7.64 mmol) was added to the solution and the solution was cooled to 0 °C using an ice water bath. TESOTf (1.45 mL, 6.41 mmol) was added to the cooled solution and the mixture was allowed to warm to rt and stir. When starting material was consumed (~1 h, TLC), aqueous $NaHCO_3$ (ca. 10 mL) was added, the product was then extracted with CH_2Cl_2, washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The resulting silanol was purified by column chromatography (95:5 hexanes/ethyl acetate, $R_f=0.64$) to provide 1.102 g (90%) of (*S*)-**3** as a colorless liquid; IR (neat): 3078 (w), 2956 (s), 2817 (s), 1416 (w), 1239 (w), 1135 (m), 1011 (m), 910 (m) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 5.81 (dddd, $J=16.8$, 10.2, 6.6, 6.6 Hz, 1H), 5.00 (dm, $J=17.2$ Hz, 1H), 4.94 (dm, $J=10.2$ Hz, 1H), 3.74–3.83 (m, 1H), 2.01–2.07 (m, 2H), 1.25–1.45 (m, 4H), 1.13 (d, $J=5.9$ Hz, 3H), 0.96 (t, $J=8.1$ Hz, 9H), 0.59 (q, $J=7.7$ Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 139.0 (o), 114.4 (e), 68.6 (o), 39.5 (e), 34.1 (e), 25.5 (e), 24.2 (o), 7.2 (o) 5.3 (e); for (*S*)-**3**: $[\alpha]_D^{25}+9.53$ (*c* 1.29, $CHCl_3$). HRMS (CI) calcd for $C_{13}H_{29}OSi$ ($[M+H]^+$): 229.1982; found: 229.1987.

4.1.4. Preparation of (±)-5-(triethylsilyloxy)hexanal, (±)-4 or (*S*)-4. Alkene **3** (0.415 g, 1.82 mmol, 1.0 equiv), CH_2Cl_2 (25 mL), and methanol (5 mL) were added to an

oven-dried round bottom flask equipped with a stir bar. The solution was cooled to −70 °C using a dry ice/acetone bath. $NaHCO_3$ (~0.1 g) was added and O_3 was bubbled through the solution for approximately 15 min. O_2 was then bubbled through the solution for 15 min followed by argon. Me_2S (excess, 0.8 mL) was added and the mixture warmed to rt and stirred for 48 h. The mixture was filtered through Celite and concentrated. The product was purified by column chromatography (9:1 hexanes/ethyl acetate, R_f=0.30) to afford 0.308 g (74%) of (±)-**4** as a colorless oil; IR (neat): 2957 (s), 2878 (s), 2814 (m), 2716 (m), 1728 (s), 1140 (m), 739 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 9.77 (t, J= 2.0 Hz, 1H), 3.77–3.86 (m, 1H), 2.44 (dt, J=7.3, 1.8 Hz, 2H), 1.68–1.78 (m, 1H), 1.58–1.62 (m, 1H), 1.40–1.50 (m, 2H), 1.15 (d, J=6.2 Hz, 3H), 0.96 (t, J=8.1 Hz, 9H), 0.60 (q, J=7.7 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 202.6 (o), 68.3 (o), 44.2 (e), 39.3 (e), 24.1 (o), 18.7 (e), 7.3 (o), 5.3 (e). For (*S*)-**4**: $[\alpha]_D^{25}$ +12.76 (*c* 1.05, $CHCl_3$). HRMS (CI) calcd for $C_{12}H_{25}O_2Si$ ($[M+H]^+$): 231.1775; found: 231.1768.

4.1.5. Preparation of ethyl 3-oxo-7-(triethylsilyl-oxy)octanoate, (±)-5 or (*S*)-5.[15] $SnCl_2$ (14.2 mg, 0.075 mmol, 0.15 equiv) was weighed into a 5 mL round bottom flask. CH_2Cl_2 (2.0 mL) and ethyl diazoacetate (63 μL, 0.60 mmol, 1.2 equiv) were then added by syringe. Hexanal **4** (0.115 g, 0.50 mmol, 1.0 equiv) was dissolved in 1.0 mL CH_2Cl_2 and added slowly by syringe. After 1.5 h, the solution was quenched with brine and extracted with Et_2O (3×10 mL). The combined organics were dried ($MgSO_4$), concentrated in vacuo and the residue purified by column chromatography (7:3 pentanes/ethyl ether, R_f=0.77) to provide 0.139 g (88%) of (±)-**5** as a colorless liquid which contained ~15% of the enol form; IR (neat): 2959 (s), 2878 (s), 1748 (s), 1721 (s), 1644 (m), 1239 (s), 1138 (m), 1036 (m) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 4.19 (q, J= 7.2 Hz, 2H), 3.75–3.84 (m, 1H), 3.43 (s, 2H), 2.55 (t, J= 7.3 Hz, 2H), 1.57–1.72 (m, 2H), 1.37–1.47 (m, 2H), 1.28 (t, J=7.2 Hz, 3H), 1.13 (d, J=6.0 Hz, 3H), 0.95 (t, J=7.8 Hz, 9H), 0.59 (q, J=7.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 202.7 (e), 167.2 (e), 68.4 (o), 61.6 (e), 49.6 (e), 43.4 (e), 39.2 (e), 24.1 (o), 20.1 (e), 14.5 (o), 7.3 (o), 5.3 (e); for the enol form; ^1H NMR (400 MHz, $CDCl_3$) δ: 12.1 (s, 1H), 4.98 (s, 1H), 4.19 (q, J=7.2 Hz, 2H), 3.75–3.84 (m, 1H), 2.19 (t, J=7.3 Hz, 2H), 1.57–1.72 (m, 2H), 1.37–1.44 (m, 2H), 1.29 (t, J=7.2 Hz, 3H), 1.13 (d, J=6.0 Hz, 3H), 0.95 (t, J= 7.8 Hz, 9H), 0.59 (q, J=7.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 178.6 (e), 94.5 (e), 89.3 (o), 68.3 (o), 60.2 (e), 39.3 (e), 35.3 (e), 24.1 (o), 22.8 (e), 14.6 (o), 7.3 (o), 5.3 (e). Anal. Calcd for $C_{16}H_{32}O_4Si$ (316.51): C, 60.72; H, 10.19. Found: C, 60.85; H, 10.43; for (*S*)-**5**: $[\alpha]_D^{25}$ +11.44 (*c* 2.71, $CHCl_3$).

4.1.6. Preparation of ethyl cis-2-(6-methyltetrahydro-2*H*-pyran-2-yl)acetate, (±)-cis-6 and/or (+)-(*S*,*S*)-cis-6. Octanoate **5** (0.158 g, 0.50 mmol, 1.0 equiv) was weighed into a 15 mL round bottom flask and CH_3CN (4.0 mL) was added by syringe. A solution of $BiBr_3$ (0.35 mL, 0.075 mmol, 0.15 equiv) (100 mg/1.0 mL in CH_3CN) and $HSiEt_3$ (0.16 mL, 1.0 mmol, 2.0 equiv) were added simultaneously. After 1 h, the solution was concentrated in vacuo, filtered through a small SiO_2 pipette column with 9:1 hexanes/ethyl acetate and concentrated in vacuo. The product was purified by column chromatography (9:1 hexanes/ethyl acetate, R_f=0.46) to provide 0.086 g (92%) of (±)-cis-**6** as a colorless liquid; the spectral data were in agreement with those for (±)-**6** described by Marotta and co-workers;[16] for (+)-(*S*,*S*)-cis-**6**: $[\alpha]_D^{25}$ +16.43 (*c* 2.13, $CHCl_3$).

4.1.7. Preparation of (±) methyl trans-2-(6-methyltetrahydro-2*H*-pyran-2-yl)acetate, trans-7. Aldehyde **4** (0.0967 g, 0.420 mmol, 1.0 equiv) was added to an oven-dried test tube equipped with a micro stir bar by preweighed syringe. Acetonitrile (4.5 mL) was added under an argon atmosphere followed by $BiBr_3$ (0.0296 g, 0.066 mmol, 15.7%). Trimethylsilyl dimethylketene acetal, (CH_2=C(OTMS)OMe) (0.167 g, 0.971 mmol) was then added by syringe in two equal portions. After the reaction mixture was stirred for 2 h, the solvent was evaporated and the reaction mixture was filtered through a plug of silica gel using CH_2Cl_2 as eluant. After removal of the solvent in vacuo, the diastereomers were separated by column chromatography (9:1 hexanes/ethyl acetate, R_f=0.34 for trans-isomer and 0.44 for cis-isomer) to afford 0.037 g (51%) of trans-**7** as a colorless oil and 0.006 g (8%) of cis-**7** as a colorless liquid; trans-**7** The spectral data for both cis-**7** and trans-**7** were consistent with those described by Banwell and co-workers.[10c]

4.1.8. Alternative preparation of (±) methyl cis-2-(6-methyltetra-hydro-2*H*-pyran-2-yl)acetate, cis-7. Ester trans-**7** (0.077 g, 0.45 mmol, 1.0 equiv) was added by a pre-weighed 1.0 mL syringe to a flame dried round bottom flask equipped with a stir bar and under an argon atmosphere. THF (2.1 mL) was added to the flask and the solution was cooled to 0 °C with an ice/salt water bath. Solid *tert*-BuOK (0.014 g, 0.13 mmol, 28%) was added to the flask and the solution turned dark orange. The reaction was stirred for 1.5 h and was quenched with aqueous NH_4Cl (~10 mL). The product was extracted with EtOAc (3× 10 mL), dried ($MgSO_4$), and concentrated in vacuo to afford 0.051 g (66%) of cis-**7** (dr≥19:1) as a colorless oil.

4.1.9. Preparation of (±)-trans-2-allyl-6-methyl-tetrahydro-2*H*-pyran, trans-8. An oven-dried test tube equipped with a micro stir bar was prepared under argon. Aldehyde **4** (0.115 g, 0.50 mmol, 1.0 equiv) was added by preweighed syringe followed by CH_3CN (4.0 mL). A solution (0.35 mL) of $BiBr_3$ (100 mg/mL $BiBr_3$ in CH_3CN) was added to the reaction mixture, then allyltrimethylsilane (0.16 mL, 1.0 mmol, 2.0 equiv) was added via syringe. The reaction was stirred for 2 h, then water (30 mL) was added and the mixture was extracted with Et_2O (2×20 mL). The combined ether layers were dried ($MgSO_4$) and concentrated in vacuo. The product was purified by column chromatography (9:1 petroleum ether/Et_2O, R_f=0.62) to afford 0.049 g (70%) of (±)-trans-**8** as a colorless oil; IR (neat): 3076 (w), 2974 (m), 2938 (s), 2864 (m), 1375 (m), 1204 (m), 1043 (m) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 5.81 (dddd, J=16.8, 9.9, 7.0, 7.0 Hz, 1H), 5.07 (overlapping dm, J=17.2 Hz, 1H), 5.04 (overlapping dm, J=10.3 Hz, 1H), 3.93 (dqd, J=13.2, 6.6, 3.7 Hz, 1H), 3.81 (tdd, J=9.2, 6.6, 3.7 Hz, 1H), 2.42 (ddddd, J=14.3, 7.0, 7.0, 3.5, 3.5 Hz, 1H), 2.21 (ddddd, J=14.1, 7.0, 7.0, 3.5, 3.5 Hz, 1H), 1.60–1.73 (m, 4H),

1.24–1.40 (m, 2H), 1.08 (d, J=6.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 135.6 (o), 116.5 (e), 70.8 (o), 67.2 (o), 38.2 (e), 31.8 (e), 29.5 (e), 20.0 (o), 18.6 (e); HRMS (CI) calcd for $C_9H_{15}O$ ($[M-H]^+$): 139.1117; found: 139.1112.

4.1.10. Preparation of (±) trans-2-(6-methyltetrahydro-2*H*-pyran-2-yl)propan-2-one, trans-9. An oven-dried test tube equipped with a micro stir bar was cooled under argon. Aldehyde **4** (0.115 g, 0.50 mmol, 1.0 equiv) was added by pre-weighed syringe followed by CH_3CN (4.0 mL). A solution (0.35 mL) of $BiBr_3$ (100 mg/mL $BiBr_3$ in CH_3CN) was added to the reaction mixture. The silyl enol ether of acetone (0.20 mL, 1.0 mmol, 2.0 equiv) was then added. The reaction was stirred overnight, the solvent was evaporated, and the reaction mixture filtered through a plug of SiO_2 with CH_2Cl_2 as eluant. The crude material was concentrated in vacuo and the product was purified by column chromatography (9:1 hexanes/ethyl acetate, R_f= 0.28) to afford 0.045 g (58%) of (±)-trans-**9** as a colorless oil. The spectral data were in agreement with those reported by Dixon and co-workers.[10a]

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Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tet.2005.09.045

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Photoreduction of imines. An environmentally friendly approach to obtain amines

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Abstract—The photoreduction of different imines to amines in alcoholic solvents is reported. The reduction involves a versatile and chemoselective methodology that is environmentally friendly in that it avoids the use of metal hydrides and other dangerous reducing agents.
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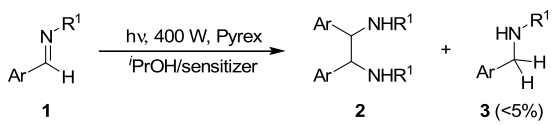
1. Introduction

One of the current challenges that faces organic synthesis, and chemistry in general, is the development of new and cleaner processes that minimise or eliminate the use of hazardous substances.[1] However, a wide variety of reactions that are not environmentally friendly are currently used in the chemical industry. One of these reactions is the reduction of imines—a useful method for the synthesis of secondary amines[2]—which are important precursors of key compounds in the pharmaceutical and agricultural industries.[3] Furthermore, amines play a significant role as pharmacophoric groups in biologically active substances. In this sense, catalytic hydrogenation,[4] metal hydrides[5] and dissolving metals[6] are usually employed for this transformation.

The photochemical behavior of compounds containing a carbon–nitrogen double bond has been the focus of our attention for almost a decade[7] and, more specifically, we have been concerned with the photoreduction of imines-a topic that has not been extensively studied.[8]

In this context, we have previously reported the sensitized photoreductive coupling of aldimines **1**[9] (Scheme 1), which leads to symmetrical vicinal diamines **2** in good-to-excellent yields. The reaction involves the formation of triplet species' that progress to give radical species' through hydrogen abstraction of the isopropyl alcohol.[9a] Interestingly, we detected the presence of secondary amines **3** as minor products ($\leq 5\%$) in this process, particularly in cases where the formation of the imine radical was less effective. This finding prompted us to modify the experimental conditions with the goal of obtaining monoamines as the major products of the photoreaction.



Scheme 1. Sensitized photocoupling of aldimines.

In this report, we present the photoreduction of a wide variety of aldimines and ketimines to amines with the aim of developing a new methodology that is compatible with the principles of green chemistry.[1] In this process the combination of light and an alcoholic solvent is used as the reducing system.

2. Results and discussion

2.1. Photoreduction of aldimines to amines

We initially studied the conditions under which the secondary amines could be obtained as the major products in the photoreaction of aldimines. Our experience in this type of process led us to believe that the control of radical formation is essential to achieve the objective. In this sense, we initially tested the effect of different features such as aldimine concentration, ^{i}PrOH/acetone ratio and filter glass on the reaction of aldimine **1a**.

When a solution of **1a** in a mixture of ^{i}PrOH and acetone was irradiated through Pyrex glass (i.e., sensitized conditions) at different imine concentrations or with different ^{i}PrOH/acetone ratios, amine **3a** was detected by NMR

Keywords: Photoreduction; Imines; Monoamines; Stereoselectivity.
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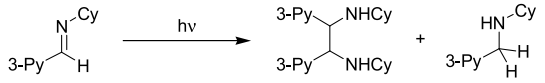
Table 1. Modulation of the photoreduction of aldimine **1a**



1a **2a** **3a**

Entry	Conditions	Concentration **1a**[a]	Ratio **2a/3a**[b]
1	^{i}PrOH/acetone (7/3), Pyrex	3	≥95/5
2	^{i}PrOH/acetone (7/3), Pyrex	1.5	94/6
3	^{i}PrOH/acetone (9/1), Pyrex	1.5	93/7
4	^{i}PrOH, Pyrex	1.5	No reaction
5	^{i}PrOH, Vycor	1.5	55/45
6	^{i}PrOH, Vycor	0.7	≤5/95

[a] 10^{-2} molar concentration.
[b] Determined by ^{1}H NMR analysis of the crude reaction mixture.

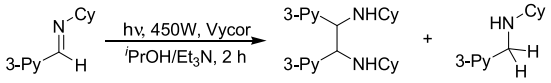
spectroscopy but diamines **2a** were the major products of the reaction (see Table 1, entries 1–3). In any case, amine **3a** could not be isolated from the reaction mixture. Furthermore, the photoreaction through Pyrex glass did not proceed in the absence of acetone and only the starting imine was recovered (Table 1, entry 4).

We therefore, decided to analyse the effect of using a different filter. When a Vycor glass filter was employed, the presence of acetone as a sensitizer was not necessary since **1a** absorbs above 260 nm and, as a result, the irradiation was carried out successfully using only isopropyl alcohol. The reaction was monitored under these conditions and a substantial decrease in the **2a/3a** ratio was observed (see Table 1, entries 5 and 6), particularly when the imine concentration was quite low. This observation demonstrates that the reaction is concentration-dependent. In fact, as shown in entry 6, the irradiation of a 7.5×10^{-3} M solution of imine **1a** in isopropyl alcohol for 2 h through Vycor glass gave amine **3a** almost exclusively (**2a/3a** ratio ≤5/95). The yield of this reaction was 32% after purification by column chromatography. However, prolonged reaction times through Vycor gave large amounts of polymeric material, which indicates that the amine formed may not be photostable under the reaction conditions described above. This photodegradation was also detected in the irradiation of other aldimines tested in this study. This situation clearly limits the utility of the photoreduction, because the yields obtained using this approach were in the range 20–35% in all cases.

It is well known that amines are involved in photoreduction processes of different unsaturated compounds, such as ketones, due to their lower ionization potential.[10] In the context of our photoreduction process, the degradation of the desired monoamines **3** can be explained in terms of a similar process, in which the amine reacts with aldimine **1** but mainly gives polymeric material and, consequently, decreases the yield of the reaction. It would be expected that the presence of a more reactive amine in the irradiation, such as triethylamine (Et_3N), would avoid the photodecomposition of the resulting secondary monoamines **3**.

In fact, the use of an excess of Et_3N in the irradiations led to a considerable improvement in the yield of the desired monoamine **3a**. As can be seen in Table 2, the yield of monoamine **3a** became higher as the amount of Et_3N in the

Table 2. Photoreduction of aldimine **1a** in the presence of Et_3N



1a **2a** **3a**

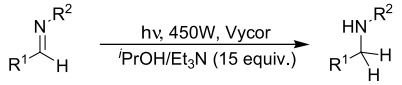
Entry	Et_3N (equiv)[a]	Ratio **2a/3a**[b]	Yield **3a** (%)[c]
1	0	≥5/95	32
2	7	16/84	41
3	10	10/90	48
4	15	15/85	62
5	25	15/85	55

[a] Refer to initial imine **1a**.
[b] Determined by ^{1}H NMR analysis of the crude reaction mixture.
[c] Isolated yield after purification by column chromatography.

photoreaction was increased. The best result was obtained on using 15 equiv of Et_3N with respect to the initial imine **1a** (see entry 4 in Table 2). Fortunately, under these modified reaction conditions, the ratio **2a/3a** was not significantly modified. In these reactions it is Et_3N, which is a tertiary amine in excess, that mainly undergoes the photodegradation and this sacrificial effect leads to an increase in the yield of the secondary amines **3**.

Bearing in mind the effect of Et_3N, we aimed at exploring the scope and synthetic possibilities of monoamine formation. Thus, we tested the reaction with a representative set of aldimines with different R^1 and R^2 groups, imines **1b–1g**, and we carried out the photoreduction of these compounds using the conditions optimized for aldimine **1a** (see Table 3).

Table 3. Photoreduction of different aldimines in the presence of Et_3N



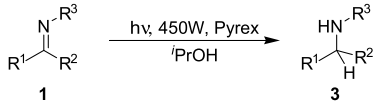
1 **3**

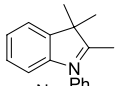
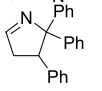
Imine	R^1	R^2	Time (h)	Yield **3** (%)[a]
1a	3-Py	Cy	2	62
1b	3-Py	^{t}Bu	2	65
1c	2-Py	Cy	2	46
1d	4-Py	Cy	2	55
1e	Ph	Cy	2	47[b]
1f	2-Naphthyl	Cy	4	50
1g	2-Quinolyl	Cy	9	30[c]

[a] Isolated yield after purification by column chromatography.
[b] The aminoalcohol resulting from addition of the isopropyl radical was isolated (23%).
[c] The irradiation was carried out through Pyrex glass.

In general, the photoreduction gave the corresponding amines **3** in moderate-to-good yields. As mentioned above, the use of Et_3N has a marked effect on the yield of the photoreaction but hardly alters the diamine/monoamine ratio. However, some points concerning these reactions should be noted. On the one hand, the irradiation of benzaldimine **1e** yielded both the desired monoamine and the aminoalcohol resulting from addition of the isopropyl radical, which is generated by hydrogen abstraction of the alcoholic solvent. On the other hand, the irradiation of aldimine **1g** warrants particular attention since it was possible in this case to carry out the irradiation through Pyrex glass. This finding shows that the UV–vis absorption

Table 4. Photoreduction of ketimines



Imine	R^1	R^2	R^3	Time (h)	Yield **3** (%)[a]
1h	Ph	CH_3	Cy	3	23[b]
1i	2-Naphthyl	CH_3	Cy	7	52[b]
1j	2-Py	CH_3	Cy	11	58
1k	Ph	Ph	H	9	80
1l	Ph	Ph	Cy	12	62
1m	Ph	Ph	*c*-Pr	10	75
1n	Ph	Ph	CH_2CN	6	70
1o	Ph	Ph	CH_2CO_2Et	13	61
1p	Ph	CO_2Et	Cy	5	60[c]
1q	Ph	CO_2Et	(*R*)-$CH(CH_3)_3Ph$	5.5	48[c]
1r				2	15[b]
1s				14	51

[a] Isolated yield after purification by column chromatography.
[b] The irradiation was carried out through Vycor glass in the presence of Et_3N.
[c] The irradiation was carried out through Vycor glass and ethanol was used as the alcoholic solvent.

characteristics of the materials depend on the nature of the substituents on the imine. It is well known that the active band in the photoreduction is an $n\rightarrow\pi^*$ electronic transition ($\lambda\approx$250–285 nm),[8b] which can experience a bathochromic shift-particularly when R^1 (aryl group) includes a heteroatom or is a group that provides conjugation to the system.

2.2. Photoreduction of ketimines and cyclic imines to amines

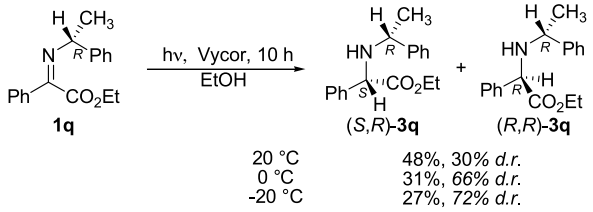
The next step in our study involved exploring the generality and versatility of the photoreduction. In order to achieve this goal we extended this investigation to other imines. As detailed in Table 4, a number of representative ketimines and cyclic imines were irradiated through Pyrex or Vycor glass, depending on the absorption spectrum of the compound in question. The desired monoamines **3** were obtained in moderate-to-good yields.

It is worth noting that the larger substituents in the imine group made the coupling reaction more difficult. As a result, formation of the monoamine is favourable and the photoreduction does not depend on imine concentration-as it did in the case of aldimines. Moreover, ultraviolet irradiation through Pyrex glass (which filters out radiation below 290 nm) of ketimines **1j**–**1o** and **1s** also gave the monoamines in good yields and without decomposition. As example, irradiation of imine **1k** through Vycor glass mainly gave polymeric material; the corresponding amine **3k** was detected but could not be isolated. However, irradiation through Pyrex led to monoamine **3k** in excellent yield. In this case, lower energy radiation is involved and clearly the reaction times required are longer. In contrast, ketimines **1h** and **1i** did not react when Pyrex-filtered light was used and these irradiations were carried out as described previously for aldimines, using Vycor glass and in the presence of Et_3N.

Interestingly, it was observed that the photochemical reduction of different cyclic imines proceeded successfully. Some of the most significant results are gathered in Table 4. As can be seen in this table, the irradiation of imines **1r** and **1s** under general conditions[11] gave the expected amines in moderate yields. Cyclic derivatives of indolenine (such as **1r**) have previously been reported as being unreactive toward hydrogen atom abstraction.[12]

As far as the chemoselectivity of the photoreaction is concerned, it should be noted that photoreduction of bifuncional imines, such as ketimine **1n** and imino esters **1o**–**1q**, was carried out in a chemoselective way and gave only the desired amines. Moreover, in the case of imino esters **1p**–**1q**, we employed Vycor-filtered light since these compounds did not absorb above 290 nm. In addition, it was observed that the resulting amino esters are more stable to the photodegradation than monoamines and, as a consequence, it was not necessary to use Et_3N. Other byproducts, such as diamines or aminoalcohols, could not be detected. Moreover, the reaction rate increased markedly when ethanol was used as the solvent.

Finally, it is worth noting that the photoreduction of chiral imino ester **1q** (Scheme 2) was carried out with moderate/high diastereoselectivity [(*R*,*S*)/(*S*,*S*) ratio=4:1]. The two diastereomers **3q** could be easily separated by simple column chromatography. As shown in Scheme 2, the



Scheme 2. Stereoselectivity in the photoreduction of ketimines.

diastereoselectivity of the reaction depends on the irradiation temperature and the best diastereomeric excess (72%) was obtained at −20 °C.

The absolute configuration of the amino ester (*R*,*R*)-**3q** was determined by reduction with $LiAlH_4$ and comparison of the resulting aminoalcohol with data reported in the literature.[13] Interestingly, the amino esters **3q** can be transformed into the corresponding α-amino acids,[14] providing an alternative method to that described in the literature[15] to obtain this type of molecule.

3. Conclusions

From the results described above, we conclude that the photoreduction of different imines bearing a hydrogen atom, alkyl or aryl groups leads to the corresponding amines through a new and versatile approach that is compatible with the environment. Moreover, we have modulated, for the first time, the photoreduction of aldimines to obtain secondary amines as reaction products instead of vicinal diamines. The use of Et_3N delays the decomposition of monoamines and improves the synthetic utility of this process.

We have also demonstrated that cyclic imines are reactive toward hydrogen atom abstraction. Moreover, the irradiation of a variety of bifunctional imines has demonstrated that photoreduction of the carbon–nitrogen double bond is chemoselective in the presence of other functional groups such as ester and cyano groups. Finally, the reduction of chiral imino esters led to enantiopure amino esters that can be easily converted into the corresponding α-amino acids, thus providing a new method for the synthesis of this kind of compound. Finally, studies to elucidate the mechanism and to extend the scope of this reaction are in progress.

4. Experimental

4.1. General aspects

^1H and ^{13}C NMR spectra were recorded on a Bruker ARX-300 spectrometer in $CDCl_3$ with TMS as internal standard. Electrospray mass spectra were obtained on an HP 5989 B apparatus with an HP 59987A interface, in positive-ion mode with methanol–water–acetic acid (60/35/5) as the mobile phase. GC–MS spectra were recorded on an HP G1800A apparatus. Elemental analyses were obtained using a CE Instrument Model 1110. Optical rotations were measured on a Perkin-Elmer 341 polarimeter in 1.0 dm cells of 1.0 and 0.35 mL capacity. All solvents were purified by standard procedures and freshly distilled prior to use. Reagents were of commercial grade (Aldrich).

4.2. General procedure for synthesis of imines 1a–s

Imines **1a–j** were prepared by condensation of different carboxaldehydes with the corresponding primary amine in chloroform or toluene under reflux according to literature procedures.[16] Imines **1l** and **1m** were prepared by condensation of benzophenone imine with the corresponding primary amine in dry toluene at 80 °C. Imino esters **1p** and **1q** were prepared by condensation of ethyl benzoylformate with the corresponding amine (1.1 equiv) in toluene at 100 °C catalyzed by $AlCl_3$. Cyclic imine **1s** was obtained by an *N*-cyclopropylimine-1-pyrroline rearrangement.[7] Imines **1k**, **1n**, **1o** and **1r** are commercially available

4.3. General procedure for irradiation of imines 1a–s

Argon was bubbled through a solution of the corresponding imine **1** (0.75 mmol for **1a–g** and **1s**, and 1.5 mmol for **1h–r**) in an alcoholic solvent (100 mL) and the solution was irradiated through Vycor or Pyrex glass (depending on the absorption spectra, see Tables 3 and 4) at room temperature using a medium-pressure mercury lamp (450 W) until complete consumption of starting material was observed (monitored by ^1H NMR spectroscopy and GC–MS chromatography). The alcoholic solvent was distilled off and the residue was purified by ion exchange column chromatography (Amberlite CG-50 I, NH_3/MeOH 0.28 M)-except amino esters **3p–q**, which were purified by silica column chromatography (hexane/AcOEt 9:1).

4.4. Irradiation of imines in presence of triethylamine

Et_3N (1.5 mL, 10 mmol) was added to a solution of the corresponding imine **1** (0.75 mmol) in isopropyl alcohol (20 mL). Isopropyl alcohol was added to give a total volume of 100 mL and argon was bubbled through the solution. The reaction mixture was irradiated through Vycor at room temperature using a medium-pressure mercury lamp (450 W) until 90% conversion of starting material was observed (monitored by ^1H NMR spectroscopy and GC–MS chromatography). The isopropyl alcohol was distilled off and the residue was purified by ion exchange column chromatography (Amberlite CG-50 I, NH_3/MeOH 0.28 M).

4.5. Characterization data of isolated monoamines 3

4.5.1. *N*-[(3-pyridinyl)methyl]cyclohexanamine (3a). ^1H NMR ($CDCl_3$): δ 1.10–1.93 (m, 11H), 2.48 (m, 1H), 3.83 (s, 2H), 7.23–7.27 (m, 1H), 7.67–7.69 (m, 1H), 8.49–8.50 (m, 1H), 8.56 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 25.1, 26.2, 33.8, 48.4, 56.4, 123.4, 135.8, 136.4, 148.4, 149.8. GC–MS: 190, 147, 92. MS-ES(+): 191.2 (M+1). Anal. Calcd for $C_{12}H_{18}N_2$: C, 75.74; H, 9.53; N, 14.72. Found: C, 75.1; H, 9.85; N, 15.05.

4.5.2. *N*-[(3-pyridinyl)methyl]*tert*-butanamine (3b). ^1H NMR ($CDCl_3$): δ 1.19 (s, 9H), 1.53 (br s, 1H), 3.75 (s, 2H), 7.22–7.28 (m, 1H), 7.70–7.73 (m, 1H), 8.48 (m, 1H), 8.5 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 29.2, 44.7, 51.1, 123.5, 136.1, 148.4, 149.9. GC–MS: 163, 149, 92. MS-ES(+): 164.2 (M+1). Anal. Calcd for $C_{10}H_{16}N_2$: C, 73.13; H, 9.82; N, 17.06. Found: C, 73.55; H, 9.75; N, 16.70.

4.5.3. *N*-[(2-pyridinyl)methyl]cyclohexanamine (3c). ^1H NMR ($CDCl_3$): δ 1.10–1.97 (m, 11H), 2.50 (m, 1H), 3.94 (s, 2H), 7.15 (m, 1H), 7.30 (m, 1H), 7.64 (m, 1H), 8.56 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 25.2, 26.3, 33.6, 52.5, 56.8, 122.0, 122.5, 136.6, 149.3, 160.2. GC–MS: 190, 147, 92. MS-

ES(+): 191.2 (M+1). Anal. Calcd for $C_{12}H_{18}N_2$: C, 75.74; H, 9.53; N, 14.72. Found: C, 75.61; H, 9.50; N, 14.73.

4.5.4. *N*-[(2-pyridinyl)methyl]cyclohexanamine (3d). ^{1}H NMR ($CDCl_3$): δ 1.02–1.93 (m, 11H), 2.46 (m, 1H), 3.84 (s, 2H), 7.27 (m, 2H), 8.54 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 25.1, 26.3, 33.7, 49.9, 56.4, 123.1, 150.0. GC–MS: 190, 147, 92. MS-ES(+): 191.2 (M+1). Anal. Calcd for $C_{12}H_{18}N_2$: C, 75.74; H, 9.53; N, 14.72. Found: C, 75.64; H, 9.52; N, 14.70.

4.5.5. *N*-benzylcyclohexanamine (3e). ^{1}H NMR ($CDCl_3$): δ 1.15–1.96 (m, 11H), 2.48–2.60 (m, 1H), 3.84 (s, 2H), 7.25–7.37 (m, 5H). ^{13}C NMR ($CDCl_3$): δ 25.0, 26.0, 33.0, 50.6, 56.1, 127.1, 128.6, 139.6. GC-MS: 189, 146, 91. MS-ES(+): 190.4 (M+1). Anal. Calcd for $C_{13}H_{19}N$: C, 82.48; H, 10.12; N, 7.40. Found: C, 82.02; H, 10.09; N, 7.36.

4.5.6. *N*-[(2-naphthyl)methyl]cyclohexanamine (3f). ^{1}H NMR ($CDCl_3$): δ 0.91–1.98 (m, 11H), 2.49–2.55 (m, 1H), 3.98 (s, 2H), 7.41–7.53 (m, 3H), 7.70–7.82 (m, 4H). ^{13}C NMR ($CDCl_3$): δ 25.2, 26.3, 33.8, 51.3, 56.3, 125.6, 126.1, 126.4, 126.7, 127.7, 127.8, 128.1, 132.7, 133.6, 138.7. GC–MS: 239, 196, 141, 115, 98. MS-ES(+): 240.3 (M+1). Anal. Calcd for $C_{17}H_{21}N$: C, 85.30; H, 8.84; N, 5.85. Found: C, 84.98; H, 8.81; N, 5.83.

4.5.7. *N*-[(2-quinolinyl)methyl]cyclohexanamine (3g). ^{1}H NMR ($CDCl_3$): δ 1.24–2.02 (m, 11H), 2.61 (m, 1H), 4.17 (s, 2H), 7.44–8.18 (m, 6H). ^{13}C NMR ($CDCl_3$): δ 25.1, 26.2, 33.4, 52.8, 57.1, 120.7, 126.2, 127.5, 127.7, 129.1, 129.6, 136.6, 147.8, 160.2. GC–MS: 238, 195, 182, 157, 129, 77, 55. MS-ES(+): 241.3 (M+1). Anal. Calcd for $C_{16}H_{20}N_2$: C, 79.96; H, 8.39; N, 11.66. Found: C, 80.15; H, 8.39; N, 11.46.

4.5.8. *N*-(1-phenylethyl)cyclohexanamine (3h). ^{1}H NMR ($CDCl_3$): δ 1.33 (d, J=7.0 Hz, 3H), 1.01–1.72 (m, 11H), 2.26 (m, 1H), 3.96 (q, J=7.0 Hz, 1H), 7.16–7.34 (m, 5H).

^{13}C NMR ($CDCl_3$): δ 14.2, 25.1, 25.4, 26.3, 33.3, 34.6, 53.8, 54.6, 126.5, 128.5, 146.4. GC–MS: 203, 188, 160, 105, 77, 56. MS-ES(+): 204.3 (M+1). Anal. Calcd for $C_{14}H_{21}N$: C, 82.70; H, 10.41; N, 6.89. Found: C, 82.34; H, 10.43; N, 6.87.

4.5.9. *N*-[1-(2′-naphthyl)ethyl]cyclohexanamine (3i). ^{1}H NMR ($CDCl_3$): δ 1.01–1.12 (m, 5H), 1.40 (d, J=6.6 Hz, 3H), 1.53–1.72 (m, 5H), 2.03 (m, 1H), 2.30 (m, 1H), 4.13 (q, J=6.6 Hz, 1H), 7.41–7.47 (m, 3H), 7.71 (s, 1H), 7.74–7.83 (m, 3H). ^{13}C NMR ($CDCl_3$): δ 25.2, 26.3, 33.4, 34.8, 53.9, 54.8, 125.0, 125.2, 125.5, 126.0, 127.8, 127.9, 128.3, 132.9, 133.6, 144.0. GC–MS: 254, 238, 155, 98, 56, 41. MS-ES(+): 254.4 (M+1). Anal. Calcd for $C_{18}H_{23}N$: C, 85.32; H, 9.15; N, 5.53. Found: C, 84.92; H, 9.11; N, 5.50.

4.5.10. *N*-[1-(2′-pyridinyl)ethyl]cyclohexanamine (3j). ^{1}H NMR ($CDCl_3$): δ 1.02–1.98 (m, 11H), 1.36 (d, J=6.6 Hz, 3H), 2.23 (m, 1H), 4.03 (q, J=6.6 Hz, 1H), 7.12–7.16 (m, 1H), 7.27–7.31 (m, 1H), 7.61–7.66 (m, 1H), 8.54–8.57 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 23.6, 25.1, 25.3, 26.3, 33.4, 34.5, 54.3, 55.9, 121.3, 121.9, 136.5, 149.4, 165.5. GC–MS: 203, 189, 161, 107, 97. MS-ES(+): 205.3 (M+1). Anal. Calcd for $C_{13}H_{20}N_2$: C, 76.42; H, 9.87; N, 13.71. Found: C, 76.81; H, 9.68; N, 13.51.

4.5.11. *N*-benzhydrylcyclohexanamine (3l). ^{1}H NMR ($CDCl_3$): δ 1.06–1.94 (m, 11H), 2.36–2.42 (m, 1H), 5.03 (s, 1H), 7.16–7.39 (m, 10H). ^{13}C NMR ($CDCl_3$): δ 25.2, 26.4, 34.1, 54.1, 63.8, 126.9, 127.5, 128.5, 144.9. GC–MS: 265, 188, 167. MS-ES(+): 266.2 (M+1). Anal. Calcd for $C_{19}H_{23}N$: C, 85.99; H, 8.74; N, 5.28. Found: C, 84.20; H, 8.70; N, 5.31.

4.5.12. *N*-benzhydrylcyclopropanamine (3m). ^{1}H NMR ($CDCl_3$): δ 0.38 (d, J=5.1 Hz, 4H), 1.98 (br s, 1H), 2.07 (m, 1H), 4.91 (s, 1H), 7.12–7.36 (m, 10 H). ^{13}C NMR ($CDCl_3$): δ 6.8, 29.7, 67.3, 127.0, 127.5, 128.5, 144.3. GC–MS: 223, 222, 167, 152. MS-ES(+): 224.3 (M+1). Anal. Calcd for $C_{16}H_{17}N$: C, 86.05; H, 7.67; N, 6.27. Found: C, 85.88; H, 7.72; N, 6.40.

4.5.13. 2-(Benzhydrylamino)acetonitrile (3n). ^{1}H NMR ($CDCl_3$): δ 1.95 (br s, 1H), 3.50 (s, 2H), 5.06 (s, 1H), 7.21–7.44 (m, 10H). ^{13}C NMR ($CDCl_3$): δ 35.4, 65.8, 117.7, 127.4, 127.8, 128.9, 141.9. GC–MS: 222, 167, 145, 104, 67. MS-ES(+): 223.4 (M+1). Anal. Calcd for $C_{15}H_{14}N_2$: C, 81.05; H, 6.35; N, 12.60. Found: C, 81.36; H, 6.52; N, 12.12.

4.5.14. 2-(Benzhydrylamino)acetic acid ethyl ester (3o). ^{1}H NMR ($CDCl_3$): δ 1.24 (t, J=7.2 Hz, 3H), 2.22 (m, 1H), 3.37 (s, 2H), 4.17 (q, J=7.2 Hz, 2H), 4.88 (s, 1H), 7.18–7.47 (m, 10H). ^{13}C NMR ($CDCl_3$): δ 14.3, 49.2, 60.8, 66.7, 127.3, 127.5, 128.6, 143.4, 172.6. GC–MS: 182, 167, 118, 91. MS-ES(+): 270.2 (M+1). Anal. Calcd for $C_{17}H_{19}NO_2$: C, 75.81; H. 7.11; N, 5.20; O, 11.88. Found: C, 75.51; H, 7.33; N, 5.02.

4.5.15. 2-Cyclohexylamino-2-phenyl acetic acid ethyl ester (3p). ^{1}H NMR ($CDCl_3$): δ 1.08–1.84 (m, 13H), 2.03–2.04 (br s, 1H), 2.33–2.37 (m, 1H), 4.08–4.22 (m, 2H), 4.51 (s, 1H), 7.26–7.39 (m, 5H). ^{13}C NMR ($CDCl_3$): δ 14.2, 25.0, 26.2, 33.4, 33.6, 54.6, 61.2, 62.5, 127.4, 128.0, 128.8, 138.9, 173.8. GC–MS: 260, 188, 106, 79. MS-ES(+): 262.1 (M+1). Anal. Calcd for $C_{16}H_{23}NO_2$: C, 73.53; H, 8.87; N, 5.36; O, 12.24. Found: C, 73.23; H, 8.69; N, 5.47.

4.5.16. 2-Phenyl-(1′-phenylethylamino)acetic acid ethyl ester [(*S*,*R*)-3q and (*R*,*R*)-3q]. The two diastereomers **3q** were separated by simple column chromatography (silica gel, hexane/AcOEt 9:1). *Major product*: (*S*,*R*)-**3q**. ^{1}H NMR ($CDCl_3$): δ 1.13 (t, J=7.2 Hz, 3H), 1.34 (d, J=6.6 Hz, 3H), 2.30–2.50 (br s, 1H), 3.56 (q, J=6.6 Hz, 1H), 4.00–4.13 (m, 2H), 4.17 (s, 1H), 7.22–7.38 (m, 10H). ^{13}C NMR ($CDCl_3$): δ 14.1, 24.5, 54.8, 61.2, 62.8, 127.1, 127.3, 127.8, 128.1, 128.6, 128.7, 138.6, 144.8, 173.0. $[\alpha]_D^{25}$ (c 0.50, MeOH) +130.6. GC–MS: 283, 210, 105, 79. MS-ES(+): 284.3 (M+1). Anal. Calcd for $C_{19}H_{23}NO_2$: C, 76.73; H, 7.80; N, 4.71; O, 10.76. Found: C, 76.13; H, 7.96; N, 4.60. *Minor product*: (*R*,*R*)-**3q**. ^{1}H NMR ($CDCl_3$): δ 1.22 (t, J=7.2 Hz, 3H), 1.39 (d, J=6.6 Hz, 3H), 2.25–2.40 (br s, 1H), 3.56 (q, J=6.6 Hz, 1H), 4.11–4.26 (m, 3H), 7.23–7.35 (m, 10H). ^{13}C NMR ($CDCl_3$): δ 14.3, 24.9, 56.7, 61.2, 63.1, 127.1, 127.2, 127.3, 128.0, 128.6, 128.7, 128.8, 138.7, 145.0, 173.9. $[\alpha]_D^{25}$ (c 0.49, MeOH) = −19.5. GC–MS: 283, 210, 105, 79. MS-ES(+): 284.3 (M+1). Anal. Calcd for $C_{19}H_{23}NO_2$: C, 76.73; H, 7.80; N, 4.71; O, 10.76. Found: C, 76.24; H, 8.06; N, 4.52.

4.5.17. 2,3,3-Trimethyl-2,3-dihydro-1*H*-indole (3r). ^{1}H NMR ($CDCl_3$): δ 1.04 (s, 3H), 1.18 (d, $J=6.6$ Hz, 3H), 1.28 (s, 3H), 3.52 (q, $J=6.6$ Hz, 1H), 3.76–3.79 (br s, 1H), 6.61–6.64 (m, 1H), 6.72–6.77 (m, 1H), 6.99–7.04 (m, 1H), 7.26 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 15.3, 22.5, 26.3, 43.5, 65.3, 109.5, 119.0, 122.4, 127.3, 139.3, 149.4. GC–MS: 161, 146, 131, 77. MS-ES(+): 162.3 (M+1). Anal. Calcd for $C_{11}H_{15}N$: C, 81.94; H, 9.38; N, 8.69. Found: C, 82.32; H, 9.19; N, 8.49.

4.5.18. 2,2,3-Triphenylpyrrolidine (3s). ^{1}H NMR ($CDCl_3$): δ 2.18–2.34 (m, 2H), 3.08–3.12 (m, 1H), 3.36–3.41 (m, 1H), 3.25 (br s, 1H), 4.12–4.17 (m, 1H), 6.90–7.08 (m, 10H), 7.22–7.35 (m, 3H), 7.54–7.57 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 33.2, 44.0, 52.9, 75.3, 126.0, 126.8, 127.2, 127.9, 128.2, 128.4, 129.4, 142.6, 144.5, 147.6. GC–MS: 299, 194, 135, 116, 91, 77. MS-ES(+): 300.4 (M+1). Anal. Calcd for $C_{22}H_{21}N$: C, 88.25; H, 7.07; N, 4.68. Found: C, 88.61; H, 6.89; N, 4.50.

4.6. Reduction of amino esters 3q

A solution of pure amino ester (*S*,*R*)-**3q** or (*R*,*R*)-**3q** (50 mg, 0.18 mmol) in dry THF (5 mL) was added to a solution of $LiAlH_4$ (40 mg, 1.05 mmoles) in dry THF (10 mL) at 0 °C under argon. The mixture was stirred at room temperature until complete formation of the corresponding aminoalcohol was observed by TLC. The reaction mixture was decomposed by the careful dropwise addition of water (2 mL) and the product extracted with ethyl acetate (3×15 mL). The combined organic layers were dried (Na_2SO_4), concentrated and purified by column chromatography (hexane/AcOEt 1:1) to give the corresponding aminoalcohol in 84% yield (38 mg, 0.15 mmol).

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Syntheses of macrocyclic bisbibenzyls on solid support

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Abstract—We describe a route for the polymer supported total synthesis of the cyclic bisbibenzyls of the isoplagiochin type found in liverworts. TentaGel® resins were used as solid support for a sequence involving Suzuki, Wittig and hydrogenation protocols. The polymer linked intermediates could be characterized by HR-MAS NMR. This route is to be extended to the synthesis of small libraries of differently halogenated derivatives.
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1. Introduction

The cyclic bisbibenzyls isoplagiochin C (**1**) and D (**2**) were isolated from the liverworts *Plagiochila fruticosa*,[1] *Plagiochila deflexa*[2] and *Herbertus sakuraii* (Fig. 1).[3] Altogether, 21 chlorinated derivatives of the type **3** were detected in the liverworts *Bazzania trilobata*,[4] *Lepidozia incurvata*,[5] *Mastigophora diclados*, *H. sakuraii*,[6] *Plagiochila peculiaris*[7] and *P. deflexa*.[8] Phenolic compounds of the bibenzyl and bisbenzyl type exhibit remarkable antitumoural, antibacterial and antimycotic activities.[9,10] The isoplagiochin framework proved to be of substantial structural interest because of the chirality of the entire molecule.[11]

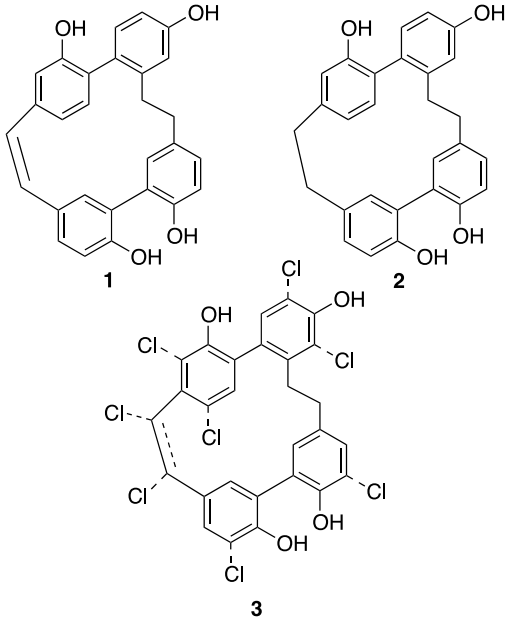

Figure 1. Structures of isoplagiochins **1**, **2** and halogenated derivatives **3**.

Conventional total syntheses ('in solution') for **1** and **2**[12] and for three examples of **3**[13] were described applying an efficient and flexible unit construction system and making extensive use of Suzuki and Wittig protocols.

Syntheses of **1** and **2** on solid support[14] especially using TentaGel® resins[15] should give valuable contributions to Suzuki and Wittig reactions on this carrier[16,17] as well as to the characterization of polymer bound intermediates by HR-MAS NMR.[18]

TentaGel resins are poly(ethylene glycol) polystyrene graft copolymers consisting of ~30% polystyrene matrix (cross-linked with ~1% divinylbenzene) and of ~70% poly(ethylene glycol). They are available in different types of functional anchor groups (Fig. 2) chargeable up to 0.40–0.60 mmol/g and are well capable of swelling in

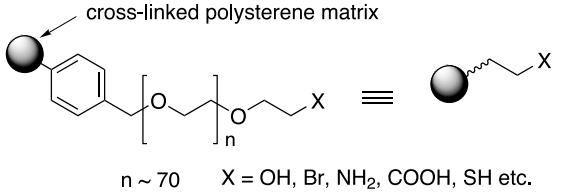

Figure 2. Chemical constitution of TentaGel resins.

Keywords: Macrocycles; Bisbibenzyls; Solid phase synthesis; TentaGel; HR-MAS NMR.
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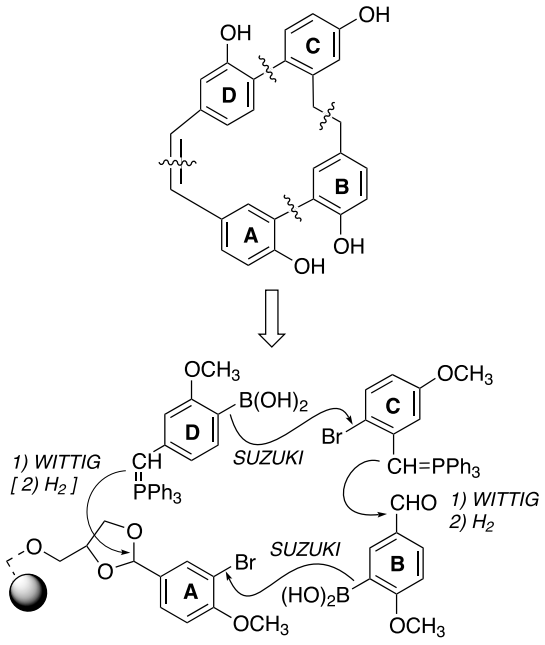


Scheme 1. Fragments and strategy for the synthesis of isoplagiochins on solid support.

dichloromethane, chloroform, dioxane, DMF, THF, water, methanol and pyridine (bad, however, in ethanol and diethylether).[15,19]

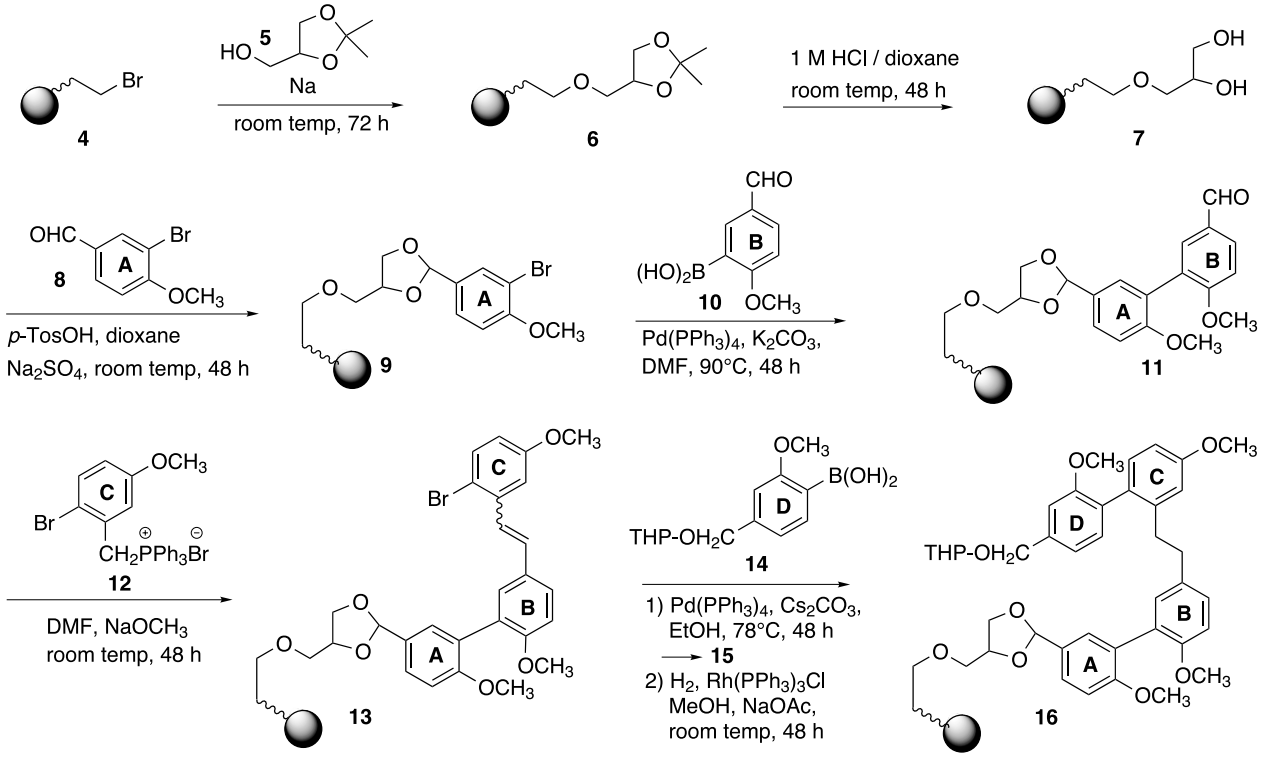
2. Results and discussion

Our strategy of synthesis (Scheme 1) was to start with a polymer bound aldehyde fragment **A**. Subsequently, Suzuki, Wittig and hydrogenation protocols should lead to an acyclic polymer bound **A–B–C–D** precursor, which has to be ring-closed after cleavage from the solid support.

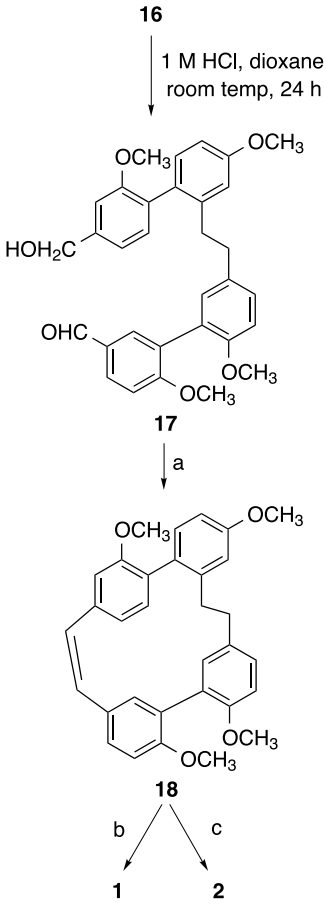
Thus, starting from TentaGel–Br (**4**) with a coverage density of 0.41 mmol/g resin a glycerol linker was introduced according to the procedure of Leznoff (formerly applied for Merrifield type resins)[20] by Williamson-coupling with the 1,3-dioxolane protected glycerol **5** (to **6**) and subsequent acidic hydrolysis to the polymer-bound 1,2-diol **7**. Coupling with the aldehyde **8** by acetal formation yielded the TentaGel linked starting subunit **9** (fragment **A**). Suzuki reaction of the polymer-bound bromoarene **9** with the boronic acid **10**[12] yielded the biaryl dialdehyde **11** (**A–B** fragment) on solid support, which was then reacted with the phosphonium salt **12**[21] (**C** fragment) to the stilbene **13** according to a Wittig protocol. After coupling with the boronic acid **14** (**D** fragment), the double bond in **15** (*E*/*Z*-mixture of isomers) was hydrogenated in presence of a homogeneous Wilkinson catalyst[22] resulting in the TentaGel linked acyclic **A–B–C–D** precursor **16** (Scheme 2).

On acidic hydrolysis of **16** the hydroxyaldehyde **17** was liberated from the solid support (Scheme 3). According to the spectroscopical data the acyclic **A–B–C–D** fragment **17** could be clearly identified as the known precursor for the ring-closing to **18** and completion of the syntheses of isoplagiochins C/D **1** and **2**.[12]

The TentaGel coupled intermediates could be unambiguously characterized by HR-MAS NMR spectroscopy as can be demonstrated for the final polymer bound compound **16** (see Fig. 3, for all intermediates see Supporting information).



Scheme 2. Formation of the TentaGel linked acyclic **A–B–C–D** precursor **16**.



Scheme 3. Cleavage from the solid support and completion of the synthesis for **1** and **2**. Reagents and conditions:[12,13] (a) (i) $Ph_3PH^+Br^-$, CH_3CN, reflux 24 h, 95%;[13] (ii) $NaOCH_3$, CH_2Cl_2, rt, 24 h, 82%; (b) BBr_3, CH_2Cl_2, −70 °C→rt, 48 h, 86%; (c) (i) H_2 3 bar, 5% Pd/C, EtOAc, 88%; (ii) BBr_3, CH_2Cl_2, −70 °C→rt, 48 h, 82%.

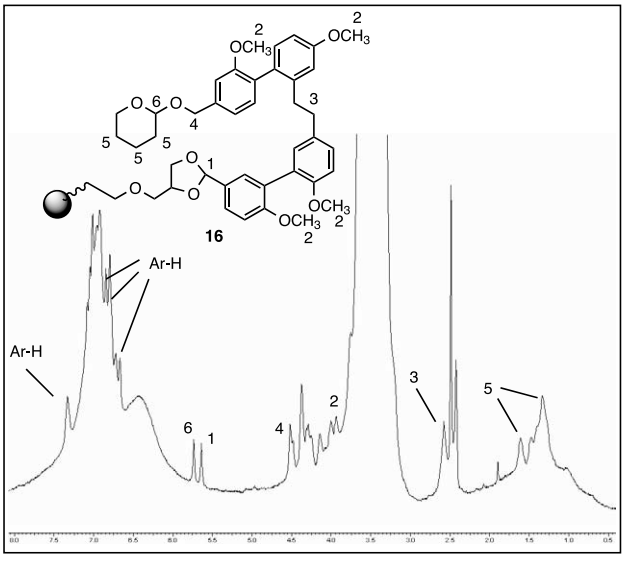
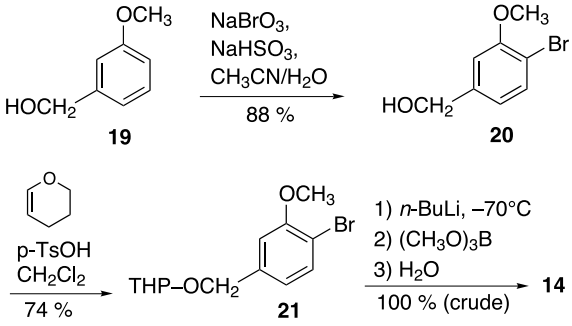


Figure 3. HR-MAS ^1H NMR spectrum of **16**.

The **D**-fragment **14** was prepared from 4-bromo-3-methoxybenzylalcohol **19** by regioselective bromination, (to **20**), formation of the THP ether **21** and transformation to the corresponding boronic acid (see Scheme 4).



Scheme 4. Preparation of the **D**-fragment **14**.

3. Conclusion

The multi-step synthesis of the target molecule could be realized with a final charging of about 0.10 mmol/g resin and a 25% overall-yield for **17** after cleavage from the solid support. The intermediates could be directly characterized by HR-MAS NMR spectroscopy. The procedure can be extended to the synthesis of small libraries of differently halogenated derivatives **3**.

4. Experimental

4.1. General

TentaGel® S–Br (0.41 mmol/g) was purchased from Rapp Polymere, Tübingen, Germany. All reactions with shaking at room temperature (rt) were performed on an IKA® Vibrax VXR basic at 500 rpm. Reactions requiring heating were performed in a Labnet® VorTemp 1550 at 600 rpm. NMR spectra in solution ($CDCl_3$, DMSO-d_6) were obtained with a Bruker DRX 500. Chemical shifts (δ) are given in ppm relative to TMS. HR-MAS NMR spectra were obtained with a Bruker DRX 500 using a 4 mm HR-MAS probe. Rotational frequency: ^1H: 8 kHz, ^{13}C: 8 kHz. Pulse repetition time: ^1H: 4 s, ^{13}C: 2 s. Solvents were commonly dried and purified by conventional methods prior to use. All air- or moisture-sensitive reactions were carried out under an argon atmosphere.

4.2. Preparation of the boronic acid 14

4.2.1. 4-Bromo-3-methoxybenzylalcohol (20). To a stirred solution of the 3-methoxybenzylalcohol (**19**) (10.0 g, 72.4 mmol, 8.98 mL) in CH_3CN/H_2O 1:1 (500 mL) were added $NaBrO_3$ (19.1 g, 127 mmol) and $NaHSO_3$ (13.2 g, 127 mmol). The reaction was stirred for 1.5 h at rt and continuously monitored by TLC. The reaction mixture was quenched with aqueous $Na_2S_2O_3$ and extracted with Et_2O (3×200 mL). The combined organic layers were washed with aqueous Na_2CO_3, H_2O, dried ($MgSO_4$) and concentrated in vacuo. The residue was chromatographed on silica gel/$CHCl_3$ to give a colourless oil; 13.9 g (88%).

^{1}H NMR ($CDCl_3$) δ (ppm) 7.40 (d, $J=8.8$ Hz, 1H, Ar-H), 7.05 (d, $J=3.1$ Hz, 1H, Ar-H), 6.70 (dd, $J_1=8.8$ Hz, $J_2=3.1$ Hz, 1H, Ar-H), 4.69 (s, 2H, Ar-CH_2O), 3.80 (s, 3H, OCH_3), 2.14 (br s, 1H, OH).

^{13}C NMR ($CDCl_3$) δ (ppm) 159.25, 140.74, 133.14, 144.77, 144.22, 112.50, 64.99, 55.52.

4.2.2. THP ether 21 of 4-bromo-3-methoxybenzylalcohol. The benzyl alcohol **20** (13.9 g, 63.9 mmol) was dissolved in anhydrous CH_2Cl_2 (250 mL). 3,4-Dihydro-2*H*-pyrane (13.4 g, 160 mmol, 14.6 mL) and toluene-4-sulfonic acid monohydrate (243 mg, 1.28 mmol) were added and the mixture was stirred at rt for 16 h. The solvent was evaporated and the residue was chromatographed on silica gel/CH_2Cl_2 to give a yellowish liquid; 14.2 g (74%).

^{1}H NMR ($CDCl_3$) δ (ppm) 7.41 (d, $J=8.5$ Hz, 1H, Ar-H), 7.09 (d, $J=3.2$ Hz, 1H, Ar-H), 6.70 (dd, $J_1=8.5$ Hz, $J_2=3.2$ Hz, 1H, Ar-H), 4.78 (t, $J=3.5$ Hz, 1H, O–CH–O), 4.78, 4.54 (2d, $J=13.6$ Hz, 2H, Ar-CH_2O), 3.96–3.89 (m, 1H, –CH_2O), 3.80 (s, 3H, OCH_3), 3.60–3.55 (m, 1H, CH_2O), 1.95–1.50 (m, 6H, –$(CH_2)_3$–).

^{13}C NMR ($CDCl_3$) δ (ppm) 159.06, 138.91, 133.00, 114.65, 114.23, 112.81, 98.43, 68.46, 62.22, 55.46, 30.54, 25.45, 19.36.

4.2.3. Boronic acid 14. The aryl bromide **21** (10.0 g, 33.2 mmol) was dissolved in THF (200 mL). *n*-Butyllithium (16.1 mL, 40.2 mmol, 2.5 M in *n*-hexane) was added at −70 °C and the reaction mixture was stirred for 30 min at −70 °C. After addition of trimethyl borate (10.4 g, 99.6 mmol, 11.1 mL) stirring was continued for 1 h while the reaction mixture was allowed to warm up to rt. H_2O (150 mL) was added, the aqueous layer was extracted with Et_2O (3×50 mL) and the combined organic layers were washed with satd aqueous NaCl, dried ($MgSO_4$) and evaporated. For purification, the product was dissolved in Et_2O, extracted with 2 M NaOH and neutralized to pH 6–7. The product was extracted with Et_2O, dried ($MgSO_4$) and the solvent was evaporated to give the crude boronic acid as a colourless solid; 8.84 g (100%).

^{1}H NMR (d_6-DMSO) δ (ppm) 7.49 (d, $J=8.2$ Hz, 1H, Ar-H), 6.92 (d, $J=2.5$ Hz, 1H, Ar-H), 6.79 (dd, $J_1=8.2$ Hz, $J_2=2.5$ Hz, 1H, Ar-H), 4.80, 4.61 (2d, $J=12.6$ Hz, 2H, Ar-CH_2O) 4.65 (t, $J=3.5$ Hz, 1H, O–CH–O), 3.83–3.77 (m, 1H, –CH_2O), 3.74 (s, 3H, OCH_3), 3.48–3.43 (m, 1H, –CH_2O), 1.80–1.40 (comb m, 6H, –$(CH_2)_3$–).

^{13}C NMR (d_6-DMSO) δ (ppm) 160.00, 144.55, 135.49, 112.83, 111.10, 97.59, 68.51, 61.21, 54.81, 30.11, 25.03, 19.02.

4.3. Preparations on solid support

For the HR-MAS NMR spectra of all polymer-bound intermediates see Supporting information.

4.3.1. Coupling of TentaGel–S–Br 4 with the hydroxy dioxolane 5. Sodium (236 mg, 10.3 mmol) was added to 4-hyroxymethyl-2,2-dimethyl-1,3-dioxolane **5** (20 mL) and the mixture was stirred and heated to 60 °C until the sodium had completely dissolved. The solution was cooled to rt and TentaGel–S–Br resin **4** (2.50 g) was added. The mixture was shaken at rt for 3 days and for additional 4 h at 80 °C. The resin was collected by filtration and washed with 1,4-dioxane (3×10 mL), H_2O (6×10 mL), EtOH/H_2O (1:1; 3×10 mL), EtOH (3×10 mL) and dry Et_2O (3×10 mL) to give the pale yellow polymer-bound dioxolane **6** (2.73 g).

4.3.2. Hydrolysis of the polymer-bound dioxolane 6. The polymer-bound dioxolane **6** (2.30 g) was suspended in a mixture of 1,4-dioxane/1 M HCl (1:1, 30 mL) and the slurry was shaken for 48 h at rt. The resin was filtered off and washed with H_2O (6×10 mL), acetone (10 mL), EtOH (3×10 mL) and dry Et_2O (3×10 mL) to give the yellow polymer-bound diol **7** (2.30 g).

4.3.3. Acetalization of the aldehyde 8 with the polymer-bound diol 7. The diol-modified TentaGel **7** (2.13 g) was suspended in anhydrous 1,4-dioxane (30 mL). 3-Bromo-4-methoxybenzaldehyde **8** (1.61 g, 7.52 mmol), toluene-4-sulfonic acid monohydrate (16.6 mg, 0.09 mmol) and anhydrous Na_2SO_4 (2.00 g) were added and the mixture was shaken at rt for 48 h with exclusion of moisture and then shaken for additional 4 h at 80 °C. The resin was filtered off and washed with anhydrous pyridine (2×10 mL), pyridine/H_2O (1:1; 2×10 mL), H_2O (10×10 mL), EtOH (3×10 mL) and dry Et_2O (3×10 mL) to give the pale yellow polymer-bound bromoarene **9** (2.00 g).

4.3.4. Suzuki-coupling of the boronic acid 10 with the polymer-bound bromo arene 9. The TentaGel-bound bromo arene **9** (1.80 g) was swelled in DMF (40 mL) for 10 min. To this slurry were added the boronic acid **10** (439 mg, 2.45 mmol), $Pd(PPh_3)_4$ (36.7 mg, 24.5 μmol) and K_2CO_3 (169 mg, 1.22 mmol). The mixture was shaken for 48 h at 90 °C under an argon atmosphere. The resin was filtered and washed alternately with DMF/H_2O (4:1, 3×10 mL), followed by MeOH (3×10 mL) and CH_2Cl_2 (3×10 mL). The solid was dried in vacuo at rt to give a yellowish brown polymer-bound biarylaldehyde **11** (1.78 g).

4.3.5. Wittig-reaction between the polymer-bound aldehyde 11 and the phosphonium salt 12. To a solution of the phosphonium salt **12** (3.55 g, 6.55 mmol) in anhydrous DMF (40 mL) was added $NaOCH_3$ (1.06 g, 19.6 mmol) under argon atmosphere. After shaking for 5 min, the polymer-bound aldehyde (1.77 g) was added to the orange solution. The mixture was shaken at rt for 48 h. Then the resin was filtered and washed alternately with DMF (3×10 mL), DMF/H_2O (1:1, 3×10 mL), H_2O (3×10 mL), followed by MeOH (3×10 mL) and CH_2Cl_2 (3×10 mL). The solid was dried in vacuo at rt to give a beige polymer-bound stilbene **13** (1.77 g).

4.3.6. Suzuki coupling of the boronic acid 14 with the polymer-bound bromide 13. The polymer-bound bromide **13** (1.18 g) was swelled in EtOH (20 mL). To the mixture was added the boronic acid **14** (606 mg, 2.10 mmol), $Pd(PPh_3)_4$ (24.4 mg, 21.1 μmol) and Cs_2CO_3 (339 mg, 1.05 mmol). The mixture was shaken for 48 h at 90 °C under an argon atmosphere. Then the resin was filtered and

washed alternately with EtOH (3×10 mL), DMF/H_2O (1:1, 3×10 mL), H_2O (3×10 mL), followed by MeOH (3×10 mL) and CH_2Cl_2 (3×10 mL). The solid was dried in vacuo at rt to give a brown polymer-bound stilbene **15** (1.20 g).

4.3.7. Catalytic hydrogenation of the stilbene 15. A two-necked round bottom flask containing the polymer-bound stilbene **15** (713 mg) was evacuated a few times and flushed with argon. After the last evacuation, a hydrogen-atmosphere was adjusted by a balloon. Wilkinson-catalyst $Rh(PPh_3)_3Cl$ (43.5 mg, 47 μmol) and NaOAc (40 mg) were dissolved in MeOH (20 mL) and added by a syringe. The mixture was shaken at rt for 24 h. The resin was filtered and washed alternately with MeOH (3×10 mL) and CH_2Cl_2 (3×10 mL). The solid was dried in vacuo at rt to give a brown polymer-bound bibenzyl **16** (700 mg).

4.3.8. Hydrolytic cleavage from the solid support. The polymer-bound bibenzyl **16** (633 mg) was swelled in dioxane/3 M HCl (1:1, 20 mL) and shaken at rt for 48 h. The resin was filtered and washed alternately with H_2O (6×5 mL), acetone (1×10 mL), EtOH (3×5 mL) and Et_2O (3×10 mL). The aqueous layer was extracted with Et_2O (3×40 mL), the combined organic layers were washed with H_2O (6×40 mL) and dried ($MgSO_4$). The solvent was evaporated to give the crude bibenzyl (81 mg). Purification by column chromatography (SiO_2/$CHCl_3$–EtOAc 3:1) yielded pure **17** as a colourless oil (32 mg, 25% overall for eight steps).

The NMR-spectroscopic data were identical with those reported in the literature:[12]

^{1}H NMR ($CDCl_3$) δ (ppm) 9.92 (s, 1H, –CHO), 7.86 (dd, J_1=8.4 Hz, J_2=2.0 Hz, 1H, Ar-H), 7.68 (d, J=2.2 Hz, 1H, Ar-H), 7.08 (d, J=8.4 Hz, 1H, Ar-H), 7.05 (d, J=8.3 Hz, 1H, Ar-H), 7.02 (d, J=7.6 Hz, 1H, Ar-H), 6.98 (d, not resolved, 1H, Ar-H), 6.93–6.91 (not resolved, 2H, Ar-H), 6.83 (d, J=2.7 Hz, 1H, Ar-H), 6.80 (d, J=8.4 Hz, 1H, Ar-H), 6.80 (dd, not resolved, 1H, Ar-H), 6.74 (d, J=2.2 Hz, 1H, Ar-H), 4.67 (s, 2H, Ar-CH_2O), 3.84 (s, 3H, –OCH_3), 3.82 (s, 3H, –OCH_3), 3.75 (s, 3H, –OCH_3), 3.72 (s, 3H, –OCH_3), 2.69 (br s, 4H, –CH_2CH_2–), 1.69 (br s, 1H, –OH).

^{13}C NMR ($CDCl_3$) δ (ppm) 191.2, 162.3, 158.9, 157.1, 155.2, 141.8, 141.7, 134.1, 132.6, 131.9, 131.6, 131.3, 131.2, 130.6, 129.6, 129.1, 128.9, 126.2, 118.6, 114.4, 111.1, 110.9, 110.7, 109.1, 65.3, 56.0, 55.8, 55.5, 55.2, 36.3, 36.1.

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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.2005.09.048. HR-MAS NMR spectra of all polymer-bound intermediates.

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Polyhydroxylated pyrrolidines, III. Synthesis of new protected 2,5-dideoxy-2,5-iminohexitols from D-fructose☆

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Abstract—The readily available 3-*O*-benzoyl-4-*O*-benzyl-1,2-*O*-isopropylidene-β-D-fructopyranose (**6**) was straightforwardly transformed into 5-azido-3-*O*-benzoyl-4-*O*-benzyl-5-deoxy-1,2-*O*-isopropylidene-β-D-fructopyranose (**8**), after treatment under modified Garegg's conditions followed by reaction of the resulting 3-*O*-benzoyl-4-*O*-benzyl-5-deoxy-5-iodo-1,2-*O*-isopropylidene-α-L-sorbopyranose (**7**) with lithium azide in DMF. *O*-debenzoylation at C(3) in **8**, followed by oxidation and reduction caused the inversion of the configuration to afford the corresponding β-D-psicopyranose derivative **11** that was transformed into the related 3,4-di-*O*-benzyl derivative **12**. Cleavage of the acetonide of **12** to give **13** followed by *O*-*tert*-butyldiphenylsilylation afforded a resolvable mixture of **14** and **15**. Compound **14** was transformed into (2*R*,3*R*,4*S*,5*R*)- (**17**) and (2*R*,3*R*,4*S*,5*S*)-3,4-dibenzyloxy-2′,5′-di-*O*-*tert*-butyldiphenylsilyl-2,5-bis(hydroxymethyl)pyrrolidine (**18**) either by a tandem Staudinger/intramolecular aza-Wittig process and reduction of the resulting intermediate Δ^2-pyrroline (**16**), or only into **18** by a high stereoselective catalytic hydrogenation. When **15** was subjected to the same protocol, (2*S*,3*S*,4*R*,5*R*)- (**21**) and (2*R*,3*S*,4*R*,5*R*)-3,4-dibenzyloxy-2′-*O*-*tert*-butyldiphenylsilyl-2,5-bis(hydroxymethyl)pyrrolidine (**22**) were obtained, respectively.
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1. Introduction

In previous papers, we have reported on the highly stereoselective synthesis of orthogonally protected derivatives of 2,5-dideoxy-2,5-imino-D-glucitol (DGDP)[1] and 2,5-dideoxy-2,5-imino-D-mannitol (DMDP),[2] from the cheap and commercially available D-fructose. Both compounds were shown to be excellent chiral key intermediates for the preparation of natural[3] and unnatural hyacinthacines,[4] potent glycosidase inhibitors.[5]

Figure 1 shows the synthetic potentiality of (2*R*,3*S*,4*R*,5*R*)-3,4-dibenzyloxy-2′-*O*-*tert*-butyldiphenylsilyl-2,5-bis(hydroxymethyl)pyrrolidine [**22**, 2,5-dideoxy-2,5-imino-D-altritol (DALDP) displaying the retrosynthesis of a great variety of hyacinthacines, recently isolated from different natural sources,[6] where clearly is shown that **22** must be considered an appropriate chiral starting material for the synthesis of such target molecules. Thus, protection interchange between the hydroxyl groups at C(2′)–C(5′), carbon-chain lengthening at C(2′) (the original C(1) of D-fructose) in a two more carbon atoms fragment suitably functionalised, followed by a further cyclization, could lead to pyrrolizidines, which stereochemistry at C(1,2,3,7a) belonging to that of the natural hyacinthacines.

Continuing with our efforts on the title topic, we describe herein, the highly stereoselective synthesis of **22** together with that of its C(2)-epimer (**21**) using a D-psicose derivative (**12**) as key intermediate, which is available from D-fructose as source of chirality and functionalization.

2. Results and discussion

In order to obtain the above mentioned intermediate **12**, it was necessary to prepare 3,4-di-*O*-benzyl-1,2-*O*-ispropylidene-β-D-psicopyranose (**4**). This was initially attempted by a well established protocol consisting in: partial deacetonation of the already known 3-*O*-benzyl-1,2:4,5-di-*O*-isopropylidene-β-D-psicopyranose (**1**)[7] to the corresponding 1,2-*O*-isopropylidene derivative (**2**), subsequent 4,5-*O*-di-*n*-butylstannylation to the not characterized **3**, and finally regioselective ring opening by benzyl bromide (see Scheme 1), but conversely to many other cases,[1,8] where the main product comes from the electrophilic attack of the reagent at the oxygen with the equatorial disposition [O(4)], no regioselectivity was observed and an unresolvable mixture of the corresponding 3,4- (**4**) and 3,5-di-*O*-benzyl (**5**) derivatives was produced in low yield. The use of any

☆ For Part II, see Ref. 2.

Keywords: D-fructose; Stereoselective síntesis; Polyhydroxylated pyrrolidines; 2,5-Dideoxy-2,5-iminohexitols; DADP; DALDP.
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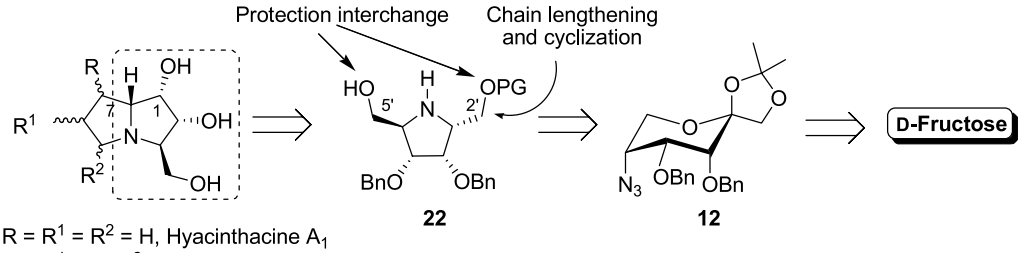
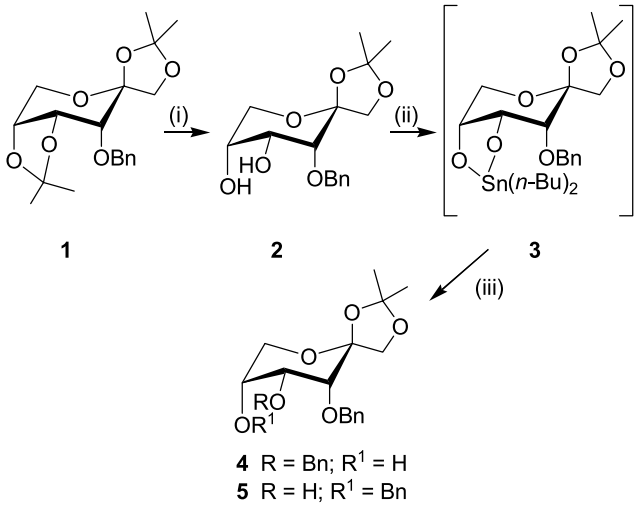


R = R^1 = R^2 = H, Hyacinthacine A$_1$
R = R^1 = H; R^2 = Me, (5*R*)-Hyacinthacine A$_6$
(5*S*)-Hyacinthacine A$_7$
R = R^1 = H, R^2 = CH$_2$OH, (5*R*)-Hyacinthacine B$_1$
(5*S*)-Hyacinthacine B$_2$
R = OH; R^1 = H; R^2 = Me, (5*R*,7*R*)-Hyacinthacine B$_3$
(5*R*,7*S*)-Hyacinthacine B$_5$
R = R^1 = OH; R^2 = Me, (5*R*,6*R*,7*R*)-Hyacinthacine C$_1$

Figure 1. Retrosynthesis of natural hyacinthacines.



Scheme 1. Synthesis of **4** and **5** from **1**. Reagents and conditions: (i) 75% aq AcOH/45 °C, 2 h; (ii) *n*-Bu$_2$SnO/MeOH/reflux; (iii) BnBr/DMF/110 °C.

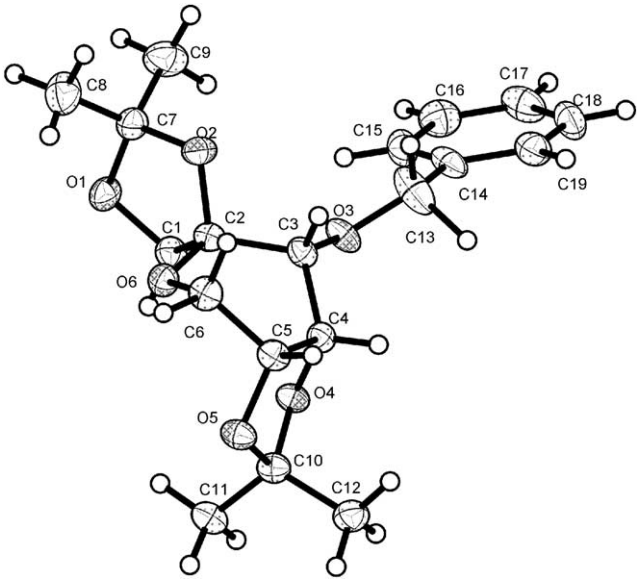
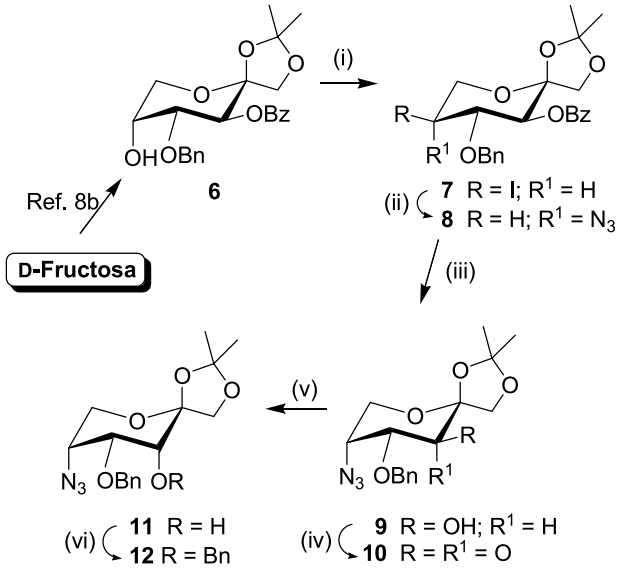


Figure 2. ORTEP view of compound **1**. Thermal ellipsoid enclosed 50% of electron density.

other electrophilic reagent (BzCl, TBDMSCl, MEMCl, etc.) also gave the same result.

These unexpected results could be explained if intermediate **3** would adopt a ^{3,6}B conformation similar to that found in **1** (see Fig. 2), and presuming that a $Me_2C<\rightarrow n$-$Bu_2Sn<$ change must not be of great stereochemical significance. It can be observed the oxygen atoms at C(4,5) adopt a parallel disposition, in such a way that both can be attacked by the electrophilic reagent giving **4** and **5**, without regioselectivity.

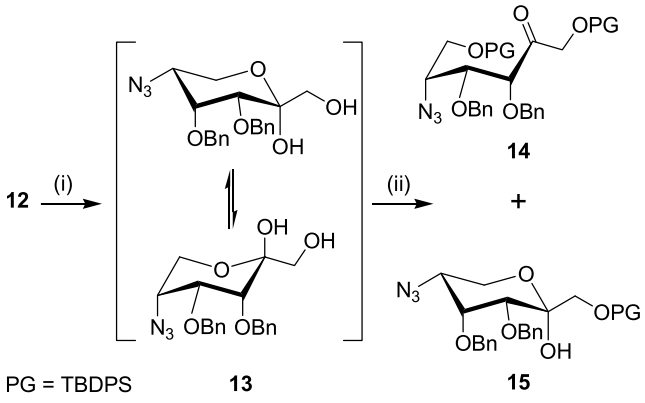
On the basis of the above results a new synthetic route was designed (see Scheme 2), consisting in the introduction of the appropriate funtionalization and stereochemistry at C(5) in 3-*O*-benzoyl-4-*O*-benzyl-1,2-*O*-isopropylidene-β-D-fructopyranose (**6**)[8b] prior to the inversion of the configuration at C(3). Thus, reaction of **6** under the Garegg's conditions[9] afforded the corresponding 5-deoxy-5-iodo-α-L-*sorbo* derivative (**7**), which treatment with LiN_3 in DMF effected S_N2 substitution to yield 5-azido-3-*O*-benzoyl-4-*O*-benzyl-5-deoxy-1,2-*O*-isopropylidene-β-D-fructopyranose (**8**).



Scheme 2. Synthesis of **12** from D-fructose. Reagents and conditions: (i) I_2/Ph_3P/imidazole/MePh, reflux; (ii) LiN_3/DMF/100 °C; (iii) MeOH/MeONa (cat), rt; (iv) Dess–Martin/Cl_2CH_2, rt; (v) $NaBH_4$/MeOH, 0 °C; (vi) NaH/DMF/BnBr, rt.

Zemplen debenzoylation of **8** gave the 3-*O*-deprotected **9**, that was subsequently oxidized to 2,3-diulose **10**, not totally characterized but reduced to 5-azido-4-*O*-benzyl-5-deoxy-1,2-*O*-isopropylidene-β-D-psicopyranose (**11**). The high stereoselectivity found was in accordance with that observed by Tipson et al. in the reduction of 1,2:4,5-di-*O*-isopropylidene-β-D-*erythro*-2,3-hexodiulo-2,6-pyranose.[10] Compound **11** was straightforwardly transformed into the totally protected derivative **12**.

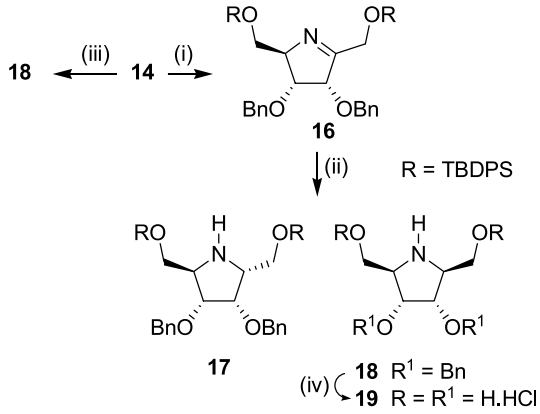
According to Scheme 3, deacetonation of **12** in acid medium gave **13**, existing as a ≈2:1 mixture of α- and β-pyranoses in $^5C_2$ and $^2C_5$ conformations, respectively, according to their ^1H and ^{13}C NMR data (see Tables 1 and 2).



Scheme 3. Synthesis of protected uloses **14** and **15**. Reagents and conditions: (i) aq 60% TFA, rt; (ii) TBDPSCl/imidazole//DMF, rt.

Attempt to protect **13** as its 1-*O*-*tert*-butyldiphenylsilyl derivative under the conventional conditions (silylating reagent/imidazole/DMF) resulted in the formation of 5-azido-3,4-di-*O*-benzyl-1-*O*-*tert*-butyldiphenylsilyl-5-deoxy-α-D-psicopyranose (**15**, 70%) together with an appreciable amount of the unexpected 5-azido-3,4-di-*O*-benzyl-1,6-di-*O*-*tert*-butyldiphenylsilyl-5-deoxy-D-psicose (**14**, 26%), that were separated by chromatographic means.

Compounds **14** and **15** were the key intermediates for the synthesis of the target 2,5-dideoxy-2,5-iminohexitols (see Scheme 4). In a first approach, submission of **14** to an intramolecular tandem Staudinger/aza-Wittig reaction[11] afforded the expected (3*S*,4*R*,5*R*)-3,4-dibenzyloxy-2′,5′-di-



Scheme 4. Synthesis of total and partially protected polyhydroxylated pyrrolidines **17** and **18**. Reagents and conditions: (i) Ph_3P/THF, reflux; (ii) $NaCNBH_3$/THF/AcOH, 0 °C; (iii) Raney-Ni/H_2/MeOH–THF; (iv) *n*-$Bu_4N^+F^-$ 3 H_2O/THF, then 10% Pd–C/H_2/MeOH/HCl.

O-*tert*-butyldiphenylsilyl-2,5-bis(hydroxymethyl)-Δ^1-pyrroline (**16**) in almost quantitative yield that could be characterized, but decomposed on standing. Conventional reduction of **16** with sodium cianoborohydride gave a 2:1 mixture of (2*R*,3*R*,4*S*,5*R*)- (**17**) and (2*R*,3*R*,4*S*,5*S*)-3,4-dibenzyloxy-2′,5′-di-*O*-*tert*-butyldiphenylsilyl-2,5-bis(hydroxymethyl)pyrrolidine (**18**). The absolute configuration at the new generated stereogenic centre [C(5)] in **17**, and hence that of **18**, was easily determined after recording their optical, ^1H and ^{13}C NMR data. Thus, **18** was optically inactive indicating the presence of a symmetry plane, confirmed by the presence of half of the resonance signals in its NMR spectra. On the other hand, hydrogenation of **14** under the presence of Raney-Ni occurred with high stereoselectivity to afford only **18**. Compound **18** was totally *O*-deprotected to the already known[12] 2,5-dideoxy-2,5-imino-D-allitol (**19** DADP) as its hydrochloride salt.

Following the above protocol but in **15** (see Scheme 5), the intramolecular Staudinger/aza-Wittig reaction afforded the expected Δ^1-pyrroline **20** (TLC evidence) that could not be characterized due to its instability, but reduced to give only one compound identified as (2*S*,3*S*,4*R*,5*R*)-3,4-dibenzyloxy-2′-*O*-*tert*-butyldiphenylsilyl-2,5-bis(hydroxymethyl)-pyrrolidine (**21**), whereas the catalytic hydrogenation of **15** gave the (2*R*,3*S*,4*R*,5*R*)-isomer (**22**). De-*O*-silylation of **22** gave the corresponding **23**, which analytical and

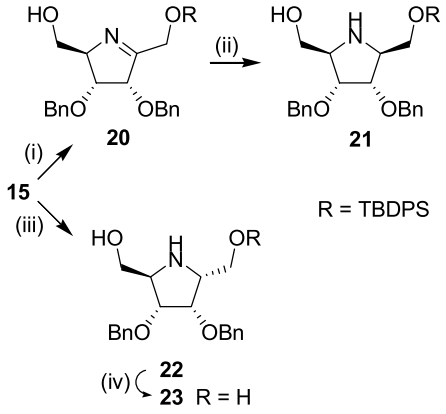
Table 1. ^1H RMN chemical shifts (δ) and *J* (Hz) values for compound **13α** and **13β**

Compound	H-1	H-1′	H-3	H-4	H-5	H-6ax	H-6eq	C*H*$_2$Ph	C*H*$_2$Ph	OH
13α	3.69d, $J_{1,1'}$ = 11.6 Hz	3.46d	3.58d, $J_{3,4}$ = 2.5 Hz	4.22br s	3.31ddd, $J_{4,5}$ = 2.5 Hz, $J_{5,6eq}$ = 5.3 Hz	4.07t, $J_{5,6ax}$ = 11.4 Hz, $J_{6,ax,6eq}$ = 11.4 Hz	3.74dd	4.85d, 4.78d, J = 10.6 Hz	4.63s	
13β	3.93d, $J_{1,1'}$ = 11.8 Hz	3.26d	3.74m	4.10br s	3.86m	3.92dd, $J_{5,6ax}$ = 2.4 Hz, $J_{6,ax,6eq}$ = 12.4 Hz	3.74m	5.00d, 4.69d, J = 11.6 Hz	4.76d, 4.68d, J = 11.9 Hz	5.57br s, 2.11br s

Table 2. ^{13}C RMN chemical shifts (δ) values for compound **13α** and **13β**

Compound	C-1	C-2	C-3	C-4	C-5	C-6	*C*H$_2$Ph
13α	64.46	97.86	76.86[a]	73.12[a]	57.09	56.77	75.98, 72.27
13β	65.91	97.67	76.46[a]	75.26[a]	56.28	62.15	74.89, 71.42

[a] Assignments may be interchanged.



Scheme 5. Synthesis of partially protected polyhydroxylated pyrrolidines **21** and **22**. Reagents and conditions: (i) Ph_3P/THF, reflux; (ii) $NaCNBH_3$/THF/AcOH, 0 °C; (iii) Raney-Ni/H_2/MeOH; (iv) n-$Bu_4N^+F^-$ 3 H_2O/THF.

spectroscopic data were consistent with the assigned stereochemistry.

Comments merit the high stereoselectivity found in the catalytic hydrogenation of **14** and **15** (see Fig. 3). Contrary to that previously reported,[13] where the authors stated that the stereochemistry of five-membered ring system is controlled by that at C(4), our results seemed to indicate that the size of the substituent at C(5′) must play an important roll in the steric course of the reaction, since either the presence, in **14**, or absence, in **15**, of bulky TBDPS protecting group at C(5′) makes either the bottom or top face the preferable for the hydrogen attack, respectively.

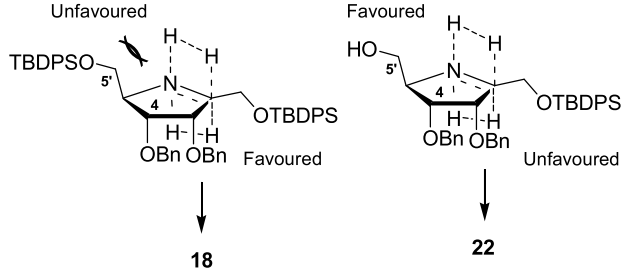


Figure 3. Transition states for catalytic hydrogenation of **14** and **15**.

3. Conclusions

D-fructose was an excellent chiral starting material for the stereoselective synthesis of polyhydroxylated pyrrolidines alkaloids. Highly diastereoselective hydrogenation of protected 5-azido-5-deoxy-D-psicose derivatives was the best synthetic route to the target molecules.

4. Experimental

4.1. Crystal structure determination

Single crystal of **1** was mounted on a Bruker-Smart Apex area detector diffractometer (Mo Kα $\lambda=0.71073$ Å). The cell parameters were determined from reflections taken from one steps in phi angle) each at 20 s exposure. The structures were solved using direct methods (SHELXS[14]) and refined against F^2 using the SHELXS12 software.[14] The absorption was non-corrected. All non-hydrogen atoms were generated according to stereochemistry and refined using a riding model in SHELXS97.

$C_{19}H_{26}O_6$: $M=350.40$ g mol^{-1}; triclinic; space group P-1; $a=5.881(10)$ Å, $b=8.052(14)$ Å, $c=10.490(18)$ Å, $\alpha=67.714(2)°$, $\beta=84.247(3)°$, $\gamma=78.551(3)°$, $V=450.39(13)$ Å^3, $Z=1$, $\rho_{calcd}=1.292$ g cm^{-3}, $\mu=0.095$ mm^{-1}, $F(000)=180$. Colourless crystal, dimensions $0.27\times0.13\times0.07$ mm^3. A total of 5288 reflections were collected with $2.10°<\theta<28.36°$; 3885 independent reflections with 3644 having $I<2\sigma<(I)$; 233 parameters; $R_1=0.0412$; $wR_2=0.1018$; Goof$=1.081$; maximum residual electronic density$=0.481$ e^{-1} Å^3.

Full data collection parameters and structural data are available as supporting information. Crystallographic data for the crystal structure have been deposited with the Cambridge Crystallographic Data Centre, CCDC 269114. Copies of this information may be obtained free of charge from The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336033; e-mail, deposit@ccdc.cam.ac.uk; web, http://www.ccdc.cam.ac.uk).

4.2. General procedures

Melting points were determined with a Gallenkamp apparatus and are uncorrected. Solutions were dried over $MgSO_4$ before concentration under reduced pressure. The ^{1}H and ^{13}C NMR spectra were recorded with Bruker AMX-300, AM-300, and ARX-400 spectrometers for solutions in $CDCl_3$ (internal Me_4Si). IR spectra were recorded with a Perkin-Elmer 782 instrument and mass spectra with a Micromass Mod. Platform II and Autospec-Q mass spectrometers. Optical rotations were measured for solutions in $CHCl_3$ (1 dm tube) with a Jasco DIP-370 polarimeter. TLC was performed on precoated E. Merck silica gel 60 F_{254} aluminium sheets with detection by charring with H_2SO_4 or employing a mixture of 10% ammonium molybdate (w/v) in 10% aqueous sulphuric acid containing 0.8% cerium sulphate (w/v) and heating. Column chromatography was performed on silica gel (E. Merck, 7734). The no crystalline compounds, for which elemental analyses were not obtained, were shown to be homogeneous by chromatography and characterized by NMR spectroscopy and FAB-HRMS with thioglycerol matrix.

4.2.1. 3-*O*-benzyl-1,2-*O*-isopropylidene-β-D-psicopyranose (2). A solution of 3-*O*-benzyl-1,2:4,5-di-*O*-isopropylidene-β-D-psicopyranose[7] (**1**, 3.50 g, 10 mmol) in 75% aqueous acetic acid (50 mL) was heated at 45 °C for 2 h. TLC (ether) then revealed a new slower running compound. The mixture was concentrated and repeatedly codistilled with water and then dissolved in ethanol (60 mL), neutralized with solid K_2CO_3 and concentrated. Column chromatography (1:1 ether/hexane) gave pure syrupy **2** (2.50 g, 79%); $[\alpha]_D^{27}-90$ (c 1); IR (neat): υ 3484 and 3451 (OH), 3031 (aromatic), 1373 and 1245 (CMe_2), 745 and 700 cm^{-1} (aromatic). ^{1}H NMR (400 MHz): δ 7.33 (m, 5H, Ph), 4.86 and 4.69 (2d, 2H, $J=11.3$ Hz, CH_2Ph), 4.06 and 3.80 (2d, 2H, $J_{1,1'}=9.3$ Hz, H-1,1′), 3.96 (t, 1H, $J_{3,4}$, $J_{4,5}=3.5$ Hz, H-4), 3.96 (dd, 1H, $J_{5,6}=2$ Hz, $J_{6,6'}=12.3$ Hz, H-6), 3.86 (dd, 1H, $J_{5,6'}=2.3$ Hz, H-6′), 3.74 (m, 1H, H-5), 3.66

(br d, 1H, H-3), 2.60 (br s, 2H, HO-4,5), 1.47 and 1.35 (2s, 6H, CMe_2). ^{13}C NMR: δ 137.15, 128.69, 128.38, and 128.16 ($PhCH_2$), 112.06 (CMe_2), 104.88 (C-2), 80.99 (C-3), 76.02 (C-1), 73.27 (CH_2Ph), 69.28 and 67.43 (C-4,5), 65.33 (C-6), 26.78 and 26.29 (CMe_2). HRMS: *m/z* 333.1319 [M^++Na]. For $C_{16}H_{22}O_6Na$ 333.1314 (deviation −1.5 ppm).

4.2.2. 3-*O*-benzoyl-4-*O*-benzyl-5-deoxy-5-iodo-1,2-*O*-isopropylidene-α-L-sorbopyranose (7). To a solution of triphenylphosphine (8.82 g, 33.62 mmol), imidazole (4.57 g, 67.20 mmol), and iodine (8.52 g, 23.57 mmol) in dry toluene (120 mL) was added 3-*O*-benzoyl-4-*O*-benzyl-1,2-*O*-isopropylidene-β-D-fructopyranose[8b] (**6**, 6.95 g, 17.79 mmol) in the same solvent (50 mL) and the mixture heated at 110 °C for 2 h. TLC (3:2 ether/hexane) then revealed a faster-running compound. The reaction mixture was cooled, washed with 10% aqueous sodium thiosulphate and brine, then concentrated. Column chromatography (1:3 ether/hexane) afforded crystalline **7** (6.7 g, 76%); mp 166–168 °C (from ether); $[\alpha]_D^{26}$ −64.5 (*c* 1.1); IR (KBr): υ 3033 (aromatic), 1727 (ester), 1382 and 1274 (CMe_2), 713 and 693 cm^{-1} (aromatic). ^1H NMR (300 MHz): δ 8.10–8.07, 7.63–7.58, 7.49–7.44, and 7.17–7.10 (4m, 10H, 2 Ph), 5.30 (d 1H, $J_{3,4}$=8.8 Hz, H-3), 4.84 and 4.59 (2d, 2H, J=10.1 Hz, CH_2Ph), 4.22–3.92 (m, 4H, H-4,5,6_{ax},6_{eq}), 3.97 and 3.92 (2d, 2H, $J_{1,1'}$=9.3 Hz, H-1,1′), 1.52 and 1.43 (2s, 6H, CMe_2). ^{13}C NMR (inter alia): δ 165.56 (COPh), 112.52 (CMe_2), 104.93 (C-2), 81.88 (C-3), 75.57 (C-1), 72.67 (C-4), 71.88 (CH_2Ph), 66.20 (C-6), 26.77 and 26.30 (CMe_2), and 25.57 (C-5). Anal. Calcd for $C_{23}H_{25}IO_6$: C, 52.68; H, 4.81. Found: C, 53.21; H, 5.04.

4.2.3. 5-Azido-3-*O*-benzoyl-4-*O*-benzyl-5-deoxy-1,2-*O*-isopropylidene-β-D-fructopyranose (8). A stirred solution of **7** (3 g, 5.72 mmol) and lithium azide (0.84 g, 17.1 mmol) in dry DMF (25 mL) was heated at 100 °C for 6 h. TLC (3:2 ether/hexane) then revealed a lower-running compound. The mixture was concentrated to a residue that was dissolved in ether (40 mL), washed with brine and concentrated. Flash column chromatography (1:1 ether/hexane) of the residue afforded **8** (2.5 g, 99%) as a colourless syrup; $[\alpha]_D^{26}$ −127 (*c* 0.7); IR (neat): υ 3033 (aromatic), 2105 (N_3), 1724 (ester), 1372 (CMe_2), 710 cm^{-1} (aromatic). ^1H NMR (300 MHz): δ 8.08–8.06, 7.63–7.57, 7.49–7.44, and 7.22–7.17 (4m, 10H, 2 Ph), 5.64 (d 1H, $J_{3,4}$=9.9 Hz, H-3), 4.65 and 4.58 (2d, 2H, J=12.1 Hz, CH_2Ph), 4.12 (dd, 1H, $J_{4,5}$=3.8 Hz, H-4), 4.02 and 3.94 (2d, 2H, $J_{1,1'}$=9.3 Hz, H-1,1′), 4.00–3.94 (m, 2H, H-5,6), 3.76 (dd, 1H, $J_{5,6'}$=2 Hz, $J_{6,6'}$=13 Hz, H-6′), 1.47 and 1.37 (2s, 6H, CMe_2). ^{13}C NMR (inter alia): δ 165.80 (COPh), 112.22 (CMe_2), 104.87 (C-2), 76.83 (C-3), 72.49 and 71.97 (C-1 and CH_2Ph), 68.93 (C-4), 62.26 (C-6), 59.86 (C-5), 26.66 and 26.27 (CMe_2). HRMS: *m/z* 462.1640 [M^++Na]. For $C_{23}H_{25}N_3O_6Na$ 462.1641 (deviation +0.3 ppm).

4.2.4. 5-Azido-4-*O*-benzyl-5-deoxy-1,2-*O*-isopropylidene-β-D-fructopyranose (9). Compound **8** (2.47 g, 5.62 mmol) in anhyd. MeOH (50 mL) was treated with 0.5 M NaMeO in MeOH (10 mL) for 24 h. TLC (3:2 ether/hexane) then revealed a slower-running compound. The mixture was neutralized with AcOH, concentrated and the residue was partitioned in Cl_2CH_2-water. The organic phase was separated, concentrated and the residue submitted to flash chromatography (hexane → 1:1 ether/hexane) to afford crystalline **9** (1.83 g, 97%); mp 76–78 °C (from ether/hexane); $[\alpha]_D^{27}$ −132 (*c* 0.9); ν (KBr) 3521 (OH), 3039 (aromatic), 2099 (N_3), 1373 (CMe_2), 741 and 697 cm^{-1} (aromatic). ^1H NMR (300 MHz): δ 7.45–7.25 (m, 5H, Ph), 4.77 and 4.72 (2d, 2H, J=11.6 Hz, CH_2Ph), 4.19 and 3.99 (2d, 2H, $J_{1,1'}$=8.8 Hz, H-1,1′), 3.93–3.86 (m, 3H, H-3,5,6), 3.76 (dd, 1H, $J_{4,5}$=3.5 Hz, $J_{3,4}$=9.5 Hz, H-4), 3.70 (dd, 1H, $J_{5,6'}$=2.2 Hz, $J_{6,6'}$=12.8 Hz, H-6′), 1.48 and 1.43 (2s, 6H, CMe_2). ^{13}C NMR: δ 137.62, 128.67, 128.16, and 127.98 (CH_2Ph), 112.27 (CMe_2), 105.76 (C-2), 79.59 (C-3), 72.73 and 72.01 (C-1 and CH_2Ph), 68.23 (C-4), 62.17 (C-6), 59.41 (C-5), 26.72 and 26.27 (CMe_2). Anal. Calcd for $C_{16}H_{21}N_3O_5$: C, 57.30; H, 6.31; N, 12.53. Found: C, 57.15; H, 6.77; N, 12.05.

4.2.5. 5-Azido-4-*O*-benzyl-5-deoxy-1,2-*O*-isopropylidene-β-D-psicopyranose (11). To a stirred suspension of Dess–Martin periodinane (3.48 g, 8.18 mmol) in dry CH_2Cl_2 (20 mL) was added dropwise a solution of **9** (1.83 g, 5.46 mmol) in the same solvent (15 mL) under Ar. The mixture was stirred at room temperature for 1 h. TLC (3:2, ether/hexane) then revealed the presence of a faster-running product. The reaction mixture was filtered and the filtrate washed with 10% aqueous Na_2CO_3, brine and water, then concentrated. The residue was percolated (3:2 ether/hexane) through a short column of silica gel to afford fractions containing presumably ketone **10** [1.7 g, 93.4%; IR: υ (neat) 1753 cm^{-1}], that was used in the next step.

To a stirred and ice-water cooled solution of **10** (1.7 g, 5.1 mmol) in dry methanol (20 mL) $NaBH_4$ (0.29 g, 7.5 mmol) was added portionwise. After 1 h, TLC (3:2 ether/hexane) showed no ketone **10** and the presence of a new product of higher mobility. The reaction mixture was neutralized with AcOH, concentrated and the residue was dissolved in Cl_2CH_2, washed with water then concentrated. Flash column chromatography (1:1 ether/hexane) afforded crystalline **11** (1.51 g, 88%); mp 60–61 °C (from ether/hexane); $[\alpha]_D^{24}$ −117 (*c* 1); ν (KBr) 3509 (OH), 3033 (aromatic), 2124 (N_3), 1368 (CMe_2), 744 and 693 cm^{-1} (aromatic). ^1H NMR (300 MHz): δ 7.42–7.26 (m, 5H, Ph), 4.80 and 4.63 (2d, 2H, J=11.8 Hz, CH_2Ph), 4.17 and 4.08 (2d, 2H, $J_{1,1'}$=9.5 Hz, H-1,1′), 3.96 (dd, 1H, $J_{5,6ax}$=2.2 Hz, $J_{6ax,6eq}$=12.8 Hz, H-6ax), 3.89 (t, 1H, $J_{3,4}$, $J_{4,5}$=3.5 Hz, H-4), 3.85–3.80 (m, 3H, H-3,5,6eq), 3.17 (br d, 1H, $J_{OH,3}$=8.8 Hz, OH), 1.45 and 1.36 (2s, 6H, CMe_2). ^{13}C NMR: δ 137.48, 128.63, 128.08, and 127.77 (CH_2Ph), 112.48 (CMe_2), 105.70 (C-2), 73.72 (C-1), 73.26 (C-4), 70.83 (C-3), 70.43 (CH_2Ph), 62.34 (C-6), 58.86 (C-5), 26.74 and 26.42 (CMe_2). Anal. Calcd for $C_{16}H_{21}N_3O_5$: C, 57.30; H, 6.31; N, 12.53. Found: C, 57.50; H, 6.29; N, 12.63. HRMS: *m/z* 358.1380 [M^++Na]. For $C_{16}H_{21}N_3O_5Na$ 358.1379 (deviation −0.2 ppm).

4.2.6. 5-Azido-3,4-di-*O*-benzyl-5-deoxy-1,2-*O*-isopropylidene-β-D-psicopyranose (12). To a stirred suspension of NaH (60% oil dispersion, 0.27 g, 6.76 mmol) in dry DMF (10 mL), compound **11** (1.51 g, 4.51 mmol) in the same solvent (10 mL) was added at room temperature. After 15 min, the mixture was cooled (ice-water) benzyl bromide (580 μL, 4.96 mmol) was added and the mixture was allowed to reach room temperature, then left for 2 h. TLC

(3:2 ether/hexane) then showed the presence of a faster-running compound. The mixture was poured into ice-water, and extracted with ether (4×30 mL). The combined extracts were washed with brine, water, and concentrated. Flash column chromatography (1:3 ether/hexane) of the residue gave **12** (1.37 g, 72%) as white crystals; mp 118–120° (from ether/hexane); $[\alpha]_D^{25}$ −101 (*c* 1); *ν* (KBr): 3031 (aromatic), 2103 (N_3), 1377 (CMe_2), 735 and 694 cm^{-1} (aromatic). ^1H NMR (300 MHz): *δ* 7.43–7.26 (m, 10H, 2 Ph), 4.86 and 4.76 (2d, 2H, *J*=11.7 Hz, CH_2Ph), 4.82 and 4.77 (2d, 2H, *J*=9.2 Hz, CH_2Ph), 4.14 and 3.98 (2d, 2H, $J_{1,1'}$=9.8 Hz, H-1,1′), 4.04 (t, 1H, $J_{3,4}$, $J_{4,5}$=3.0 Hz, H-4), 3.87 (dd, 1H, $J_{5,6ax}$=4.1 Hz, $J_{6ax,6eq}$=11.9 Hz, H-6ax), 3.80 (dd, 1H, $J_{5,6eq}$=6.0 Hz, H-6eq), 3.66 (d, 1H, H-3), 3.61 (m, 1H, H-5), 1.48 and 1.35 (2s, 6H, CMe_2). ^{13}C NMR: *δ* 137.99, 137.68, 128.65, 128.54, 128.50, 128.26, 127.97, and 127.85 (CH_2Ph), 111.39 (CMe_2), 105.69 (C-2), 77.22 and 77.04 (C-3,4), 74.50 (C-1), 74.49 and 73.21 (CH_2Ph), 62.01 (C-6), 56.47 (C-5), 27.20 and 26.03 (CMe_2). Anal. Calcd for $C_{23}H_{27}N_3O_5$: C, 64.93; H, 6.40; N, 9.88. Found: C, 64.49; H, 6.90; N, 10.06.

4.2.7. 5-Azido-3,4-di-*O*-benzyl-5-deoxy-D-psicopyranose (13). A solution of **12** (1.37 g, 3.22 mmol) in 60% aqueous TFA (5 mL) was kept at room temperature for 2 h. TLC (ether) then revealed two nearby and slower running compounds. The mixture was concentrated and repeatedly codistilled with water and then dissolved in dichloromethane, washed with 10% aqueous sodium carbonate and water, then concentrated. Flash column chromatography (1:1 ether/hexane) gave pure syrupy **13** (1.05 g, 85%) as ~2:1 mixture of its α and β-anomers, respectively. ^1H and ^{13}C NMR data (400 Hz), see Tables 1 and 2. HRMS: *m/z* 408.1538 [M^++Na]. For $C_{20}H_{23}N_3O_5Na$ 408.1535 (deviation −0.6 ppm).

4.2.8. Silylation of (13). To an ice-water cooled and stirred solution of **13** (4.90 g, 12.7 mmol) in dry DMF (30 mL) were added imidazole (1 g, 14.7 mmol) and *tert*-butylchlorodiphenylsilane (3.7 mL, 14.5 mmol) and the mixture was left at room temperature for 20 h. TLC (ether) then showed a complex mixture. MeOH (1 mL) was added and after 15 min the reaction mixture was diluted with water (100 mL) and extracted with ether (2×40 mL), then concentrated to a residue that was submitted to flash-chromatography (1:6, ether/hexane→ether) to afford first 5-azido-3,4-di-*O*-benzyl-1,6-di-*O*-*tert*-butyldiphenylsilyl-5-deoxy-D-psicose (**14**, 2.31 g, 26%) as a colourless syrup; $[\alpha]_D^{26}$ −1.2, $[\alpha]_{405}^{26}$ +7 (*c* 1.2); *ν* (neat) 2101 (N_3), 1735 (ketone), and 701 cm^{-1} (aromatic). ^1H NMR (300 MHz): *δ* 7.70–7.00 (4m, 30H, 6 Ph), 4.58 and 4.49 (2d, 2H, $J_{1,1'}$= 18.6 Hz, H-1,1′), 4.45 and 4.39 (2d, 2H, *J*=11.8 Hz, CH_2Ph), 4.45 and 4.33 (2d, 2H, $J_{1,1'}$=11.3 Hz, CH_2Ph), 4.20 (d, 1H, $J_{3,4}$=3 Hz, H-3), 3.92 (q, 1H, H-5), 3.82 (dd, 1H, $J_{4,5}$=7.2 Hz, H-4), 3.73 (t, 1H, $J_{5,6}$=$J_{6,6'}$=6.4 Hz, H-6), 3.71 (t, 1H, $J_{5,6'}$=6.7 Hz, H-6′), and 1.06 (s, 9H, CMe_3). ^{13}C NMR (inter alia): *δ* 206.67 (C-2), 81.77 (C-3), 79.43 (C-4), 73.27 (2 CH_2Ph), 69.11 (C-6), 64.55 (C-1), 62.94 (C-5), 26.81 (CMe_3) and 19.36 (CMe_3). HRMS: *m/z* 884.3896 [M^++Na]. For $C_{52}H_{59}N_3O_5NaSi_2$ 884.3891 (deviation −0.6 ppm).

Eluted second was 5-azido-3,4-di-*O*-benzyl-1-*O*-*tert*-butyldiphenylsilyl-5-deoxy-α-D-psicopyranose (**15**, 4.55 g, 70%) as a colourless syrup; $[\alpha]_D^{25}$ −37 (*c* 1.4); *ν* (neat) 3468 (OH), 2102 (N_3), and 701 cm^{-1} (aromatic). ^1H NMR (300 MHz): *δ* 7.75–7.25 (2m, 20H, 4 Ph), 5.40 (s, 1H, OH-2), 4.86 and 4.79 (2d, 2H, *J*=10.7 Hz, CH_2Ph), 4.69 (s, 2H, CH_2Ph), 4.28 (br t, 1H, H-4), 4.10 (t, 1H, $J_{5,6ax}$=$J_{6ax,6eq}$=11.3 Hz, H-6ax), 3.96 and 3.60 (2d, 2H, $J_{1,1'}$=10.5 Hz, H-1,1′), 3.90 (d, 1H, $J_{3,4}$=2.6 Hz, H-3), 3.78 (dd, 1H, $J_{5,6eq}$=5.0 Hz, H-6eq), 3.36 (ddd, 1H, $J_{4,5}$=2.5 Hz, H-5), and 1.08 (s, 9H, CMe_3). ^{13}C NMR (inter alia): *δ* 98.52 (C-2), 77.32 and 73.97 (C-3,4), 75.92 and 72.59 (2 CH_2Ph), 65.47 (C-1), 57.54 (C-5), 56.92 (C-6), 27.04 (CMe_3) and 19.43 (CMe_3). Starting material (0.87 g,) was finally recovered.

4.2.9. (2*R*,3*R*,4*S*,5*R*)- (17) and (2*R*,3*R*,4*S*,5*S*)-3,4-Dibenzyloxy-2′,5′-di-*O*-*tert*-butyldiphenylsilyl-2,5-bis(hydroxymethyl)pyrrolidine (18). To a solution of **14** (418 mg, 0.48 mmol) in dry THF (10 mL) was added triphenylphosphine (250 mg, 0.95 mmol) and the mixture refluxed with stirring for 5 h. TLC (1:2 ether/hexane) then revealed a slower-running compound. The reaction mixture was supported on silica gel previously treated with ether/hexane/TEA 1:3:0.1 and chromatographed (1:3:0.1 ether/hexane/TEA) to afford (3*S*,4*R*,5*R*)-3,4-dibenzyloxy-2′,5′-di-*O*-*tert*-butyldiphenylsilyl-2,5-bis(hydroxymethyl)-δ^2-pyrroline (**16**, 380 mg, 97%). $[\alpha]_D^{24}$ −39 (*c* 1.3); *ν* (neat) 3070, 3049, and 3031 (aromatic), 1659 (C=N), and 701 cm^{-1} (aromatic). ^1H NMR (300 MHz): *δ* 7.72–7.20 (3m, 30H, 6 Ph), 4.83 (br d, 1H, $J_{3,4}$=5.8 Hz, H-3), 4.72 and 4.63 (2d, 2H, *J*=11.2 Hz, CH_2Ph), 5.57 and 4.51 (2d, 2H, *J*=11.7 Hz, CH_2Ph), 4.61–4.54 (m, 2H, H-2′a,2′b), 4.25 (m, 1H, H-5), 4.17 (dd, 1H, $J_{4,5}$=1.9 Hz, H-4), 3.87 (dd, 1H, $J_{5,5'a}$=3.5 Hz, $J_{5'a,5'b}$=10.5 Hz, H-5′a), 3.81 (dd, 1H, $J_{5,5'b}$=4.1 Hz, H-5′b), 1.06 and 1.03 (2s, 18H, 2 CMe_3). ^{13}C NMR (inter alia): *δ* 176.40 (C-2), 82.29, 77.63 and 73.23 (C-3,4,5), 73.39 and 71.89 (2 CH_2Ph), 64.03 and 62.81 (C-2′,5′), 27.01 (2 CMe_3), 19.33 and 19.27 (2 CMe_3). Compound **16** decomposed on standing.

To a stirred and ice-water cooled solution of **16** (340 mg, 0.41 mmol) in THF (5 mL) containing acetic acid (50 μL), $NaCNBH_3$ (70 mg, 1.11 mmol) was added portionwise. After 15 min, the reaction mixture was allowed to reach room temperature. TLC (3:2 ether/hexane) then showed two slower-running compounds. The reaction mixture was slightly basified by addition of aqueous ammonia solution, then concentrated to a residue that was partitioned into ether/water, the organic phase was separated and concentrated. Column chromatography (1:3:0.1 ether/hexane/TEA) afforded first **17** (145 mg, 43%) as a colourless syrup. $[\alpha]_D^{29}$ +19 (*c* 1.5); *ν* (neat) 3070, 3049, and 701 cm^{-1} (aromatic). ^1H NMR (300 MHz): *δ* 7.70–7.20 (3m, 30H, 6 Ph), 4.69 and 4.61 (2d, 2H, *J*=11.9 Hz, CH_2Ph), 4.52 and 4.42 (2d, 2H, *J*=12.0 Hz, CH_2Ph), 4.11 (t, 1H, $J_{3,4}$=$J_{4,5}$= 4.4 Hz, H-4), 3.94 (dd, 1H, $J_{5,5'a}$=6.9 Hz, $J_{5'a,5'b}$=10.2 Hz, H-5′a), 3.93 (dd, 1H, $J_{2,3}$=6.4 Hz, H-3), 3.82 (dd, 1H, $J_{5,5'b}$=7.4 Hz, H-5′b), 3.70 (dd, 1H, $J_{2,2'a}$=4.6 Hz, $J_{2'a,2'b}$=10.7 Hz, H-2′a), 3.66 (dd, 1H, $J_{2,2'b}$=4.4 Hz, H-2′b), 3.45 (dt, 1H, H-5), 3.33 (dt, 1H, H-2), 1.90 (br s, 1H, NH), 1.09 and 1.06 (2s, 18H, 2 CMe_3). ^{13}C NMR (inter alia): *δ* 80.49 (C-3), 78.00 (C-4), 73.23 and 72.18 (2 CH_2Ph), 64.58 (C-2′), 63.72 (C-5′), 61.70 (C-2), 61.20 (C-5),

27.09 and 27.07 (2 C*Me*$_3$), and 19.39 (2 *C*Me$_3$). Anal. Calcd for $C_{52}H_{61}NO_4Si_2$: C, 76.15; H, 7.50; N, 1.71. Found: C, 75.87; H, 7.34; N, 2.05.

Eluted second was **18** (77 mg, 23%) as a colourless syrup. ν (neat) 3070, 3048, 3030 and 701 cm^{-1} (aromatic). ^{1}H NMR (300 MHz): δ 7.70–7.21 (2m, 30H, 6 Ph), 4.51 and 4.47 (2d, 4H, J=12.4 Hz, 2 CH$_2$Ph), 3.81 (br d, 2H, $J_{2,3}=J_{4,5}=$ 4.7 Hz, H-3,4), 3.76 (dd, 2H, $J_{2,2'a}=J_{5,5'a}=4.5$ Hz, $J_{2'a,2'b}=J_{5'a,5'b}=10.5$ Hz, H-2′a,5′a), 3.71 (dd, 2H, $J_{2,2'b}=J_{5,5'b}=4.4$ Hz, H-2′b,5′b), 3.41 (br q, 2H, H-2,5), 1.95 (br s, 1H, NH), and 1.03 (s, 18H, 2 CMe$_3$). ^{13}C NMR (inter alia): δ 78.43 (C-3,4), 71.87 (2 *C*H$_2$Ph), 64.47 (C-2′,5′), 63.07 (C-2,5), 27.05 (2 C*Me*$_3$), and 19.35 (2 *C*Me$_3$). Anal. Calcd for $C_{52}H_{61}NO_4Si_2$: C, 76.15; H, 7.50; N, 1.71. Found: C, 76.43; H, 7.46; N, 1.67.

4.2.10. Hydrogenation of 14. Compound **14** (1.76 g, 2.04 mmol) in MeOH/THF (2:1 v/v, 60 mL) was hydrogenated at 60 psi over wet Raney-Ni (3 g) for 5 h. TLC (3:1 ether/hexane) then revealed the presence of a slower-running compound. The catalyst was filtered off, washed with MeOH, and the combined filtrate and washings were concentrated to a residue that was submitted to column chromatography (1:3:0.1 ether/hexane/TEA) to afford **18** (1.06 g, 73%).

4.2.11. (2*R*,3*R*,4*S*,5*S*)-3,4-dihydroxy-2,5-bis(hydroxymethyl)pyrrolidine hydrochloride [2,5-dideoxy-2,5-imino-D-allitol (DADP, 19)]. Compound **18** (990 mg, 1.2 mmol) in THF (25 mL), was treated with a solution of tetra-*n*-butylammonium fluoride trihydrate (915 mg, 2.9 mmol) for 1 h at room temperature TLC (ether) then showed the presence of a non mobile compound. The reaction mixture was concentrated and the residue dissolved in AcOEt and washed with brine, then concentrated. Column chromatography (ether→5:1:0.1 ether/methanol/NH$_4$OH) of the residue afford fractions containing 3,4-dibenzyloxy derivative of **18** (NMR evidence), contaminated with tetra-*n*-butylammonium hydroxide, that was subsequently hydrogenated in MeOH (15 mL) and concd HCl (5 drops) over 10% Pd–C (200 mg) in an H$_2$ atmosphere for 24 h. TLC (5:1:0.1 ether/methanol/NH$_4$OH) then showed the presence of a compound of lower mobility. The catalyst was filtered off, washed with MeOH and the combined filtrate and washings concentrated to a residue that was repeatedly washed with Cl$_2$CH$_2$ to yield **19** hydrochloride (90 mg, 38%) as a colourless foam. ^{1}H NMR (300 MHz, MeOH-d_4)): δ 4.10 (m, 2H, H-3,4), 3.86 (dd, 2H, $J_{2,2'a}=J_{5,5'a}=3.9$ Hz, $J_{2'a,2'b}=J_{5'a,5'b}=12.0$ Hz H-2′a,5′a), 3.77 (dd, 2H, $J_{2,2'b}=J_{5,5'b}=5.7$ Hz, H-2′b,5′b), and 3.59 (m, 2H, H-2,5). ^{13}C NMR: δ 72.44 (C-3,4), 66.21 (C-2,5), and 59.46 (C-2′,5′).

4.2.12. (2*S*,3*S*,4*R*,5*R*)-3,4-Dibenzyloxy-2′-*O*-*tert*-butyldiphenylsilyl-2,5-bis(hydroxymethyl)pyrrolidine (21). Treatment of **15** (1.2 g, 1.93 mmol) in dry THF (30 mL) with triphenylphosphine (760 mg, 2.9 mmol) as above for 7 h, afforded after work-up and column chromatography (2:1 ether/hexane→ether) (3*S*,4*R*,5*R*)-3,4-dibenzyloxy-2′-*O*-*tert*-butyldiphenylsilyl-2,5-bis(hydroxymethyl)-δ^2-pyrroline (**20**, 850 mg, 77%) as an unstable pale yellow syrup, that was not characterized but used in the next step.

To a stirred and ice-water cooled solution of **20** (815 mg, 1.41 mmol) in THF (15 mL) containing acetic acid (180 μL), NaCNBH$_3$ (273 mg, 4.35 mmol) was added portionwise. After 1 h, the reaction mixture was allowed to reach room temperature. TLC (ether) then showed a non-mobile compound. The reaction mixture was concentrated and the residue dissolved in EtAcO and washed with brine, then concentrated. Column chromatography (10:1 ether/methanol) gave **21** (335 mg, 41%) as a colourless syrup. $[\alpha]_D^{27}+32$ (*c* 1.6). ν (neat) 3338 (OH), 3069, 3031, and 701 cm^{-1} (aromatic). ^{1}H NMR (300 MHz): δ 7.70–7.20 (2m, 20H, 4 Ph), 4.68 and 4.56 (2d, 2H, J=11.7 Hz, CH$_2$Ph), 4.60 and 4.48 (2d, 2H, J=11.9 Hz, CH$_2$Ph), 4.03 (t, 1H, $J_{2,3}=J_{3,4}=4.3$ Hz, H-3), 3.92 (dd, 1H, $J_{2,2'a}=$ 6.6 Hz, $J_{2'a,2'b}=10.2$ Hz, H-2′a), 3.86 (dd, 1H, $J_{2,2'b}=$ 7.3 Hz, H-2′b), 3.80 (dd, 1H, $J_{4,5}=6.1$ Hz, H-4), 3.51 (dd, 1H, $J_{5,5'a}=3.5$ Hz, $J_{5'a,5'b}=10.5$ Hz, H-5′a), 3.46 (dd, 1H, $J_{5,5'b}=3.5$ Hz, H-5′b), 3.38 (m, 1H, H-2), 3.32 (m, 1H, H-5), 2.28 (br s, 1H, NH), and 1.08 (s, 9H, CMe$_3$). ^{13}C NMR (inter alia): δ 80.89 (C-4), 78.47 (C-3), 73.23 and 72.58 (2 *C*H$_2$Ph), 62.96 (C-2′,5′), 61.46 (C-2), 61.09 (C-5), 27.05 (C*Me*$_3$), and 19.32 (*C*Me$_3$). Anal. Calcd for $C_{36}H_{43}NO_4Si$: C, 74.32; H, 7.45; N, 2.41. Found: C, 74.48; H, 7.43; N, 2.28.

4.2.13. Hydrogenation of 15. Compound **15** (3.3g, 5.3 mmol) in MeOH (40 mL) was hydrogenated at 60 psi over wet Raney-Ni (3 g) for 20 h. TLC (15:1 ether/methanol) then revealed the presence of a slower-running compound. The catalyst was filtered off, washed with MeOH, and the combined filtrate and washings were concentrated to a residue that was submitted to column chromatography (ether→15:1 ether/methanol) to afford crystalline (2*R*,3*S*,4*R*,5*R*)-3,4-dibenzyloxy-2′-*O*-*tert*-butyldiphenylsilyl-2,5-bis(hydroxymethyl)pyrrolidine (**22**, 1.9 g, 62%); mp 92–94° (from ether); $[\alpha]_D^{27}+16$ (*c* 1); ν (KBr) 3270 (OH, NH), 3087, 3030, and 700 cm^{-1} (aromatic). ^{1}H NMR (400 MHz): δ 7.68–7.62 and 7.44–7.25 (2m, 20H, 4 Ph), 4.56 and 4.49 (2d, 2H, J=11.8 Hz, CH$_2$Ph), 4.52 (s, 2H, CH$_2$Ph), 3.86 (t, 1H, $J_{3,4}$, $J_{4,5}=4.5$ Hz, H-4), 3.78 (br t, 1H, $J_{2,3}=5.2$ Hz, H-3), 3.52 (br d, 2H, H-2′,2′), 3.49–3.39 (m, 4H, H-2,5,5′a,5′b), 2.05 (br s, 2H, NH,OH), and 1.05 (s, 9H, CMe$_3$). ^{13}C NMR (inter alia): δ 78.71 (C-3), 78.59 (C-4), 72.28 and 71.83 (2 *C*H$_2$Ph), 65.46 (C-2′), 62.72 and 61.50 (C-2,5), 62.05 (C-5′), 27.00 (C*Me*$_3$), and 19.34 (*C*Me$_3$). Mass spectrum (LSIMS): *m*/*z* 582.3038 [M$^+$+H] for $C_{36}H_{44}NO_4Si$ 582.3040 (deviation +0.2 ppm). Anal. Calcd for $C_{36}H_{43}NO_4Si$: C, 74.32; H, 7.45; N, 2.41. Found: C, 74.35; H, 7.29; N, 2.22.

4.2.14. (2*R*,3*R*,4*S*,5*R*)-3,4-dibenzyloxy-2,5-bis(hydroxymethyl)pyrrolidine (23). Compound **22** (110 mg, 0.2 mmol) in THF (10 mL), was treated with a solution of tetra-*n*-butylammonium fluoride trihydrate (120 mg, 0.4 mmol) for 2 h at room temperature. TLC (5:1:0.1 ether/methanol/TEA) then showed the presence of a more polar compound. The reaction mixture was concentrated and the residue submitted to column chromatography (5:1:0.1 ether/methanol/TEA) to yield **23** (50 mg, 73%) as a colourless syrup; $[\alpha]_D^{26}-3$ (*c* 1). ^{1}H NMR (400 MHz): δ 7.24–7.15 (m, 10H, 2 Ph), 4.50–4.40 (m, 4H, 2 CH$_2$Ph), 3.92 (br s, 2H, OH,NH), 3.70 (br d, 1H, $J_{2,7}=2.7$ Hz, H-3 or H-4), 3.62–3.55 and 3.43–3.30 (2m, 6H, H-4 or H-3, H-2′a,2′b,5′a,5′b, H-5 or

H-2), and 3.05 (m, 1H, H-5 or H-2). ^{13}C NMR (inter alia): δ 78.52 and 77.74 (C-3,4), 72.11 and 71.97 (2 *C*H$_2$Ph), 70.16 and 62.38 (C-2,5), 62.51 and 62.42 (C-2′,5′). Mass spectrum (LSIMS): *m/z* 366.1680 [M$^+$+Na] for $C_{20}H_{25}NO_4Na$ 366.1681 (deviation+0.2 ppm).

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Synthesis of ferrocenylcarbodiimide as a convenient electrochemically active labeling reagent for nucleic acids

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Abstract—Ferrocenylcarbodiimides carrying different redox potentials, **1** and **2**, were designed and synthesized as convenient electrochemically active labeling reagents for nucleic acids, which may be used as dually labeling reagents of nucleic acids like Cy3 and Cy5 dyes. These reagents could react with the imino unit of thymine or guanine base on DNA or of uracil base on RNA under a basic buffer condition to yield a labeled product quantitatively in a short period of time. The current responses of the labeled DNAs in square wave voltammetric (SWV) measurement showed a good linear correlation with the amount of the hybridized ones. DNAs labeled with the two different reagents, **1** and **2**, could be detected electrochemically at different potentials after hybridization with a DNA probe-immobilized gold electrode.
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1. Introduction

Recently, conventional DNA detecting systems are required in the gene diagnosis and many DNA sensing methods based on electrochemical techniques have been reported,[1–19] as they are expected to realize a convenient gene testing system, which enables direct electronic readout and miniaturization with good cost performance and high sensitivity. Furthermore, they are expected as one of the solutions for the carrier-type gene testing chip and can be applied to various purposes of gene testing such as the point-care test.[20] A variety of electrochemical DNA detecting techniques have been reported by using electrochemically active DNA ligands such as intercalating molecules,[4,7–8,21] or DNA labeling with electrochemically active reagents.[2,5,14–15,19,24–28] Direct electrochemical DNA detection has been achieved under limited conditions using special electrodes and electrolytes.[1,22–23] Ferrocene is often used as an electrochemically active reagent,[2,5,7–9,11,14–15,19,21,24–29] as its reversible redox potential appears where dissolved and atmospheric oxygen does not interfere with the measurement. Oligonucleotides labeled with ferrocene have been used as a DNA probe in electrochemical DNA sensing. Introduction of a ferrocence moiety to oligonucleotides was achieved by a ferrocenyl amide reagent for automated DNA synthesis,[5,19,25–27,30–34] ferrocenyl nucleotide triphosphate as substrate of DNA polymerase,[9,35–38] and the reaction of an activated ester of ferrocene with the amino linked oligonucleotides.[2,11,14,39–41] Direct modification of DNA with ferrocene was also reported by using the Sonogashira reaction of ferrocenyl-propargylamide with halogenated nucleic base of DNA.[42,43] However, all of these methods described here, suffer time-consuming steps and therefore, simpler and more effecient ferrocenylation methods for DNA need to be devised.

To achieve a simple labeling method for nucleic acids, ferrocenylcarbodiimide derivatives, **1** and **2**, carrying different redox potentials were designed and synthesized. Water-soluble carbodiimide derivatives are known to react with the imino moiety of thymine and guanine bases on DNA or of uracil base on RNA reversibly under basic conditions[44–47] with excellent yield and therefore, ferrocenylcarbodiimide derivatives should react with DNA and RNA in the same manner, thereby rapidly labeling natural single stranded DNA or RNA fragments with ferrocene as depicted in Scheme 1. Since the redox potential of ferrocene can be altered readily by changing the nature of its substituent, ferrocenylcarbodiimide derivatives having different redox potentials may be prepared by designing a linker connecting ferrocene with carbodiimide parts. Once prepared, such compounds will serve as an important tool to enable competitive analysis of two different samples labeled differentially with the ferrocenyl groups with a different redox potential.

Keywords: Ferrocenylcarbodiimide; Electrochemical detection; DNA; RNA; Dual labeling; Competitive hybridization.
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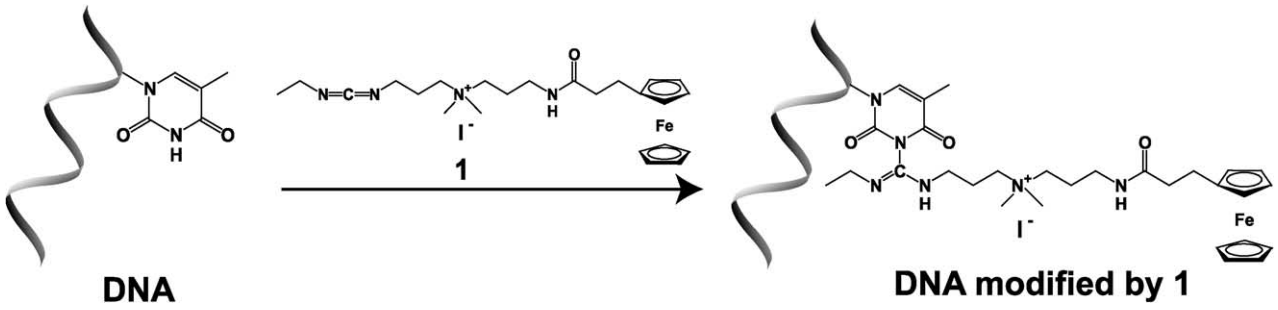


Scheme 1. Example of a ferrocene-modification reaction for thymine base of nucleic acid with **1**.

The principle of this method is illustrated in Figure 1, which is similar to the expression analysis based on a DNA microarray coupled with a dual labeling of standard and sample DNAs with Cy3 and Cy5 dyes, respectively. We assessed the feasibility of electrochemical gene expression analysis by using ferrocenyl oligonucleotides, which are labeled by the activated ester of ferrocenecarboxylic acid and ferrocenepropionic acid.[48] Other electrochemical gene expression analyses were reported by using 7-deaza guanine and adenine bases incorporated by PCR[49] and nucleoside triphosphate derivatives carrying ferrocene and anthraquinone units.[50] These reports underscored the potential importance of the electrochemical gene expression analysis and usefulness of ferrocenylation reagents having different redox potentials.

In this paper, the synthetic methods of **1** and **2** were established by surveying an effective condition for the reaction with single stranded DNA or RNA. The stability of the ferrocenyl oligonucleotides thus obtained was also evaluated by the melting curve analysis. Finally, we succeeded in the electrochemical detection of target DNA by using **1** and **2**, to suggest that the electrochemical gene expression analysis based on DNA or RNA labeled with **1** or **2** is promising for practical use.

2. Results and discussion

2.1. Synthesis of ferrocenylcarbodiimides and their stability in aqueous solution

Ferrocenylcarbodiimides **1** and **2** were synthesized as shown in Scheme 2. The carbodiimide function was generated by the method described previously.[51] Integrity of **1** and **2** was assessed by ^{1}H NMR and FT IR measurements, in which characteristic IR absorption at 2129 cm^{-1} shown in Figure 2 due to the carbodiimide group was strong evidence for the structure. Cyclic voltammograms determined in 20 mM NaH_2PO_4/Na_2HPO_4 buffer (pH 7.0) containing 100 mM $NaClO_4$ revealed a one-electron redox reaction with $E_{1/2}=$ 207 mV and ΔE peak $=63$ mV for **1** and $E_{1/2}=443$ mV and $\Delta E=71$ mV for **2** (Fig. 3). These half-wave currents were shifted toward the positive potential side from those of ferrocenylpropionic acid ($E_{1/2}=171$ mV) and ferrocenecarboxylic acid ($E_{1/2}=328$ mV), due presumably to a difference in their cationic and anionic characters. The stability of carbodiimides **1** and **2** was tested in several kinds of buffer by monitoring a change in the intensity of the absorption at 2130 cm^{-1} in FT IR. The carbodiimide function was destroyed within 4 or 12 h in acetic acid buffer (pH 5.6) or phosphate buffer (pH 7.0), respectively (data not

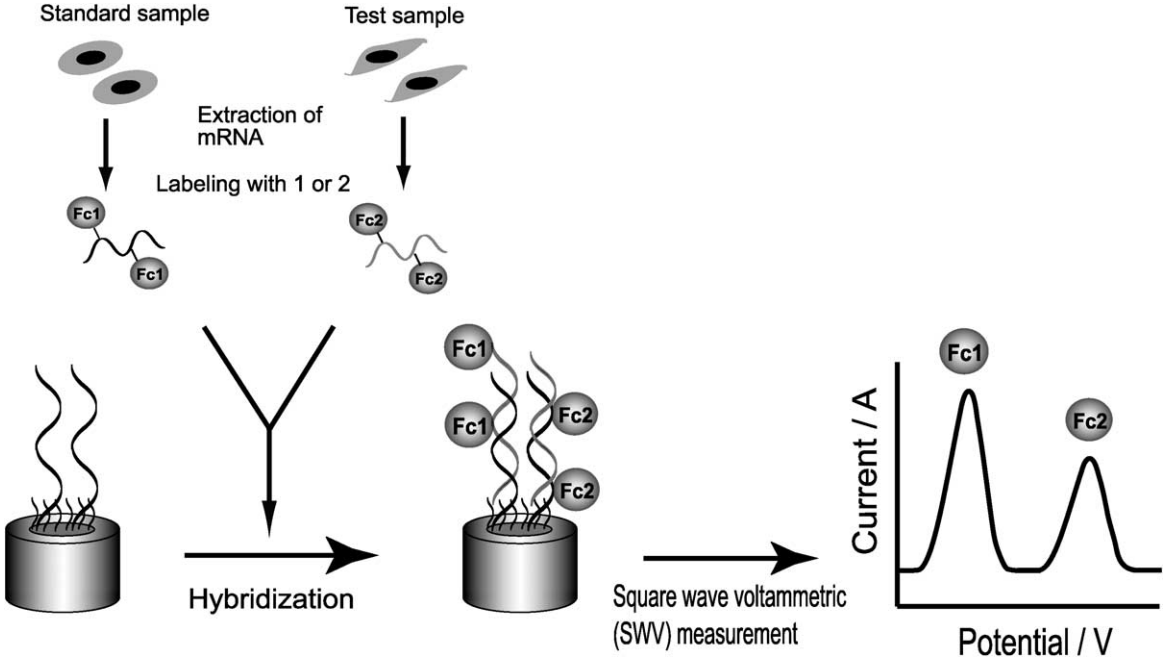
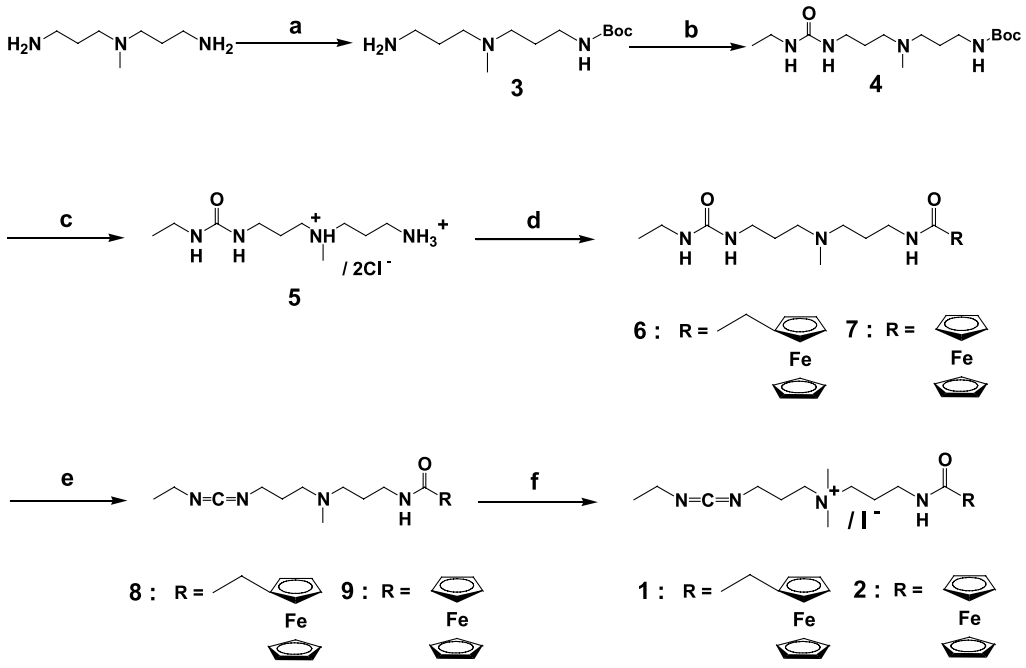


Figure 1. Principle of the electrochemical differential hybridization assay.



Scheme 2. Synthetic route to **1** and **2**. The reaction conditions are as follows: (a) *S-tert*-butoxycarbonyl-4,6-dimethyl-2-mercaptopyrimidine/1,4-dioxane; (b) ethylisocyanate/dry ether; (c) HCl/1,4-dioxane; (d) ferrocenepropionic acid or ferrocenecarboxylic acid/PyBOP/HOBt/TEA/$CHCl_3$; (e) TsCl/TEA/dryCH_2Cl_2, reflux; (f) CH_3I/dry ether.

shown). However, it was stable over 1 day in borate buffer (pH 8.5, 9.0 and 9.5). These data are in good agreement with the results on carbodiimide derivatives described previously.[47]

2.2. Reactivity for DNA

The reactivity of **1** and **2** was studied with several kinds of DNAs, namely **D1** and **D2** carrying one thymine or guanine base at the 5′-terminus of a decamer, respectively, **D3** carrying one thymine base in the middle of a decamer, and **D4** carrying a thymine base at the terminus of a 20-mer (Table 1). The reactivity was assessed from the peak intensities of the modified and unmodified DNA in HPLC. Typical HPLC traces before and after reaction of **1** with **D1** at pH 8.5 for 12 h are shown in Figure 4. The peak at 23 min of retention time was a 1:1 adduct of **1** with **D1** as deduced by MALDI TOF MS measurement shown in Figure 5. Products of **D2**–**D4** with **1** or **2** could also be assigned as 1:1 adduct analogously (data not shown). No HPLC change was observed upon reaction of **1** or **2** with **D9** as negative control (Table 1). These results demonstrated that **1** and **2** could react with thymine and guanine bases on DNA.

In the next step, the reactivity of **1** and **2** with DNA was studied at different pH. The time course of the reaction of **1** with **D1** or **D9** at pH 8.5, 9.0 or 9.5 is shown in Figure 6A. Quantitative reaction occurred with **D1** carrying a thymine moiety and **1** within 10 h, whereas no reaction was observed with **D9**. Since the imino moiety of thymine or guanine base

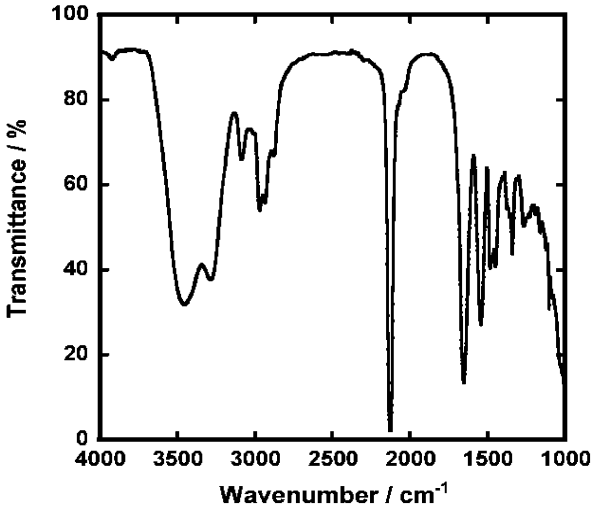


Figure 2. FT IR spectrum of **1**.

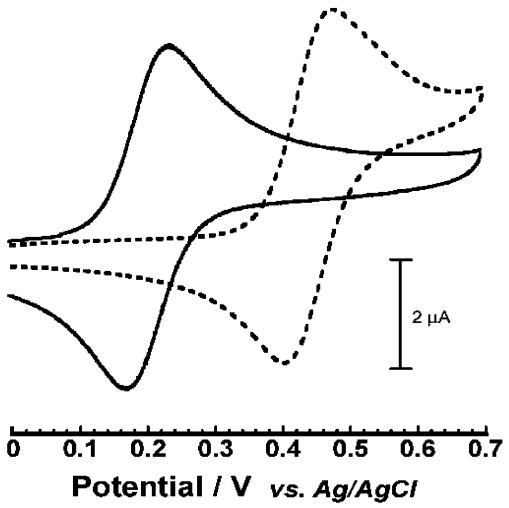


Figure 3. Cyclic voltammogram of 0.1 mM **1** (solid line) or **2** (dotted line) in 20 mM NaH_2PO_4/Na_2HPO_4 buffer (pH 7.0) containing 100 mM $NaClO_4$. The scan rate was 100 mV/s.

Table 1. Synthetic DNAs used in this study

Abbreviation	Sequence
D1	5′-TAA AAA AAA A-3′
D2	5′-GAA AAA AAA A-3′
D3	5′-AAA ATA AAA A-3′
D4	5′-TAA AAA AAA AAA AAA AAA AA-3′
D5	5′-AAA ATA AAA AAA AAA AAA AA-3′
D6	5′-AAA AAA AAA TAA AAA AAA AA-3′
D7	5′-AAA ATA AAA TAA AAA AAA AA-3′
D8	5′-AGG GGT AAG GTT CAT TAG TTG GAA-3′
HS-D8(−)	5′ HS-$(CH_2)_6$-TTC CAA CTA ATG AAC CTT ACC CCT-3′
rUA$_9$	5′-UAA AAA AAA A-3′
D9	5′-AAA AAA AAA A-3′
D10	5′-TTC CAA CTA ATG AAC CTT ACC CCT-3′
D11	5′-TTT TTT TTT TAT TTT TTT TT-3′
D12	5′-TTT TTT TTT TGT TTT TTT TT-3′
D13	5′-TTT TTT TTT TCT TTT TTT TT-3′
D14	5′-TTT TTT TTT TTT TTT TTT TT-3′

of DNA reacts with carbodiimide, the reactivity depends on pH.[47] The pK_a of the imino moiety of thymine or guanine base is 9.9–10.5 or 9.4–10.0, respectively,[52] and therefore, the reactivity of the imino moieties increased with pH and the labeling reaction progressed quantitatively in a short period of time at higher pH. For example, **1** reacted with **D1** quantitatively within 4 h at pH 9.5. On the other hand, **D2** carrying guanine base reacted with **1** faster than that of thymine base (Fig. 6B). However, prolongation of the reaction time resulted in a decrease in the yield. Since the peak of the starting **D1** increased at the expense of the product, the labeled product of **1** at the guanine base should have hydrolyzed under alkaline conditions. Similar behavior was reported in the reaction of carbodiimide with nucleic bases.[47]

In the third step, the reactivity of **1** was tested at 37 or 50 °C. The reaction at 50 °C progressed quantitatively in a shorter period than that at 37 °C as shown in Figure 7. However, extension of the reaction time brought about a decrease in yield, because of hydrolysis of the labeled product at the higher temperature. The same behavior was also observed for **2**. The reactivity of **1** was not influenced by the position of thymine base in the sequence or the length of DNA (compare **D1** with **D3** or **D4** in Fig. 9).

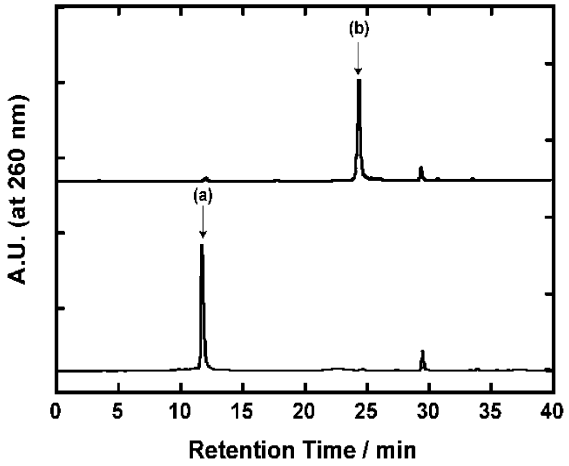


Figure 4. Reversed phase HPLC before (a) and after (b) reaction of 0.5 mM **D1** with 50 mM **1** in 20 mM borate buffer (pH 8.5) containing 30% DMSO at 37 °C for 12 h.

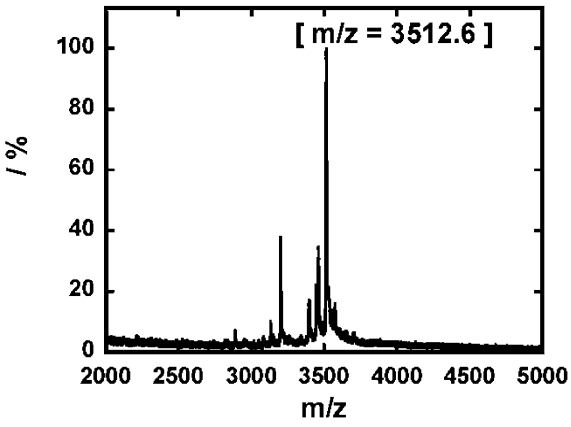


Figure 5. MALDI TOF MS of the HPLC fraction at 23 min in Figure 4. Matrix, 3-HPA; mode, negative. *m/z* [M−H]=3512.6 (theory for $C_{124}H_{152}N_{54}O_{54}P_{10}Fe$, 3512.1).

2.3. Reactivity for RNA

The reactivity of **1** with rUA$_9$ as RNA was also studied. The product was identified by MALDI TOF MS and the

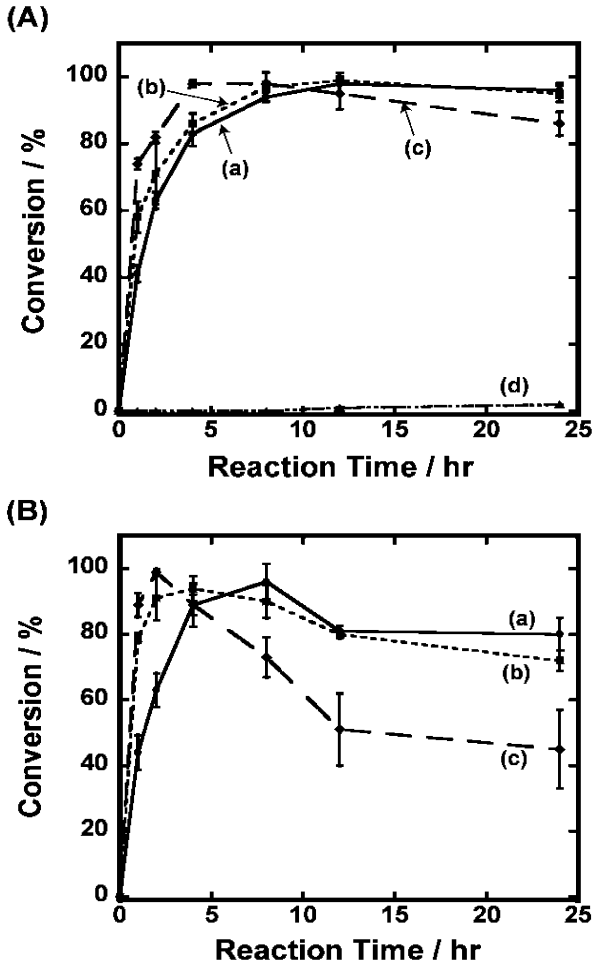


Figure 6. (A) pH dependence of reactivity of 0.5 mM **D1** with 50 mM **1** in 20 mM borate buffer containing 30% DMSO at pH 8.5 (a), pH 9.0 (b) or pH 9.5 (c). The reactivity of 0.5 mM **D9** with 50 mM **1** in 20 mM borate buffer at pH 9.0 is also shown by trace (d). (B) pH dependence of the reactivity of 0.5 mM **D2** with 50 mM **1** in 20 mM borate buffer containing 30% DMSO at pH 8.5 (a) pH 9.0 (b) or pH 9.5 (c). All experiments were conducted at 37 °C.

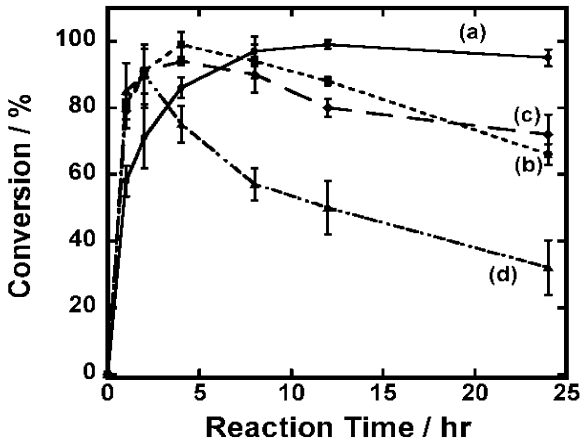


Figure 7. Temperature dependence of the reactivity of 0.5 mM **D1** with 50 mM **1** in 20 mM borate buffer (pH 9.0) containing 30% DMSO at 37 °C (a) or 50 °C (b). Temperature dependence for **D2** is shown for reaction at 37 °C (c) or 50 °C (d).

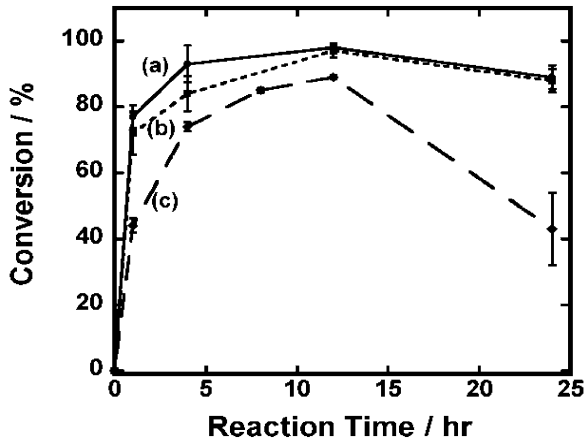


Figure 9. Sequence dependence of the reactivity of 0.5 mM **D3** (a) or **D4** (b) with 50 mM **1** in 20 mM borate buffer (pH 9.5) containing 30% DMSO at 37 °C. The reactivity of 0.1 mM rUA_9 with 10 mM **1** is also shown by trace (c) in the same buffer at pH 8.5.

reactivity was evaluated from the peak intensity of HPLC. A new peak was observed at 23 min with the progress of reaction in addition to the peak of rUA_9 at 9 min. The former was collected and subjected to MALDI TOF MS analysis to reveal a 1:1 adduct of rUA_9 with **1** as shown in Figure 8. Ferrocene-labeling reagent **1** gave the labeled product for rUA_9 in 80% yield in 10 h at pH 8.5 and 37 °C and prolongation of the reaction time resulted in a poorer yield (Fig. 9). Precipitation was observed during the reaction of **1** and rUA_9 at pH 9.5.

2.4. Stability of double stranded DNA labeled with 1

Since the imino moiety of thymine or guanine is involved in the hydrogen bonding of a DNA duplex, its modification with carbodiimide could destabilize the duplex structure of DNA or RNA. Bucci et al. reported the stability of a 17-meric DNA duplex having thymine modified with a ferrocenylmethyl group at the imino moiety.[30] Terminal modification with a ferrocenylmethyl moiety exerted only a small effect on the stability of the duplex, whereas modification in the middle of the sequence brought about larger destabilization by −14 °C as assessed by T_m measurement.

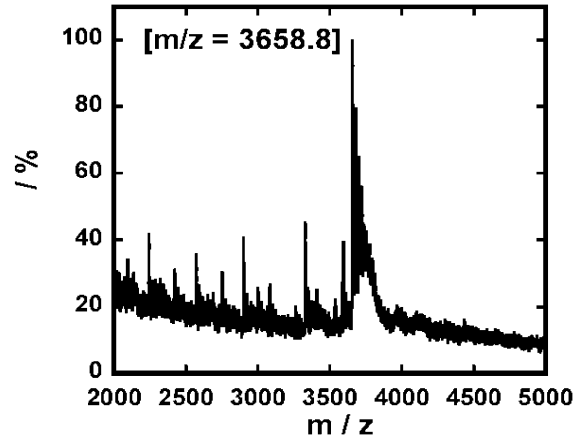


Figure 8. MALDI TOF MS spectrum of rUA_9 after reaction with **1**. Matrix, 3-HPA; mode, negative. *m/z* [M−H]=3658.8 (theory for $C_{123}H_{139}N_{54}O_{64}P_{10}Fe$, 3660.5).

To evaluate the effect of the modification with **1** on the stability of a DNA duplex, **D4**–**D8** modified with **1** and their adducts were purified by HPLC. After hybridization of these modified **D4**–**D8** with their complementary DNAs, T_m was measured in 20 mM KH_2PO_4/K_2HPO_4 buffer (pH 7.0) containing 100 mM KCl (Fig. 10). The T_m values calculated from the melting curves are summarized in Table 2. Terminal modification of **D4** with **1** did not destabilize its DNA duplex appreciably (Fig. 10A, entry 1 in Table 2), whereas middle modification in **D5** and **D6** caused considerable destabilization of their DNA duplexes (entries 2 and 3). Modification of **D7** with **1** at two sites destabilized its DNA duplex further (entry 4). Modification with **1** in the middle destabilized the DNA by −10 °C in T_m values (entries 2 and 3), which is in agreement with the previous paper.[30] Nevertheless, all of the modified DNAs could still form a DNA duplex at low temperature and therefore hybridization was monitored there.

Compound **D8** carrying many thymine and guanine bases in its sequence was also modified with **1** possibly in more than one position. In fact, many peaks including three main peaks were observed in reversed phase HPLC upon reaction of **D8** with **1**. These three peaks were collected separately and analyzed by MALDI TOF MS. It turned out that they were **D8** modified by one, two or three molecules of **1**. The melting curves of the DNA duplex of **D8** labeled with one to three molecules of **1** with its complementary DNA are shown in Figure 10B and entries 5–7 in Table 2. The T_m curve shown in Figure 10B was broader than that in Figure 10A. This is reasonable given the fact that the fraction of **D8** modified by one molecule of **1** could still be a mixture carrying one molecule of **1** in a different position. As the number of modified **1** increased, the T_m values were lowered. Nonetheless, all of these DNA duplexes were stable at 10 °C, as proven by the circular dichroism (CD) spectra of **D8** unlabeled or labeled triply with **1** before and after hybridization with its complementary DNA (Fig. 11).

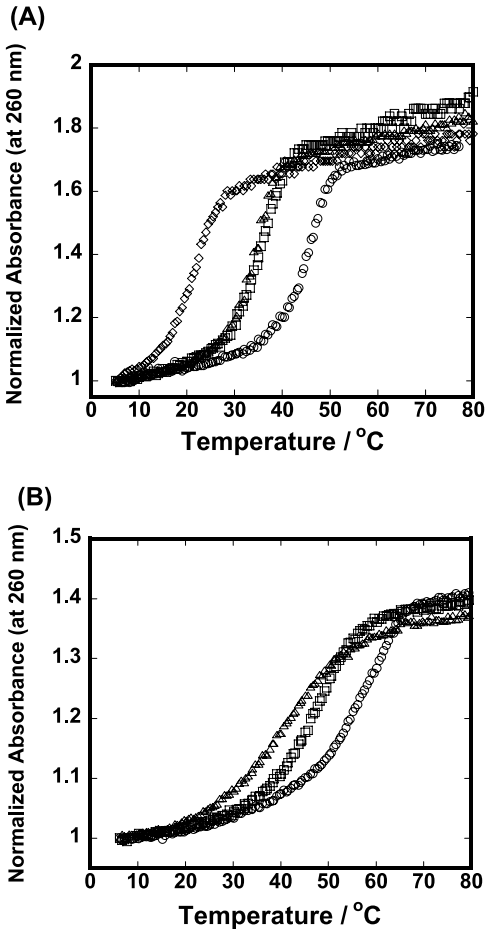


Figure 10. (A) Melting curves of the DNA duplex of 5 μM **D4** (○), **D5** (□), **D6** (△), or **D7** (◇) modified with **1** with respective complementary DNA in 20 mM KH_2PO_4/K_2HPO_4 buffer (pH 7.0) containing 0.1 M KCl. (B) Melting curves of DNA duplex of 5 μM **D8** modified with one (○), two (□), or three (△) molecules of **1** with 5 μM its complementary DNA in 20 mM KH_2PO_4/K_2HPO_4 buffer (pH 7.0) containing 0.1 M KCl.

The effect of introduction of **1** to the thymine base on the stability of the DNA duplex was studied (Table 3). Before the modification, occurrence of a mismatch in the middle of the sequence lowered T_m by ca. 4.0 °C (entries 1–4). Upon modification of thymine with **1**, the T_m was lowered by ca. 10 °C (entry 5), and the magnitude of this T_m lowering was barely dependent on the type of mismatch (entries 6–8), demonstrating that modification in the middle of the sequence with bulky **1** impairs duplex formation to a larger extent than ordinary mismatches. Nonetheless, the fact that the duplex stability is nearly independent of the type of mismatch is advantageous, as hybridization can be carried out under uniform conditions, in this case at low temperature.

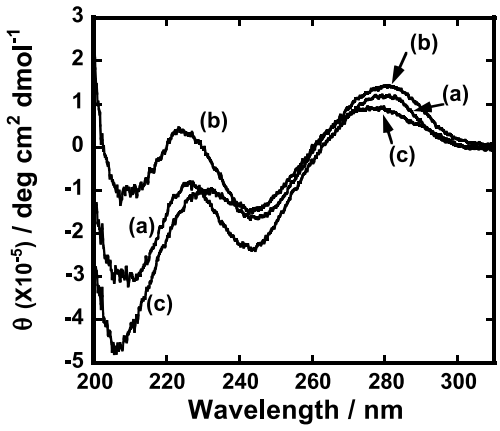


Figure 11. CD spectra of 3 μM **D8** unmodified (a) or modified (b) with three molecules of **1** after hybridization with its complementary DNA in 20 mM KH_2PO_4/K_2HPO_4 buffer (pH 7.0) containing 0.1 M KCl at 10 °C. The CD spectrum of 3 μM **D8** before hybridization is also shown (c).

2.5. Electrochemical detection of D8 labeled with 1 or 2

Electrochemical DNA detection was carried out by using a **HS-D8**(−)-immobilized electrode. Target DNA of **D8** was allowed to react with **1** or **2** and **D8** modified with three molecules of **1** or **2** was used for hybridization reaction (Fig. 12). The SWV method was used in this experiment because of its low background current.[53] The current peak based on the ferrocene moieties of **D8** modified with **1** was observed at 0.20 V and its intensity increased with an increase in the amount of **D8** modified with **1**, whereas no peak current was observed for **D10** modified with three molecules of **1** as negative control (Fig. 12A). This result indicated that **D8** modified with **1** hybridized indeed with the complementary **HS-D8**(−) on the electrode.

Analogously, the current peak was observed at 0.43 V for **D8** modified with **2** and the peak intensity was proportional to its concentration (Fig. 12B). Since the peak currents started to level off above 1 μM **D8** (Figs. 12A and B), the DNA on the electrode seemed to be covered by around 1 μM **D8**. It was estimated from the peak currents that the electrode is covered with 4.9×10^{11}–9.9×10^{12} molecules of 24-meric DNA/cm^2 at saturation. This value is in good agreement with the data previously described by Tarlov and Georgiadis groups.[54–61] In conclusion, the DNA hybridization was quantitative at **D8** concentrations lower than 1.0 μM.

Table 2. Melting temperature of several combinations of DNA duplexes between **1**-modified DNAs and their complementary DNAs

Entry	Sequence[a]	T_m (°C)	ΔT_m (°C)[b]
1	5′-T^{Fc}AA AAA AAA AAA AAA AAA AA-3′ 3′-A TT TTT TTT TTT TTT TTT TT-5′	44.2	−0.9
2	5′-AAA AT^{Fc}A AAA AAA AAA AAA AA-3′ 3′-TTT TA T TTT TTT TTT TTT TT-5′	34.2	−10.5
3	5′-AAA AAA AAA T^{Fc}AA AAA AAA AA-3′ 3′-TTT TTT TTT A TT TTT TTT TT-5′	34.1	−10.3
4	5′-AAA AT^{Fc}A AAA T^{Fc}AA AAA AAA AA-3′ 3′-TTT TA T TTT A TT TTT TTT TT-5′	22.1	−22.2
5	5′-AGG GGT AAG GTT CAT TAG TTG GAA-3′(1Fc) 3′-TCC CCA TTC CAA GTA ATC AAC CTT-5′	55.3	−4.5
6	5′-AGG GGT AAG GTT CAT TAG TTG GAA-3′(2Fc) 3′-TCC CCA TTC CAA GTA ATC AAC CTT-5′	47.8	−12.0
7	5′-AGG GGT AAG GTT CAT TAG TTG GAA-3′(3Fc) 3′-TCC CCA TTC CAA GTA ATC AAC CTT-5′	39.1	−20.7

[a] Fc represents ferrocene of **1** modifying the site(s) marked in the sequence.
[b] $\Delta T_m = T_m$(modified with **1**) − T_m(unmodified).

Table 3. Effect of the type of mismatch on the DNA duplex stability

Entry	Sequence	X	T_m (°C)	ΔT_m (°C)[a]
1	5′-AAA AAA AAA TAA AAA AAA AA-3′ 3′-TTT TTT TTT XTT TTT TTT TT-5′	A	44.4	—
2		G	42.0	−2.4
3		C	40.0	−4.4
4		T	39.8	−4.6
5	5′-AAA AAA AAA T^{Fc}AA AAA AAA AA-3′ 3′-TTT TTT TTT X TT TTT TTT TT-5′	A	34.1	−10.3
6		G	35.2	−9.2
7		C	34.9	−9.5
8		T	36.3	−8.1

[a] $\Delta T_m = T_m$(T/X or T^{Fc}/X) − T_m(T/A).

2.6. Electrochemical gene expression analysis

As a model gene expression experiment was successful with **D8** modified with **1** or **2**, competitive hybridization was attempted with a **HS-D8**(−)-immobilized electrode. **D8** modified with **1** or **2** was mixed at various ratios and allowed to hybridize with the **HS-D8**(−)-immobilized electrode. As shown in Figure 13A, two current peaks were obtained at 0.20 and 0.43 V in SWV measured in 20 mM NaH_2PO_4/Na_2HPO_4 buffer (pH 7.0) containing 100 mM $NaClO_4$. The intensity of these peaks was proportional to the amount of **D8** modified with **1** or **2** and the total peak current was ca. 40 nA, which correspond to ca. 4.0×10^{12} molecules/cm^2. This result shows that **D8** modified with **1** or **2** can hybridize on the electrode competitively.

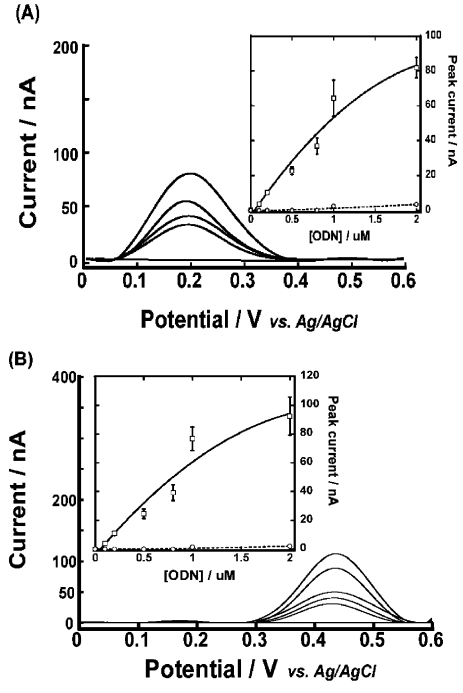


Figure 12. (A) Square wave voltammogram of **HS-D8**(−) immobilized on the electrode after hybridization with different concentrations of **D8** modified with three molecules of **1** (□) or **D10** (○) in 20 mM NaH_2PO_4/Na_2HPO_4 buffer (pH 7.0) containing 100 mM $NaClO_4$ at 10 °C. ΔE_p = 50 mV, ΔE_s = 10 mV, f = 10 Hz. A standard line for the current peak at 0.20 V was plotted against the concentration of **D8**. (B) Square wave voltammogram of **HS-D8**(−) immobilized on the electrode after hybridization with different concentrations of **D8** modified with three molecules of **2** (□) or **D10** (○) in 20 mM NaH_2PO_4/Na_2HPO_4 buffer (pH 7.0) containing 100 mM $NaClO_4$ at 10 °C. ΔE_p = 50 mV, ΔE_s = 10 mV, f = 10 Hz. A standard line for the current peak at 0.43 V was plotted against the concentration of **D8**.

Figure 13B shows a plot of the observed ratio of peak current of **D8** modified with **1** or **2** against the mixed ratio of **D8** modified with **1** or **2**. A good correlation obtained suggested the feasibility of electrochemical expression analysis by using **D8** modified with **1** and **2** coupled with a **HS-D8**(−)-immobilized electrode.

3. Conclusion

Ferrocenylcarbodiimide derivatives **1** and **2** having a different redox potential were designed and synthesized. They could react with DNA or RNA quantitatively under basic conditions. Although the labeling of DNA and RNA with **1** and **2** destabilized their DNA duplex, a DNA duplex could still form at low temperature. The ferrocene-labeled

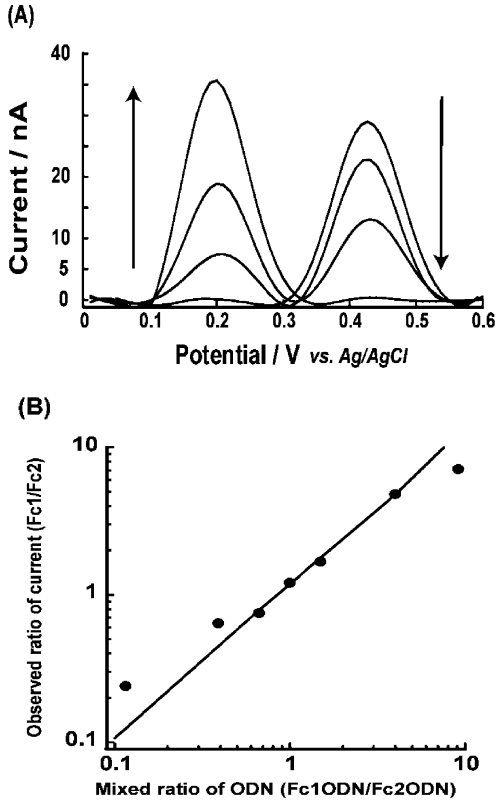


Figure 13. (A) Square wave voltammogram of a **HS-D8**(−)-immobilized electrode after hybridization with a mixture of **D8** modified with **1** or **2** (1.5:0.5, 1.2:0.8, 0.8:1.2, 0.5:1.5 μM/μM) in 20 mM NaH_2PO_4/Na_2HPO_4 buffer (pH 7.0) containing 100 mM $NaClO_4$ at 10 °C. ΔE_p = 50 mV, ΔE_s = 10 mV, f = 10 Hz. (B) Plot of the observed ratio of the peak current of **D8** modified with **1** to that with **2** against the mixed ratio of **D8** modified with **1** to that with **2**.

DNA could hybridize with its complementary DNA probe-immobilized electrode and the peak current was proportional to the amount of the target DNA. When DNA was labeled differentially with **1** or **2**, the resulting DNA gave rise to signals competitively on the electrode, making electrochemical gene expression analysis promising with the detection limit being ca. 0.05 μM DNA sample in 1 μl (ca. 50 fmol).

4. Experimental

4.1. Chemicals

N,*N*-bis(3-aminopropyl)methylamine, *S*-*tert*-butoxycarbonyl-4,6-dimethyl-2-mercaptopyrimidine, ethylisocyanate, ferrocenylcarboxylic acid, benzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate (PyBOP), 1-hydroxybenzotriazole (HOBt), *p*-toluenesulfonyl chloride (TsCl), and iodomethane were purchased from Tokyo Kasei Co., (Tokyo, Japan). HCl/1,4-dioxane was purchased from Watanabe Chemical Inc. (Hiroshima, Japan). Solvents used in this paper, were purchased from Wako Chemicals Inc. (Osaka, Japan). Ferrocenepropionic acid was synthesized according to the method reported previously.[35] Synthetic DNAs, rUA_9 of RNA, and thiolated DNA were custom-synthesized by Genenet Co., (Fukuoka, Japan). The sequences of these DNAs are listed in Table 1. **D8**(−) carried a sequence complementary to that of mRNA coding for the cytochrome c gene of Xenopus. MilliQ water was used throughout (Millipore, Billerica, MA). Buffers were prepared from the following chemicals: boric acid, disodium hydrogenphosphate dodecahydrate, sodium dihydrogenphosphate dihydrate, dipotassium hydrogenphosphate, potassium dihydrogenphosphate, sodium hydroxide, triethylamine, and hydrochloric acid were purchased from Wako. Tris(hydroxymethyl)aminomethane was purchased from Nacalai Tesque (Kyoto, Japan). Buffers 20×SSC (0.3 M sodium citrate containing 3 M NaCl) and 2×SSC for hybridization were purchased from Wako. Buffer for reaction of **1** and **2** with DNA and RNA was prepared as 50 mM NaH_2BO_3/NaOH buffers (pH 8.5, 9.0 or 9.5) and used after dilution. Two hundred mM KH_2PO_4/K_2HPO_4 buffer (pH 7.0) containing 1 M $NaClO_4$ or 1 M KCl was prepared and used after dilution in electrochemical or spectrophotometric measurements, respectively, and 100 mM triethylammonium acetate (TEAA) buffer (pH 7.0) was used as an eluent in high performance liquid chromatography (HPLC).

4.2. Instruments

4.2.1. Identification of product. Compounds **1** and **2** were characterized mainly by ^{1}H NMR (250 MHz spectrometer, Bruker, Rheinstetten, Germany) and Fourier transform infrared (FT IR, Spectrum One FT IR, Perkin Elmer Co., Wellesley, MA). Tetramethylsilane (TMS) was used as a standard in ^{1}H NMR measurement. IR was measured with 4 cm^{-1} resolution after sandwiching the sample between CaF_2 single crystal plates.

4.2.2. HPLC analysis. The HPLC system used in this experiment, was composed of the following components: Hitachi C-7300 column oven, L-7450H diode array detector, L-7100 pump and D-7000 interface chromatograph. Reversed phase HPLC was run using a Lichrospher RP-18 (Cica-Merck, Kanto Chemicals Co., Tokyo, Japan) column with the gradient condition where the acetonitrile content in 100 mM TEAA buffer (pH 7.0) was linearly changed from 0 to 40% over 30 min at a flow rate of 1.0 ml/min with detection at 260 nm. The reactivity of **1** and **2** with single stranded DNAs or RNA was assessed from the ratio of peak heights for unmodified and modified DNAs or RNA.

4.2.3. MALDI TOF MS analysis. DNAs or RNA modified with **1** or **2** were characterized by matrix-assisted laser desorption ionization time-of-flight mode mass spectrometry (MALDI TOF MS, Voyager™ Linear-SA, PerSeptive Biosystems Inc., Fostercity, CA) measurement of the products separated by HPLC. They were desalted by Dowex 50WX8 cation exchange resin and dissolved in a solution of 50 mg/ml 3HPA (3-hydroxypicolinic acid) in 0.1% TFA/50% CH_3CN and dried. Mass spectra were measured by the negative mode.

4.2.4. Melting curve measurement. Melting curves of DNA duplexes were measured on a Hitachi 3300 spectrometer equipped with an SPR 10 temperature controller. The concentration of DNAs unmodified or modified with **1** or **2** was estimated from the molar absorptivity at 260 nm, 6229 or 6201 $cm^{-1}\,M^{-1}$ for **1** or **2**, respectively. Melting temperature was measured in 20 mM KH_2PO_4/K_2HPO_4 buffer (pH 7.0) containing 100 mM KCl. A mixture of 5 μM DNAs modified with **1** or **2** and 5 μM their complementary DNA was placed in the cell of 1 cm in light path length (total 200 μl) and absorbance change at 260 nm was monitored with raising to 85 °C at a rate of 0.5 °C/min.

4.2.5. Electrochemical measurement. Electrochemical measurement was made on an ALS Electrochemical Analyzer Model 900 (CH Instrument Inc., Austin, TX). The redox behavior of 100 μM **1** or **2** was monitored by cyclic voltammetric (CV) measurement over a scan range of 0–0.7 V at a scan rate of 100 mV/s. The Osteryoung square wave voltammetry (SWV) method was used in the experiments for DNA-immobilized electrodes before and after hybridization with an amplitude of 50 mV, applied potential of 10 mV, and frequency of 10 Hz. The electrolyte used was 20 mM NaH_2PO_4/Na_2HPO_4 buffer (pH 7.0) containing 100 mM $NaClO_4$, as it is known that the stable redox reaction of ferrocence occurs in this buffer.[62] The cell was furnished with three electrodes of Ag/AgCl as reference electrode, Pt wire as counter electrode, and DNA-immobilized electrode as working electrode. All measurements were conducted at 10 °C where the double stranded structure of DNA used is stable on the working electrode.

4.3. Synthesis of 1 and 2

Ferrocenylcarbodiimides **1** and **2** were synthesized according to the route shown in Scheme 2.

4.3.1. [3-[(3-Aminopropyl)methylamino]propyl]carbamic acid *tert*-butyl ester (3). *N*,*N*-bis(3-aminopropyl)-methylamine (10 ml, 60 mmol) was dissolved in 20 ml of

1,4-dioxane and a solution of *S-tert*-butoxycarbonyl-4,6-dimethyl-2-mercaptopyrimidine (7.7 g, 30 mmol) in 50 ml of 1,4-dioxane was dropped to the solution over 10 h at room temperature and the mixture was stirred for 20 h. After filtration of the yellow solid, the solvent was removed under reduced pressure and 50 ml of water were added. The white precipitates formed were removed by filtration and NaCl was added to the filtrate. Further precipitates and remaining solid NaCl were removed by filtration and mono Boc derivative **3** was extracted with ethyl acetate (30 ml×4). The extract was dried over magnesium sulfate and **3** was obtained as a yellow oil (4.7 g, 32% yield) after evaporation and drying under reduced pressure. ^{1}H NMR (250 MHz, $CDCl_3$) δ=1.44 (9H, s, $C(CH_3)_3$), 1.66 (4H, m, CH_2CH_2-CH_2), 2.19 (3H, s, NCH_3), 2.36 (4H, m, NCH_2CH_2), 2.75 (2H, m, $CH_2CH_2NH_2$), and 3.16 (2H, m, CH_2CH_2NHCO) ppm.

4.3.2. [3-[[3-(3-Ethylureido)propyl]methylamino]propyl]carbamic acid *tert*-butyl ester (4). A solution of ethylisocyanate (1.5 g, 20 mmol) in 13 ml of diethyl ether was added slowly to a solution of **3** (4.7 g, 9.0 mmol) in 7 ml of diethyl ether at 0 °C with stirring. The reaction mixture was stirred for 3 h at room temperature after standing for 10 min at 0 °C. The mixture became negative to ninhydrin in 3 h and the solvent was removed and the residue dried under reduced pressure to give **4** as a yellow oil (5.5 g, 95% yield). ^{1}H NMR (250 MHz, $CDCl_3$) δ=1.11 (3H, t, $NHCONHCH_2CH_3$), 1.45 (9H, s, $C(CH_3)_3$), 1.64 (4H, m, $CH_2CH_2CH_2$), 2.18 (3H, s, NCH_3), 2.37 (4H, m, NCH_2CH_2), and 3.18 (6H, m, CH_2CH_2NHCO, CH_2CH_2-$NHCOO$(tBu), $CONHCH_2CH_3$) ppm.

4.3.3. 1-[3-[(3-Aminopropyl)methylamino]propyl]-3-ethylurea (5). Compound **4** (5.5 g, 17 mmol) was dissolved in 4 N HCl/1,4-dioxane (13 ml, 52 mmol) and stirred for 3 h. The precipitates formed were collected and dried under reduced pressure to give **5** as a yellow gum-like solid (5.2 g, 95% yield). ^{1}H NMR (250 MHz, DMSO-d_6) δ=1.00 (3H, t, $NHCONHCH_2CH_3$), 1.80 (2H, m, CH_2CH_2NHCO), 2.03 (2H, m, $CH_2CH_2N^+H_3$) 2.70 (3H, s, NCH_3), and 3.03 (10H, m, $N^+HCH_2CH_2$, $CH_2CH_2N^+H_3$, CH_2CH_2NHCO, $CONHCH_2CH_3$) ppm.

4.3.4. *N*-[3-[[3-(3-Ethylureido)-propyl]-methylamino]-propyl]-3-ferrocenylpropionamide (6). Compound **5** (2.5 g, 8.8 mmol) was dissolved in TEA (5.1 ml, 36 mmol) and chloroform (30 ml). Ferrocenepropionic acid (2.6 g, 10 mmol), PyBOP (5.2 g, 10 mmol), and HOBt (1.3 g, 10 mmol) were added to the solution and stirred for 10 h at room temperature. The progress of reaction was monitored by the spot of R_f=0.22 on thin-layer chromatography (TLC) ($CHCl_3/CH_3OH$/TEA=95:5:1) on silica gel. The solution was washed with saturated $NaHCO_3$ aqueous solution (30 ml×2) and the solvent was removed under reduced pressure. After collection of the R_f=0.22 fraction on silica gel chromatography with the eluent ($CHCl_3/CH_3OH$/TEA=95:5:1), compound **6** was obtained as a pale yellow oil (2.8 g, 70% yield). ^{1}H NMR (250 MHz, $CDCl_3$) δ=1.11 (3H, t, $NHCONHCH_2CH_3$), 1.80 (4H, q, $CH_2CH_2CH_2$), 2.14 (3H, s, NCH_3) 2.38 (4H, m, CH_2NCH_3), 2.68 (2H, t, $FcCH_2CH_2$), 3.02–3.35 (8H, m, $FcCH_2CH_2$, CH_2NHCO), and 4.12 (9H, m, $C_5H_5FeC_5H_4CH_2$) ppm.

4.3.5. *N*-[3-[[3-(3-Ethylureido)-propyl]-methylamino]-propyl]-3-ferrocenylamide (7). Compound **5** (2.5 g, 8.8 mmol) was dissolved in TEA (5.1 ml, 36 mmol) and chloroform (30 ml), ferrocenecarboxylic acid (2.3 g, 10 mmol), PyBOP (5.2 g, 10 mmol), and HOBt (1.3 g, 10 mmol) were added to the solution and stirred for 18 h at room temperature. The progress of reaction was monitored by the spot of R_f=0.20 on TLC ($CHCl_3/CH_3OH$/TEA=95:5:1). The reaction mixture was washed with saturated $NaHCO_3$ aqueous solution (30 ml×2) and the solvent was removed under reduced pressure. The yellow fraction of R_f=0.20 ($CHCl_3/CH_3OH$/TEA=95:5:1) was collected from silica gel chromatography. After removing the solvent and drying under reduced pressure **7** was obtained as a pale yellow oil (1.9 g, 51% yield). ^{1}H NMR (250 MHz, $CDCl_3$) δ=1.11 (3H, t, $NHCONHCH_2CH_3$), 1.80 (4H, q, CH_2CH_2-CH_2), 2.14 (3H, s, NCH_3) 2.38 (4H, m, CH_2NCH_3), 3.05–3.43 (6H, m, CH_2NHCO), 4.15 (5H, s, $(C_5H_5)Fe(C_5H_4)$-CONH), 4.33 (2H, m, $(C_5H_5)Fe(C_2H_2C_2H_2C)CONH$), and 4.63 (2H, m, $(C_5H_5)Fe(C_2H_2C_2H_2C)CONH$) ppm.

4.3.6. *N*-[3-[(3-Ethyliminomethyleneaminopropyl) methylamino]propyl]-3-ferrocenylpropionamide (8). The reaction was carried out under the nitrogen atmosphere. Compound **6** (2.0 g, 4.4 mmol) was dissolved in TEA (2.4 ml, 18 mmol) and dry dichloromethane (20 ml) and stirred for 15 min. The solution was kept for 15 min at −20 °C. A dry dichloromethane solution (15 ml) of *p*-toluenesulfonyl chloride (1.7 g, 8.8 mmol) was added to the solution slowly. After standing at room temperature, the reaction mixture was refluxed for 4 h and the progress of reaction was monitored by the spot of R_f=0.48 on TLC ($CHCl_3$/TEA=100:0.5). The reaction mixture was washed with 40% potassium carbonate aqueous solution (20 ml×4) and the solvent was removed. The solid obtained was dissolved in 30 ml of diethyl ether and insoluble material was removed by filtration. The solvent was evaporated under reduced pressure and the residue was dried to give **8** as an orange viscous oil (0.81 g, 41% yield). ^{1}H NMR (250 MHz, $CDCl_3$) δ=1.23 (3H, t, $N{=}C{=}NCH_2CH_3$), 1.68 (4H, m, $CH_2CH_2CH_2$), 2.16 (3H, s, NCH_3) 2.38 (4H, m, CH_2NCH_3), 2.68 (2H, t, $FcCH_2CH_2$), 3.32–3.49 (8H, m, $FcCH_2CH_2$, $CH_2N{=}C{=}N$, CH_2NHCO), and 4.10 (9H, m, $C_5H_5FeC_5H_4CH_2$) ppm.

4.3.7. *N*-[3-[(3-Ethyliminomethyleneaminopropyl) methylamino]propyl]-3-ferrocenylamide (9). The reaction was carried out under the nitrogen atmosphere. Compound **7** (1.5 g, 3.5 mmol) was dissolved in TEA (1.9 ml, 14 mmol) and dry dichloromethane (15 ml) and stirred for 15 min. The solution was kept for 15 min at −20 °C. A dry dichloromethane solution (10 ml) of *p*-toluenesulfonyl chloride (1.3 g, 7.0 mmol) was added to the solution slowly. After standing at room temperature, the reaction mixture was refluxed for 4 h. The progress of reaction was monitored by the spot of R_f=0.42 on TLC ($CHCl_3$/TEA=100:0.5). The reaction mixture was washed with 40% potassium carbonate aqueous solution (20 ml×4) and the solvent was removed. The solid left was dissolved in 30 ml of diethyl ether and insoluble material was removed

by filtration. The solvent was evaporated under reduced pressure and the residue was dried to give **9** as an orange viscous oil (0.55 g, 38% yield). ^{1}H NMR (250 MHz, $CDCl_3$) δ=1.11 (3H, t, N=C=NCH$_2$C*H*$_3$), 1.70 (4H, q, CH$_2$C*H*$_2$CH$_2$), 2.14 (3H, s, NC*H*$_3$) 2.38 (4H, m, C*H*$_2$NCH$_3$), 3.02–3.35 (6H, m, C*H*$_2$N=C=N, C*H*$_2$NHCO), 4.15 (5H, s, (C$_5$*H*$_5$)Fe(C$_5$H$_4$)CONH), 4.33 (2H, m, (C$_5$H$_5$)Fe(C$_2$*H*$_2$C$_2$H$_2$C)CONH), and 4.63 (2H, m, (C$_5$H$_5$)Fe(C$_2$H$_2$C$_2$*H*$_2$C)CONH) ppm.

4.3.8. (3-Ethyliminomethyleneaminopropyl)dimethyl[3-(3-ferrocenylpropionylamino)propyl]ammonium iodide (1). Compound **8** (0.81 g, 1.8 mmol) was dissolved in diethyl ether (3 ml) and iodomethane (1.1 ml, 3.6 mmol) was added and stirred for 18 h. The precipitates formed were collected by filtration and dried under reduced pressure to give **1** as a yellow solid (0.76 g, 72% yield). ^{1}H NMR ($CDCl_3$) δ=1.23 (3H, t, N=C=NCH$_2$C*H*$_3$), 1.96–2.16 (4H, m, CH$_2$C*H*$_2$CH$_2$), 2.68 (2H, m, FcC*H*$_2$CH$_2$), 3.21 (6H, s, N$^+$(C*H*$_3$)$_2$), 3.24–3.48 (12H, m, C*H*$_2$N$^+$(CH$_3$)$_2$, FcCH$_2$-C*H*$_2$, C*H*$_2$N=C=N, C*H*$_2$NHCO), and 4.10 (9H, m, C$_5$*H*$_5$FeC$_5$*H*$_4$CH$_2$) ppm, FT IR (CaF_2) 2129 cm^{-1} (–N=C=N–), 1643 cm^{-1} (–NH–CO–), 1559 cm^{-1} (–NH–CO–).

4.3.9. (3-Ethyliminomethyleneaminopropyl)dimethyl[3-(3-ferrocenylamino)propyl]ammonium iodide (2). Compound **9** (0.55 g, 1.3 mmol) was dissolved in diethyl ether (3 ml) and iodomethane (0.80 ml, 2.6 mmol) was added and stirred for 18 h. The precipitates formed were collected by filtration and dried under reduced pressure to give **2** as a yellow solid (0.49 g, 68% yield). ^{1}H NMR ($CDCl_3$) δ=1.24 (3H, t, N=C=NCH$_2$C*H*$_3$), 1.93–2.14 (4H, m, 2×CH$_2$-C*H*$_2$CH$_2$), 3.19 (6H, s, N$^+$(C*H*$_3$)$_2$), 3.20–3.49 (10H, m, C*H*$_2$N$^+$(CH$_3$)$_2$, C*H*$_2$N=C=N, C*H*$_2$NHCO), 4.19 (5H, s, (C$_5$*H*$_5$)Fe(C$_5$H$_4$)CONH), 4.42 (2H, m, (C$_5$H$_5$)Fe(C$_2$*H*$_2$C$_2$H$_2$C)CONH), and 4.75 (2H, m, (C$_5$H$_5$)Fe(C$_2$H$_2$C$_2$*H*$_2$C)CONH) ppm, FT IR (CaF_2) 2127 cm^{-1} (–N=C=N–), 1642 cm^{-1} (–NH–CO–), 1557 cm^{-1} (–NH–CO–).

4.4. Labeling reaction of DNAs with 1 or 2

Ten microliters of a solution of 1 mM DNA in 20 mM borate buffer (pH 8.5, 9.0 or 9.5) were mixed with 10 μl of a solution of 100 mM **1** or **2** in 20 mM borate buffer containing 60% DMSO at proper temperature for a specified period of time. The mixture was diluted to 1 ml with 0.1 M TEAA buffer (pH 7.0) and then loaded on a NAP-10 column (Pharmacia Sephadex G-25, Amersham Biosciences Co., Uppasala, Sweden). After discarding the first 1 ml of the flow-through, DNA was eluted with 1.5 ml of 0.1 M TEAA buffer and lyophilized. After addition of 100 μl of water, 20 μl of this solution were subjected to reversed phase HPLC to evaluate the composition.

4.5. Labeling reaction of RNA with 1 or 2

Ten microliters of a solution of 0.2 mM rUA$_9$ in 20 mM borate buffer (pH 8.5) were mixed with 10 μl of a solution of 20 mM **1** or **2** in 20 mM borate buffer containing 60% DMSO at 37 °C for a specified period of time. The mixture was diluted to 1 ml with 0.1 M TEAA buffer (pH 7.0) and then loaded on a NAP-10 column. After discarding the first 1 ml of the flow-through, RNA was eluted with 1.5 ml of 0.1 M TEAA buffer and lyophilized. After addition of 100 μl of water, 20 μl of this solution were subjected to reversed phase HPLC to assess the composition.

4.6. Preparation of a DNA-immobilized electrode and its hybridization with complementary DNA

A gold electrode having 2.0 mm^2 in area was polished with 6 μm, 1 μm of diamond slurry, and 0.05 μm of alumina slurry in this order and sonicated in MilliQ water for 10 min. This electrode was electrochemically polished by scanning 40 times from −0.2 to 1.5 V at a scan rate of 100 mV/s in 1 M H_2SO_4 aqueous solution and sonicated in MilliQ water for 15 min. One microliter of 1 M NaCl solution containing 2 μM thiolated DNA (see Table 1) was placed on the gold electrode held upside down and kept in a closed container under high humidity for 24 h at room temperature. After washing with MilliQ water, 1 μl of 1 mM 6-mercaptohexanol was placed on the electrode for 1 h at 45 °C.

One microliter of 2×SSC containing 0.1, 0.2, 0.5, 0.8, 1 or 2 μM DNA modified with **1** or **2** was placed on the electrode for 6 h at 10 °C to allow hybridization to proceed. The electrode was kept in 20 mM NaH_2PO_4/Na_2HPO_4 buffer (pH 7.0) containing 100 mM $NaClO_4$ and SWV was measured with an ALS model 900 Electrochemical Analyzer. Competitive hybridization of two different DNA samples was carried out as follows. One microliter of a mixture of DNAs modified with **1** or **2** (1.8:0.2, 1.5:0.5, 1.2:0.8, 1.0:1.0, 0.8:1.2, 0.5:1.5, or 0.2:1.8 μM/μM) was placed on the DNA-probe immobilized electrode for 8 h at 10 °C to allow hybridization to proceed. These electrodes were dipped in the same electrolyte as above for 1 min and SWV was measured at 10 °C.

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1-*C*-(2′-Oxoalkyl) glycosides as latent α,β-unsaturated conjugates. Synthesis of aza-*C*-glycosides by an intramolecular hetero-Michael addition

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Abstract—1-*C*-(2′-Oxoalkyl)-5-azido-5-deoxy-glycofuranosides were used as latent substrates for intramolecular hetero-Michael addition. Reduction of the azido groups by catalytic hydrogenation followed by base treatment produced 2′-ester and 2′-ketone aza-*C*-glycopyranosides. The conjugation addition was stereoselective in favor of aza-*C*-glycosides with equatorial substitutions at the pseudo anomeric center.
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1. Introduction

Azasugars (iminosugars)[1] are powerful glyco-processing enzyme inhibitors and potential therapeutics for the treatment of diabetic, cancer, viral infection and other diseases.[2] For example, *N*-hydroxyethyl-1-deoxynojirimycin (Miglitol)[3] and *N*-butyl-1-deoxynojirimycin (Zavestca)[4] have been approved, respectively, for the treatment of type 2 diabetes and type 1 Gaucher disease. Numerous synthetic methods towards azasugars have been developed,[2a,b,5] which include various reductive and double-reductive amination between an azido/amino group and carbonyl groups,[6] and S_N2 substitutions by nucleophilic amine to epoxides,[7] halides,[8] and other leaving groups.[9] Aza-*C*-glycosides, azasugars with a *C*-linked aglycon, often possess improved inhibition specificity and membrane permeability. Besides the natural occurrences they have been synthesized from azasugars via elimination, followed by addition of organometallic reagent to C=N,[10] and from properly constructed substrates, by intramolecular and intermolecular conjugation additions of nucleophilic amines. For example, Compernolle et al.[11] synthesized aza-*C*-glycosides from 7-amino-2,3-unsaturated ketones and esters, by an intramolecular Michael addition, which were prepared from 1-amino-1-deoxy-D-glucitol by regioselective *O*-isopropylidenation, selective deprotection, oxidation of 5,6-diol to aldehyde, and Wittig reaction. In another example, Dhavale et al.[12] prepared a conjugated ester from 3-*O*-benzyl-1,2-*O*-isopropylidene-glucofuranose through oxidation of 5,6-diol to 5-aldehyde, followed by a Wittig reaction. An amino group was introduced at C5 by an intermolecular Michael addition, and the azasugar was then obtained following removal of 1,2-*O*-isopropylidene and subsequent reductive amination. The major limitation associated with these procedures, however, is the accessibility of these intermediates, the formation of which is highly dependent on the stereochemistry of the hydroxy groups in the starting materials in order to achieve the regioselective protection. Therefore, easy access to molecules with both amine and α,β-unsaturated ester (ketone) functionalities is desired.

Previously, we had found that 1-*C*-(2′-oxoalkyl)-glycosides, following base-mediated β-elimination, formed acyclic α,β-conjugates,[13] which enabled an intramolecular addition by a thiol group to form *C*-thioglycoside.[14] Thus, 1-*C*-(2′-oxoalkyl)-glycosides can be considered as latent substrates for Michael addition. We envisioned that 1-*C*-(2′-oxoalkyl)-glycosides with an amino group could undergo a similar intramolecular Michael addition to produce aza-*C*-glycosides. Additionally, the 2′-oxoalkyl group, presented as a *C*-aglycon, could be further condensed with various amines for library constructions. The library approach has led to the discovery of azasugars with improved inhibition activities against fucosidase,[15] *N*-acetyl-β-hexosaminidase,[16] α-mannosidase,[7e,17] and β-glucosidase.[18]

Keywords: Aza-*C*-glycosides; Inhibitors; Michael addition; Synthesis.
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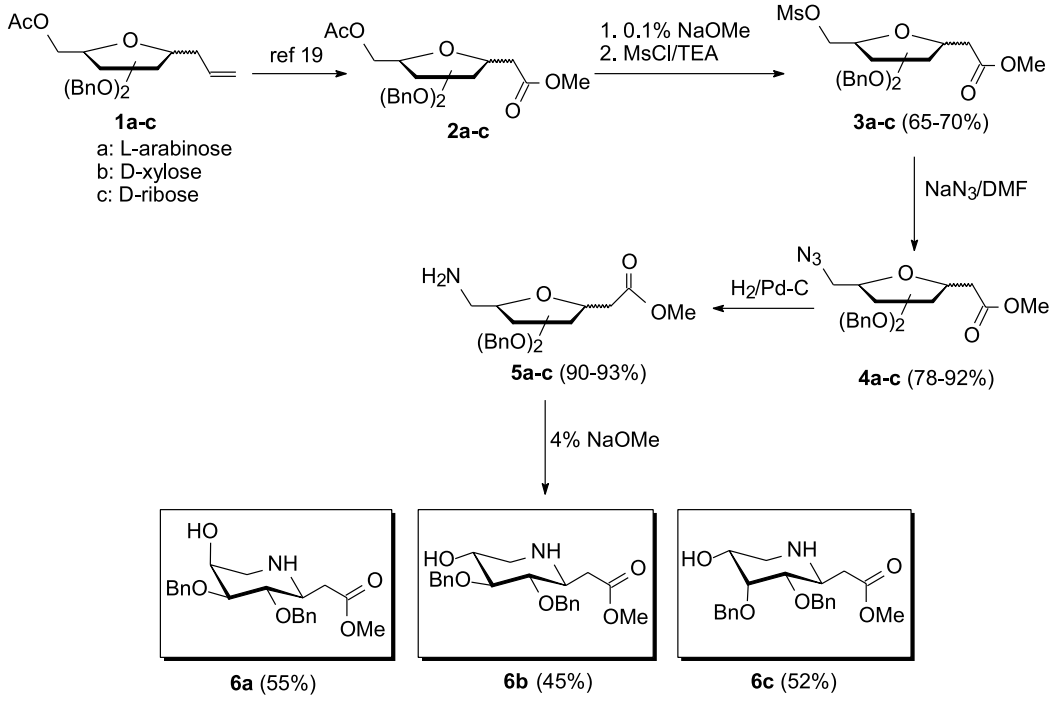
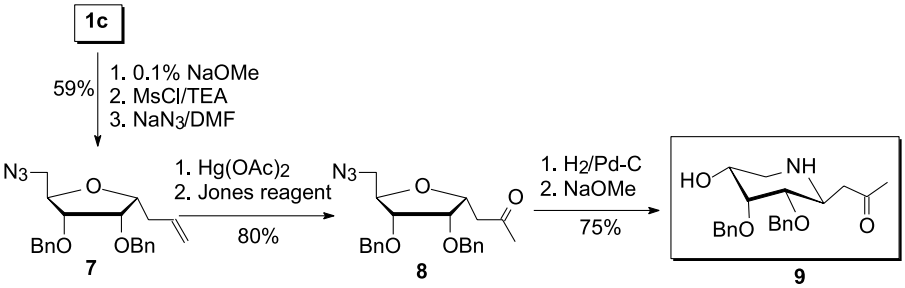
2. Results and discussion

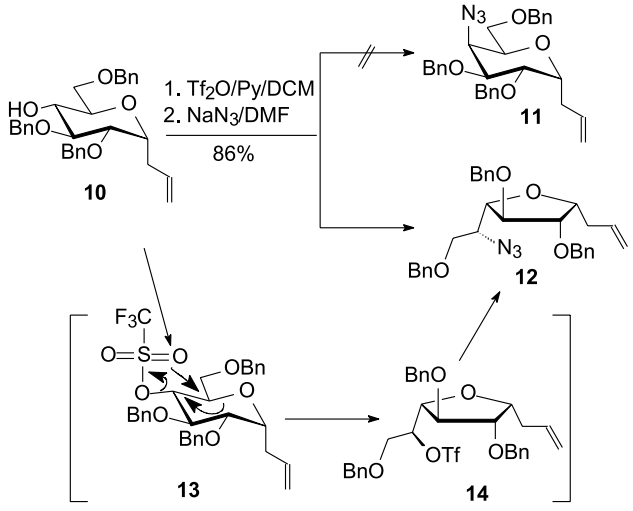
2′-Esters (**2a–c**) were prepared previously from three allyl *C*-glycosides (**1a–c**) (L-arabinose, D-ribose, and D-arabinose),[19] which can also be synthesized by Wittig reaction to sugar lactols.[20] Removal of the 5-*O*-acetyl group in **2a–c** followed by mesylation, afforded **3a–c** (Scheme 1). Displacement of 5-*O*-Ms by an azido group led to



Scheme 1.



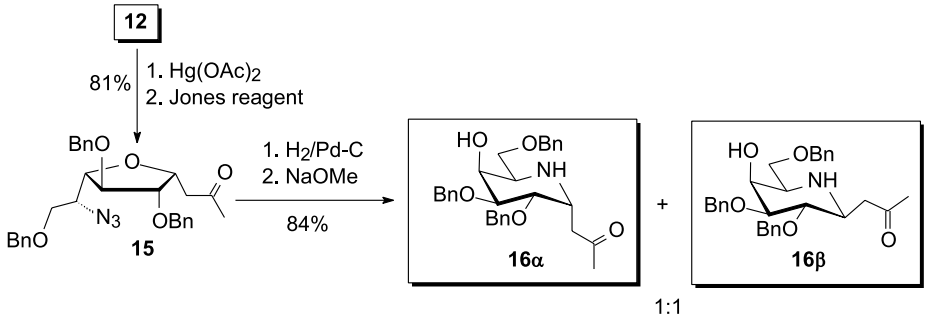
Scheme 2.



Scheme 3.

compounds **4a–c**. Catalytic hydrogenation under balloon pressure for 20 min selectively reduced the 5-azido groups to 5-amines (**5a–c**) in 90% yield without affecting the *O*-benzyl groups. Subsequent treatment of **5a–c** with 4% NaOMe overnight produced, via β-elimination and hetero-Michael addition, aza-*C*-glycosides (**6a–c**) in 45–55% yield. This conjugate addition was highly stereoselective to give 1-*C*-equatorial aza-*C*-glycosides, which were assigned based on NMR anlysis. The stereochenmistry is in agreement with the previous observation.[11] No 1-*C*-axial product was isolated. The moderate yields were the result of competing side reactions such as amidation, as evidenced by the isolatation of a cyclized amide dimer (ca. 10%) with *m/z* 707.3 (MH^+).

In order to prepare 2′-ketonyl aza-*C*-glycosides, 1-*C*-allyl glycoside **1c** was converted to 5-azido-*C*-glycoside **7** in three steps similar to those descibed for **4a–c** (Scheme 2).



Scheme 4.

The 1-*C*-allyl group was in turn oxidized with $Hg(OAc)_2$ and Jones reagent[21] to 1-*C*-acetylmethyl glycoside **8**. Reduction of the azido group by catalytic hydrogenation to amine was followed by a base-mediated Michael addition, to produce, as expected, 2′-ketonyl aza-*C*-glycoside **9** in 75% yield. Similar to the observation with 2′-ester aza-*C*-glycosides (**6a**–**c**), the 1-*C*-(2′-ketone) group was equatorial at the pseudo anomeric carbon.

We attempted to extend this approach to the synthesis of aza-*C*-glycofuranosides by introducing an azido group at the 4-position of the pyranosides. However, sulfonation (Tf_2O) at the 4-OH of glucoside **10** followed by treatment of NaN_3 in DMF did not yield 4-azido-galactoside **11**. Instead, this azidolysis produced 5-azido galactofuranoside **12** in excellent yield.[22] The 5-azido substitution was unambigously confirmed by various 2D NMR analysis including HMBC, HSQC, and COSY. The transformation involves participation of the ring-oxygen atom resulting in a concurrent 4,5-migration of TfO-group and ring contraction to intermediate **14** (Scheme 3) prior to the displacement of its 5-OTf by an azido group. Although this type of rearrangement has never been observed with *C*-glycosides, it is known to occur in *O*-glycosides as a result of steric hindrance in bimolecular displacement from axial direction.[23]

The oxidation of 1-*C*-allyl glycoside **12** with $Hg(OAc)_2$ and Jones reagent[21] afforded azide **15**, which was reduced to amine. Without purification, the crude product was subsequently subjected to a base-mediated Michael addition to produce a mixture of aza-*C*-galactosides, **16α** and **16β**, in 84% yield in a 1:1 ratio. Pure forms of **16α** and **16β** were obtained by chromatography, and their stereochemistry was assigned based on the NOEs between H1 and H5 (H3) in **16β** and between H3 and H1′ in **16α** (Scheme 4).

In summary, we have described a synthetic method toward aza-*C*-glycosides from 1-*C*-(2′-oxoalkyl)-5-amino-5-deoxy-*C*-glycofuranosides via an intramolecular hetero-Michael addition. This tandem β-elimination and conjugate addition under basic conditions provides an easy access to biologically important aza-*C*-glycosides. The core azasugar structures are suitable for further development as potential glycosidase inhibitors.

3. Experimental

3.1. General methods

^1H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively, with a Varian instrument at 293 K. Chemical shifts were given in ppm downfield to the signal of internal TMS, and were assigned on the basis of 2D ^1H-COSY, TOCSY, and ^1H–^{13}C chemical-shift correlated experiments. High-resolution fast-atom bombardment mass spectrometry (HRFABMS) was carried out on a JEOL JMS-AX505Hmass spectrometer using a 6 kV xenon beam at an accelerating voltage of 3 kV. *m*-Nitrobenzyl alcohol (*m*-NBA) was used as the matrix, and polyethylene glycol (PEG) was the internal calibrant. All chemicals are purchased from Aldrich Co. without further purification.

3.1.1. Methyl 2-*C*-(2,3-di-*O*-benzyl-5-*O*-Ms-α/β-L-arabinofuranosyl)acetate (3a). A solution of **2a** (6.1 g, 14.3 mmol) in 0.1% MeONa–MeOH solution (50 mL) was stirred for 1 h, and then neutralized by the addition of Dowex 50WX2-100 (H^+) resin. The filtrate was concentrated to a syrup (5.5 g), which was dissolved in 100 mL of CH_2Cl_2 and triethyl amine (10 mL, 69 mmol). To the solution was dropwise added MsCl (3.3 g, 28.8 mmol) at 0 °C, and the solution was stirred overnight at 0 °C to room temperature. The reaction was quenched by the addition of aq $NaHCO_3$. Routine work-up and purification by chromatography (Hexane/EtOAc 2:1) gave **3a** (5.1 g, 70% in two steps) as a mixture of two anomers ($\alpha/\beta=3:1$). *For α-anomer.* ^1H NMR ($CDCl_3$): δ 7.37–7.24 (m, 10H), 4.55–4.51 (m, 5H), 4.29–4.22 (m, 3H), 3.99 (m, 1H), 3.93 (m, 1H), 3.64 (s, 3H), 2.96 (s, 3H), 2.74–2.63 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 171.0, 137.3, 137.2, 128.6, 128.5, 128.0, 127.9, 127.7, 85.7, 83.9, 80.9, 79.9, 72.1, 71.7, 69.0, 51.7, 37.7, 37.4; HRFABMS: Calcd for $C_{23}H_{29}O_8S$ (MH^+), *m/z* 465.1583; Found: 465.1627.

3.1.2. Methyl 2-*C*-(2,3-di-*O*-benzyl-5-*O*-Ms-α/β-D-xylofuranosyl)acetate (3b). Following the same procedures as described above **3b** was obtained from **2b** as a mixture of two anomers ($\alpha/\beta=2:1$) in 65% yield. *For α-anomer.* ^1H NMR ($CDCl_3$): δ 7.39–7.24 (m, 10H), 4.60–4.51 (m, 3H), 4.42–4.31 (m, 5H), 4.05 (d, 1H, $J=3.6$ Hz), 4.03 (d, 1H, $J=3.2$ Hz), 3.66 (s, 3H), 2.99 (s, 3H), 2.74–2.59 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 171.7, 137.5, 137.4, 128.6, 128.2, 128.0, 127.9, 127.8, 84.5, 82.4, 80.5, 77.6, 72.4, 71.7, 68.9, 51.8,

38.8, 37.5. *For β-anomer*. ^{1}H NMR ($CDCl_3$): δ 7.39–7.24 (m, 10H), 4.60–4.51 (m, 2H), 4.42–4.31 (m, 6H), 4.01 (dd, 1H, J=4.0, 1.2 Hz), 3.90 (dd, 1H, J=2.4, 1.2 Hz), 3.66 (s, 3H), 2.99 (s, 3H), 2.74–2.59 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 171.7, 137.5, 137.4, 128.6, 128.1, 128.0, 127.0, 127.8, 81.2, 78.8, 77.0, 72.4, 71.7, 68.7, 51.7, 34.0, 37.5; HRFABMS: Calcd for $C_{23}H_{29}O_8S$ (MH^+), *m/z* 465.1583; Found: 465.1547.

3.1.3. Methyl 2-*C*-(2,3-di-*O*-benzyl-5-*O*-Ms-α/β-D-ribofuranosyl)acetate (3c). Following the same procedures as described above **3c** was obtained from **2c** as a mixture of two anomers (α/β=4:1) in 69% yield. *For α-anomer*. ^{1}H NMR ($CDCl_3$): δ 7.36–7.32 (m, 10H), 4.80 (d, 1H, J=11.6 Hz), 4.69 (d, 1H, J=12.0 Hz), 4.56 (d, 1H, J=11.2 Hz), 4.53 (d, 1H, J=11.2 Hz), 4.47–4.45 (m, 1H), 4.35 (d, 1H, J=8.8 Hz), 4.24–4.20 (m, 1H), 4.12 (t, 1H, J=4.0 Hz), 4.05 (dd, 1H, J=7.2, 4.4 Hz), 3.63 (s, 3H), 2.99 (s, 3H), 2.76 (d, 1H, J=2.8 Hz), 2.74 (d, 1H, J=3.2 Hz); ^{13}C NMR ($CDCl_3$): δ 171.6, 137.8, 137.2, 128.6, 128.5, 128.3, 128.1, 128.0, 127.9, 127.8, 79.3, 77.9, 77.0, 76.8, 73.6, 73.1, 69.1, 51.8, 37.4, 34.7; HRFABMS: Calcd for $C_{23}H_{29}O_8S$ (MH^+), *m/z* 465.1583; Found 465.3067.

3.1.4. Methyl 2-*C*-(5-azido-2,3-di-*O*-benzyl-5-deoxy-α/β-L-arabinofuranosyl)acetate (4a). A mixture of **3a** (5.1 g, 10.4 mmol) and NaN_3 (2.1 g, 32 mmol) in DMF (80 mL) was stirred overnight at 80 °C. Upon cooling to room temperature the reaction mixture was diluted by the addition of ethyl ether (200 mL) and the resulted solution was washed with water and brine. Purification by chromatography (Hexane/EtOAc 4:1) gave **4a** (4.1 g, 92%) as a mixture of two anomers. *For α-anomer*. ^{1}H NMR ($CDCl_3$): δ 7.39–7.22 (m, 10H), 4.58 (m, 1H), 4.61–4.40 (m, 4H), 4.19 (m, 1H), 3.96 (m, 2H), 3.67 (s, 3H), 3.41–3.30 (m, 2H), 2.79 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 171.1, 137.6, 137.4, 128.6, 128.1, 128.0, 127.8, 127.7, 85.2, 82.2, 79.5, 77.8, 72.2, 71.9, 52.4, 51.8, 38.0; *For β-anomer*. ^{1}H NMR ($CDCl_3$): δ 7.39–7.22, 4.61–4.40 (m, 4H), 4.45 (m, 1H), 4.04 (m, 2H), 3.88 (m, 1H), 3.64 (s, 3H), 3.41–3.30 (m, 2H), 2.79 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 171.6, 137.6, 137.5, 128.6, 128.5, 128.1, 127.8, 86.2, 83.9, 82.7, 72.2, 71.9, 52.6, 51.7, 34.0; HRFABMS: Calcd for $C_{22}H_{26}O_5N_3$ (MH^+), *m/z* 412.1872; Found: 412.1995.

3.1.5. Methyl 2-*C*-(5-azido-2,3-di-*O*-benzyl-5-deoxy-α/β-D-xylofuranosyl)acetate (4b). Obtained from **3b** as a mixture of two anomers in 80% yield. *For α-anomer*. ^{1}H NMR ($CDCl_3$): δ 7.39–7.22 (m, 10H), 4.60–4.42 (m, 4H), 4.38 (dt, 1H, J=6.8, 2.4 Hz), 4.26–4.17 (m, 1H), 3.96 (m, 1H), 3.91 (m, 1H), 3.66 (s, 3H), 3.55–3.38 (m, 2H), 2.73 (dd, 1H, J=13.6, 6.8 Hz); ^{13}C NMR ($CDCl_3$): δ 171.8, 137.6, 137.4, 128.6, 128.2, 128.1, 129.9, 127.8, 84.8, 82.6, 80.2, 79.8, 72.5, 71.7, 51.9, 50.2, 38.9; *For β-anomer*. ^{1}H NMR ($CDCl_3$): δ 7.39–7.22, 4.60–4.42 (m, 4H), 4.57 (m, 1H), 4.26–4.17 (m, 1H), 4.07 (d, 1H, J=3.6 Hz), 3.96 (m, 1H), 3.66 (s, 3H), 3.55–3.38 (m, 2H), 2.73 (dd, 1H, J=13.6, 6.8 Hz), 2.64 (dd, 1H, J=13.6, 6.8 Hz); ^{13}C NMR ($CDCl_3$): δ 171.3, 137.6, 137.4, 128.6, 128.2, 128.0, 128.9, 127.8, 81.5, 81.3, 78.8, 76.8, 72.5, 71.7, 51.7, 50.2, 34.1; HRFABMS: Calcd for $C_{22}H_{26}O_5N_3$ (MH^+), *m/z* 412.1872; Found: 412.1826.

3.1.6. Methyl 2-*C*-(5-azido-2,3-di-*O*-benzyl-5-deoxy-α/β-D-ribofuranosyl)acetate (4c). Obtained from **3c** as a mixture of two anomers (α/β=4:1) in 78% yield. *For α-anomer*. ^{1}H NMR ($CDCl_3$): δ 7.33–7.32 (m, 10H), 4.79 (d, 1H, J=11.6 Hz), 4.68 (d, 1H, J=11.6 Hz), 4.54–4.52 (m, 3H), 4.18–4.15 (m, 2H), 4.02 (m, 1H), 3.63 (s, 3H), 3.53 (d, 1H, J=13.2 Hz), 3.16 (d, 1H, J=12.8 Hz), 2.84–2.75 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 171.7, 138.0, 137.4, 128.5, 128.3, 128.0, 127.7, 80.1, 78.9, 77.2, 76.7, 73.5, 72.9, 52.0, 51.6, 34.8; HRFABMS: Calcd for $C_{22}H_{25}O_5N_3$ (MH^+), *m/z* 412.1872; Found 412.1907.

3.1.7. Methyl 2-*C*-(5-amino-2,3-di-*O*-benzyl-5-deoxy-α/β-L-arabinofuranosyl)acetate (5a). A mixture of **4a** (0.5 g, 1.2 mmol) and 10% Pd–C (50 mg) in methanol (20 mL) was stirred under H_2 atmosphere (balloon pressure) for 20 min when the starting material was completely disappeared. The reaction mixture was filtered and the filtrate was concentrated. Purification by chromatography (CH_2Cl_2/MeOH 10:1) gave **5a** (0.4 g, 93%) as a mixture of two anomers (α/β=3:1). *For α-anomer*. ^{1}H NMR ($CDCl_3$): δ 7.39–7.21 (m, 10H), 4.59–4.50 (m, 4H), 4.41 (m, 1H), 3.99 (m, 1H), 3.90 (m, 1H), 3.86 (m, 1H), 3.66 (s, 3H), 2.83 (m, 2H), 2.65 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 171.4, 137.8, 137.7, 128.6, 128.0, 127.8, 127.7, 86.7, 85.7, 84.6, 79.0, 72.0, 71.8, 51.9, 44.3, 37.9; HRFABMS: Calcd for $C_{22}H_{28}O_5N$ (MH^+), *m/z* 386.1967; Found: 386.1992.

3.1.8. Methyl 2-*C*-(5-amino-2,3-di-*O*-benzyl-5-deoxy-α/β-D-xylofuranosyl)acetate (5b). Obtained from **4b** following the same procedures as described above as a mixture of two anomers (α/β=2:1) in 90% yield. *For α-anomer*. ^{1}H NMR ($CDCl_3$): δ 7.39–7.22 (m, 10H), 4.58–4.41 (m, 4H), 4.33 (dt, 1H, J=7.2, 2.8 Hz), 4.09 (m, 1H), 3.97 (d, 1H, J=4.4 Hz), 3.87 (dd, 1H, J=2.4, 1.2 Hz), 3.66 (s, 3H), 3.05 (m, 2H), 2.85 (br, 2H), 2.73 (dd, 1H, J=15.6, 7.2 Hz), 2.64 (dd, 1H, J=15.6, 6.8 Hz); ^{13}C NMR ($CDCl_3$): δ 171.5, 137.7, 137.5, 129.0, 128.7, 128.1, 127.9, 127.7, 85.4, 83.1, 81.8, 79.6, 71.9, 71.7, 51.8, 41.1, 38.9; *For β-anomer*. ^{1}H NMR ($CDCl_3$): δ 7.39–7.22 (m, 10H), 4.60 (m, 1H), 4.58–4.41 (m, 4H), 4.17 (dd, 1H, J=10.4, 5.6 Hz), 4.09 (m, 1H), 3.99 (d, 1H, J=4.4 Hz), 3.63 (s, 3H), 2.99 (m, 2H), 2.85 (br, 2H), 2.69 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 172.0, 137.7, 137.6, 128.7, 128.6, 128.0, 127.0, 127.7, 81.5, 80.0, 76.4, 72.3, 72.2, 51.7, 41.1, 34.2; HRFABMS: Calcd for $C_{22}H_{28}O_5N$ (MH^+), *m/z* 386.1967; Found: 386.2032.

3.1.9. Methyl 2-*C*-(5-amino-2,3-di-*O*-benzyl-5-deoxy-α/β-D-ribofuranosyl)acetate (5c). Obtained from **4c** following the same procedures as described above as a mixture of two anomers (α/β=4:1) in 92% yield. *For α-anomer*. ^{1}H NMR ($CDCl_3$): δ 7.36–7.27 (m, 10H), 4.64–4.51 (m, 4H), 4.39 (dt, J=6.0, 5.6 Hz, 1H), 4.07 (m, 1H), 3.84 (dd, J=5.2, 5.2 Hz, 1H), 3.75 (dd, J=5.2, 5.2 Hz, 1H), 3.65 (s, 3H), 2.94 (m, 1H), 2.71 (m, 1H), 2.61 (dd, J=15.6, 5.2 Hz, 1H), 2.51 (dd, J=16.6, 7.2 Hz, 1H), 2.35 (br, 2H); ^{13}C NMR ($CDCl_3$): δ 173.1, 138.8, 137.7, 128.5, 128.4, 128.0, 127.8, 127.7, 82.6, 80.2, 77.5, 72.1, 51.9, 43.7, 38.4; HRFABMS: Calcd for $C_{22}H_{28}NO_5$ (MH^+), *m/z* 386.1967; Found 386.2029.

3.1.10. Methyl 2-*C*-(2,3-di-*O*-benzyl-5-imino-5-deoxy-α-L-arabinopyranosyl)acetate (6a). A solution of **5a** (83 mg,

0.21 mmol) in 4% NaOMe/MeOH (10 mL) was stirred overnight. NH_4Cl (0.2 g) was added and the solvent was evaporated under diminished pressure. The remaining residue was dissolved in water, extracted with EtOAc. The organic phase was dried and concentrated. Purification by chromatography (EtOAc/MeOH 10:1) gave **6a** (44 mg, 55%) as a colorless syrup. $[\alpha]_D$ +4.1 (*c* 1.15, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.37–7.24 (m, 10H), 4.64 (d, 1H, J= 11.6 Hz), 4.57 (d, 1H, J=11.6 Hz), 4.52 (d, 1H, J= 11.6 Hz), 4.46 (d, 1H, J=11.6 Hz), 3.85 (m, 1H), 3.75 (dd, 1H, J=3.6 Hz), 3.62 (s, 3H), 3.60 (m, 1H), 3.41 (m, 1H), 2.94 (dd, 1H, J=12.8, 4.4 Hz), 2.78 (dd, 1H, J=13.2, 6.8 Hz), 2.43 (m, 2H), 1.93 (br s, 2H); ^{13}C NMR ($CDCl_3$): δ 172.7, 137.9, 137.8, 128.7, 128.6, 128.3, 128.2, 128.0, 76.3, 75.3, 72.9, 66.6, 51.8, 51.2, 47.2, 35.6; HRFABMS: Calcd for $C_{22}H_{28}O_5N$ (MH^+), *m*/*z* 386.1967; Found: 386.1991.

3.1.11. Methyl 2-*C*-(2,3-di-*O*-benzyl-5-imino-5-deoxy-β-D-xylopyranosyl)acetate (6b). The same procedures as described above were used for the preparation of **6b** (45%). $[\alpha]_D$ +3.1 (*c* 0.23, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.39–7.24 (m, 10H), 4.68–4.39 (m, 4H), 4.48 (br s, 2H), 3.79 (m, 2H), 3.62 (s, 3H), 3.62–3.58 (m, 2H), 3.23 (m, 1H), 3.12 (m, 1H), 2.69 (dd, 1H, J=16.0, 6.8 Hz), 2.52 (dd, 1H, J=16.0, 6.4 Hz); ^{13}C NMR ($CDCl_3$): δ 172.2, 137.9, 136.6, 128.8, 128.7, 128.5, 128.2, 127.9, 75.2, 73.0, 72.4, 66.3, 51.9, 51.4, 47.2, 35.3; HRFABMS: Calcd for $C_{22}H_{28}O_5N$ (MH^+), *m*/*z* 386.1967; Found: 386.1997.

3.1.12. Methyl 2-*C*-(2,3-di-*O*-benzyl-5-imino-5-deoxy-β-D-ribopyranosyl)acetate (6c). The same procedures as described above were used for the preparation of **6c** (52%). $[\alpha]_D$ −10.2 (*c* 0.21, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.35–7.34 (m, 10H), 5.00 (d, 1H, J=11.6 Hz), 4.73 (d, 1H, J= 11.6 Hz), 4.58 (d, 1H, J=11.2 Hz), 4.52 (d, 1H, J= 11.2 Hz), 4.06 (s, 1H), 3.64 (s, 3H), 3.60 (m, 1H), 3.39–3.35 (dt, 1H, J=8.4, 2.6 Hz), 3.25 (d, 1H, J=8.8 Hz), 2.88 (m, 1H), 2.82–2.75 (m, 2H), 2.33 (dd, 1H, J=16.2, 8.4 Hz), 1.94 (br s, 2H); ^{13}C NMR ($CDCl_3$): δ 172.8, 138.5, 137.5, 128.5, 128.4, 127.9, 127.8, 127.7, 80.8, 76.0, 74.0, 71.7, 69.0, 51.6, 51.2, 47.2, 36.0; HRFABMS: Calcd for $C_{22}H_{28}NO_5$ (MH^+), *m*/*z* 386.1967; Found 386.2025.

3.1.13. 1-*C*-Allyl 5-azido-2,3-di-*O*-benzyl-5-deoxy-α-D-ribofuranoside (7). A solution of **1c** (300 mg, 0.76 mmol) in 0.1% MeONa–MeOH (10 mL) was stirred for 1 h, and then neutralized by the addition of Dowex 50WX2-100 (H^+) resin. The filtrate was concentrated to a residue, which was dissolved in CH_2Cl_2 (15 mL) and triethyl amine (1 mL, 6.9 mmol). To the solution was added MsCl (174 mg, 1.52 mmol) at 0 °C. The mixture was stirred overnight at 0 °C to room temperature, and then quenched by the addition of aq $NaHCO_3$. Routine work-up and chromatographic purification (Hexane/EtOAc 4:1) afforded 5-OMs product. A mixture of the above product and NaN_3 (210 mg, 3.2 mmol) in DMF (10 mL) was stirred at 80 °C overnight. Routine work-up and chromatographic purification (Hexane/EtOAc 6:1) gave **7** as a syrup (170 mg, 59%). $[\alpha]_D$ +83.7 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.36–7.28 (m, 10H), 5.79 (m, 1H), 5.10 (m, 2H), 4.83 (d, 1H, J= 11.6 Hz), 4.68 (d, 1H, J=11.6 Hz), 4.61 (d, 1H, J=11.6 Hz), 4.51 (d, 1H, J=11.6 Hz), 4.21 (dt, 1H, J=7.6, 3.6 Hz), 4.06 (dt, 1H, J=3.2, 7.2 Hz), 4.02 (dd, 1H, J=4.0 Hz), 3.98 (m, 1H), 3.59 (dd, 1H, J=12.8, 3.2 Hz), 3.16 (dd, 1H, J= 13.2, 3.6 Hz), 2.49 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 138.4, 137.7, 134.7, 128.6, 128.4, 128.1, 127.9, 127.8, 117.1, 80.7, 80.4, 78.8, 77.5, 73.6, 73.0, 52.1, 34.3; HRFABMS: Calcd for $C_{22}H_{25}O_3N_3Na$ ($M+Na^+$), *m*/*z* 402.1794; Found 402.1874.

3.1.14. 1-*C*-Acetylmethyl 5-azido-2,3-di-*O*-benzyl-5-deoxy-α-D-ribofuranoside (8). To a solution of **7** (160 mg, 0.42 mmol), $Hg(OAc)_2$ (96 mg, 0.3 mmol), and water (1 mL) in acetone (4 mL) was added 2 mL of Jones reagent (prepared by dissolving 2 mL of H_2SO_4 and 2 g of $Na_2Cr_2O_7 \cdot 2H_2O$ in 6 mL of water). The reaction mixture was stirred at room temperature for 4 h. The reaction mixture was poured into water (10 mL) and extracted by EtOAc, the organic phase was washed with water and brine. Purification by column (Hexane/EtOAc 4:1) gave **8** as a syrup (133 mg, 80%). $[\alpha]_D$ +83.3 (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.36–7.28 (m, 10H), 4.80 (d, 1H, J=11.2 Hz), 4.69 (d, 1H, J=12.0 Hz), 4.54 (d, 1H, J=12.0 Hz), 4.48 (m, 1H), 4.46 (d, 1H, J=11.2 Hz), 4.15 (m, 2H), 3.99 (dd, 1H, J=8.0, 3.6 Hz), 3.52 (dd, 1H, J=12.8, 3.2 Hz), 3.18 (dd, 1H, J=12.8, 3.6 Hz), 2.92 (dd, 1H, J=17.6, 7.6 Hz), 2.82 (dd, 1H, J=17.6, 6.0 Hz), 2.07 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 207.2, 138.2, 137.6, 128.7, 128.5, 128.2, 128.0, 127.9, 80.5, 78.6, 77.5, 76.5, 73.9, 73.1, 52.2, 43.9, 30.6; HRFABMS: Calcd for $C_{22}H_{25}O_4N_3Na(M+Na^+)$, *m*/*z* 418.1743; Found 418.1674.

3.1.15. 1-*C*-Acetylmethyl 2,3-di-*O*-benzyl-5-imino-5-deoxy-β-D-ribopyranoside (9). A mixture of **8** (120 mg, 0.3 mmol) and 10% Pd–C (50 mg) in methanol (10 mL) was stirred under H_2 atmosphere (balloon pressure) for 20 min when the starting material was disappeared. The reaction mixture was filtered and the filtrate was concentrated to a residue. The above residue was dissolved in 4% NaOMe/MeOH (10 mL) and stirred overnight. NH_4Cl (0.2 g) was added and the mixture was evaporated. The remaining residue was dissolved in water, extracted with EtOAc. Purification by chromatography (EtOAc) produced **9** as a syrup (83 mg, 75%). $[\alpha]_D$ −38.8 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.39–7.23 (m, 10H), 5.00 (d, 1H, J=11.2 Hz), 4.74 (d, 1H, J=11.6 Hz), 4.59 (d, 1H, J=11.2 Hz), 4.50 (d, 1H, J=11.6 Hz), 4.05 (s, 1H), 3.59 (m, 1H), 3.36 (dt, 1H, J=8.8, 3.2 Hz), 3.23 (d, 1H, J=9.2 Hz), 2.88–2.76 (m, 3H), 2.39 (dd, 1H, J=17.2, 8.8 Hz), 2.20 (br, 2H), 2.09 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 208.6, 138.7, 138.1, 137.7, 128.6, 128.1, 127.8, 127.7, 81.0, 76.2, 74.2, 71.8, 69.1, 50.7, 47.4, 45.4, 30.5; HRFABMS: Calcd for $C_{22}H_{28}O_4N$ (MH^+), *m*/*z* 370.2019; Found: 370.2042.

3.1.16. 1-*C*-Allyl 5-azido-5-deoxy-2,3,6-tri-*O*-benzyl-α-D-galactofuranoside (12). To a solution of **10** (450 mg, 0.95 mmol) and pyridine (210 mg, 2.65 mmol) in CH_2Cl_2 (10 mL) was added Tf_2O (490 mg, 1.73 mmol) at 0 °C and the solution was stirred at room temperature for 5 h. The reaction was quenched by the addition of water, extracted with CH_2Cl_2. The organic phase was washed with water, dried, and concentrated to yellowish oil. A mixture of the above oil and sodium azide (330 mg, 5.08 mmol) in DMF (10 mL) was stirred overnight at room temperature. The reaction mixture was diluted by ethyl ether (50 mL) and the organic phase was washed with water and brine. Purification

by chromatography (Hexane/EtOAc 8:1) gave **12** as a syrup (410 mg, 86%). $[\alpha]_D$ −63.0 (*c* 0.4, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.39–7.21 (m, 15H), 5.81 (m, 1H), 5.09 (m, 2H), 4.56–4.41 (m, 6H), 3.98 (m, 2H), 3.92 (dd, 1H, J=4.8, 3.6 Hz), 3.81 (d, 1H, J=3.6 Hz), 3.61 (m, 3H), 2.50 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 137.9, 137.7, 137.6, 134.7, 128.6, 128.5, 128.2, 128.0, 127.9, 127.8, 127.7, 117.1, 84.0, 83.6, 82.6, 81.3, 73.5, 71.8, 71.6, 70.0, 62.1, 33.2; HRFABMS: Calcd for $C_{30}H_{34}O_4N$ ($MH^+ - N_2$), *m*/*z* 472.2488; Found: 472.2407.

3.1.17. 1-*C*-Acetylmethyl 5-azido-5-deoxy-2,3,6-tri-*O*-benzyl-α-D-galactofuranoside (15). The same procedures as described for the preparation of **8** were used to obtain **15** in 81% yield. $[\alpha]_D$ −40.6 (*c* 1.3, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.38–7.21 (m, 15H), 4.51 (m, 4H), 4.44–4.34 (m, 3H), 4.03 (d, 1H, J=4.0 Hz), 4.01 (dd, 1H, J=4.0, 0.4 Hz), 3.90 (m, 1H), 3.58 (m, 3H), 2.88 (m, 2H), 2.12 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 207.0, 137.7, 137.5, 128.7, 128.6, 128.1, 128.0, 127.9, 127.8, 83.88, 83.81, 82.8, 77.5, 73.5, 72.0, 71.8, 69.9, 61.9, 42.9, 30.8; HRFABMS: Calcd for $C_{30}H_{33}O_5N_3Na$ ($M+Na^+$), *m*/*z* 538.2318; Found: 538.2320.

3.1.18. 1-*C*-Acetylmethyl 2,3,6-tri-*O*-benzyl-5-imino-5-deoxy-α/β-D-galactopyranoside (16α/16β). The same procedures as described for the preparation of **9** were used for the transformation of **15** to a mixture of **16α** and **16β**, with a 1:1 ratio, in 84% yield. For **16α**: $[\alpha]_D$ +9.3 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.38–7.21 (m, 15H), 4.69 (s, 2H), 4.64 (d, 1H, J=11.6 Hz), 4.56 (d, 1H, J=11.6 Hz), 4.48 (s, 2H), 3.99 (s, 1H), 3.84 (m, 2H), 3.62 (dd, 1H, J=9.2, 5.6 Hz), 3.52 (m, 1H), 3.45 (dd, 1H, J=8.8, 2.8 Hz), 2.94 (dd, 1H, J=5.6 Hz), 2.77 (dd, 1H, J=17.2, 4.4 Hz), 2.59 (br s, 1H), 2.55 (dd, 1H, J=16.8, 8.4 Hz), 2.08 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 207.9, 138.5, 138.4, 138.1, 128.5, 128.4, 128.2, 128.0, 127.9, 127.8, 79.1, 77.3, 73.6, 73.2, 72.4, 70.9, 68.0, 52.0, 50.6, 40.4, 30.9; HRFABMS: Calcd for $C_{30}H_{36}O_5N$ (MH^+), *m*/*z* 490.2593; Found: 490.2641. For **16β**: $[\alpha]_D$ −3.8 (*c* 0.4, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.39–7.22 (m, 15H), 4.96 (d, 1H, J=11.2 Hz), 4.75 (d, 1H, J=11.6 Hz), 4.65 (d, 1H, J=10.4 Hz), 4.57–4.50 (m, 3H), 4.05 (s, 1H), 3.61 (dd, 1H, J=9.2, 5.2 Hz), 3.53 (m,1H), 3.47 (m, 2H), 2.95 (dt, 1H, J=8.8, 2.8 Hz), 2.90–2.83 (m, 2H), 2.50 (br, 1H), 2.43 (dd, 1H, J=17.2, 8.8 Hz), 2.06 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 208.1, 138.5, 138.1, 128.6, 128.5, 128.2, 128.0, 127.9, 84.5, 79.3, 75.3, 73.6, 71.6, 70.8, 66.9, 57.4, 55.9, 46.1, 30.4; HRFABMS: Calcd for $C_{30}H_{36}O_5N$ (MH^+): *m*/*z* 490.2593; Found: 490.2611.

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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.2005.09.037. NMR spectra for compounds **5a–c**, **6a–c**, **7–9**, **12**, **15**, **16α** and **16β**.

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Isolation and structure of five lyngbyabellin derivatives from a Papua New Guinea collection of the marine cyanobacterium *Lyngbya majuscula*

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Abstract—Five new lyngbyabellin analogs along with a known compound, dolabellin, have been isolated from the marine cyanobacterium *Lyngbya majuscula* collected from Papua New Guinea. The structures of lyngbyabellins E–I were elucidated through extensive spectroscopic analysis, including HR-FABMS and 1D and 2D NMR experiments. The absolute configurations of lyngbyabellin E and H were ascertained by chiral HPLC and GC/MS analysis of degradation products, in combination with NMR experiments. All five lyngbyabellins showed cytotoxicity to NCI-H460 human lung tumor and neuro-2a mouse neuroblastoma cell lines with LC_{50} values between 0.2 and 4.8 μM.
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1. Introduction

Cyanobacteria are phenomenal producers of structurally intriguing and biologically active secondary metabolites.[1,2] The pantropical marine cyanobacterium *Lyngbya majuscula* Gomont (Oscillatoriaceae) is one of the more prolific producers of interesting secondary metabolites, yielding no fewer than 200 reported compounds.[1] As part of an effort to discover new and biologically active natural products, we now report the isolation and structure elucidation of a series of new lyngbyabellin analogs, lyngbyabellins E–I (**1**–**5**), and the known compound dolabellin (**6**), originally isolated from the sea hare *Dolabella auricularia*.[3] The sea hare, *D. auricularia* has yielded several cytotoxic agents structurally similar to cyanobacterial counterparts, including the anticancer agent dolastatin-10 and the cytotoxic agent, dolabellin.[3,4] The isolation of dolastatin-10 and related compounds, including symplostatin-1, from the marine cyanobacterium *Symploca spp.*,[5] and the dolabellin-like compounds, lyngbyabellins A–D and hectochlorin (**7**)[6–10] from *L. majuscula*, indicates that these sea hares sequester bioactive metabolites from their cyanobacterial diet.

2. Results and discussion

A shallow water (1–3 m) strain of *L. majuscula* was collected by hand from Alotau Bay, Papua New Guinea, in 2002. The alga was extracted with CH_2Cl_2/MeOH 2:1 and fractionated by silica gel vacuum liquid chromatography. Preliminary bioassay showed strong toxicity in the brine shrimp model ($LD_{50} \sim 1$ ppm) in the EtOAc/hexanes 9:1 eluted fraction. Guided by this assay, this fraction was further chromatographed over a Mega Bond RP_{18} solid-phase extraction (SPE) cartridge and then via reversed-phase HPLC to afford five new lyngbyabellin analogs E–I (**1**–**5**), plus dolabellin (**6**) (Fig. 1).

The molecular formula of lyngbyabellin E (**1**) was determined as $C_{37}H_{51}Cl_2N_3O_{12}S_2$ on the basis of HR-FABMS and NMR spectral data (Table 1). The ratio of $[M+H]^+$ isotope peaks, 5:4:1, at *m/z* 862/864/866, clearly indicated the presence of two chlorine atoms. From the ^1H and ^{13}C NMR data, six carbonyls, two carbon–carbon double bonds, and two additional carbon-heteroatom double bonds accounted for ten of the thirteen degrees of unsaturation implied by the molecular formula. Two downfield ^1H singlets at δ 8.12 (H-12) and 8.27 (H-18), HSQC-correlated to carbons at δ_C 128.7 and 130.1, were consistent with the presence of two 2,4-disubstituted thiazole rings, which assigned two of the remaining three degrees of unsaturation. The final degree of unsaturation

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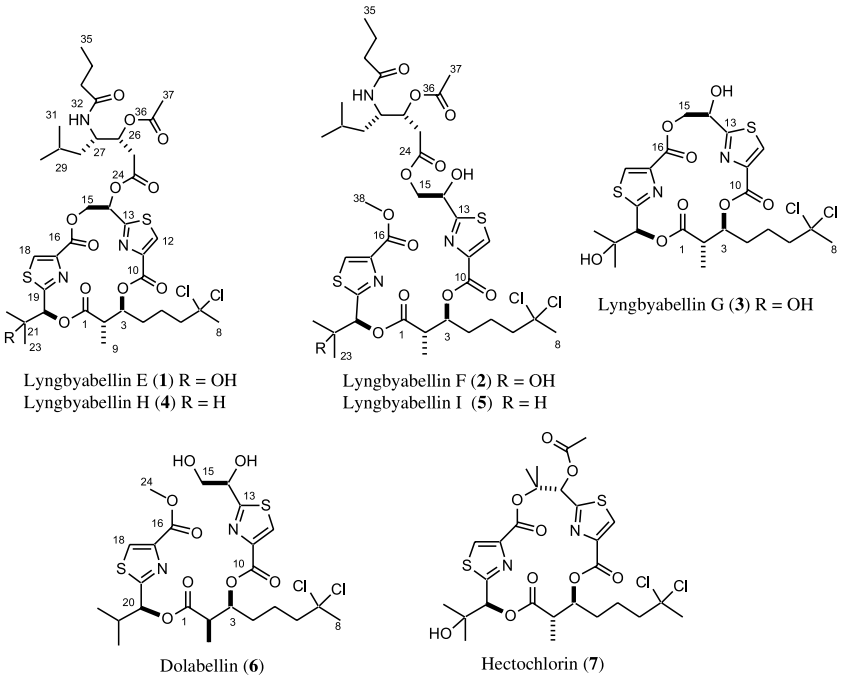

Figure 1. Structures of lynbyabellins E–I (**1**–**5**), dolabellin (**6**), and hectochlorin (**7**) [numbering scheme for compounds **1**, **2**, **4**, and **5** based on lyngbyabellin D].[9]

could be accounted for by an additional ring within the structure of **1**.

The two thiazole ring structures were confirmed by HMBC correlations from H-12 to C-11 (δ_C 146.4) and C-13 (δ_C 165.9), and H-18 to C-17 (δ_C 145.9) and C-19 (δ_C 165.0), respectively (Table 1). Furthermore, three-bond correlations from H-12 and H-18 to conjugated carbonyl carbons at δ_C 161.2 (C-10), and 161.9 (C-16), respectively, were indicative of carboxylic acid derived functionalities attached to these heterocycles. HMBC correlations from a methine triplet at δ 6.45 (H-14) to the thiazole C-13 (δ_C 165.9) and an oxymethylene carbon at δ 64.4 (C-15) established a 2-(1,2-dihydroxyethyl)thiazole-4-carboxylate unit. The second thiazole ring formed a 2-(1,2-dihydroxy-2-methylpropyl)thiazole-4-carboxylate unit as well based on HMBC correlations from an oxymethine singlet at δ 5.65 (H-20) to thiazole C-19 (δ_C 165.0) and an oxygenated quaternary carbon at δ 72.1 (C-21), which in turn showed three-bond correlations to two methyl singlets (δ 1.18 and 1.34, H_3-22 and H_3-23, respectively).

Inspection of the ^{1}H NMR spectrum of **1** revealed a series of upfield and highly coupled resonances indicative of an aliphatic chain. A downfield methyl singlet at δ_H 2.09 (H_3-8) showed HMBC correlations to a quaternary carbon at δ_C 90.4 (C-7) and a methylene carbon at δ_C 49.3 (C-6). The chemical shift of C-7 was indicative of a *gem*-dichloro subtituent as observed in dolabellin (**6**), hectochlorin (**7**) (Fig. 1), and lyngbyabellins A–D. HSQC-TOCSY was used to extend this moiety to include an additional six carbons (C-2–C-6, and C-8), identifying this unit as 7,7-dichloro-3-acyloxy-2-methyloctanoate (DCAMO). Further analysis of 2D NMR data identified acetate and butyrate moieties as well.

The remaining unassigned $C_8H_{14}NO_2$ was clearly attached to C-14 via an ester linkage, based on a $^3J_{CH}$ correlation from H-14 to C-24. A $^1J_{CH}$ from H-26 (δ 5.17) to a downfield carbon at 72.6 ppm indicated that this carbon was oxygenated, while COSY correlation to the methylene protons (H_2-25) and two HMBC correlations from H-26 to carbonyls at 169.2 and 170.6 (C-24, C-36) established the acetylated structure depicted (Fig. 1). An exchangeable amide proton signal at δ 5.60 (27-NH) showed a strong COSY correlation to H-27, which was expanded into a modified leucine unit (C-24 to C-31) based on the 2D NMR correlations.

The HMBC data summarized in Table 1 allowed connection of the partial structures and functional groups described above. Although the ester linkage between C-3 and C-10 was not indicated by the HMBC experiments, the ^{1}H and ^{13}C NMR shift data were strongly supportive of this remaining linkage, and were needed to account for the final degree of unsaturation indicated by the molecular formula. The planar structure of lyngbyabellin E is thus, represented by **1**, and closely related to the recently reported structure of lyngbyabellin D.[9]

Compound **1** posed a number of interesting problems in the assignment of stereochemistry. (*S*)- and/or (*R*)-α,β-dihydroxyisovaleric acid (DHIV) could not be detected in the acid hydrolyzate by comparison with the retention times of synthetic standards,[11] and was apparently unstable under the conditions of acid hydrolysis. However, ozonolysis and subsequent base hydrolysis at 90 °C afforded (*S*)-α,β-dihydroxyisovaleric acid, without any detectable racemization, as demonstrated by chiral GC–MS analysis of both synthetic (*S*)- and (*R*)-DHIV methyl esters and the hydrolyzed/methylated natural product. Thus, C-20 in compound **1** possessed an *S* configuration, which is identical to that found in the DHIV unit of lyngbyabellin A[6] and hectochlorin.[10] Chiral HPLC following base hydrolysis of **1** firmly established the stereochemistry of the glyceric acid

Table 1. NMR Data for Lyngbyabellin E (**1**) in $CDCl_3$

Lyngbyabellin E (**1**)			
Position	δ_C	δ_H (J in Hz)	HMBC[a]
1	174.4		
2	43.3	3.00, dq (9.5, 7.5)	1, 3, 9
3	74.6	5.26, m	2, 4
4	31.4	1.80, 1.75, m	3, 5
5	20.9	1.75, 1.73, m	6
6	49.3	2.23, 2.15, m	5, 7
7	90.4		
8	37.6	2.09, s	6, 7
9	14.4	1.25, d (7.5)	1, 2, 3
10	159.6		
11	146.4		
12	128.7	8.12, s	10, 11, 13
13	165.9		
14	70.6	6.45, t (5.7)	13, 15, 24
15	64.4	4.99, dd (11.4, 5.4); 4.50, dd (11.4, 7.6)	13, 14, 16
16	160.5		
17	145.9		
18	130.1	8.27, s	16, 17, 19
19	165.0		
20	76.0	5.65, s	1, 19, 21
21	72.1		
21-OH		3.63, br s	
22	27.4	1.18, s	
23	26.1	1.34, s	20, 21, 22
24	169.1		
25	36.5	2.81, dd (16.0, 4.9); 2.74, dd (16.1, 7.7)	24, 26, 27
26	72.6	5.17, ddd (4.9, 5.5, 7.7)	27, 36
27	49.2	4.32, m	26, 28
27-NH		5.60, d (9.4)	
28	40.0	1.28, m	27, 29, 30
29	25.0	1.65, m	28, 30, 31
30	21.6	0.90, d (6.5)	28, 29, 31
31	23.8	0.93, d (6.3)	28, 29, 30
32	173.1		
33	38.9	2.12, t (7.2)	32, 34, 35
34	19.4	1.62, m	32, 33, 35
35	13.9	0.89, t (7.2)	33, 34
36	170.6		
37	21.2	2.05, s	36

[a] Proton showing HMBC correlation to indicated carbon.

moiety, and thus, lyngbyabellin E possessed an *R* configuration at C-14. There are few reports of and no general method for determining the configuration of γ-amino-β-hydroxy acids. However, reports in the literature have indicated that 4-amino-3-hydroxy-5-methylhexanoic acid, such as found in the lyngbyabellin D, undergoes an epimerization at C-3 via an acid-catalyzed dehydration/hydration sequence, and that significant quantities of the intermediate α,β-unsaturated acid are produced upon prolonged hydrolysis.[9,12] This suggested that the absolute configuration of C-27 could be determined by acid hydrolysis, subsequent ozonolysis, and oxidative work-up to D- or L-leucine. Indeed, this reaction sequence gave leucine, and chiral HPLC showed unambiguously that C-27 was derived from L-leucine. Knowing the absolute configuration at C-27, the stereochemistry at C-26 was defined using homonuclear coupling constant information and selective 1D NOE experiments in combination with a systematic analysis of all energetically reasonable (staggered) rotamers (Fig. 2). NOE enhancement of the resonances for H-26 and H_2-25 was observed following low power irradiation at δ 4.32 (H-27), ruling out model A2, A3, B2, and B3 (Fig. 2). The ^1H–^1H coupling constant of 5.5 Hz between H-26 and H-27 suggested a *gauche* relationship between these two protons, consequently again ruling out models A3 and B2. Irradiation at δ 5.60 (NH-27) led to NOE enhancement of the signals for H-26 (ruling out model A1 and again B3), H-27, H_2-28, H-29 and H_2-33. The remaining model B1 fulfilled all criteria, and thus, indicated a 26*R*, 27*S* configuration for the γ-amino-β-hydroxy acid residue of lyngbyabellin E (**1**).

The relative stereochemistry of the polyketide-derived β-hydroxy acid (DCAO) was assigned from NOE and hetero half-filtered TOCSY (HETLOC) experiments.[13] NOE correlations were observed between H-3 and CH_3-9, and between CH_3-9 and H_2-4. In combination with a large ^3J_H coupling of 9 Hz between H-2 and H-3, these data defined an anti relationship between H-2 and H-3 (e.g., -*S*,*S* or *R*,*R* configuration). In the HETLOC spectrum, a small heteronuclear coupling of $^3J_{H3-C9} = -3.0$ Hz was consistent with a gauche arrangement of H-3 and CH_3-9, while a larger $^2J_{H2-C3}$(5.2 Hz) also implied a gauche relationship between H-2 and the oxygen of the ester linked (dihydroxyethyl)thiazole carboxylate moiety. Other J_{CH} values for **1** were 0.3 Hz (H-3/C-2), −3.0 Hz (H-3/C-9), 2.6 Hz (H-9/C-2), and −5.3 Hz (H-9/C-3). All of these data were comparable to those for hectochlorin (**7**), the 2*S*,3*S* configuration of which was determined by X-ray crystallographic analysis.[10] Conformational analysis of both **1** and **7** by standard methods (MM2*), and based on the crystal structure of **7**, resulted in a good structural overlay with the energetically favored 2*S*, 3*S* configuration in **1**. Therefore, and recognizing that all other members of this natural product family possess a 3*S* configuration,[7–10,12] these data support the stereochemical assignment of lyngbyabellin E (**1**) as 2*S*, 3*S*, 14*R*, 20*S*, 26*R*, and 27*S*.

Lyngbyabellin F (**2**) was isolated by RP-HPLC from a slightly more polar fraction than that containing lyngbyabellin E (**1**). Its high structural homology to **1** was evident from nearly identical ^1H and ^{13}C NMR chemical shifts (see Supplementary Material). However, the molecular formula of **2** was established as $C_{38}H_{55}Cl_2N_3O_{13}S_2$ by HR-FABMS, indicating twelve degrees of unsaturation. These could be ascribed to six carbonyls, two carbon–carbon double bonds, two additional carbon-heteroatom double bonds, and two rings. The presence of a methoxy singlet at δ 3.95 (H-38) in conjunction with a free hydroxyl group at C-14 (Supplementary Material), suggested the linear nature of **2**. Moreover, C-24 (169.4 ppm) was clearly attached to the β-hydroxyl group of the glyceric acid-derived moiety via an ester linkage, rather than to the α-hydroxyl group as in structure **1**, based on an HMBC correlation from H-15 to C-24. Because this structure accounted for all twelve degrees of unsaturation, **2** was a linear lipopeptide, structurally similar to the previously isolated lyngbyabellin D.[9]

High-resolution FABMS analysis of lyngbyabellin G (**3**) revealed a $[M+H]^+$ at 595.0733, consistent with a molecular formula of $C_{23}H_{29}Cl_2N_2O_8S_2$, thus, requiring 10 degrees of unsaturation. Careful examination of 1D and 2D NMR data for **3** indicated that it possessed the same structure as **1** for C-1 through C-23, but lacked the C-24 to C-37 side chain. In this respect, the planar structure of **3** was almost identical to the previously reported lynbyabellin C,[8] which has an α,β-dihydroxy-β-methylpentanoic acid unit

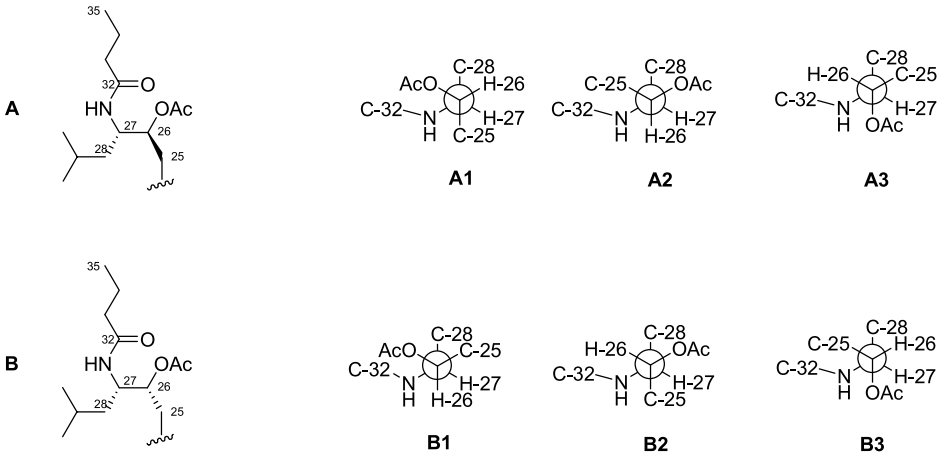

Figure 2. Diagram of all energetically reasonable rotamers (staggered) for the two possible C-26 epimers 26*S*,27*S* (A series) and 26*R*,27*S* (B series) of lyngbyabellin E (**1**).

instead of the α,β-dihydroxyisovaleric acid present in **3**. Intriguingly, one ester linkage of lyngbyabellin E (**1**) appeared to be particularly prone to methanolysis, as treatment of **1** with MeOH and H_2O caused it to slowly convert to **3** by regioselective ester cleavage at C-14. Hence, lyngbyabellin G may be an artifact of **1** from storage of the collection in ethanol and seawater. Hydrolysis and stereoanalysis of **2** and **3** were not undertaken due to their limited quantity, however, because of the comparable spectroscopic properties of **1**, **2**, and **3**, we propose that they are of the same configurational series.

HR-FABMS of lyngbyabellin H (**4**) indicated its molecular formula of $C_{37}H_{51}Cl_2N_3O_{11}S_2$, which is one less oxygen than in lyngbyabellin E (**1**). 1D NMR data comparisons indicated that lyngbyabellin H (**4**) was also closely related to **1**. However, it displayed obvious differences in the α,β-dihydroxyisovaleric acid (dhiv) unit in that the singlet at δ 5.65 for H-20 in **1** was replaced by a doublet at δ 5.52 in the spectrum for **4**. Moreover, the oxygenated quaternary carbon C-21 (δ 72.1) in **1** was replaced by an upfield methine carbon (δ 32.5) in **4**, thus indicating that this residue was 2-hydroxyisovaleric acid (hiva). From extensive 2D NMR analysis, the planar structure of lyngbyabellin H (**4**) was otherwise the same as lyngbyabellin E (**1**), and conceptually, also a precursor of dolabellin (**6**), the original compound in the series discovered from the sea hare *D. auricularia*. Ester bond cleavage at C-24 in **4** may occur in the acidic digestive glands of the sea hare to first yield the cyclized form of dolabellin, and subsequent methanolysis at any stage of the storage or isolation procedure would yield dolabellin (**6**). Chemical conversion of diet-derived metabolites in the digestive glands of sea hares has been observed previously (e.g., laurinterol into aplysin in *Aplysia californica*).[14] In partial support of this hypothesis, we isolated trace quantities of dolabellin (**6**) or its C-2 stereoisomer, along with these new lyngbyabellins E (**1**)–I (**5**). Due to the very small quantity of **6** isolated, 0.08 mg, its identity was established only by comparison of ^{1}H NMR and HR-TOFMS with literature data[3] and by MS/MS experiments; stereochemical aspects were not investigated. Hence, we are uncertain if the dolabellin obtained in this study has the 2*S* stereochemistry present in the co-metabolites **1**–**5**, or the 2*R* stereochemistry observed in the originally reported dolabellin (**6**).

Lyngbyabellin I (**5**), the proposed methanolysis product of lyngbyabellin H (**4**)(see discussion below), was also isolated from this *L. majuscula* crude extract. From NMR data in conjunction with HR FABMS *m/z* $[M+H]^+$880.2639 (calcd for $C_{38}H_{56}Cl_2N_3O_{12}S_2$, 880.2682), the planar structure of **5** appeared to be nearly identical to lyngbyabellin F (**2**), except for a residue replacement of DHIV by HIVA as was the case for the structure of **4**. The absolute configurations of **4** at C-14, 20, 26 and 27 were determined as described above for lyngbyabellin E (**1**) by chiral HPLC and GC/MS analysis of degradation products, in combination with NMR analysis, which assigned the stereochemistry as 14*R*, 20*S*, 26*R*, and 27*S*. The absolute configurations at C-2 and C-3 of **4** were determined as 2*S*, 3*S* by comparison of coupling constants and chemical shifts with that of compound **1**. Due to the limited amount of compound **5**, the absolute stereochemistry was not established by chemical methods, however, we propose that it belongs to the same enantiomeric series as these other co-occurring metabolites.

The lyngbyabellins were tested for cytotoxicity to NCI-H460 human lung tumor and neuro-2a mouse neuroblastoma cells and had LC_{50} values between 0.2 and 4.8 μM (Table 2). Intriguingly, lyngbyabellin E (**1**) and H (**4**) appeared to be more active against the H460 cell line with LC_{50} values of 0.4 and 0.2 μM, respectively, compared to LC_{50} values of 1.2 and 1.4 μM in the neuro-2a cell line. Lynbyabellin I (**5**) was the most toxic to neuro-2a cells (LC_{50} 0.7 μM), whereas lyngbyabellin G (**3**), was the least cytotoxic of all compounds to either cell line. On the basis of this limited screening, it appears that lung tumor cell

Table 2. Cytotoxicity of compounds **1**–**5**

Compound	H460 LC_{50} (μM)	Neuro-2a LC_{50} (μM)
1	0.4	1.2
2	1.0	1.8
3	2.2	4.8
4	0.2	1.4
5	1.0	0.7

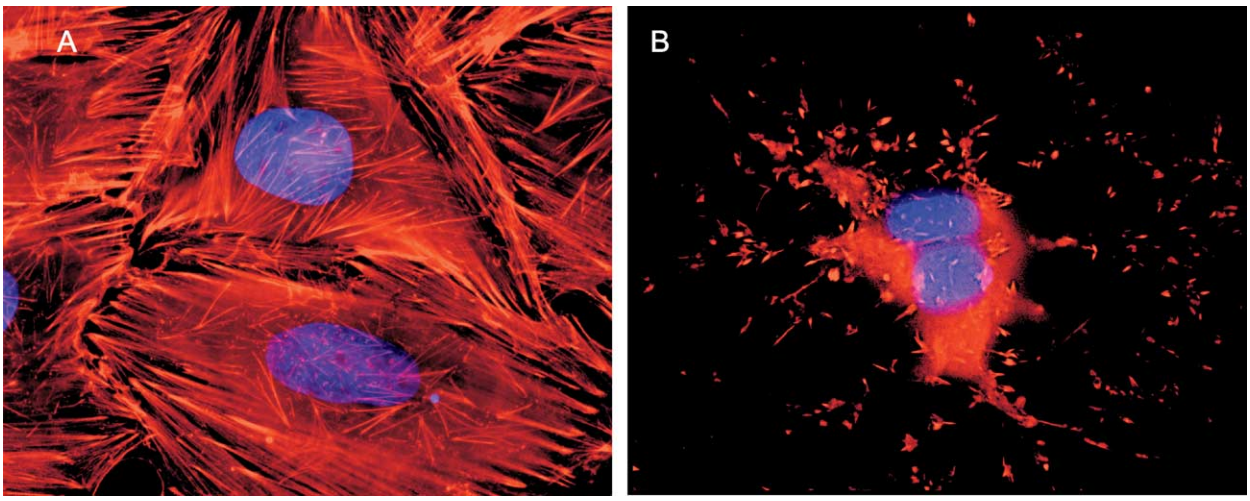

Figure 3. Effect of lyngbyabellin E (**1**) on the actin cytoskeleton of A-10 cells. After 24 h, cells were processed and exposed to the microfilament staining reagent TRITC-phalloidin (visualized as red in the figure) and to the DNA-reactive compound DAPI (visualized as blue). (A) control cells; (B) treatment of the cells with lyngbyabellin E (**1**) at 60 nM, which caused complete loss of the cellular microfilament network and generated binucleated cells.

toxicity is enhanced in the cyclic representatives with an elaborated side chain. However, that the acyclic compounds (lyngbyabellin F and I and dolabellin) are still relatively potent cytotoxins suggests that either 1) they may adopt a conformation similar to the cyclic forms at their biomolecular target, or 2) the acyclic forms are produced in vitro by intracellular esterases and represent the truly bioactive forms of these molecules. If this latter hypothesis is correct, then differences in potency between the cyclic and acylic forms could be due to differences in their cell permeability. Unfortunately, our results are difficult to compare with those obtained for lyngbyabellins A–D, dolabellin, and a few other analogs produced synthetically or semi-synthetically because different cell lines were used (e.g., much of this work is reported using the KB cell line, which is considerably more drug sensitive than those used in this report, the H-460 and neuro-2a cell lines).[3,6–10] However, the trend in the data supports the conclusion drawn above that an overall cyclic constitution is not required for potent cytotoxic properties in this drug class.

Because lyngbyabellin A and hectochlorin have been reported to be strong actin-disrupting agents,[6,10] the cytoskeletal-disrupting effects of **1** were also tested in this study. Lyngbyabellin E (**1**), at concentrations of 0.01–6.0 μM, disrupted the cellular microfilament network in A-10 cells (Fig. 3). Additionally, at the higher concentrations tested, many cells contained two nuclei, consistent with the inhibition of cytokinesis that often occurs after disruption of the microfilament network. The effects were specific for microfilaments, as there was no evidence of microtubule loss at these concentrations.

Biosynthetically, these lyngbyabellins may derive from an assembly by nonribosomal peptide synthetases (NRPS) and polyketide synthases (PKS).[1] Thus, the structural variation of the lyngbyabellins is intriguing and suggests the involvement of adenylation domains with relaxed substrate specificity, as observed from molecular genetics and biochemical studies of the closely related compound hectochlorin (**7**).[15] Hence, it can be envisaged that the appropriate adenylation domain accepts a dhiv unit as its substrate to form lyngbyabellins E–G (**1**–**3**) or it processes hydroxyisovaleric acid to give the non-hydroxylated lyngbyabellin derivatives H (**4**) and I (**5**). While speculative, it is intriguing to propose that lyngbyabellin F (**2**) may derive from methanolysis of lyngbyabellin E (**1**). If correct, then initial methanolysis of the ester bond would necessarily be followed by acyl group migration from C-14 to C-15. Moreover, this hypothesis supports the origin of lyngbyabellin I (**5**) from lyngbyabellin H (**4**), and dolabellin (**6**) from lyngbyabellin G (**3**).[8] The occurrence of dolabellin (**6**) or its C-2 stereoisomer in this cyanobacterial extract, as well as the close relationship of lyngbyabellins E–G (**1**–**5**) to dolabellin, further consolidates the hypothesis that these secondary metabolites are biosynthesized by marine cyanobacteria, and that sea hares incorporate these compounds as a consequence of their diet of cyanobacteria, occasionally with biotransformations occurring in this process.

3. Experimental

3.1. General

Optical rotations were measured on a Perkin–Elmer 141 polarimeter. IR and UV spectra were recorded on Nicolet 510 and Beckman DU640B spectrophotometers, respectively. NMR spectra were recorded on Bruker Avance DPX 400 MHz and Bruker Avance DPX 300 MHz spectrometers with the solvent $CDCl_3$ used as an internal standard (δ_H at 7.26, δ_C at 77.2). High resolution mass spectra were recorded on a Kratos MS-50 TC mass spectrometer while MS/MS experiments were conducted on a Waters Micromass LCT Classic. For chiral GC–MS, analysis was accomplished on a Hewlett-Packard gas chromatograph 5890 series II with a Hewlett-Packard 5971 mass selective detector using an Alltech capillary column (CHIRASIL-VAL phase 25 m×0.25 mm). HPLC was performed using Waters 515 HPLC pumps and a Waters 996 photodiode array detector. Asymmetric dihydroxylation reagents, AD-mix-α and AD-mix-β, were purchased from Aldrich.

3.2. Collection

The marine cyanobacterium *L. majuscula* (voucher specimen available from WHG as collection number PNG5-27-02-1) was collected by hand from shallow waters (1–3 m) in Alotau Bay, Papua New Guinea, on May 7, 2002. The material was stored in 2-propanol at −20 °C until extraction.

3.3. Extraction and isolation

Approximately 138 g (dry wt) of the alga were extracted repeatedly with CH_2Cl_2/MeOH 2:1 to produce 3.05 g of crude organic extract. The extract (3.0 g) was fractionated by silica gel vacuum liquid chromatography using a stepwise gradient solvent system of increasing polarity starting from 10% EtOAc in hexanes to 100% MeOH. The fraction eluting with 100% MeOH was found to be active at 1 ppm in the brine shrimp toxicity assay. This fraction was further chromatographed on Mega Bond RP_{18} solid-phase extraction (SPE) cartridges using a stepwise gradient solvent system of decreasing polarity starting from 80% aqueous MeOH to 100% MeOH. The most active fractions after SPE (85% toxicity at 1 ppm to brine shrimp) were then purified by HPLC [Phenomenex Sphereclone 5 μ ODS (250×10 mm), 9:1 MeOH/H_2O, detection at 211 nm] giving compounds **1** (7.0 mg), **2** (0.7 mg), **3** (0.7 mg), **4** (0.7 mg), **5** (0.5 mg), and **6** (0.08 mg).

3.3.1. Lyngbyabellin E (1). Colorless amorphous solid; $[\alpha]^{26}_D$ −31 (*c* 0.70, MeOH); UV (MeOH) λ_{max} 240 nm (log ε 4.18); IR (neat) 3365, 2926, 2875, 1739, 1644, 1454, 1243, 1030 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HR FABMS *m/z* $[M+H]^+$ 864.2373 (calcd for $C_{37}H_{52}Cl_2N_3O_{12}S_2$, 864.2370).

3.3.2. Lyngbyabellin F (2). Colorless amorphous solid; $[\alpha]^{26}_D$ −6.5 (*c* 0.20, MeOH); UV (MeOH) λ_{max} 238 nm (log ε 4.0); IR (neat) 3365, 2928, 2873, 1731, 1650, 1455, 1232, 1098 cm^{-1}; ^1H and ^{13}C NMR data, see Supplementary Material; HR FABMS *m/z* $[M+H]^+$ 896.2633 (calcd for $C_{38}H_{56}Cl_2N_3O_{13}S_2$, 896.2631).

3.3.3. Lyngbyabellin G (3). Colorless amorphous solid; $[\alpha]^{26}_D$ −26 (*c* 0.20, MeOH); UV (MeOH) λ_{max} 238 nm (log ε 3.75); IR (neat) 3398, 2925, 2853, 1732, 1484, 1233, 1097 cm^{-1}; ^1H and ^{13}C NMR data, see Supplementary Material; HR FABMS *m/z* $[M+H]^+$ 595.0733 (calcd for $C_{23}H_{29}Cl_2N_2O_8S_2$, 595.0742).

3.3.4. Lyngbyabellin H (4). Colorless amorphous solid; $[\alpha]^{26}_D$ −53 (*c* 0.08, MeOH); UV (MeOH) λ_{max} 242 nm (log ε 4.15); IR (neat) 3305, 2959, 2852, 1735, 1649, 1470, 1238, 1073 cm^{-1}; ^1H and ^{13}C NMR data, see Supplementary Material; HR FABMS *m/z* $[M+H]^+$ 848.2458 (calcd for $C_{37}H_{52}Cl_2N_3O_{11}S_2$, 848.2420).

3.3.5. Lyngbyabellin I (5). Colorless amorphous solid; $[\alpha]^{26}_D$ −25 (*c* 0.04, MeOH); UV (MeOH) λ_{max} 238 nm (log ε 4.28); IR (neat) 3309, 2960, 2852, 1740, 1650, 1461, 1233, 1096 cm^{-1}; ^1H and ^{13}C NMR data, see Supplementary Material; HR FABMS *m/z* $[M+H]^+$ 880.2639 (calcd for $C_{38}H_{56}Cl_2N_3O_{12}S_2$, 880.2682).

3.4. Absolute stereochemistry of Lyngbyabellin E (1)

A stream of ozone was bubbled through 0.8 mg of **1** dissolved in 2 mL of CH_2Cl_2 at −78 °C until the solution turned pale blue (ca. 1 min). The solvent was removed under a stream of N_2, and 0.3 mg of the sample was hydrolyzed with 2 N NaOH at 90 °C for 7 h. This was analyzed by chiral HPLC [column Phenomenex Chirex 3126 (D), 4.6× 50 mm; 0.8 mL/min; detection at 254 nm]. The retention times (min) of the commercially available D-glyceric and L-glyceric acid were 8.2 and 6.2 min, respectively, with a solvent system of 0.5 mM $CuSO_4$ in MeCN/H_2O 5:95. The base hydrolysate was found to contain D-glyceric acid (8.2 min), which was confirmed by coinjection of the appropriate standard. The presence of *S*-α,β-dihydroxyisovaleric acid in **1** was determined by chiral GC–MS analysis. Standard *R*- and *S*-dhiv methyl esters were synthesized by asymmetric dihydroxylation of methyl 3,3-dimethylacrylate using AD-mix-α and AD-mix-β, respectively.[9,11] Capillary GC–MS analysis was conducted using a Chirasil-Val column (Alltech, 25 m×0.25 mm) using the following conditions: column temperature was set at 70 °C for 3 min, and was increased from 70 to 100 °C (3 °C/min), then 100 to 200 °C (15 °C/min). The retention time of the methylated dhiv residue derived from ozonolysis and saponification of **1**, followed by methylation (CH_2N_2) matched that of the methylated *S*-dhiv standard (25.0 min) but not the methylated *R*-dhiv standard (24.7 min).

Another portion of the ozonized sample of **1** (0.5 mg) was hydrolyzed at 118 °C for 24 h in 6 N HCl. The acid was removed under a stream of N_2 and the residue ozonized for 30 min in 2 mL of methanol at −78 °C. After removal of the solvent, the residue was dissolved in 2:1 98% formic acid and 30% H_2O_2, and left to stir overnight before refluxing for 1 h at 100 °C. The solvent was removed and the sample was analyzed by chiral HPLC [Phenomenex Chirex 3126 (D), 4.6×250 mm; 0.8 mL/min; detection at 254 nm]. The retention time (min, % CH_3CN/2 mM $CuSO_4$) of the standards, were L-Leu (15.2, 15%), D-Leu (16.5, 15%). The hydrolysate showed a peak coincident with the L-Leu standard (15.2 min).

3.5. Absolute stereochemistry of Lyngbyabellin H (4)

A sample of **4** (0.4 mg) was ozonized as described above for the lyngbyabellin E (**1**). Approximately one half of the sample was hydrolyzed with 2 N NaOH, followed by chiral HPLC analysis to confirm the assignments of D-glyceric acid (8.2 min), and L-hiva (9.2 min). The remainder of the sample was subjected to acid hydrolysis, ozonolysis, and oxidative workup to give L-leucine (15.2 min) by chiral HPLC analysis as described above.

3.6. Brine shrimp toxicity

Brine shrimp (*Artemia salina*) toxicity was measured as previously described.[16] After a 24 h hatching period, aliquots of a 10 mg/mL stock solution of compounds **1–5** were added to test wells containing 5 mL of artificial seawater and brine shrimp to achieve a range of final concentrations from 0.1 to 100 ppm. After 24 h, the live and dead shrimp were tallied.

3.7. Cytotoxicity

Cytotoxicity was measured in NCI-H460 human lung tumor cells and neuro-2a mouse neuroblastoma cells using the method of Alley et al.[17] with cell viability being determined by MTT reduction.[18] Cells were seeded in 96-well plates at 6000 cells/well in 180 μL medium. Twenty-four hours later, the test chemicals were dissolved in DMSO and diluted into medium without fetal bovine serum and then added at 20 μL/well. DMSO was less than 0.5% of the final concentration. After 48 h, the medium was removed and cell viability determined.

3.8. Microfilament disrupting assay

Lyngbyabellin E (**1**) was tested for microfilament-disrupting activity using the rhodamine-phalloidin assay. A-10 cells (rat aortic smooth muscle cell line) were grown on glass coverslips in Basal Medium Eagle (BME) containing 10% fetal calf serum. The cells were incubated with the test compound for 24 h and then fixed with 3% paraformaldehyde for 20 min, permeabilized with 0.2% Triton X-100 for 2 min, and chemically reduced with sodium borohydride (1 mg/mL in PBS) three times for 5 min each. Following a 45 min incubation with 100 nM TRITC-phalloidin in phosphate buffered saline (to visualize the actin cytoskeleton), the coverslips were washed, stained with 4,6-diamidino-2-phenylindole (DAPI) to visualize DNA, mounted on microscope slides, and examined and photographed using a Nikon E800 Eclipse fluorescence microscope with a Photometrics Cool Snap FX3 camera. The images were colorized and overlayed using Metamorph® software.

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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.2005.09.036. ^{1}H NMR, ^{13}C NMR, and 2D NMR spectra in $CDCl_3$ for lyngbyabellins E (**1**). ^{1}H NMR and ^{13}C NMR data and spectra in $CDCl_3$ for lyngbyabellins F (**2**)–I (**5**). ^{1}H NMR spectrum, HR-TOFMS and MS/MS fragmentation of dolabellin (**6**).

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Spectrokinetic studies on new bi-photochromic molecules containing two naphthopyran entities

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Abstract—A range of new bi-photochromic molecules containing two identical (**3a–d**) or two distinct naphthopyran units (**6a–d**), linked through the phenyl substituents located on the sp^3 hybridised pyran ring carbon atom, using conjugated and non-conjugated spacers, have been synthesised from bis-propynols and (substituted)naphthols. Study of the spectrokinetic properties of these compounds under near UV–vis continuous irradiation conditions revealed that the two naphthopyran units are stimulated independently leading to open forms with higher colourabilities but without affecting the individual bleaching kinetics. Compared to the individual photochromic components and to model mono-photochromes it was observed that the nature of the bridge has a small effect on the photochromic properties of each system.
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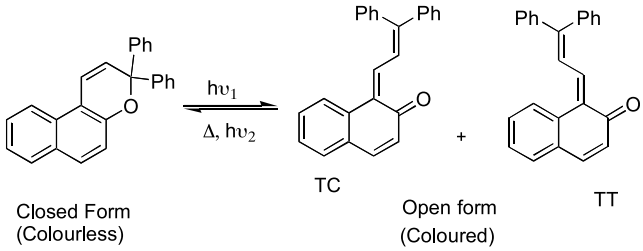
1. Introduction

The photochromic properties of naphthopyrans have been extensively studied in the last decade due to the wide range of applications with prominence in the manufacture of ophthalmic plastic lenses and solar protection glasses.[1] Under near-UV light irradiation these uncoloured or faintly coloured molecules, either in solution or incorporated in polymeric matrices, undergo an electrocyclic pyran-ring opening with cleavage of the $C(sp^3)$–O bond and a subsequent structural reorganisation allowing the photogenerated species to adopt more planar structures (the so-called 'open form', OF) with greater conjugation, which is responsible for the increased absorption in the visible part of the spectrum (Scheme 1). The OF is constituted by a set of coloured stereoisomers, with similar absorption characteristics but with diverse thermal stabilities.[2] Under continuous near-UV irradiation a photostationary equilibrium is attained between the uncoloured 'closed form' (CF) and the OF leading to a colour change of the system. When the light source is removed the equilibrium shifts and the system returns to the original colourless state, either via a thermal or a photoinduced process, with light of different wavelength from the first, unveiling the reversible colour behaviour that is characteristic of this photochromic system. UV and NMR studies revealed that at room temperature the main photoproducts are the TC and TT isomers (Scheme 1), the latter being the most thermally stable.[3]



Scheme 1.

A great number of substitutions and/or annelations have been studied in the naphthopyran family in order to increase the ability to produce intense coloured forms (colourability), to tailor chromatic properties or to change fading rates of these systems.[4–6] Consequently, there has been a significant increase in patenting of new molecules and systems, although many exaggerated claims were made about their performances. Nevertheless, significant improvements were attained and many commercially interesting photochromic systems were discovered. An important advancement is related to the colour range achievable with naphthopyrans that, initially, was limited to orange/yellow colours and nowadays is extended to virtually any colour.

This feature is particularly important for photochromic lens manufacturers because the optical lens market is dominated by neutral colours (browns and greys). In principle, neutral

Keywords: Naphthopyrans; Photochromism; bi-Photochromic; bi-Naphthopyrans.
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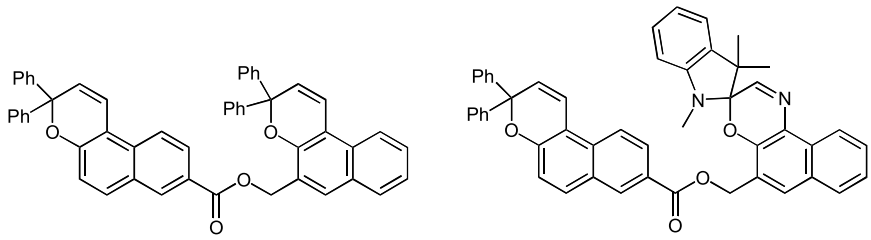


Scheme 2.

colours can be produced by mixing two or more photochromic dyes that allow a constant coverage of an extended range of the visible spectrum (usually 420–620 nm is acceptable). This approach meets the goal, although it opens a set of serious problems, beyond the position of the absorption maxima and the shape of the absorption bands in the visible and UV (activating) spectral range. To prevent photochromic lenses from displaying different colours under varying exposure conditions the photochromic dyes should have similar (within some tolerance limits) photochromic properties. These include the colouring and fading kinetics, temperature dependence, solvatochromic effects and fatigue resistance. The mixture of the lesser possible number of photochromes belonging to the same family seems to be recommended. Several interesting formulations were developed and can be found in the patent literature.[7]

A second and highly desired approach to obtain neutral-colouring articles, because all the matching problems are overcome and the formulation processes would obviously be simpler, involves the use of a single photochromic dye with the desired absorption properties: at least two absorption bands conveniently spaced ($\Delta\lambda \sim 100$–130 nm) in the visible spectrum, of approximately equal intensity. Such molecules can be, in principle, obtained through appropriate substitution/annelation of the naphthopyran moieties, but in the literature few examples are described.[8–10]

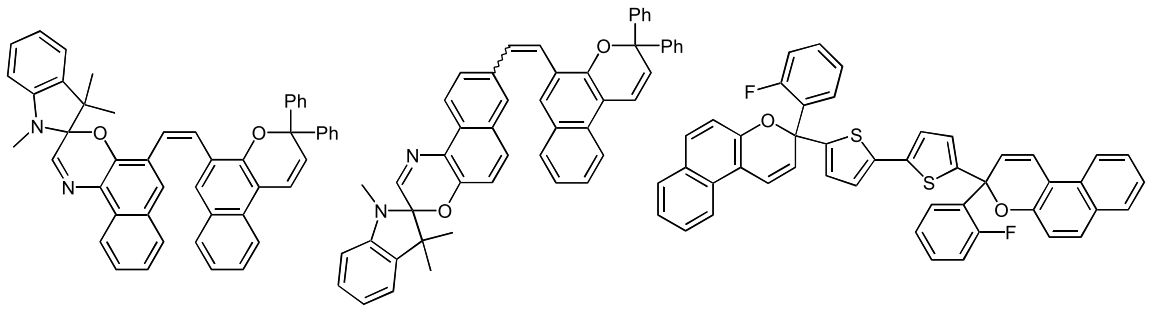
Recently, molecular structures involving the coupling of conveniently selected photochromes (e.g., naphthopyran–diarylethene,[11] naphthacene–diarylethene,[12–13] diarylethene–dihydroazulene,[14] naphthopyran–naphthopyran,[15–18] spirooxazine–spirooxazine,[17,19–20] naphthopyran–spirooxazine[15,17,21]) through a covalent linkage have received some attention.

Studies on bi-photochromic supermolecules including naphthopyran units, linked through their naphthalene units, revealed that although the kind of molecular spacer nature is critical and fading rates are sometimes affected, the simultaneous activation of both photochromic systems is possible. Alkyl (ethane), ester (Scheme 2), or acetylenic bridges did not substantially change the photochromic behaviour exhibited by the components taken individually and an additive effect was observed.[15–16]

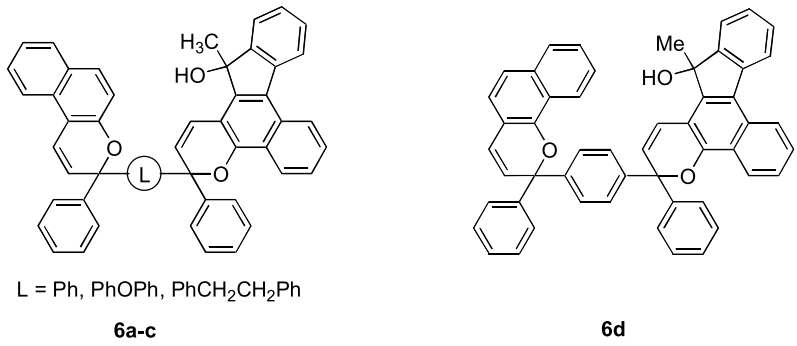
On the other hand, ethylenic spacers lead to supermolecules, which exhibit a complex and contradictory behaviour (Scheme 3).[2,17–21] Some compounds with (*Z*)-ethylenic bonds exhibited thermally irreversible photochromic properties, and upon UV irradiation thermally stable coloured forms that persist from days to several weeks in the dark were formed, while others exhibited a thermally reversible behaviour, which can be considered as the superposition of the properties of the individual systems taken separately. With some bi-naphthopyrans it was observed that only one pyran unit was opened under UV irradiation, indicating a possible de-activation of one photochrome due to the presence of the other in the same framework.

Another approach involves the linkage of the two naphthopyrans thought one of the geminal aryl aromatic substituents. Recently, some interesting results have been obtained on bi-photochromic supermolecules with two naphthopyrans sharing a bi-thiophene unit linked through the two pyran ring sp^3 C atoms (Scheme 3). These molecules showed a very significant bathochromic shift of the maximum wavelength of absorption of the OF and high colourabilities due to the extension of π-conjugation and the opening of both photochromic systems.[22–23]

In the present paper, we report our results on the study of new bi-photochromic molecules obtained through the linkage of the naphthopyran sp^3 phenyl substituents, using conjugated and non-conjugated spacers, including a shared aromatic ring (Scheme 4).



Scheme 3.

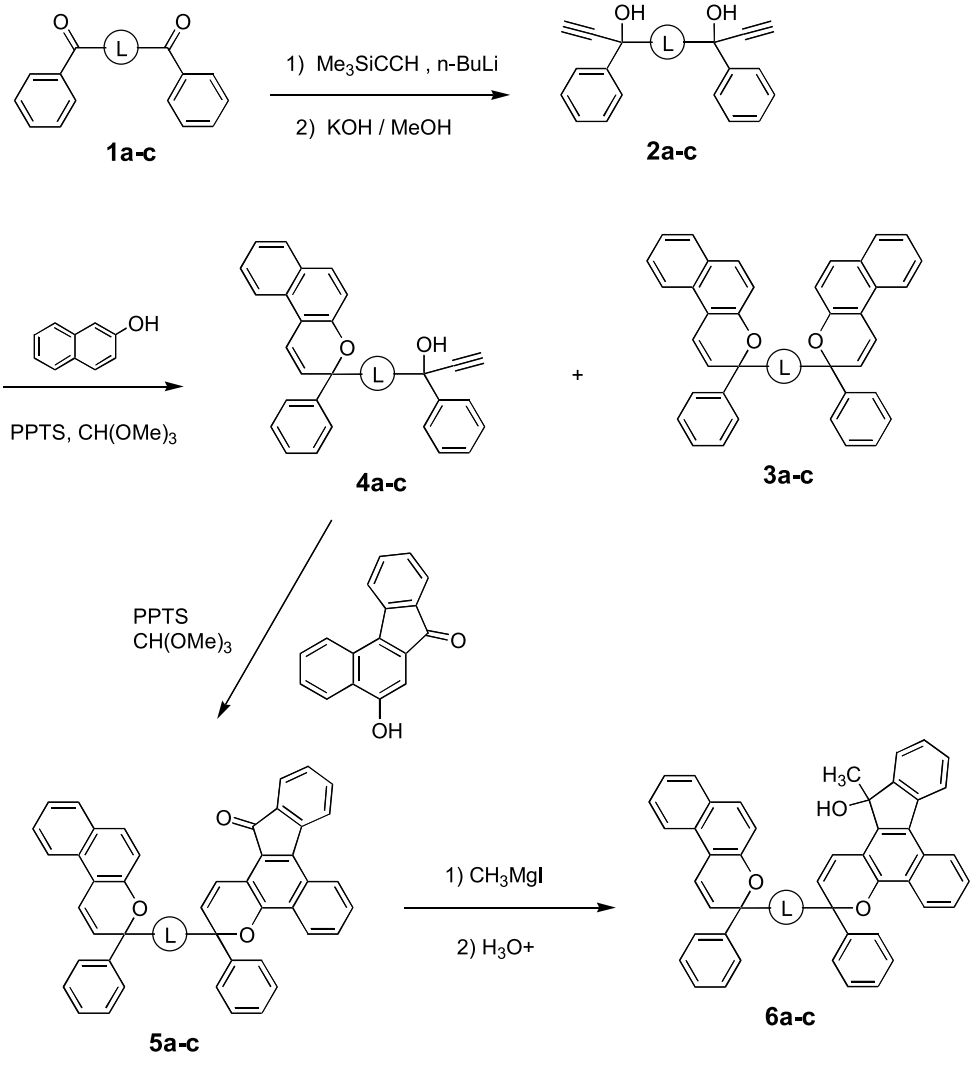


Scheme 4.

2. Results and discussion

2.1. Synthesis

The bi-photochromic compounds **6a–d** were prepared in four steps starting from aromatic diketones **1a–c**, which were commercially available (**1b**) or readily prepared by Friedel-Crafts benzoylation of diphenyl ether or dibenzyl (Scheme 5).[24] Treatment of diketones **1a–c** with lithium trimethylsilylacetylide in THF followed by basic hydrolysis

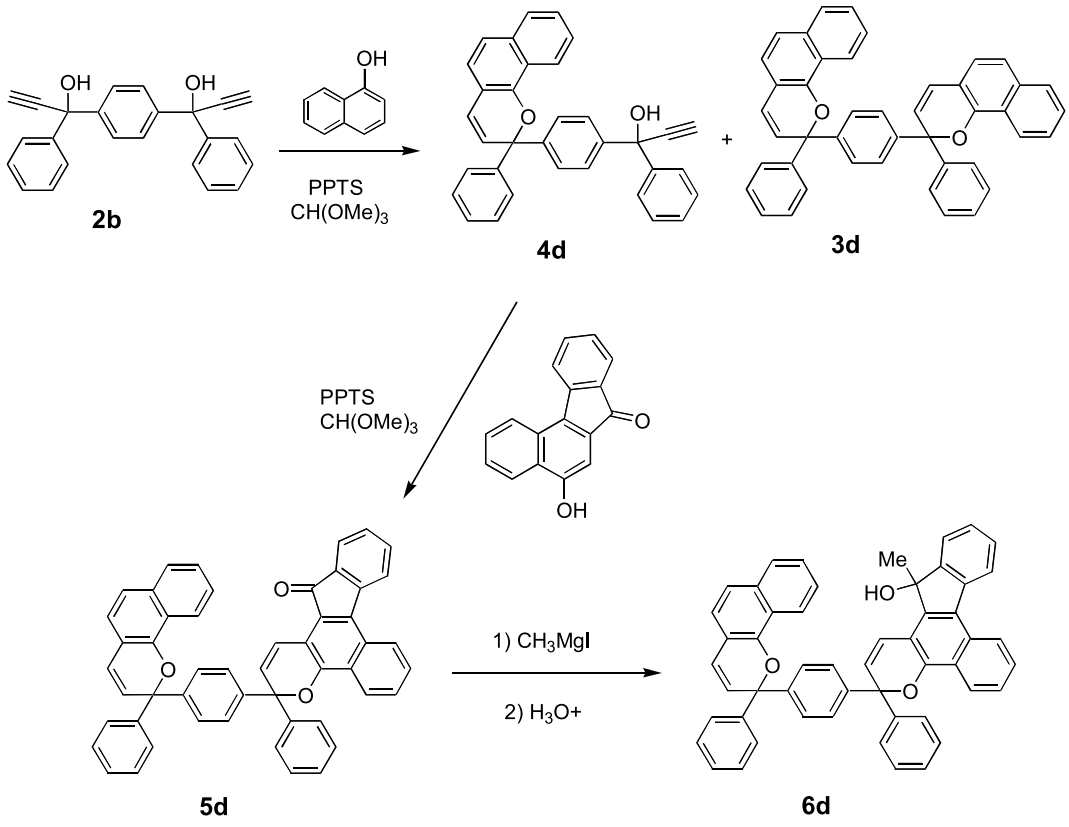


a: L = PhOPh

b: L = Ph

c: L = $PhCH_2CH_2Ph$

Scheme 5.



Scheme 6.

(KOH/MeOH) gave almost pure propargylic diols **2a–c** in excellent yields (91–97%).

Heating these diols with 0.8 equiv of 2-naphthol in 1,2-dichloroethane in the presence of an acid catalyst (pyridinium *p*-toluenesulphonate) and 2 equiv of trimethyl orthoformate, added as a dehydrating agent,[25] gave a mixture of symmetrical bi-naphthopyrans **3a–c** (minor) and mono naphthopyrans **4a–c** (major), with one reactive propargylic alcohol function still present, which were easily separated by column chromatography. Both compounds exhibit photochromic properties in solution at room temperature. Treating the mono naphthopyrans **4a–c** with 1.1 equiv of 5-hydroxy-7*H*-benzo[*c*]fluoren-7-one, under the same reaction conditions, gave the naphthopyrans **5a–c**. These compounds are highly coloured and possess two different covalently linked naphthopyrans entities, however, they show very weak photochromic properties due to the presence of the carbonyl function in the indene ring.[4] Treatment of these compounds with a CH_3MgI solution gave, after hydrolysis, the new photochromic bi-naphthopyrans **6a–c** in good yield (70–79%).

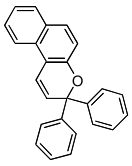
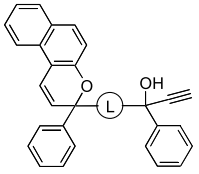
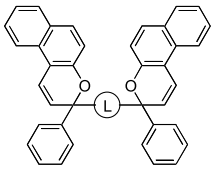
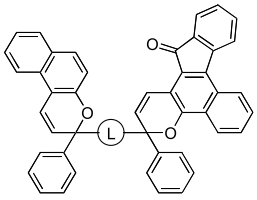
The same methodology was used to prepare the bi-naphthopyran **6d** from diol **2b** except that 1-naphthol was used instead of 2-naphthol (Scheme 6).

2.2. Photochromic properties

The photochromic behaviour of compounds **3–6** was studied in 10^{-4} M toluene solutions at 20 °C under continuous near UV–vis irradiation (150 W ozone free Xe lamp, light flux of 40 W m^{-2}, quartz cells, 1 cm light path, 3.5 ml of solution). Three spectrokinetic parameters, normally quoted when describing the properties of photochromic compounds, were evaluated: maximum wavelength of absorption of the open form (λ_{max}), thermal bleaching rates (k_Δ) and colourability (A_{eq}), estimated by the absorbance of the solution after reaching a photostationary equilibrium under the experimental conditions. The bleaching kinetics were studied in the dark, under thermal conditions. The data are summarised in Tables 1–4 where three reference compounds were included for comparison: **Ref1** (=3,3-diphenyl-3*H*-naphtho[2,1-*b*]pyran), **Ref2** (=3,3-diphenyl-13-methyl-13-hydroxy-3*H*-indeno[2,1-*f*]naphtho[1,2-*b*]pyran) and **Ref3** (=2,2-diphenyl-2*H*-naphtho[1,2-*b*]pyran).

2.2.1. Compounds 4a–d. Compounds **4a–c** are 3,3-diphenylnaphtho[2,1-*b*]pyran derivatives substituted at the *para* position of one of the 3-phenyl groups by an aryl substituted alkyl chain, **4b–c**, or a conjugative electron donating group (OAr), **4a**. The observed photochromic behaviours are characteristic of coloured forms of this family of compounds and, thus, similar to **Ref1** (Table 1, Fig. 1): under continuous irradiation conditions, compounds **4a–c** exhibit a single wide absorption band centered between 434–446 nm and weak colourabilities (0.2–0.3). After the removal of the UV irradiation source these compounds exhibit a bi-exponential colour decay with two very different fading rates: one fast (0.07–0.09 s^{-1}) and another very slow (2×10^{-5}–6×10^{-5} s^{-1}). As already referred these rate constants can be attributed, respectively, to the TC and TT open form isomers.[3] Considering similar molar absorptivities for these isomers,[2] from the amplitudes

Table 1. Spectrokinetic properties under continuous irradiation: maxima wavelengths of the coloured form (λ_{max}), colourability (A_{eq}), thermal bleaching rate (k_{Δ}) of compounds **3a–c**, **4a–c**, **5a–c** and **Ref1** in toluene solutions

Compounds			λ_{max} (nm)	A_{eq}	Residual colour[a]	k_{Δ} (s^{-1})
	**Ref1**		432	0.27	0.06	6×10^{-2} (73) 7×10^{-6} (27)
	**4a**	L=PhOPh	446	0.19	0.03	9×10^{-2} (74) 1×10^{-5} (26)
	4b	L=Ph	434	0.25	0.06	7×10^{-2} (72) 2×10^{-5} (28)
	4c	L=$PhCH_2CH_2Ph$	438	0.25	0.05	7×10^{-2} (73) 6×10^{-5} (27)
	**3a**	L=PhOPh	446	0.30	0.06	9×10^{-2} (72) 3×10^{-5} (28)
	3b	L=Ph	433	0.33	0.08	6×10^{-2} (72) 4×10^{-5} (28)
	3c	L=$PhCH_2CH_2Ph$	438	0.42	0.09	8×10^{-2} (78) 4×10^{-5} (22)
	**5a**	L=PhOPh	438[b]	0.14	0.02	6×10^{-2} (74) 8×10^{-4} (14) 2×10^{-4} (12)
	5b	L=Ph	430[b]	0.21	0.05	5×10^{-2} (55) 2×10^{-2} (16) 8×10^{-6} (29)
	5c	L=$PhCH_2CH_2Ph$	436[b]	0.17	0.03	8×10^{-2} (66) 2×10^{-2} (14) 4×10^{-5} (20)

[a] Absorbance after 2000 s in the dark.
[b] Shoulder. These measurements were not made at the λ_{max} because these solutions of compounds were already orange/red coloured before irradiation.

of the fading kinetics it can be deduced that the coloured OF is a mixture constituted mainly by the short lived TC isomer (72–73%), responsible for the initial fast colour decay, and by the more stable TT isomer (26–27%), which is responsible for the persistence of colour for a long time (Fig. 1). As expected, due to the presence of the *p*-substituent at the 3-phenyl group, the open forms are slightly less thermally stable than **Ref1** and the observed lower colourabilities can be related to the faster ring closure kinetics, as these two parameters are inversely related.[26]

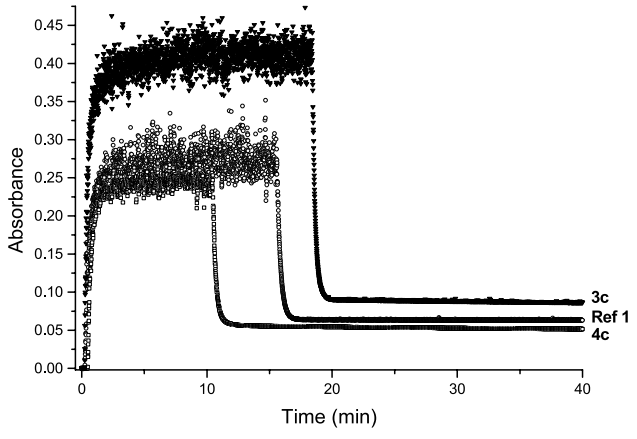


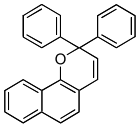
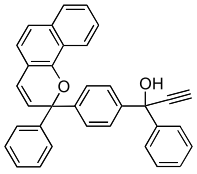
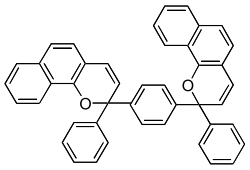
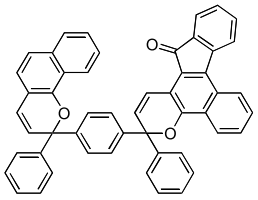
Figure 1. Colour forming and colour bleaching at λ_{max} of **Ref1**, **3c** and **4c**.

Considering **Ref1**, compound **4a** exhibit the more pronounced bathochromic shift to the λ_{max} (+14 nm), although smaller that the one observed when the *p*-substituent is a methoxy group (+36 nm), reflecting the weaker electron donating character of the OAr group.[27]

Similar results were obtained for compound **4d**. This compound is a 2,2-diphenylnaphtho[1,2-*b*]pyran substituted at the *para* position of a 2-phenyl group and, as expected, its photochromic properties are very similar to **Ref3** (Table 2). It exhibits a maximum wavelength of absorption, λ_{max}, at 471 nm and two slow fading rates (7×10^{-4} and 2×10^{-6} s^{-1}) that are responsible for a very slow colour decay and therefore a high colourability ($A_{eq}=1.1$) (Fig. 2). The maximum absorbance obtained under UV exposition ($A_{eq}=1.0$) was, however, considerably lower than the one obtained with **Ref3** ($A_{eq}=1.5$).

2.2.2. Compounds 3a–d. Compounds **3a–c** are bi-photochromic supermolecules with two naphtho[2,1-*b*]pyrans linked through conjugated (Ph) or non-conjugated chains

Table 2. Spectrokinetic properties under continuous irradiation: maxima wavelengths of the coloured form (λ_{max}), colourability (A_{eq}), thermal bleaching rate (k_{Δ}) of compounds **3d**, **4d**, **5d**, and **Ref3** in toluene solutions

Compounds		λ_{max} (nm)	A_{eq}	Residual colour[a]	k_{Δ} (s^{-1})
	**Ref3**	470	1.5	0.36	6×10^{-4} (76) 1×10^{-6} (24)
	**4d**	471	1.1	0.26	7×10^{-4} (75) 2×10^{-6} (25)
	**3d**	480	2.7	0.94	5×10^{-4} (65) 7×10^{-7} (35)
	**5d**[b]	479	1.0	0.25	6×10^{-4} (75) 2×10^{-6} (25)

[a] After 10000 s in the dark.
[b] Solutions of this compound were already orange/red coloured before irradiation.

(PhOPh, $PhCH_2CH_2Ph$). Since the two sp^3 aryl substituents are not equivalent many different coloured photoisomers can be expected to form. Relative to the λ_{max} and fading kinetics, it was observed a behaviour very similar to that recorded for the mono-photochromic molecules **4a–c** (Table 1, Figs. 1 and 3).

However, the maximum absorbances attained at the photostationary state (colourability) are significantly higher (Fig. 1) and as under our experimental conditions the colourability of 3,3-diphenyl-naphtho[2,1-*b*]pyran, **Ref1**, is not linearly related to concentration (Fig. 4) one can infer that probably both pyran units were opened under exposure to UV. The increase of the residual absorbance at the end of the experiments corroborates this inference. Regarding the absorption wavelength of coloured forms of the model compounds **4a–c** we can conclude that no additional extension of conjugation was achieved in the open forms of the bi-photochromic molecules, even considering **3b** where a phenyl group is shared by the two parts. This may indicate that, upon UV irradiation, both photochromic moieties are opened and the molecule adopts a conformation where each 'planar' open form is completely out of

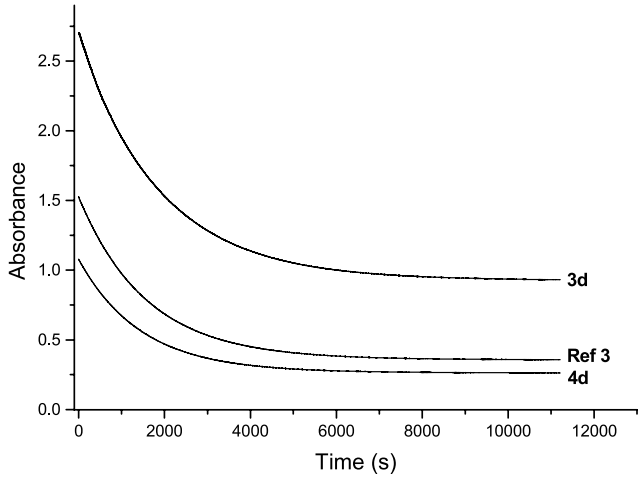


Figure 2. Colour bleaching at λ_{max} of **Ref3**, **3d** and **4d**.

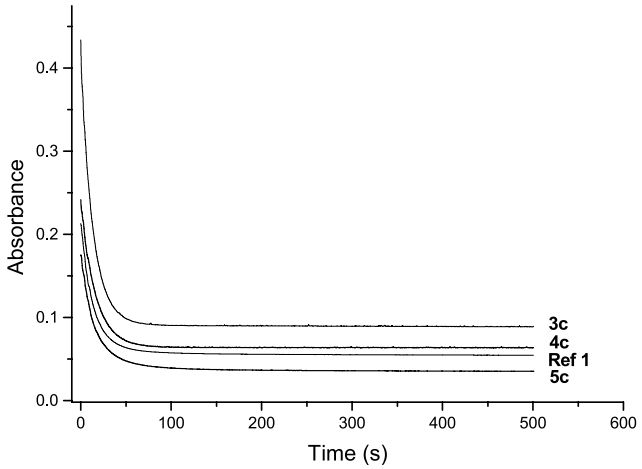
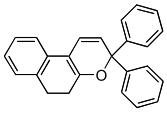
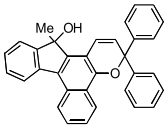
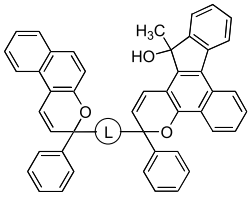
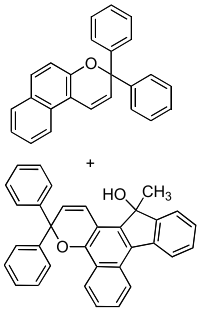


Figure 3. Normalized colour bleachings of **Ref1**, **3c**, **4c** and **5c**.

Table 3. Spectrokinetic properties under continuous irradiation: maxima wavelengths of the coloured form (λ_{max}), colourability (A_{eq}) thermal bleaching rate (k_{Δ}) of compounds **6a–c**, **Ref1**, **Ref2** and a equimolar mixture of **Ref1** and **Ref2**

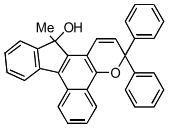
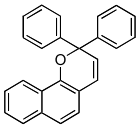
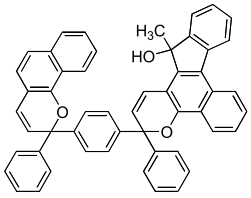
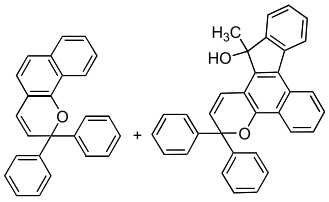
Compounds			λ_{max} (nm)	A_{eq}	Residual colour[a]	k_{Δ} (s^{-1})
		**Ref1**	432	0.27	0.06	6×10^{-2} (73) 7×10^{-6} (27)
		**Ref2**	533 419	1.3 0.71	0.21	3×10^{-3} (82) 5×10^{-6} (18)
	**6a**	L=PhOPh	425	0.85	0.10	7×10^{-2} (4) 5×10^{-3} (83) 1×10^{-5} (13)
			536	1.4	0.19	5×10^{-3} (86) 1×10^{-5} (14)
	6b	L=Ph	423	0.76	0.12	4×10^{-2} (6) 3×10^{-3} (78) 2×10^{-5} (16)
			533	1.3	0.22	3×10^{-3} (82) 8×10^{-6} (18)
			600[b]	0.62	0.07	3×10^{-3} (87) 4×10^{-5} (13)
	6c	L=$PhCH_2CH_2Ph$	423	0.84	0.13	6×10^{-2} (6) 4×10^{-3} (78) 2×10^{-5} (16)
			533	1.3	0.20	4×10^{-3} (83) 2×10^{-5} (17)
	**Ref1**+**Ref2**		420	0.85	0.15	6×10^{-2} (11) 3×10^{-3} (70) 2×10^{-6} (19)
			528	1.2	0.22	5×10^{-2} (2) 3×10^{-3} (80) 5×10^{-6} (18)

[a] Absorbance after 2000 s in the dark.
[b] Wavelengths that do not correspond to λ_{max}.

plane of the other. The nature of the assayed bridges linking the two photochromic parts seems to have a negligible influence in the photochromic behaviour of these compounds.

Compound **3d** has two naphtho[1,2-*b*]pyran units linked by a common phenyl group. Exposure of this compound to UV light produced a band with a λ_{max} initially at 470 nm that gradually shifts to 480 nm until the photostationary state is attained (an inverse behaviour was observed during the bleaching). Compared to **Ref3** and the model compound **4d**, it can be observed a bathochromic shift (+10 nm) of the maximum wavelength of absorption that suggests that a phenyl (conjugative) bridge allows some extension of conjugation in the open form of this compound. The analysis of the bleaching kinetics revealed a bi-exponential behaviour, with close values for kinetic constants although with slightly different amplitudes (Table 2, Fig. 2). Relative to **Ref3** and **4d**, for compound **3d** the amplitude of the slower fading rate increased from 24 to 35% that points to an increase in the concentration of the TT species at the photostationary equilibrium. The colourability attained by this compound is nearly twice the one achieved by **Ref3** (A_{eq}=1.5) and the residual colour (0.94) is much higher than those observed for **Ref3** (0.36) and **4c** (0.26) (Fig. 2). This additive behaviour unambiguously suggests the

Table 4. Spectrokinetic properties under continuous irradiation: maxima wavelengths of the coloured form (λ_{max}), colourability (A_{eq}) thermal bleaching rate (k_Δ) of compounds **6d**, **Ref2**, **Ref3** and a equimolar mixture of **Ref2** and **Ref3**

Compounds		λ_{max} (nm)	A_{eq}	Residual colour[a]	k_Δ (s^{-1})
	**Ref2**	533 419	1.3 0.71	0.21	3×10^{-3} (82) 5×10^{-6} (18)
	**Ref3**	470	1.5	0.36	6×10^{-4} (76) 1×10^{-6} (24)
	**6d**	472[a]	1.7	0.47	3×10^{-3} (23) 6×10^{-4} (49) 4×10^{-6} (28)
		530	2.0	0.39	3×10^{-3} (48) 6×10^{-4} (31) 7×10^{-6} (21)
		620[a]	0.91	0.06	3×10^{-3} (78) 6×10^{-4} (14) 1×10^{-5} (8)
	**Ref2**+**Ref3**	470[a]	2.1	0.48	4×10^{-3} (27) 6×10^{-4} (50) 1×10^{-6} (23)
		494	2.2	0.47	4×10^{-3} (35) 6×10^{-4} (44) 2×10^{-6} (21)
		530[a]	2.1	0.50	4×10^{-3} (27) 6×10^{-4} (50) 1×10^{-6} (23)

[a] Wavelengths that do not correspond to λ_{max}.

independent opening of the two pyran rings in this molecule and the displacement of the λ_{max} with the irradiation time points to a consecutive opening of the pyran rings.

Considering **Ref3** a study of the effect of the concentration on the observed colourability, under our experimental conditions, displayed a linear relationship between A_{eq} and concentration over a more extended concentration range (until 2×10^{-4} M) than for **Ref1** (Fig. 4). Obviously, at these concentrations, deviations from direct proportionality are strongly dependent upon discolouration kinetics values. No measurements could be performed at higher concentrations due to instrumental limitations related to the very high absorbances attained (>4 absorbance units).

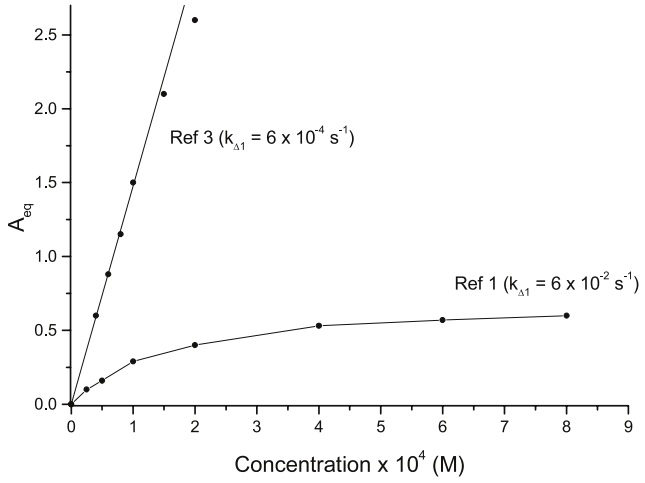


Figure 4. Colourability versus concentration for **Ref1** and **Ref3**.

2.2.3. Compounds 5a–d. Compounds **5a–d** have a naphtho[1,2-*b*]pyran or a naphtho[2,1-*b*]pyran linked to a non-photochromic 13-oxoindeno[2,1-*f*]naphtho[1,2-*b*]-pyran.[4] Before UV irradiation they all show a small absorption band with a maximum between 489–503 nm (Abs: 0.20–0.23; ε=2000–2300 mol^{-1} L cm^{-1}), which is responsible for their orange/red colourations. Upon irradiation of compounds **5a–c** with UV light a new band appears as a shoulder between 430–438 nm that can be assigned to the opening of the naphtho[2,1-*b*]pyran ring (Table 1). From the position of these bands it is apparent that no enhancement in the extension of conjugation is achieved even with a conjugative bridge. The observed A_{eq} for these compounds (0.14–0.21) are lower than the colourabilities observed for the mono-photochromic **4a–c** (0.19–0.25) and for **Ref1** (0.27) (Fig. 3), although there is no apparent increase in the bleaching kinetics. The analysis of the fading kinetics of **5a–c** shows a good fit to

a tri-exponential model indicating that upon UV irradiation a more complex mixture is obtained.

Compound **5d**, also already orange/red ($\lambda_{max}=489$ nm) coloured before UV-irradiation, associates a 2,2-diphenyl-naphtho[1,2-*b*]pyran and the non-photochromic 2,2-diphenyl-13-oxoindeno[2,1-*f*]naphtho[1,2-*b*]pyran through a *para* substituted phenyl group. Upon irradiation with UV light this compound develops a stronger and wider band with a λ_{max} at 479 nm indicating the opening of the non-fused naphtho[1,2-*b*]pyran ring (Table 2). This bathochromic shift, relative to **Ref3** and the model compound **4d**, points to an increase of the π-conjugation. The usual dual kinetic of thermal bleaching of naphthopyrans, indicating the presence of TT and TC isomers, was observed. The similarity of the kinetic constants and amplitudes with the mono-photochromic model compound **4d** and **Ref3** indicates that the presence of the non-photochromic moiety has a negligible effect on the thermal stability of **5d**.

2.2.4. Compounds 6a–d. Compounds **6a–c** have two different photochromic units, a naphtho[2,1-*b*]pyran and a 13-methyl-13-hydroxy-indeno[2,1-*f*]naphtho[1,2-*b*]pyran, linked through PhOPh, Ph, and $PhCH_2CH_2Ph$ bridges, respectively. Taken independently, upon irradiation with UV light, one residue (**Ref1**), generates an open form with a λ_{max} centered at 432 nm ($A_{eq}=0.27$) while the other (**Ref2**) generates an open form with two absorption maxima in the visible region: one at 530 nm ($A_{eq}=1.3$) and the other near 419 nm ($A_{eq}=0.72$) (Fig. 5). Moreover, the two photochromic units exhibit biexponential bleaching kinetics, although with quite different values in the initial fading rate: **Ref1** (6×10^{-2} s^{-1}, 73%) fades initially about 20 times faster than **Ref2** (3×10^{-3} s^{-1}, 82%).

The covalent linkage of these two units yielded molecules that exhibit thermally reversible photochromic properties. As expected, due to the very different colourabilities of the associated units, the photochromic properties of the indeno-fused naphtho[1,2-*b*]pyran unit practically overlap those observed for the other naphtho[2,1-*b*]pyran and consequently, for the bi-photochromic compounds **6a–c**, it is very difficult to distinguish between their behaviour and those of **Ref2.** In what it concerns the visible spectra of the open forms of bi-photochromic compounds **6a–c** only subtle differences are observed even when compared with a solution of the same concentration of an equimolar mixture of **Ref1** and **Ref2** (see Fig. 5).

However, colourabilities obtained at the lower λ_{max} (where the maximum contribution of the **Ref1** residue is expected) are higher than for **Ref2** alone and kinetic curves constants and amplitudes at this λ_{max}, show a good fit to a tri-exponential model with values in a range that may indicate that both pyran units are opened. The faster kinetic constant (4×10^{-2}–7×10^{-2} s^{-1}), representing 4–6% of the absorbance decay can be attributed to the TC isomer resulting from the opening of the naphtho[2,1-*b*]pyran (**Ref1**) while the second slower kinetic constant (3×10^{-3}–5×10^{-3} s^{-1}) representing 78–83% is probably due to the fading of the TC isomer resulting from the opening of the indeno[2,1-*f*]-naphtho[1,2-*b*]pyran moiety.

Although the diphenyl ether bridge of series **a** (L=Ph–O–Ph) induced an expected small bathochromic shift in the visible spectra and some thermal instability of the open forms, it is apparent that the nature of the covalent bridge has no significant effect on the photochromic performances of the bi-naphthopyrans **6a–c**.

Compound **6d** is a bi-photochromic molecule associating two different naphthopyrans (**Ref2** and **Ref3**) with similar absorbances at the λ_{max} of the open forms (Table 4), linked through a shared *p*-phenyl substituted ring. Taken independently the two photochromes exhibit quite different fading kinetics although with similar amplitudes: the first fading rate of **Ref2** (3×10^{-3} s^{-1}, 82%), is about five times faster than the first fading rate of **Ref3** (6×10^{-4} s^{-1}, 76%). When solutions of each compound were submitted to UV irradiation, under identical experimental conditions, similar absorbance values were attained at the photostationary state: **Ref3**: $A_{eq}=1.5$ (470 nm); **Ref2**: $A_{eq}=1.3$ (533 nm). Compound **6d** led to the formation of a single wide absorption band with a maximum centred at 530 nm and higher colourability ($A_{eq}=2.0$), suggesting the opening of both photochromic entities (Fig. 6).

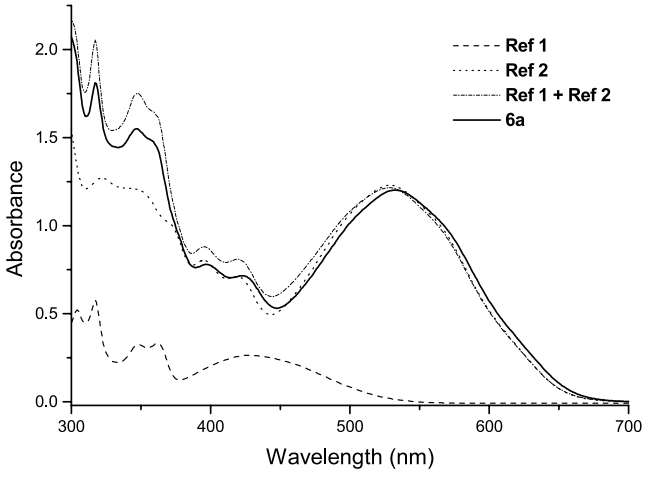


Figure 5. Absorption spectra of **Ref1**, **Ref2**, mixture **Ref1**+**Ref2** and **6a** after UV irradiation.

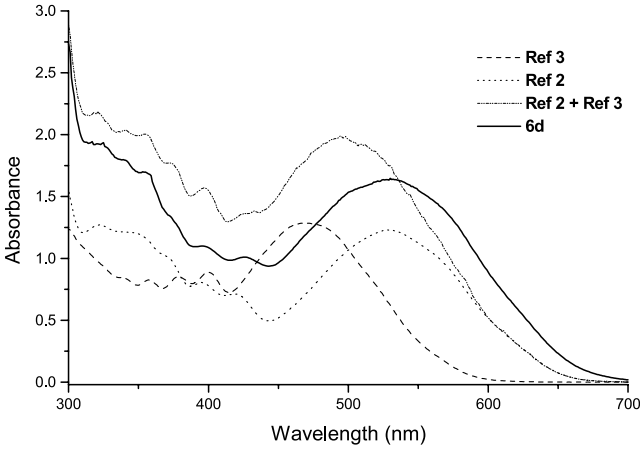


Figure 6. Absorption spectra of **Ref2**, **Ref3**, mixture **Ref2**+**Ref3** and **6d** after UV irradiation.

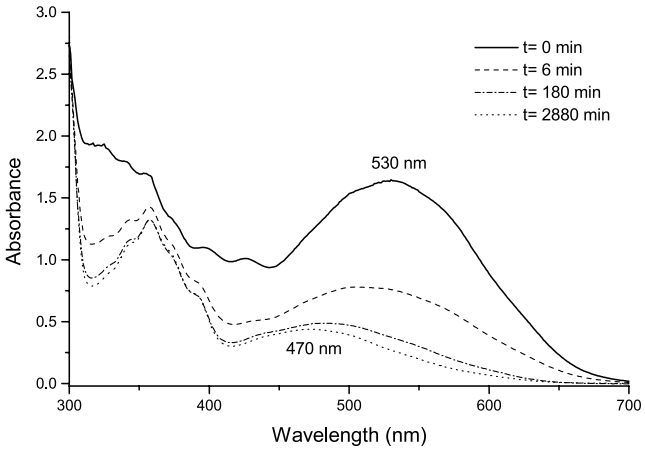


Figure 7. Time evolution of the absorption spectrum of the opened form of compound **6d**.

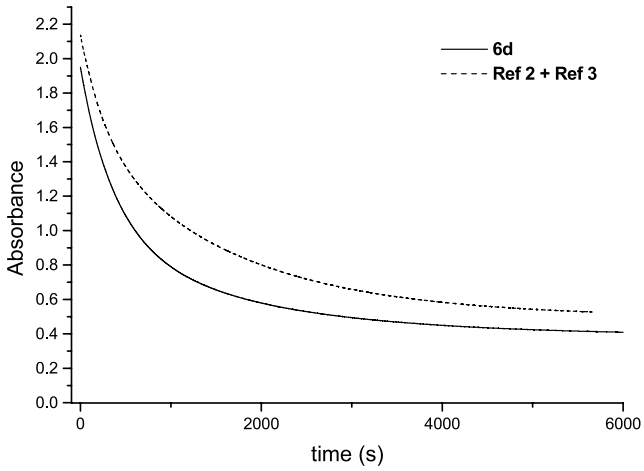


Figure 8. Colour bleaching of a mixture of **Ref2**+**Ref3** and **6d**.

For this compound, fading kinetics were measured at 472 nm (major contribution of the naphtho[1,2-*b*]pyran residue), 530 nm (major contribution of the indeno-fused naphtho[1,2-*b*]pyran residue) and 620 nm (sole absorption contribution from the indeno-fused naphtho[1,2-*b*]pyran residue) and although the same values for the constants were found (Table 4), amplitudes varied in an expected way: the amplitude of the kinetic increased along with the increase of the contribution of the naphthopyran residue that is responsible for it.

Spectral evolution of the UV–vis spectrum of compound **6d** was also recorded during the thermal bleaching of solutions (Fig. 7). Clearly, it can be observed a gradual displacement with time, of the λ_{max} toward shorter wavelengths until λ_{max} of **Ref3** (the slower photochromic entity) was attained. Indeed, photochromic properties of **6d** can be considered as the superposition of the properties of the two naphthopyran residues taken separately.

A final comparison was made between compound **6d** and an equimolar mixture of **Ref2** and **Ref3** of the same concentration (1×10^{-4} M). The absorption spectrum of the open form of **6d** shows a $\lambda_{max}=530$ nm while the mixture exhibits a $\lambda_{max}=494$ nm. This difference in the λ_{max} (+36 nm) suggests that the phenyl spacer allows some conjugation between the OF of the two photochromic entities, although a completely planar structure is probably not obtained.

Comparison of the fading kinetics observed for compound **6d** and an equimolar mixture of **Ref2** and **Ref3** (Fig. 8) revealed that **6d** constitutes a system that bleaches faster. This suggests that the opened naphthopyran may act as an electron donor substituent.

3. Conclusion

New bi-photochromic molecules, containing two identical or two distinct diphenylnaphthopyran units, were obtained by generally applicable methods. The strategy involved the linkage of one of the geminal aryl aromatic substituents, using covalent bridges, namely *p*-Ph–, *p*-PhOPh– and *p*-$PhCH_2CH_2Ph$–. All compounds exhibited photochromic behaviour in toluene solutions at room temperature. Compared to the individual photochromic components and to model monophotochromes it was observed that the nature of the bridge has a small effect on the photochromic properties of each system.

Under continuous near UV–vis irradiation the two naphthopyran units of the bi-photochromic molecules **3a–d** and **6a–d**, were stimulated independently leading to open forms with higher colourabilities but without affecting the individual bleaching kinetics.

Considering the absorbance spectra of the open forms of the bi-photochromic molecules no additional extension of the π-conjugation was observed for 3,3-diphenylnaphtho-[2,1-*b*]pyran derivatives while for 2,2-diphenylnaphtho-[1,2-*b*]naphthopyran derivatives (**3d** and **6d**) it was evident a bathochromic shift of the λ_{max}, suggesting that for these last derivatives, under UV irradiation, structures with a better global planarity are obtained.

4. Experimental

4.1. Spectrokinetic studies under continuous irradiation

For measurements of λ_{max}, A_{eq} and k_{Δ} under continuous UV–vis irradiation, 1×10^{-4} M toluene solutions were used. Irradiation experiments were made using a CARY 50 Varian spectrometer coupled to a 150 W Ozone free Xenon lamp (6255 Oriel Instruments). The light from the UV–vis lamp was filtered using a water filter (61945 Oriel Instruments) and then carried to the spectrophotometer holder at the right angle to the monitoring beam using a fiber-optic system (77654 Oriel Instruments). A 40 W m^{-2} light flux, measured with a Goldilux Photometer with a UV-A probe, was used. A thermostated (20 °C) 10 mm quartz cell, containing the sample solution (3.5 ml), equipped with magnetic stirring was used. In a preliminary experiment, the visible absorption spectrum of the closed form and the λ_{max} of the open form were determined. In a second experiment, the absorbance at photostationary

equilibrium, A_{eq}, was measured at λ_{max} and then the decrease in the absorbance with the time was monitored. The thermal bleaching rate constants, k_{Δ}, were calculated fitting the absorbance curve obtained in the dark, at 20 °C, to a multi exponential model.

4.2. General remarks

^{1}H and ^{13}C spectra were recorded for $CDCl_3$ solutions on a Bruker Avance 400 MHz instrument. IR spectra were obtained on a Perkin-Elmer FTIR 1600 spectrometer using KBr disks (wavenumbers in cm^{-1}). Electronic impact mass spectra were measured on a AutoSpecE spectrometer. Melting points (°C) measured at a Büchi 535 apparatus are uncorrected. The melting points of photochromic naphthopyrans **6a–d** were not determined because thermochromism was observed at high temperatures. Column chromatography (CC) was performed on silica gel 60 (70–230 mesh). THF was pre-dried under sodium/benzophenone and distilled before use. All new compounds were determined to be >95% pure by ^{1}H NMR spectroscopy.

4.3. General procedure for the synthesis of diols 2a–c

n-Buthyllithium in hexanes (7.5 ml, 12 mmol) was added slowly with a syringe to a cold (−10 °C) stirred solution of trimethylsilylacetylene (1.7 ml, 12 mmol) in anhydrous THF (50 ml). The cold solution was stirred 1 h and then a solution of the diketone **1a–c** (4 mmol) in anhydrous THF was added and the mixture stirred at room temperature for 12 h. The reaction mixture was then cooled to 0 °C and a solution of KOH (0.84 g) in MeOH (10 ml) was added in a single portion. After stirring at room temperature for 30 min, the reaction mixture was poured into 50 ml of water and acidified to pH ~ 7 with glacial acetic acid. The organic layer was separated and the aqueous layer extracted with ethyl acetate (3×50 ml). The combined organic phases were washed with water (2×50 ml) and dried (Na_2SO_4). Removal of the solvent gave diols **2a–c**, which were sufficiently pure for subsequent use.

4.3.1. 4,4′-Bis(1-hydroxy-1-phenyl-prop-2-yn-1-yl) diphenyl ether 2a. Light yellow oil. Yield 93%. IR: 3428, 3284, 2113, 1596, 1498, 1240; ^{1}H NMR: 7.61 (m, 4H), 7.54 (m, 4H), 7.40–7.22 (m, 6H), 6.93 (m, 4H), 2.97 (large s, 2H), 2.87 (s, 2H); MS: *m/z* (%): 430 (100), 413 (15), 353 (50), 207 (24), 131 (12), 105 (17), 77 (17).

4.3.2. 1,4-Bis(1-hydroxy-1-phenyl-prop-2-yn-1-yl)benzene 2b. Light yellow solid. Yield: 91%. Mp 143–145. IR: 3426, 3286, 2115, 1486, 1448, 1166; ^{1}H NMR: 7.59 (m, 4H), 7.57 (s, 4H), 7.32 (m, 4H), 7.27 (m, 2H), 2.90 (large s, 2H), 2.86 (s, 2H); MS: *m/z* (%): 338 (100), 321 (32), 261 (70), 207 (24), 131 (55), 105 (60), 77 (17).

4.3.3. 4′,4″-Bis(1-hydroxy-1-phenyl-prop-2-yn-1-yl)-1,2-diphenylethane 2c. Light yellow oil. Yield: 97%. IR: 3421, 3288, 2956, 2115, 1448, 1178; ^{1}H NMR: 7.59 (d, *J*=7.2 Hz, 4H), 7.49 (d, *J*=8.2 Hz, 4H), 7.33 (dd, *J*=7.2 Hz, 4H), 7.27 (dd, *J*=7.2 Hz, 2H), 7.14 (d, *J*=8.2 Hz, 4H), 2.86 (m, 8H); MS: *m/z* (%): 442 (30), 424 (7), 297 (20), 221 (60), 204 (55), 105 (100).

4.4. General procedure for the reaction of 2-naphthol with diols 2a–c

A solution of diol **2a–c** (4.0 mmol), 2-naphthol (0.504 g, 3.5 mmol), PPTS (10 mg), $CH(OMe)_3$ (0.8 ml, 8 mmol) and 1,2-dichloroethane (50 ml) was refluxed for 2 h under nitrogen. Solvent evaporation gave a brown oil, which was purified by CC (3–15% ethyl acetate/hexane). Two compounds were obtained: bi-naphthopyrans **3a–c** (fraction 1 minor product) and naphthopyrans **4a–c** (fraction 2, major product). The following compounds were obtained using this protocol.

4.4.1. From 2-naphthol and diol 2a: 4,4′-bis[3-phenyl-3*H*-naphtho[2,1-*b*]pyran-3-yl]diphenyl ether 3a. White solid. Yield 16%. Mp 99–101. IR: 3056, 2923, 1590, 1496, 1240; ^{1}H NMR: 7.94 (d, *J*=8.4 Hz, 2H), 7.70 (d, *J*=8.4 Hz, 2H), 7.64 (d, *J*=8.8 Hz, 2H), 7.45 (m, 6H), 7.39 (m, 4H), 7.33–7.27 (m, 8H), 7.26–7.22 (m, 2H), 7.17 (d, *J*=8.8 Hz, 2H), 6.90 (m, 4H), 6.22 (d, *J*=10 Hz, 2H); ^{13}C NMR: 156.3, 150.4, 144.8, 139.7, 129.8, 129.3, 128.9, 128.6, 128.5, 128.3, 128.1, 127.6, 126.9, 126.6, 125.9, 123.6, 121.3, 119.5, 118.3, 113.9, 82.2; MS: *m/z* (%): 682 (5), 608 (4), 570 (5), 460 (5), 391 (7), 307 (35), 154 (100), 137 (68). **4-[3-Phenyl-3H-naphtho[2,1-b]pyran-3-yl]-4′-(1-hydroxy-1-phenyl-prop-2-yn-1-yl)diphenyl ether 4a**. Yellow oil. Yield 42%. IR: 3444, 3280, 3058, 1590, 1494, 1238; ^{1}H NMR: 7.95 (d, *J*=8.4 Hz, 1H), 7.71 (d, *J*=8.0 Hz, 1H), 7.66 (d, *J*=8.8 Hz, 1H), 7.59 (m, 2H), 7.55–7.40 (m, 7H), 7.37–7.22 (m, 8H), 7.18 (d, *J*=9.0 Hz, 1H), 6.92 (m, 4H), 6.24 (d, *J*=10 Hz, 1H), 2.87 (s, 1H), 2.77 (s, 1H); ^{13}C NMR: 156.6, 156.4, 150.4, 144.8, 144.3, 139.8, 139.4, 129.9 (two signals), 128.6, 128.5, 128.3, 128.1, 127.9, 127.62, 127.58, 127.54, 126.9, 126.6, 125.9, 123.6, 121.3, 119.5, 118.6, 118.3, 113.9, 86.3, 82.2, 75.5, 73.9; MS: *m/z* (%): 556 (100), 530 (5), 479 (40), 257 (40).

4.4.2. From 2-naphthol and diol 2b: 1,4-bis[3-phenyl-3*H*-naphtho[2,1-*b*]pyran-3-yl]benzene 3b. White solid. Yield 20%. Mp >250. IR: 3060, 2921, 1627, 1583, 1213; ^{1}H NMR: 7.97 (d, *J*=8.4 Hz, 2H), 7.72 (d, *J*=8.4 Hz, 2H), 7.66 (d, *J*=8.8 Hz, 2H), 7.58–7.40 (m, 10H), 7.38–7.22 (m, 10H), 7.20 (d, *J*=8.8 Hz, 2H), 6.26 (d, *J*=10 Hz, 2H); ^{13}C NMR: 150.5, 144.6, 144.2, 129.81, 129.77, 129.31, 128.5, 128.2, 128.0, 127.5, 127.0, 126.8, 126.6, 123.6, 121.3, 119.4, 118.3, 113.9, 82.4; MS: *m/z* (%): 590 (35), 478 (70), 447 (50), 391 (60), 257 (100), 154 (95), 136 (80). **3-Phenyl-3-[p-(1-hydroxy-1-phenyl-prop-2-yn-1-yl)phenyl]-3H-naphtho[2,1-b]pyran 4b**. White solid. Yield 43%. Mp 173–174. IR: 3548, 3438, 3272, 3056, 1631, 1587, 1444, 1216; ^{1}H NMR: 7.94 (d, *J*=8.4 Hz, 1H), 7.71 (d, *J*=8 Hz, 1H), 7.65 (d, *J*=8.8 Hz, 1H), 7.59 (m, 2H), 7.55 (d, *J*=8.0 Hz, 2H), 7.50–7.40 (m, 5H), 7.37–7.20 (m, 8H), 7.18 (d, *J*=8.8 Hz, 1H), 6.24 (d, *J*=10 Hz, 1H), 2.86 (s, 1H), 2.77 (s, 1H); ^{13}C NMR: 150.4, 144.54 (two signals), 144.0, 143.5, 129.9, 129.7, 129.3, 128.5, 128.3, 128.1, 127.9, 127.6, 127.4, 127.0, 126.9, 126.6, 125.9, 125.8, 123.6, 121.3, 119.5, 118.3, 113.9, 86.3, 82.3, 75.5, 74.1; MS: *m/z* (%): 464 (100), 387 (45), 333 (20), 257 (70), 131 (12), 105 (16), 77 (13).

4.4.3. From 2-naphthol and diol 2c: 4′,4″-bis[3-phenyl-3*H*-naphtho[2,1-*b*]pyran-3-yl]-1,2-diphenylethane 3c.

White solid. Yield 22%. Mp 204–205. IR: 3056, 3023, 2921, 1629, 1587, 1511, 1446, 1222; ^{1}H NMR: 7.95 (d, J=8.4 Hz, 2H), 7.70 (d, J=8.0 Hz, 2H), 7.65 (d, J=8.8 Hz, 2H), 7.5–7.4 (m, 6H), 7.36 (d, J=7.8 Hz, 4H), 7.30 (m, 8H), 7.25 (m, 2H), 7.19 (d, J=8.8 Hz, 2H), 7.11 (m, 4H), 6.24 (dd, J=1.2 Hz, 9.9 Hz, 2H), 2.84 (s, 4H); ^{13}C NMR: 150.5, 144.9, 142.4, 141.1, 129.8, 129.3, 128.5, 128.1, 127.8, 127.5, 127.1 (two signals), 126.9 (two signals), 126.6, 123.5, 121.3, 119.4, 118.4, 114.0, 82.4, 37.3; MS: *m*/*z* (%): 694 (5), 582 (20), 505 (10), 446 (15), 318 (35), 257 (22), 227 (55), 131 (34), 119 (50), 91 (100). **4′-[3-Phenyl-3H-naphtho[2,1-b]pyran-3-yl]-4″-(1-hydroxy-1-phenyl-prop-2-yn-1-yl)-1,2-diphenylethane 4c**. Yellow oil. Yield 38%. IR: 3419, 3284, 3056, 1648, 1589, 1446, 1243; ^{1}H NMR: 7.93 (d, J=8.5 Hz, 1H), 7.68 (d, J=8.0 Hz, 1H), 7.63 (d, J=8.0 Hz, 1H), 7.58 (m, 2H), 7.50–7.40 (m, 5H), 7.37 (d, J=8.3 Hz, 2H), 7.32–7.15 (m, 9H), 7.12 (m, 4H), 6.24 (d, J=10 Hz, 1H), 2.86 (large s, 1H), 2.82 (m, 5H); ^{13}C NMR: 150.5, 144.9, 144.4, 142.5, 142.1, 141.4, 141.0, 129.7, 129.2, 128.5, 128.3, 128.1, 127.8, 127.4, 127.1, 126.9, 126.6, 126.0, 125.9, 123.5, 121.3, 119.4, 118.3, 114.0, 86.4, 82.5, 75.5, 74.0, 37.3, 37.2; MS: *m*/*z* (%): 568 (50), 551 (10), 542 (17), 491 (20), 345 (25), 257 (50), 105 (32), 91 (100).

4.4.4. Procedure for the reaction of 1-naphthol with diol 2b. A solution of diol **2b** (4.0 mmol), 1-naphthol (0.504 g, 3.5 mmol), PPTS (10 mg), $CH(OMe)_3$ (0.8 mL, 8 mmol) and 1,2-dichloroethane (50 ml) was refluxed for 2 h under nitrogen. Solvent evaporation gave a brown oil, which was purified by CC (3–15% ethyl acetate/hexane). Two compounds were isolated. **1,4-Bis[2-phenyl-2H-naphtho[1,2-b]pyran-2-yl]benzene 3d**. Pale pink solid. Yield 8%. IR: 3052, 1644, 1617, 1446, 1394, 1371, 1267, 1097; ^{1}H NMR: 8.31 (dd, J=7.5, 1.8 Hz, 2H), 7.70 (dd, J=7.2, 1.8 Hz, 2H), 7.50–7.38 (m, 12H), 7.35–18 (m, 8H), 7.13 (d, J=8.3 Hz, 2H), 6.68 (d, J=9.7 Hz, 2H), 6.12 (d, J=9.7 Hz, 2H); ^{13}C NMR: 147.7, 144.9, 144.4, 134.6, 128.1, 127.6, 127.5, 127.1, 126.8, 126.64, 126.59, 126.3, 125.5, 124.6, 123.7, 122.0, 120.4, 115.3, 83.0; MS: *m*/*z* (%): 590 (100), 513 (10), 447 (10), 436 (10), 322 (16), 257 (41), 185 (40), 105 (24), 77 (27). **2-Phenyl-2-[p-(1-hydroxy-1-phenyl-prop-2-yn-1-yl)]phenyl-2H-naphtho[1,2-b]pyran 4d**. Yellow oil. Yield 32%. IR: 3415, 3282, 3027, 2923, 1446, 1371; ^{1}H NMR: 8.31 (d, J=8.1 Hz, 1H), 7.73 (d, J=8.6 Hz, 1H), 7.60–7.38 (m, 12H), 7.30–7.18 (m, 5H), 7.11 (d, J=8.3 Hz, 1H), 6.68 (d, J=9.7 Hz, 1H), 6.12 (d, J=10 Hz, 1H), 2.85 (large s, 1H), 2.80 (m, 1H); ^{13}C NMR: 147.6, 144.9, 144.8, 144.0, 143.5, 134.6, 128.3, 128.2, 128.1, 127.8, 127.6, 127.5, 126.8, 126.3, 125.90, 125.88, 125.84, 125.82, 125.6, 124.5, 123.8, 121.9, 120.5, 115.3, 86.3, 82.8, 75.5, 74.1; MS: *m*/*z* (%): 464 (100), 446 (70), 387 (37), 333 (27), 257 (52), 105 (24), 77 (19).

4.5. General procedure for the synthesis of bi-naphthopyrans 5a–d

A solution of compounds **4a**–**d** (1.0 mmol), 5-hydroxy-7*H*-benzo[*c*]fluoren-7-one (0.246 g, 1.1 mmol), PPTS (10 mg), $CH(OMe)_3$ (0.2 mL, 2 mmol) and 1,2-dichloroethane (50 ml) was refluxed for 2 h under nitrogen. Solvent evaporation gave a red oil, which was purified by CC (3–10% ethyl acetate/hexane). Recrystallization from hexane/$CHCl_3$ gave crystalline materials.

4.5.1. 4-[3-Phenyl-3*H*-naphtho[2,1-*b*]pyran-3-yl]-4′-[13-oxo-3-phenyl-indeno[2,1-*f*]naphtho[1,2-*b*]pyran-3-yl] diphenyl ether 5a. Red crystals. Yield 77%. Mp 150 d. IR: 3058, 2954, 1702, 1598, 1496, 1240, 1170; ^{1}H NMR: 8.35 (m, 2H), 7.94 (d, J=8.4 Hz, 1H), 7.89 (d, J=9.9 Hz, 1H), 7.86 (d, J=7.6 Hz, 1H), 7.70 (d, J=8.1 Hz, 1H), 7.64 (d, J=8.8 Hz, 1H), 7.55 (m, 3H), 7.50–7.35 (m, 10H), 7.32–7.18 (m, 9H), 7.17 (d, J=8.8 Hz, 1H), 6.90 (m, 4H), 6.32 (d, J=9.9 Hz, 1H), 6.22 (d, J=9.9 Hz, 1H); ^{13}C NMR: 195.9, 156.5, 156.2, 150.4, 149.1, 144.9, 144.8, 144.6, 139.8, 139.4, 135.5, 134.5, 134.4, 129.84, 129.8, 129.7, 129.32, 129.29, 128.6, 128.5, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 126.9, 126.8, 126.6, 126.2, 124.7, 123.7, 123.6, 123.5, 122.3, 121.3, 119.6, 119.5, 118.44, 118.38, 118.3, 113.9, 113.3, 105.7, 83.3, 82.2; MS (FAB): *m*/*z* (%): 785 ([M+1]$^+$, 7), 784 (M$^+$, 6), 707 (2), 659 (2), 391 (14), 338 (25), 154 (100), 136 (74); HRMS: $[C_{57}H_{36}O_4]^+$, found 784.2628, requires 784.2614.

4.5.2. 13-Oxo-3-phenyl-3-[*p*-(3-phenyl-3*H*-naphtho[2,1-*b*]pyran-3-yl)]phenyl-indeno[2,1-*f*]naphtho[1,2-*b*]pyran 5b. Red crystals. Yield 67%. Mp 135–138 d. IR: 3058, 2923, 1702, 1602, 1461, 1369; ^{1}H NMR: 8.36 (m, 2H), 7.93 (d, J=8.4 Hz, 1H), 7.87 (d, J=10 Hz, 1H), 7.86 (d, J=7.6 Hz, 1H), 7.69 (d, J=7.9 Hz, 1H), 7.63 (d, J=8.8 Hz, 1H), 7.55 (m, 3H), 7.50–7.40 (m, 10H), 7.30–7.18 (m, 9H), 7.16 (d, J=8.7 Hz, 1H), 6.32 (d, J=9.9 Hz, 1H), 6.23 (d, J=9.9 Hz, 1H); ^{13}C NMR: 195.7 (two signals), 150.4, 149.1, 144.8 (two signals), 144.5 (two signals), 144.4 (two signals), 144.0, 135.5, 134.5 (two signals), 134.3 (two signals), 129.8 (two signals), 129.7 (two signals), 129.3, 128.5, 128.4, 128.2, 128.1, 127.8 (two signals), 127.7, 127.6, 127.5, 127.4, 126.94, 126.9 (two signals), 126.64, 126.6 (two signals), 126.2 (two signals), 124.7, 123.6, (two signals), 123.5 (two signals), 122.3 (two signals), 121.24, 122.19, 119.6 (two signals), 119.5, 118.3 (two signals), 113.8, 113.3 (two signals), 83.4 (two signals), 82.3; MS: *m*/*z* (%): 692 (5), 583 (6), 555 (12), 527 (5), 391 (90), 149 (100); HRMS: $[C_{51}H_{32}O_3]^+$, found 692.2363, requires 692.2351.

4.5.3. 4′-[3-Phenyl-3*H*-naphtho[2,1-*b*]pyran-3-yl]-4″-[13-oxo-3-phenyl-indeno[2,1-*f*]naphtho[1,2-*b*]pyran-3-yl]-1,2-diphenylethane 5c. Red crystals. Yield 60%. Mp 108–111 d. IR: 3054, 2921, 1702, 1600, 1455, 1367; ^{1}H NMR: 8.38 (m, 2H), 7.94 (d, J=8.4 Hz, 1H), 7.87 (m, 2H), 7.70 (d, J=8.2 Hz, 1H), 7.64 (d, J=8.8 Hz, 1H), 7.60–7.14 (m, 27H), 6.33 (m, 1H), 6.24 (d, J=9.9 Hz, 1H), 2.84 (s, 4H); ^{13}C NMR: 195.9, 158.6, 150.5, 149.2, 144.9 (two signals), 144.7, 142.5, 142.2, 141.3, 141.0, 135.4, 134.5, 134.4, 130.0, 129.7, 129.3 (two signals), 128.5, 128.2 (three signals), 128.0, 127.9 (two signals), 127.8, 127.6, 127.5, 127.4, 127.1, 127.0, 126.9 (two signals), 126.8, 126.6, 126.5, 126.0, 124.7, 123.6, 123.5, 122.3, 121.3, 119.5, 119.4, 118.3, 114.0, 113.4, 83.5, 82.4, 37.30, 37.26; MS (FAB) *m*/*z* (%): 797 ([M+1]$^+$, 35), 796 (M$^+$, 33), 663 (35), 641 (65), 608 (45), 580 (72), 555 (100), 527 (44), 419 (53); HRMS: $[C_{59}H_{40}O_3]^+$, found 796.3011, requires 796.2977.

4.5.4. 13-Oxo-3-phenyl-3-[*p*-(2-phenyl-2*H*-naphtho[1,2-*b*]pyran-2-yl)]phenyl-indeno[2,1-*f*]naphtho[1,2-*b*]pyran 5d. Red crystals. Yield 69%. Mp 138–143 d. IR: 3052, 2921, 1702, 1579, 1461, 1400, 1367; ^{1}H NMR: 8.36–8.29 (m, 4H), 7.86 (m, 1H), 7.80 (m, 2H), 7.68 (m, 1H), 7.60–7.32

(m, 15H), 7.32–7.10 (m, 6H), 6.68 (m, 1H), 6.32 (d, $J=10$ Hz, 1H), 6.14 (d, $J=9.8$ Hz, 1H); ^{13}C NMR: 195.8, 149.1, 147.6, 144.9, 144.7, 144.3, 143.9, 135.5, 134.6, 134.5, 134.4, 129.7, 129.3, 128.2, 128.1, 128.0, 127.81, 127.75, 127.61, 127.56, 127.5, 127.0, 126.9, 126.8, 126.70, 126.66, 126.5, 126.4, 126.3, 126.2, 125.6, 124.7, 124.6, 124.5, 123.8, 123.6, 123.5, 122.3, 122.0, 120.5, 115.3, 113.2, 83.4, 83.0; HRMS: $[C_{51}H_{32}O_3]^+$, found 692.2367, requires 692.2351.

4.6. General procedure for the synthesis of photochromic naphthopyrans 6a–d

A solution of CH_3MgI in dry Et_2O (1 ml, 1 mmol) was added slowly to a cold solution (0 °C) of naphthopyrans **5a–d** (0.25 mmol) in THF (10 ml). After stirring at room temperature for 1 h, the solution was quenched in aqueous satd NH_4Cl, extracted with Et_2O (3×40 ml), dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by CC (3–10% ethyl acetate/light petroleum). Recrystallization from hexane/$CHCl_3$ gave a crystalline material.

4.6.1. 4-[3-Phenyl-3*H*-naphtho[2,1-*b*]pyran-3-yl]-4′-[13-hydroxy-13-methyl-3-phenyl-indeno[2,1-*f*]naphtho[1,2-*b*]pyran-3-yl]diphenyl ether 6a. Pale red crystals. Yield 79%. IR: 3355, 3055, 2956, 1596, 1495, 1235; ^{1}H NMR: 8.51 (d, $J=8.5$ Hz, 1H), 8.42 (d, $J=8.3$ Hz, 1H), 8.05 (d, $J=7.7$ Hz, 1H), 7.93 (d, $J=8.4$ Hz, 1H), 7.70 (d, $J=8.1$ Hz, 1H), 7.64 (d, $J=8.5$ Hz, 1H), 7.60–7.15 (m, 24H), 6.93–6.86 (m, 4H), 6.25–6.18 (m, 2H), 2.04 (s, OH), 1.78 (m, 3H, CH_3); ^{13}C NMR: 158.3, 156.6, 156.3, 156.1, 151.0, 150.4, 148.6, 145.3, 144.8, 144.6, 143.8, 140.2, 139.82, 139.75, 139.5, 139.3, 129.84, 129.79, 129.3, 128.9, 128.6, 128.5, 128.4, 128.22, 128.16, 128.1, 127.8, 127.7, 127.6, 127.5, 127.3, 126.9, 126.65, 126.63, 126.2, 125.4, 123.9, 123.6, 123.2, 122.4, 122.1, 121.3, 120.6, 119.5, 118.5, 118.4, 118.3, 118.2, 113.9, 113.0, 82.74, 82.69, 82.2, 80.67, 80.64, 26.6; HRMS: $[C_{58}H_{40}O_4]^+$, found 800.2938, requires 800.2927.

4.6.2. 13-Hydroxy-13-methyl-3-phenyl-3-[*p*-(3-phenyl-3*H*-naphtho[2,1-*b*]pyran-3-yl)]phenyl-indeno[2,1-*f*]naphtho[1,2-*b*]pyran 6b. Pale red crystals. Yield 75%. IR: 3388, 3264, 2923, 1633, 1365; ^{1}H NMR: 8.52 (m, 1H), 8.42 (d, $J=8.4$ Hz, 1H), 8.06 (m, 1H), 7.92 (m, 1H), 7.72–7.10 (m, 26H), 6.24 (d, $J=9.9$ Hz, 1H), 6.23 (m, 1H), 2.00 (s, 1H, OH), 1.7 (m, 3H, CH_3); ^{13}C NMR: 151.0 (two signals), 150.4 (two signals), 148.6, 145.6, 144.6, 144.4 (two signals), 144.3 (two signals), 144.1, 144.0 (two signals), 143.9, 143.8, 139.5, 129.8 (two signals), 129.3, 128.9, 128.5, 128.2, 128.1, 128.05, 128.01, 127.7, 127.5 (two signals), 127.3, 127.0 (two signals), 126.9 (two signals), 126.8 (two signals), 126.7, 126.6, 126.5, 126.1, 125.4, 123.9, 123.6, 123.3, 122.4, 122.0, 121.3, 120.6, 120.5, 119.4, 119.3 (two signals), 118.31, 118.27, 113.9, 113.8, 113.0, 112.8, 82.8, 82.4 (two signals), 80.6 (two signals), 26.4 (two signals); MS (FAB): *m*/*z* (%): 708 (1), 691 (1), 663 (1), 53 (2), 555 (4), 460 (5), 307 (35), 154 (100), 137 (67); HRMS: $[C_{52}H_{36}O_3]^+$, found 708.2684, requires 708.2664.

4.6.3. 4′-[3-Phenyl-3*H*-naphtho[2,1-*b*]pyran-3-yl]-4″-[13-hydroxy-13-methyl-3-phenyl-indeno[2,1-*f*]naphtho[1,2-*b*]pyran-3-yl]-1,2-diphenylethane 6c. Pale red crystals. Yield 70%. IR: 3330, 3023, 2924, 1633, 1366; ^{1}H NMR: 8.51 (d, $J=8.3$ Hz, 1H), 8.44 (d, $J=8.3$ Hz, 1H), 8.06 (d, $J=7.7$ Hz, 1H), 7.92 (m, 1H), 7.69 (d, $J=8.1$ Hz, 1H), 7.63 (d, $J=7.5$ Hz, 1H), 7.60–7.00 (m, 28H), 6.27–6.18 (m, 2H), 2.8 (m, 4H), 2.05 (s, OH), 1.78 (m, 3H, CH_3); ^{13}C NMR: 151.0, 150.5, 148.7, 145.5, 144.9, 144.7, 143.9, 143.0, 142.5, 141.2, 141.1, 139.6, 129.8, 129.3, 128.9, 128.5, 128.2, 128.11, 128.05, 128.0, 127.8, 127.6, 127.44, 127.36, 127.3, 127.04, 127.01, 126.8, 126.7, 126.6, 126.12, 126.06, 125.43, 125.36, 123.9, 123.6, 123.3, 122.4, 122.1, 121.3, 120.6, 119.4, 118.4, 114.0, 113.0, 82.9, 82.4, 80.7, 37.32, 37.31, 26.4; HRMS: $[C_{60}H_{44}O_3]^+$, found 812.3273, requires 812.3290.

4.6.4. 13-Hydroxy-13-methyl-3-phenyl-3-[*p*-(2-phenyl-2*H*-naphtho[1,2-*b*]pyran-2-yl)]phenyl-indeno[2,1-*f*]naphtho[1,2-*b*]pyran 6d. Pale red crystals. Yield 73%. IR: 3371, 3286, 3053, 2923, 1642, 1393, 1367; ^{1}H NMR: 8.51 (m, 1H), 8.42 (m, $J=8.4$ Hz, 1H), 8.31 (m, 1H), 8.05 (m, 1H), 7.68 (m, 1H), 7.60–7.32 (m, 15H), 7.32–7.18 (m, 8H), 7.12 (m, 1H), 6.68 (m, 1H), 6.23 (m, 1H), 6.16 (d, $J=9.7$, 0.5 Hz), 6.13 (d, $J=9.7$, 0.5 Hz), 2.03 (s, 1H, OH), 1.80 (m, 3H, CH_3); ^{13}C NMR: 151.01, 150.95, 148.6, 147.65, 147.63, 145.05, 144.96, 144.9, 144.8, 144.6, 144.5, 144.3, 143.9, 143.8, 139.5, 134.6, 129.8, 128.9, 128.2, 128.1, 128.0, 127.7, 127.6, 127.50, 127.47, 127.3, 127.1, 127.0, 126.8, 126.74, 126.71, 126.64, 126.60, 126.57, 126.3, 126.1, 125.8 (two signals) 125.40, 125.36, 124.5 (two signals), 123.9, 123.3, 123.1, 123.0, 122.4, 122.1, 122.0, 120.6, 120.4 (two signals), 115.3, 115.2, 113.0, 112.9, 112.8 (two signals), 83.0 (two signals), 80.6 (two signals), 26.4, 26.3; HRMS: $[C_{52}H_{36}O_3]^+$, found 708.2652, requires 708.2664.

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Efficient Pd(0)-catalyzed synthesis of 1,2,3-triazolo-3′-deoxycarbanucleosides and their analogues

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Abstract—The racemic synthesis of hitherto unknown 5-substituted-[1,2,3]-triazolo-3′-deoxycarbanucleosides and [1,2,3]-triazolo-[4,5-*c*] pyridin-4-one analogues is described. The key iodinated intermediate **10** was prepared in 10 steps using a malonic synthesis. Various alkynes were introduced at the C-5 position of **10** under optimized Pd(0)-catalyzed Sonogashira cross-coupling alkynylation to yield after deprotection **12a–i**. The synthesis of their 8-aza-3-deazapurine analogues (**13a–h**) was also accomplished through the heteroannulation of internal alkynes under aqueous dimethylamine.
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1. Introduction

1,2,3-Triazoles are an important class of heterocycles due to their range of applications as synthetic intermediates and pharmaceuticals.[1] Many 1,2,3-triazoles are found to be potent antimicrobial,[2] antiviral,[3] and anti-proliferative agents,[4] and to act as potassium channel activators.[5] The pharmaceutical importance of nucleoside analogues has prompted the design and synthesis of various 1,2,3-triazolonucleosides (Fig. 1). For instance, compound **1** exhibited an anti-HIV activity,[6] meanwhile **2** has a cytostatic activity,[7] and **3** was used as a radiosensitizer.[8] Recently, we have reported on the synthesis of carbocyclic and phosphonocarbocyclic analogues of the ribavirin (**4**), an anti-HCV inhibitor, which were evaluated for their antiviral activity.[9] Compounds **5** and **6** displayed a moderate IC_{50} against HIV-1 of 43.8 and 37 μM, respectively, (Fig. 1).

As part of our drug discovery program, we report herein a full account of the synthesis of 4-substituted-[1,2,3]-triazolo analogues (**12a–i**), through an optimized Pd(0) catalyzed alkynylation of the heterocycle. Those compounds were converted into their 8-aza-3-deazapurine analogues (**13a–h**).

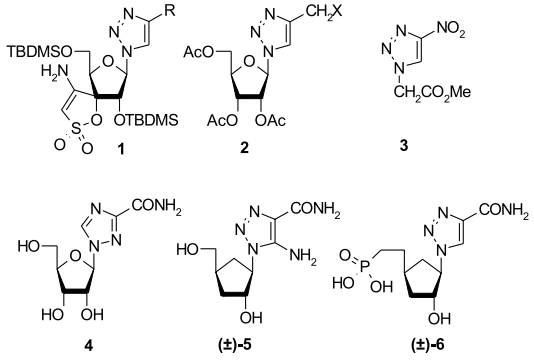

Figure 1. Bioactive triazoles.

2. Results and discussion

The synthetic strategy of the present study is depicted in Scheme 1. The known 2-azido-4-hydroxymethyl-cyclopentanol (±)-**7**, obtained from a malonic synthesis,[10] was reacted through an 1,3-dipolar cycloaddition,[11] with the 2-cyanoacetamide to yield the (±)-5-amino-1-(2-hydroxy-4-hydroxymethylcyclopentyl)-1*H*-[1,2,3]triazole-4-carboxy-lic acid amide (**8**). To achieve that cycloaddition, we first applied our previously described protocol,[9] using cyanoacetamide, K_2CO_3 and a solution of the azide in DMSO. But the difficulties encountered to entirely remove the DMSO push us to apply other known methods,[12] in which the cycloaddition takes place in anhydrous ethanol with sodium ethoxyde, in presence of cyanoacetamide and the azide compound. The regioselectivity of this ligation

Keywords: Nucleosides; Pd(0); Heteroannulation; 1,2,3-Triazole.
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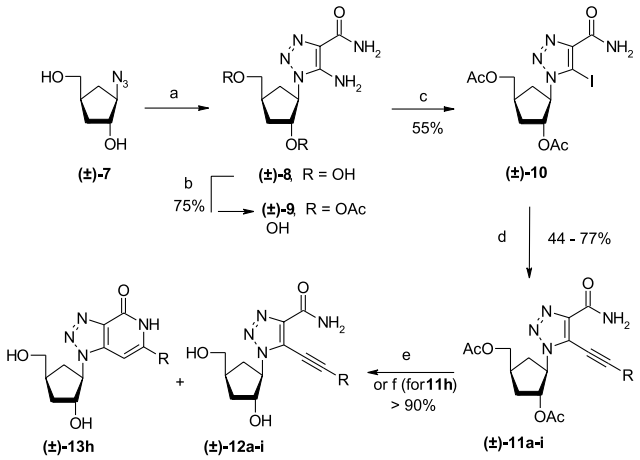


Entry	R	Compounds	Yield (%)
1	C_3H_7	**11a**	58
2	C_4H_9	**11b**	52
3	C_5H_{11}	**11c**	62
4	C_8H_{17}	**11d**	47
5	Ph	**11e**	74
6	$PhCH_3$	**11f**	77
7	PhC_3H_7	**11g**	64
8	PhC_5H_{11}	**11h**	63
9	CH_2OH	**11i**	44

Scheme 1. Reagents and conditions: (a) cyanoacetamide, EtONa, EtOH; (b) Ac_2O, pyridine, rt, 24 h; (c) isoamylnitrite, CH_2I_2, 100 °C, 2 h; (d) in sealed tube: alkyne, Et_3N, $(PhCN)_2PdCl_2$, DMF, 155 °C, 4 h; (e) MeONa/MeOH, rt, 2–24 h; (f) MeONa/MeOH, reflux.

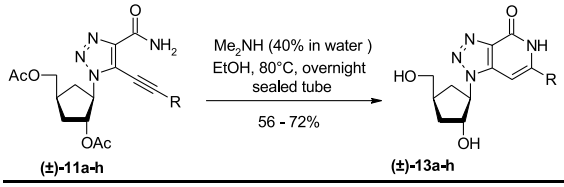
leading to the 1,2,3-triazolo moiety was confirmed by NMR using a ^1H,^{13}C-long range correlation spectra (gHMBC).

In order to apply a Pd(0)-catalyzed reaction to further modify the heterocycle, after an acetylation of **8** to **9**, the C-5-amino group of **9** was directly converted into an iodine group, by using isoamyl nitrite in diiodomethane.[13] The iodinated analogue **10** was isolated in 55% yield.

We then turned our attention to optimization of synthesis of **11a** by an alkynylation of **10** with the *n*-pentynyl under Sonogashira condition's (Table 1).[14] It appears that the reactivity of 1,2,3-triazole for Pd(0) cross-coupling is different to that of the known imidazo- or 1,2,4-triazolo heterocycles. In fact, the application of typical Sonogashira procedures afforded no reaction (entries 1–3), incomplete reaction (entry 4) or degradation (entry 5). However, by heating the mixture overnight in DMF at 155 °C in a sealed tube, the cross-coupling occurred in a moderate but encouraging yield (entry 6). Finally, when the iodinated nucleoside **10** (0.52 mmol) in a mixture of dry DMF (3 mL), dry Et_3N (370 L, 2.6 mmol), pentynyl (2.6 mmol, 5 equiv) and $(PhCN)_2PdCl_2$ (19.9 mg, 0.05 mmol), was reacted at 155 °C, in sealed tube and under argon, the Sonogashira cross-coupling reaction takes place in only 4 h. With these optimized experimental conditions, we enlarged that procedure to various alkyl and aryl groups. The yields of the desired 5-substituted 1,2,3-triazoles **11a–i** ranged from moderate to good yields (Scheme 1).

The subsequent deacetylation of **11a–i** was performed in a methanolic solution of sodium methoxide at rt (except for **11h** where the reaction occurred at reflux of MeOH), and led quantitatively to the desired nucleosides **12a–i**, respectively. In the case of **11h**, a by-product was found to be the 8-aza-3-deazapurine analogue (±)-**13h**. This compound is structurally closely related to the family of 3-deazapurine nucleosides, which includes some potent antiviral,[15] antitumoral,[16] or antibacterian[17] agents.

Related compounds have been obtained through a Pd(0)-catalyzed heteroannulation of an allylic amide with a C-5-iodo group.[18] In our case, we successfully applied a procedure reported by Minakawa et al.[19] based on the ring closure of internal alkynes in presence of aqueous



Entries	R	Compounds	Yield (%)
1	C_3H_7	**13a**	56
2	C_4H_9	**13b**	60
3	C_5H_{11}	**13c**	62
4	C_8H_{17}	**13d**	51
5	Ph	**13e**	72
6	$PhCH_3$	**13f**	51
7	PhC_3H_7	**13g**	66
8	PhC_5H_{11}	**13h**	67

Scheme 2.

Table 1. Optimized synthesis of **11a** through a Sonogashira cross-coupling reaction

Entry	Catalyst	*n*-Pentyne (equiv)	*T* (°C)	Time (h)	Observations[a]	Yield (%)
1	$Pd(PPh_3)_4+CuI$	1.2	100	8	SM[b] recovered	nd[c]
2	$Pd(PPh_3)_4$	1.2	100	8	SM recovered	nd
3	$Pd(OAc)_2+PPh_3$	1.2	100	8	SM recovered	nd
4	$(PhCN)_2PdCl_2$	1.2	100	8	Incomplete	nd
5	$Pd(PPh_3)_2Cl_2$	1.2	rt	8	Degradation	nd
6	$(PhCN)_2PdCl_2$	1.2	155	8	Completed by TLC	50
7	$(PhCN)_2PdCl_2$	5	155	4	Completed by TLC	62

[a] Assays 1–5 were performed in a flask, whereas assays 6–7 were performed in a sealed tube.
[b] SM, starting material.
[c] nd, not determined.

dimethylamine (Scheme 2). The treatment of 1,2,3-triazolo **11a–h** in a sealed tube with aqueous dimethylamine in reflux ethanol led both to the deacylation and to the ring closure. The desired 8-aza-3-deazapurines (or 6-substituted-[1,2,3]-triazolo[4,5-*c*]pyridine-4-one) (±)-**13a–h** were isolated in 51–72% yield, respectively. We failed for the ring closure of **11i**.

It is interesting to note that this is the first example of a successful heteroannulation on a nucleoside bearing an alkynyl or aryl-alkynyl chain. In fact, each assays attempted by Minakawa et al. on an imidazole ring failed. Only cases of success were reported when the reaction was applied on phenyl or pyridinic derivatives instead of imidazole.[20]

3. Conclusion

In summary, the first synthesis of hitherto unknown [1,2,3] triazolo-3′-deoxycarbanucleosides have been accomplished using a Pd(0)-catalyzed cross-coupling reaction under optimized Sonogashira conditions. Additionally, we synthesized 8-aza-2-substituted-3-deazapurine analogues. The synthesized compounds were evaluated in human PBM cells infected with HIV-1_{LAI}.[21] Except for the 1,2,3-triazole-4-carboxylic acid amide (**12h**) which was found to exhibit moderate anti-HIV activity, with an $EC_{50}=41.9$ μM, no significant antiviral activities were found; the toxicities were also assessed, and these compounds did not exhibit any significant toxicity at concentration up to 100 μM in CEM, PBM, and Vero cells.[22]

4. Experimental

4.1. General

Commercially available chemicals were reagent grade and used as received. Dry pyridine was obtained from distillation over Na, *N*,*N*-dimethylformamide and ethanol over CaH_2. Dimethylamine was dried by passage through a KOH filled tower. Methanol was dried with CaH_2 and triethylamine with KOH. The reactions were monitored by thin-layer chromatography (TLC), analysis using silica gel plates (Kieselgel 60 F_{254}, E. Merck). Compounds were visualized by UV irradiation and/or spraying with 20% H_2SO_4 in EtOH, followed by charring at 150 °C. Column chromatography was performed on Silica Gel 60 M (0.040–0.063 mm, E. Merck). The ^1H and ^{13}C NMR spectra were recorded on a Brucker AVANCE DPX 250 Fourier Transform spectrometer at 250 MHz for ^1H and 62.9 MHz for ^{13}C, in (*D*) chloroform, (D_4) methanol and (D_6)-DMSO, shift values in ppm relative to $SiMe_4$ as internal reference, unless otherwise stated; signals are reported as s (singlet), d (doublet), t (triplet), m (multiplet); *J* in Hz. High-resolution mass spectra (HRMS) were performed by the Centre Regional de Mesures Physiques de l'Ouest (University of Rennes, France), using fast atom bombardment (FAB) or electron spray ionization (ESI). The nomenclature of the obtained compounds is in accordance with the IUPAC rules and was checked with autonome.

4.2. Preparation of the iodinated key intermediate

4.2.1. (±)-5-Amino-1-(2-hydroxy-4-hydroxymethyl-cyclopentyl)-1*H*-[1,2,3]triazole-4-carboxylic acid amide (8). To a solution of **7** (10.0 mg, 0.64 mmol) in EtOH (1 mL) at rt was added a freshly prepared solution of EtONa (130.7 mg, 1.9 mmol) in EtOH (3 mL) under an argon atmosphere, then cyanoacetamide (58.9 mg, 0.70 mmol). The reaction mixture was heated at 50 °C for 20 h. After evaporation of the solvent, the residue was purified by flash silica gel column chromatography (19:1 CH_2Cl_2/MeOH) to give **8** (52%, 80.0 mg) as a colorless solid. ^1H NMR (CD_3OD) δ: 1.70–2.17 (m, 3H), 2.25–2.62 (m, 2H), 3.55 (d, $J=6.2$ Hz, 2H), 4.30–4.67 (m, 2H); ^{13}C NMR (CD_3OD) δ: 32.7, 35.9, 37.8, 65.4, 66.4, 77.5, 122.8, 146.6, 166.7; HRMS: $C_9H_{15}N_5O_3Na$, calcd for *m*/*z* 264.2397, found: *m*/*z* 264.2399.

4.2.2. (±)-Acetic acid 3-acetoxy-4-(5-amino-4-carbamoyl-[1,2,3]triazol-1-yl)-cyclopentylmethyl ester (9). To a solution of **8** (228.0 mg, 0.95 mmol) in pyridine (20 mL) at 0 °C was added Ac_2O (4 mL). The mixture was stirred at rt for 48 h. Then, MeOH (4 mL) was added at 0 °C and the stirring was continued for 4 h at rt. Solvents were evaporated, and the residue was purified by flash silica gel column chromatography (19:1 CH_2Cl_2/MeOH) to give **9** (75%, 229.0 mg) as a yellow oil. ^1H NMR (CD_3OD) δ: 1.85–2.25 (m, 9H), 3.35–2.80 (m, 2H), 4.14 (d, $J=6.2$ Hz, 2H), 4.60–4.90 (m, 1H), 5.20–5.45 (m, 1H); ^{13}C NMR (CD_3OD) δ: 20.8, 20.9, 33.8, 34.7, 36.9, 62.6, 68.0, 80.2, 123.1, 146.6, 166.9, 172.5, 172.9; HRMS: $C_{13}H_{19}N_5O_5Na$, calcd for *m*/*z* 348.3143, found: *m*/*z* 348.3144.

4.2.3. (±)-Acetic acid 3-acetoxy-4-(4-carbamoyl-5-iodo-[1,2,3]triazol-1-yl)-cyclopentylmethyl ester (10). To a solution of **9** (205.0 mg, 0.63 mmol) in CH_2I_2 (2 mL, 6.65 mmol) at rt was added isoamyl nitrite (254 L, 1.89 mmol) under an argon atmosphere. The mixture was stirred and heated at 100 °C for 2 h, and was then cooled at rt to be purified by flash silica gel column chromatography (100:1 CH_2Cl_2/MeOH) to give **10** (55%, 158.7 mg) as a yellow oil. ^1H NMR (CD_3OD) δ: 1.90–2.33 (m, 9H), 2.45–2.85 (m, 2H), 4.15 (d, $J=6.2$ Hz, 2H), 5.01–5.25 (m, 1H), 5.45–5.78 (m, 1H); ^{13}C NMR (CD_3OD) δ: 20.8, 34.8, 35.6, 36.7, 67.5, 67.8, 80.1, 86.1, 144.0, 164.1, 171.9, 172.8; HRMS: $C_{13}H_{17}IN_4O_5Na$, calcd for *m*/*z* 459.1962, found: *m*/*z* 459.1964.

4.3. General procedure for Pd(0)-catalyzed cross-coupling

Iodinated nucleoside (±)-**10** (0.52 mmol) was dissolved under argon in a mixture of dry DMF (3 mL), dry Et_3N (370 μL, 2.6 mmol) and alkyne (2.6 mmol). Then $(PhCN)_2PdCl_2$ (19.9 mg, 0.05 mmol) was added, the tube was sealed under argon and the reaction mixture was heated at 155 °C for 4 h. Solvents were evaporated under reduce pressure and the crude product was submitted to a flash silica gel column chromatography (hexanes then hexanes/EtOAc 5:5) to afford the pure alkyne compound.

4.3.1. (±)-Acetic acid 3-acetoxy-4-(4-carbamoyl-5-pent-1-ynyl-[1,2,3]triazol-1-yl)cyclopentylmethyl ester (11a).

Prepared from compound **10** by the typical procedure described before, using pentyne as alkyne, to give **11a** (58%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ: 1.09 (t, J= 7.4 Hz, 3H), 1.61–1.83 (m, 2H), 1.88–2.30 (m, 9H), 2.40–2.76 (m, 4H), 412 (d, J=6.2 Hz), 5.01 (m, 1H), 5.50 (td, J= 4.4, 7.4 Hz), 5.78 (br s, 1H), 6.96 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ: 13.6, 21.0, 21.7, 22.1, 34.3, 34.4, 35.6, 64.6, 65.5, 66.7, 78.5, 106.8, 123.6, 141.8, 161.5, 170.0, 171.1; HRMS: $C_{18}H_{24}N_4O_5Na$, calcd for m/z 399.1644, found: m/z 399.1647.

4.3.2. (±)-Acetic acid 3-acetoxy-4-(4-carbamoyl-5-hex-1-ynyl-[1,2,3]triazol-1-yl)-cyclopentylmethyl ester (11b). Prepared from compound **10** by the typical procedure described before, using hexyne as alkyne, to give **11b** (52%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ: 0.95 (t, J=7.3 Hz, 3H), 1.40–1.82 (m, 4H), 1.85–2.28 (m, 9H), 2.35–2.77 (m, 4H), 4.12 (d, J=6.0 Hz, 2H), 5.00 (ddd, J=5.2, 7.8, 9.6 Hz, 1H), 5.50 (td, J=5.0, 7.5 Hz, 1H), 5.81 (br s, 1H), 6.96 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ: 13.7, 19.8, 21.0, 22.1, 30.2, 34.3, 34.4, 35.6, 64.6, 65.4, 66.8, 78.5, 107.0, 123.6, 141.7, 161.5, 170.0, 171.1; HRMS: $C_{19}H_{26}N_4O_5Na$, calcd for m/z 413.1801, found: m/z 413.1798.

4.3.3. (±)-Acetic acid 3-acetoxy-4-(4-carbamoyl-5-hept-1-ynyl-[1,2,3]triazol-1-yl)cyclopentylmethyl ester (11c). Prepared from **10** by the typical procedure described before, using heptyne as alkyne, to give **11c** (62%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ: 0.91 (t, J=7.2 Hz, 3H), 1.21–1.53 (m, 4H), 1.55–1.77 (m, 2H), 1.78–2.30 (m, 9H), 2.35–2.77 (m, 4H), 4.11 (d, J=6.2 Hz), 4.99 (ddd, J=5.2, 8.0, 9.5 Hz), 5.50 (td, J=4.4, 7.5 Hz, 1H), 5.96 (br s, 1H), 6.98 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ: 14.1, 20.1, 21.0, 22.3, 27.9, 31.2, 34.3, 34.5, 35.6, 64.6, 65.4, 66.8, 78.5, 107.2, 123.5, 158.3, 161.4, 170.0, 171.1; HRMS: $C_{20}H_{28}N_4O_5Na$, calcd for m/z 427.1957, found: m/z 427.1957.

4.3.4. (±)-Acetic acid 3-acetoxy-4-(4-carbamoyl-5-dec-1-ynyl-[1,2,3]triazol-1-yl)-cyclopentylmethyl ester (11d). Prepared from compound **10** by the typical procedure described before, using decyne as alkyne, to give **11d** (47%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ: 0.88 (t, J=7.0 Hz, 3H), 1.12–1.39 (m, 8H), 1.40–1.55 (m, 2H), 1.56–1.77 (m, 2H), 1.89–2.29 (m, 9H), 2.35–2.75 (m, 4H), 4.13 (d, J=6.4 Hz), 5.01 (ddd, J=5.2, 8.3, 9.6 Hz, 1H), 5.52 (td, J=4.6, 7.3 Hz, 1H), 5.58 (br s, 1H), 6.93 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ: 14.2, 20.1, 21.0, 22.8, 28.2, 29.1, 29.2, 31.9, 34.3, 34.4, 35.6, 64.6, 65.4, 66.8, 78.5, 107.0, 123.6, 141.7, 161.5, 170.0, 171.1; HRMS: $C_{23}H_{34}N_4O_5Na$, calcd for m/z 469.2427, found: m/z 469.2427.

4.3.5. (±)-Acetic acid 3-acetoxy-4-(4-carbamoyl-5-phenylethynyl-[1,2,3]triazol-1-yl)cyclopentylmethyl ester (11e). Prepared from compound **10** by the typical procedure described before, using phenylacetylene as alkyne, to give **11e** (74%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ: 1.92–2.12 (m, 7H), 2.13–2.34 (m, 3H), 2.46–2.08 (m, 2H), 4.20 (d, J=6.0 Hz, 2H), 5.11 (ddd, J=5.2, 8.0, 9.6 Hz, 1H), 5.55 (td, J=4.2, 7.5 Hz, 1H), 5.86 (br s, 1H), 7.02 (br s, 1H), 7.52 (m, 5H); ^{13}C NMR ($CDCl_3$) δ: 21.0, 34.3, 35.6, 65.1, 66.6, 73.4, 78.6, 104.4, 121.0, 123.2, 128.7, 132.0, 142.3, 145.4, 161.3, 170.0, 171.0; HRMS: $C_{21}H_{22}N_4O_5Na$, calcd for m/z 433.1488, found: m/z 433.1487.

4.3.6. Acetic acid 3-acetoxy-4-(4-carbamoyl-5-p-tolyl-ethynyl-[1,2,3]triazol-1-yl)cyclopentylmethyl ester (11f). Prepared from compound **10** by the typical procedure described before, using phenylmethylacetylene as alkyne, to give **11f** (77%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ: 1.93–2.11 (m, 7H), 2.12–2.33 (m, 2H), 2.39 (s, 3H), 2.47–2.79 (m, 4H), 4.13–4.24 (m, 2H), 5.11 (ddd, J=5.0, 7.9, 9.7 Hz, 1H), 5.55 (td, J=4.4, 7.9 Hz, 1H), 5.70 (br s, 1H), 6.99 (br s, 1H), 7.20 (d, J=8.2 Hz, 2H), 7.51 (d, J=8.2 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ: 21.0, 21.8, 34.3, 35.6, 65.0, 66.7, 73.0, 78.5, 104.4, 118.0, 123.4, 125.6, 129.5, 132.1, 140.8, 142.1, 161.3, 170.0, 171.1; HRMS: $C_{22}H_{24}N_4O_5Na$, calcd for m/z 447.1644, found: m/z 447.1640.

4.3.7. (±)-Acetic acid 3-acetoxy-4-[4-carbamoyl-5-(4-propyl-phenylethynyl)-[1,2,3]triazol-1-yl]-cyclopentyl-methyl ester (11g). Prepared from compound **10** by the typical procedure described before, using phenylpropyl-acetylene as alkyne, to give **11g** (64%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ: 0.91 (t, J=7.4 Hz, 3H), 1.64 (sxt, J= 7.6 Hz, 2H), 1.90–2.09 (m, 7H), 2.10–2.30 (m, 3H), 2.20–2.79 (m, 4H), 4.12 (d, J=6.0 Hz, 2H), 5.10 (ddd, J=5.3, 7.8, 9.7 Hz, 1H), 5.54 (td, J=4.5, 7.8 Hz, 1H), 6.15 (br s, 1H), 7.05 (br s, 1H), 7.22 (d, J=8.2 Hz, 2H), 7.54 (d, J= 8.2 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ: 13.8, 20.9, 21.0, 24.3, 34.3, 35.6, 38.1, 65.0, 66.6, 72.9, 78.5, 104.4, 118.2, 123.3, 128.8, 132.0, 142.2, 145.4, 161.5, 170.0, 171.1; HRMS: $C_{24}H_{28}N_4O_5Na$, calcd for m/z 475.1957, found: m/z 475.1961.

4.3.8. (±)-Acetic acid 3-acetoxy-4-[4-carbamoyl-5-(4-pentyl-phenylethynyl)-[1,2,3]triazol-1-yl]-cyclopentyl-methyl ester (11h). Prepared from compound **10** by the typical procedure described before, using phenylpentyl-acetylene as alkyne, to give **11h** (63%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ: 0.89 (t, J=6.6 Hz, 3H), 1.21–1.43 (m, 4H), 1.52–1.69 (m, 2H), 1.93–2.11 (m, 8H), 2.12–2.33 (m, 2H), 2.46–2.79 (m, 4H), 4.14 (d, J=6.0 Hz, 2H), 5.11 (ddd, J=4.0, 5.4, 9.3 Hz, 1H), 5.56 (td, J=4.8, 7.4 Hz, 1H), 5.62 (br s, 1H), 6.97 (br s, 1H), 7.21 (d, J=8.0 Hz, 2H), 7.53 (d, J=8.0 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ: 14.4, 20.7, 20.8, 23.5, 32.0, 32.5, 34.7, 34.8, 36.6, 36.9, 66.5, 67.8, 73.9, 79.8, 105.2, 119.5, 124.3, 130.0, 133.0, 143.4, 147.0, 163.8, 171.9, 172.7; HRMS: $C_{26}H_{32}N_4O_5Na$, calcd for m/z 503.2270, found: m/z 503.2270.

4.3.9. (±)-Acetic acid 3-acetoxy-4-[4-carbamoyl-5-(3-hydroxy-prop-1-ynyl)-[1,2,3]triazol-1-yl]-cyclopentyl-methyl ester (11i). Prepared from compound **10** by the typical procedure described before, using propargyl alcohol as alkyne, to give **11i** (44%) as a yellow oil. ^{1}H NMR (CD_3OD) δ: 1.88–2.30 (m, 9H), 2.40–2.76 (m, 2H), 3.82 (s, 2H), 4.12 (d, J=6.2 Hz, 2H), 5.01 (m, 1H), 5.50 (td, J=4.4, 7.4 Hz, 1H); ^{13}C NMR (CD_3OD) δ: 21.0, 34.3, 34.4, 35.6, 64.6, 65.5, 66.7, 76.4, 78.5, 106.8, 123.6, 141.8, 161.5, 170.0, 171.1; HRMS: $C_{16}H_{20}N_4O_6Na$, calcd for m/z 387.3479, found: m/z 387.3480.

4.4. General procedure for deprotection

Acetylated nucleoside **11a–i** (0.15 mmol) was dissolved in a solution of sodium methoxide 0.1 N in methanol (3 mL, 0.3 mmol) and the mixture was stirred at rt (except at reflux for **11h**) until completion (typically 2–24 h, checked by TLC). The reaction mixture was neutralized with DOWEX 50X2-200, and then filtered through a fritted glass funnel. Solvent was evaporated in vacuo to give the pure deprotected nucleoside **12a–i**, respectively, without further purification.

4.4.1. (±)-1-(2-Hydroxy-4-hydroxymethyl-cyclopentyl)-5-pent-1-ynyl-1*H*-[1,2,3]triazole-4-carboxylic acid amide (12a). Prepared from compound **11a** by the typical procedure described before to give **12a** (96%) as a pale yellow solid. UV (MeOH) λ_{max} 250 nm; ^{1}H NMR (CD_3OD) δ: 1.10 (t, J=7.4 Hz, 3H), 1.62–2.15 (m, 5H), 2.33–2.73 (m, 4H), 3.57 (d, J=6.2 Hz, 2H), 4.67 (q, 2H, J=6.6 Hz), 4.77–5.01 (m, 1H); ^{13}C NMR (CD_3OD) δ: 13.9, 22.5, 22.6, 34.6, 36.2, 38.5, 66.5, 66.6, 69.0, 76.9, 107.7, 124.6, 143.0, 164.1; HRMS: $C_{14}H_{20}N_4O_3Na$, calcd for m/z 315.1433, found: m/z 315.1429.

4.4.2. (±)-5-Hex-1-ynyl-1-(2-hydroxy-4-hydroxymethyl-cyclopentyl)-1*H*-[1,2,3]triazole-4-carboxylic acid amide (12b). Prepared from compound **11b** by the typical procedure described before to give **12b** (94%) as a pale yellow oil. UV (MeOH) λ_{max} 251 nm; ^{1}H NMR (CD_3OD) δ: 0.98 (t, J=7.4 Hz, 3H), 1.46–1.77 (m, 4H), 1.79–2.12 (m, 3H), 2.32–2.72 (m, 4H), 3.57 (d, J=6.2 Hz, 2H), 4.67 (q, 2H, J=6.6 Hz), 4.75–5.00 (m, 1H); ^{13}C NMR (CD_3OD) δ: 13.9, 20.2, 23.1, 31.2, 34.6, 36.3, 38.5, 66.3, 66.6, 69.0, 76.8, 107.9, 124.6, 143.0, 164.1; HRMS: $C_{15}H_{22}N_4O_3Na$, calcd for m/z 329.1590, found: m/z 329.1594.

4.4.3. (±)-5-Hept-1-ynyl-1-(2-hydroxy-4-hydroxymethyl-cyclopentyl)-1*H*-[1,2,3]triazole-4-carboxylic acid amide (12c). Prepared from compound **11c** by the typical procedure described before to give **12c** (90%) as a pale yellow oil. UV (MeOH) λ_{max} 251 nm; ^{1}H NMR (CD_3OD) δ: 0.95 (t, J=7.0 Hz, 3H), 1.26–1.58 (m, 4H), 1.60–1.78 (m, 2H), 1.79–2.12 (m, 3H), 2.32–2.72 (m, 4H), 3.56 (d, J=6.2 Hz, 2H), 4.67 (q, 2H, J=6.4 Hz), 4.75–5.00 (m, 1H); ^{13}C NMR (CD_3OD) δ: 14.3, 20.5, 23.3, 28.8, 32.2, 34.6, 36.3, 38.5, 66.4, 66.6, 69.0, 76.8, 107.9, 124.6, 143.0, 164.1; HRMS: $C_{16}H_{24}N_4O_3Na$, calcd for m/z 343.1746, found: m/z 343.1741.

4.4.4. (±)-5-Dec-1-ynyl-1-(2-hydroxy-4-hydroxymethyl-cyclopentyl)-1*H*-[1,2,3]triazole-4-carboxylic acid amide (12d). Prepared from compound **11d** by the typical procedure described before to give **12d** (94%) as a pale yellow solid. UV (MeOH) λ_{max} 292 nm; ^{1}H NMR (CD_3OD) δ: 0.80–1.00 (m, 3H), 1.18–1.58 (m, 10H), 1.60–1.79 (m, 2H), 1.80–2.12 (m, 3H), 2.32–2.72 (m, 4H), 3.56 (d, J=6.2 Hz, 2H), 4.67 (q, 2H, J=6.6 Hz), 4.75–5.00 (m, 1H); ^{13}C NMR (CD_3OD) δ: 14.4, 20.5, 23.7, 29.1, 30.1, 30.2, 30.4, 33.0, 34.6, 36.3, 38.5, 66.4, 66.6, 69.0, 76.8, 107.9, 124.6, 143.0, 164.1; HRMS: $C_{19}H_{30}N_4O_3Na$, calcd for m/z 385.2216, found: m/z 385.2215.

4.4.5. (±)-1-(2-Hydroxy-4-hydroxymethyl-cyclopentyl)-5-phenylethynyl-1*H*-[1,2,3]triazole-4-carboxylic acid amide (12e). Prepared from compound **11e** by the typical procedure described before to give **12e** (94%) as a pale yellow solid. UV (MeOH) λ_{max} 289 nm; ^{1}H NMR (CD_3OD) δ: 1.83–2.20 (m, 3H), 2.41–2.65 (m, 2H), 3.58 (d, J=6.0 Hz, 2H), 4.71 (q, 2H, J=6.4 Hz), 4.80–5.08 (m, 1H), 7.35–7.80 (m, 5H); ^{13}C NMR (CD_3OD) δ: 34.5, 36.3, 38.5, 66.5, 69.4, 74.5, 77.1, 104.6, 122.4, 124.2, 129.8, 131.2, 133.0, 143.5, 164.0; HRMS: $C_{17}H_{18}N_4O_3Na$, calcd for m/z 349.1277, found: m/z 349.1278.

4.4.6. (±)-1-(2-Hydroxy-4-hydroxymethyl-cyclopentyl)-5-*p*-tolylethynyl-1*H*-1,2,3]triazole-4-carboxylic acid amide (12f). Prepared from compound **11f** by the typical procedure described before to give **12f** (93%) as a pale yellow solid. UV (MeOH) λ_{max} 292 nm; ^{1}H NMR (CD_3OD) δ: 1.80–2.20 (m, 3H), 2.27–2.67 (m, 5H), 3.58 (d, J=6.0 Hz, 2H), 4.71 (q, 2H, J=6.6 Hz), 4.80–5.08 (m, 1H), 7.27 (d, J=7.8 Hz, 2H), 7.58 (d, J=7.8 Hz, 2H); ^{13}C NMR (CD_3OD) δ: 21.6, 34.5, 36.3, 38.5, 66.5, 69.3, 74.0, 77.0, 105.0, 119.4, 124.3, 130.4, 133.0, 141.9, 143.3, 164.0; HRMS: $C_{18}H_{20}N_4O_3Na$, calcd for m/z 363.1433, found: m/z 363.1425.

4.4.7. (±)-1-(2-Hydroxy-4-hydroxymethyl-cyclopentyl)-5-(4-propylphenylethynyl)-1*H*-[1,2,3]-triazole-4-carboxylic acid amide (12g). Prepared from compound **11g** by the typical procedure described before to give **12g** (99%) as a yellow solid. UV (MeOH) λ_{max} 295 nm; ^{1}H NMR (CD_3OD) δ: 0.97 (t, J=7.5 Hz, 3H), 1.66 (sxt, J=7.4 Hz, 2H), 1.82–2.20 (m, 3H), 2.37–2.75 (m, 4H), 3.58 (d, J=5.9 Hz, 2H), 4.71 (q, 2H, J=6.9 Hz), 4.80–5.08 (m, 1H), 7.26 (d, J=8.4 Hz, 4H), 7.60 (d, J=8.4 Hz, 2H); ^{13}C NMR (CD_3OD) δ: 14.0, 25.4, 34.5, 36.3, 38.5, 39.0, 66.6, 69.3, 74.0, 77.0, 105.0, 119.7, 124.3, 129.9, 133.0, 143.3, 146.6, 164.1; HRMS: $C_{20}H_{24}N_4O_3Na$, calcd for m/z 391.1746, found: m/z 391.1749.

4.4.8. (±)-1-(2-Hydroxy-4-hydroxymethyl-cyclopentyl)-5-(4-pentyl-phenylethynyl)-1*H*-[1,2,3]-triazole-4-carboxylic acid amide (12h). Prepared from compound **11h** by the typical procedure described before to give **12h** (62%) as a white solid. UV (MeOH) λ_{max} 283 nm; ^{1}H NMR (CD_3OD) δ: 0.91 (t, J=7.5 Hz, 3H), 1.22–1.50 (m, 4H), 1.55–1.75 (m, 2H), 1.82–2.17 (m, 3H), 2.40–2.60 (m, 2H), 2.67 (t, J=7.4 Hz, 2H), 3.58 (d, J=6.2 Hz, 2H), 4.71 (q, 2H, J=6.6 Hz,), 4.81–5.05 (m, 1H), 7.27 (d, J=8.4, 2H), 7.59 (d, J=8.4 Hz, 2H); ^{13}C NMR (CD_3OD) δ: 14.3, 23.5, 32.1, 32.5, 34.5, 36.3, 36.7, 38.5, 66.6, 69.3, 74.0, 77.0, 105.0, 119.7, 124.3, 129.9, 133.0, 143.3, 146.9, 164.1; HRMS: $C_{22}H_{28}N_4O_3Na$, calcd for m/z 419.2059, found m/z 419.2053.

4.4.9. (±)-1-(2-Hydroxy-4-hydroxymethyl-cyclopentyl)-5-(3-hydroxy-prop-1-ynyl)-1*H*-[1,2,3]-triazole-4-carboxylic acid amide (12i). Prepared from compound **11i** by the typical procedure described before to give **12i** (99%) as a colorless solid. ^{1}H NMR (CD_3OD) δ: 1.62–2.15 (m, 3H), 2.33–2.73 (m, 2H), 3.57 (d, J=6.2 Hz, 2H), 3.83 (s, 2H), 4.67 (m, 1H), 4.77–5.01 (m, 1H); ^{13}C NMR (CD_3OD) δ: 34.7, 36.5, 38.2, 66.7, 67.1, 69.0, 76.9, 78.5, 107.7, 124.6, 143.0, 164.1; HRMS: $C_{12}H_{16}N_4O_3Na$, calcd for m/z 287.2739, found: m/z 287.2741.

4.5. General procedure for the one-pot hetero-annulation–deprotection

Nucleoside **11a**–**h** (0.32 mmol) was dissolved under argon in a mixture of ethanol and a solution of dimethylamine in water 40% (0.02 mmol, 2.4 mL): the tube was sealed and the reaction mixture was heated at 80 °C overnight (completion checked by TLC). Solvents were removed in vacuo and the residue was submitted to a flash silica gel column chromatography, using an appropriate eluent (typically 5:5 hexanes/EtOAc then EtOAc) to let pure **13a**–**h**, respectively.

4.5.1. (±)-1-(2-Hydroxy-4-hydroxymethyl-cyclopentyl)-6-propyl-1,5-dihydro-[1,2,3]-triazolo[4,5-*c*]pyridin-4-one (13a). Prepared from compound **11a** by the typical procedure described before to give **13a** (56%) as a yellow oil. UV (MeOH) λ_{max} 274 nm; ^{1}H NMR (CD_3OD) δ: 1.00 (t, J=7.6 Hz, 3H), 1.73 (sxt, J=7.5 Hz, 2H), 1.82–2.29 (m, 2H), 2.38–2.70 (m, 4H), 3.60 (d, J=6.0 Hz, 2H), 4.56 (q, 2H, J=7.0 Hz), 4.75–5.00 (m, 1H), 6.61 (s, 1H); ^{13}C NMR (CD_3OD) δ: 13.8, 23.3, 33.6, 36.1, 36.2, 38.0, 66.7, 68.2, 77.3, 91.2, 136.2, 141.9, 149.2, 159.5; HRMS: $C_{14}H_{20}N_4O_3Na$, calcd for m/z 315.1433, found: m/z 315.1429.

4.5.2. (±)-6-Butyl-1-(2-hydroxy-4-hydroxymethyl-cyclopentyl)-1,5-dihydro-[1,2,3]-triazolo[4,5-*c*]pyridin-4-one (13b). Prepared from compound **11b** by the typical procedure described before to give **13b** (60%) as a yellow oil. UV (MeOH) λ_{max} 275 nm; ^{1}H NMR (CD_3OD) δ: 0.98 (t, J=7.4 Hz, 3H), 1.42 (sxt, J=7.5 Hz, 2H), 1.60–1.79 (m, 2H), 1.80–2.29 (m, 2H), 2.35–2.57 (m, 2H), 2.64 (t, J=7.6 Hz, 2H), 3.60 (d, J=6.0 Hz, 2H), 4.57 (q, 2H, J=7.3 Hz), 4.72–4.96 (m, 1H), 6.60 (s, 1H); ^{13}C NMR (CD_3OD) δ: 14.1, 23.2, 32.1, 33.6, 34.0, 36.1, 38.0, 66.7, 68.2, 77.3, 91.1, 136.2, 141.9, 149.4, 159.5; HRMS: $C_{15}H_{22}N_4O_3Na$, calcd for m/z 329.1590, found: m/z 329.1579.

4.5.3. (±)-1-(2-Hydroxy-4-hydroxymethyl-cyclopentyl)-6-pentyl-1,5-dihydro-[1,2,3]triazolo[4,5-*c*]pyridin-4-one (13c). Prepared from compound **11c** by the typical procedure described before to give **13c** (62%) as a yellow oil. UV (MeOH) λ_{max} 274 nm; ^{1}H NMR (CD_3OD) δ: 0.80–1.15 (m, 3H), 1.24–1.50 (m, 4H), 1.55–1.79 (m, 2H), 1.80–2.29 (m, 3H), 2.35–2.74 (m, 4H), 3.60 (d, J=6.0 Hz, 2H), 4.56 (q, 2H, J=6.9 Hz), 4.72–4.96 (m, 1H), 6.60 (s, 1H); ^{13}C NMR (CD_3OD) δ: 14.3, 23.4, 29.7, 32.4, 33.6, 34.3, 36.1, 38.0, 66.7, 68.2, 77.3, 91.1, 136.2, 141.9, 149.4, 159.5; HRMS: $C_{16}H_{24}N_4O_3Na$, calcd for m/z 343.1746, found: m/z 343.1737.

4.5.4. (±)-1-(2-Hydroxy-4-hydroxymethyl-cyclopentyl)-6-octyl-1,5-dihydro-[1,2,3]triazolo[4,5-*c*]pyridin-4-one (13d). Prepared from compound **11d** by the typical procedure described before to give **13d** (51%) as a white solid. UV (MeOH) λ_{max} 275 nm; ^{1}H NMR (CD_3OD) δ: 0.80–1.00 (m, 3H), 1.18–1.50 (m, 10H), 1.60–1.79 (m, 2H), 1.80–2.29 (m, 3H), 2.35–2.74 (m, 4H), 3.60 (d, J=6.0 Hz, 2H), 4.55 (m, 2H), 4.72–4.96 (m, 1H), 6.60 (s, 1H); ^{13}C NMR (CD_3OD) δ: 14.4, 23.7, 30.0, 30.1, 30.3, 30.4, 32.9, 33.5, 34.3, 36.1, 38.0, 66.7, 68.2, 77.3, 91.1, 136.2, 141.9, 149.4, 159.5; HRMS: $C_{19}H_{30}N_4O_3Na$, calcd for m/z 385.2216, found: m/z 385.2218.

4.5.5. (±)-1-(2-Hydroxy-4-hydroxymethyl-cyclopentyl)-6-phenyl-1,5-dihydro-[1,2,3]triazolo[4,5-*c*]pyridin-4-one (13e). Prepared from compound **11e** by the typical procedure described before to give **13e** (72%) as a white solid. UV (MeOH) λ_{max} 298 nm; ^{1}H NMR (DMSO-d_6) δ: 1.63–2.17 (m, 3H), 2.23–2.45 (m, 2H), 2.35–3.55 (m, 1H), 4.37–4.45 (m, 1H), 4.72 (br s, 1H), 4.91 (td, J=7.4, 9.8 Hz, 1H), 5.15–5.25 (m, 1H), 7.06 (s, 1H), 7.35–7.85 (m, 5H); ^{13}C NMR (DMSO-d_6) δ: 32.1, 35.1, 36.4, 64.8, 66.4, 75.4, 89.8, 125.4, 128.8, 129.9, 133.4, 135.4, 139.8, 144.5, 156.9; HRMS: $C_{17}H_{18}N_4O_3Na$, calcd for m/z 349.1277, found: m/z 349.1275.

4.5.6. (±)-1-(2-Hydroxy-4-hydroxymethyl-cyclopentyl)-6-*p*-tolyl-1,5-dihydro-[1,2,3]triazolo[4,5-*c*]pyridin-4-one (13f). Prepared from compound **11f** by the typical procedure described before to give **13f** (51%) as a pale yellow solid. UV (MeOH) λ_{max} 297 nm; ^{1}H NMR (DMSO-d_6) δ: 1.63–2.75 (m, 3H), 2.23–2.45 (m, 5H), 3.20–3.60 (m, 2H), 4.39 (q, 2H, J=6.9 Hz), 4.74 (br s, 1H), 4.83–5.02 (m, 1H), 5.28 (br s, 1H), 7.04 (s, 1H), 7.33 (d, J=8.2 Hz, 2H), 7.69 (d, J=8.2 Hz, 2H), 11.30–11.90 (br s, 1H); ^{13}C NMR (DMSO-d_6) δ: 20.8, 32.1, 35.1, 36.4, 54.9, 64.8, 66.3, 75.4, 89.2, 127.2, 129.3, 130.5, 135.3, 139.7, 139.9, 144.5, 147.0; HRMS: $C_{18}H_{20}N_4O_3Na$, calcd for m/z 363.1433, found: m/z 363.1430.

4.5.7. (±)-1-(2-Hydroxy-4-hydroxymethyl-cyclopentyl)-6-(4-propyl-phenyl)-1,5-dihydro-[1,2,3]triazolo[4,5-*c*]pyridin-4-one (13g). Prepared from compound **11g** by the typical procedure described before to give **13g** (66%) as a pale yellow solid. UV (MeOH) λ_{max} 297 nm; ^{1}H NMR (CD_3OD) δ: 0.96 (t, J=7.4 Hz, 3H), 1.68 (sxt, J=7.6 Hz, 2H), 1.81–2.32 (m, 3H), 2.36–2.73 (m, 4H), 3.61 (d, J=5.6 Hz, 2H), 4.59 (q, 2H, J=7.4 Hz), 4.70–5.02 (m, 1H), 6.98 (s, 1H), 7.33 (d, J=8.2 Hz, 2H), 7.63d, J=8.2 Hz, 2H); ^{13}C NMR (CD_3OD) δ: 14.0, 25.5, 33.5, 36.1, 38.0, 38.7, 66.7, 68.3, 77.4, 91.1, 128.2, 130.2, 132.5, 136.5, 141.8, 146.5, 146.9, 159.5; HRMS: $C_{20}H_{24}N_4O_3Na$, calcd for m/z 391.1746, found: m/z 391.1744.

4.5.8. (±)-1-(2-Hydroxy-4-hydroxymethyl-cyclopentyl)-6-(4-pentyl-phenyl)-1,5-dihydro-[1,2,3]-triazolo[4,5-*c*]pyridin-4-one (13h). Prepared from compound **11h** by the typical procedure described before to give **13h** (67%) as a white solid. UV (MeOH) λ_{max} 298 nm; ^{1}H NMR (CD_3OD) δ: 0.91 (t, J=6.6 Hz, 3H), 1.25–1.47 (m, 4H), 1.57–1.75 (m, 2H), 1.83–2.35 (m, 3H), 2.42–2.61 (m, 2H), 2.69 (t, J=6.6 Hz, 2H), 3.61 (d, J=6.8 Hz, 2H), 4.59 (q, 2H, J=7.2 Hz), 4.80–5.00 (m, 1H), 6.99 (s, 1H), 7.35 (d, J=8.4 Hz, 2H), 7.65 (d, J=8.4 Hz, 2H); ^{13}C NMR (CD_3OD) δ: 14.4, 23.6, 32.2, 32.6, 33.5, 36.1, 36.6, 38.3, 66.7, 68.3, 77.4, 91.1, 128, 130.2, 132.5, 136.6, 141.8, 146.8, 146.9, 159.5; HRMS: $C_{22}H_{28}N_4O_3Na$, calcd for m/z 419.2059, found: m/z 419.2058.

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I_2-catalyzed Michael addition of indole and pyrrole to nitroolefins

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Abstract—An easy and efficient method to generate indolyl nitroalkane **5** and pyrrolyl nitroalkane **7** in high yields using β-nitrostyrene and indole/pyrrole at room temperature in the presence of catalytic amount of iodine is reported. The short reaction times and high yields of product are noteworthy. Molecular iodine promoted Michael addition is operationally simple and efficient method compared to the known Lewis acids or rare earth metal catalysts to generate different indolyl/pyrrolyl nitroalkanes in high yield.
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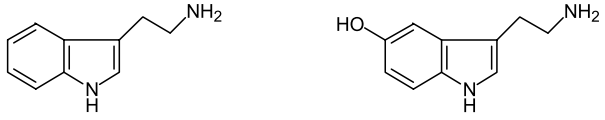
1. Introduction

Indole and many of its derivatives are most important units in many naturally occurring compounds, because of a wide variety of their pharmacological and biological properties.[1] The hapalindole alkaloids, which exhibit significant antibacterial and antimycotic activity, and several indole alkaloids such as uleine, aspidospermidine, ibophyllidine alkaloids, and numerous tryptamine derivatives are also associated with important biological activity.[2] Likewise, important pyrrole derivatives also present in compounds such as bile pigments, vitamin B_{12}, haemin, chlorophyll, and related natural products.[3] In addition, several pyrrole derivatives are important intermediates not only for the synthesis of drugs, pigments and pharmaceuticals but also for the development of organic functional groups.[4] Therefore, development of new synthetic methods of indole and pyrrole derivatives have been widely studied using various Lewis acids as well as Bronsted acids.[5] Since the 3-position of the indole is the ideal site for electrophilic attack, 3-substituted indoles are versatile intermediates for the synthesis of a wide variety of indole derivatives. Conversely, C-2 position of pyrrole is indeed the electron-rich site for Michael addition. Michael addition of indoles and pyrroles to various nucleophiles has been well documented in the literature using either protic or Lewis acids.[6–9] However, Lewis acid-catalyzed Michael addition of indole and pyrrole necessitate careful control over the acidity to avoid the undesirable side reactions such as dimerization and polymerization.[10] Incidentally, the polymerized products of indole and pyrrole derivatives involves troublesome isolation procedures to obtain the desired product with some of the Lewis acids. Furthermore, many procedures require longer reaction times, expensive and toxic reagents in stoichiometric amounts, air sensitive conditions, difficult workup procedures.

The use of molecular iodine in organic synthesis has been known for a long time. In recent years molecular iodine has received considerable attention as an inexpensive, nontoxic, readily available catalyst for various organic transformations under mild and convenient conditions to afford the corresponding products in excellent yields with high selectivity.[11] Another advantage in using iodine as catalyst, will not influence the nitro group, which is very significant for β-nitrostyrene **3**. Herein, some important pharmaceutical compounds, tryptamine **1** and serotonin **2**, which can be obtained as a result of Michael addition between indole and nitroalkene (Fig. 1). Especially serotonin, a simple derivative of indole is a major neurotransmitter and many indole derivatives also mimic the binding of neurotransmitter to its receptors also have been synthesized.[12] In continuation of our work in exploring the methods using β-nitrostyrene, we had the opportunity to focus on the iodine catalyzed Michael addition of



1 (tryptamine) 2 (serotonin)

Figure 1.

Keywords: β-Nitrostyrene; Indole; Pyrrole; Iodine; Michael addition.
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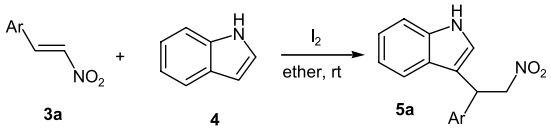
β-nitrostyrene **3** with indole, because the resultant products are analogs of **1** and **2** after reducing the nitro group (Fig. 1).

In this paper, we wish to report that elemental iodine can be used as a mild and efficient catalyst for the Michael addition of β-nitrostyrene **3** with indole **4** and pyrrole **6** at room temperature to afford products 2-indolyl-2-phenyl-1-nitroalkane **5** and 2-pyrrolyl-2-phenyl-1-nitroalkane **7** in high to excellent yields.

2. Results and discussion

In the beginning, Michael reaction between indole and β-nitrostyrene **3** was carried out using iodine (30 mol%) in chloroform (0.5 mL) led to the formation of 2-indolyl-2-phenyl-1-nitroalkane **5** in 95% yield. With this encouraging result, next we investigated the fate of reaction in different solvents. Conducting the reaction in DMSO did not proceed and DMF as a solvent afforded only 10% of the product with several unwanted side products. After substantial experimentation with different solvents (CH_2Cl_2, 82% yield; $CHCl_3$, 95% yield; EtOAc, 91% yield), diethylether came out as a solvent of choice. The iodine catalyzed reaction in ether not only improved the product yields, but also reduced the reaction times. We next, investigated the amount of iodine required to catalyze the transformation. As less as 10 mol% of iodine afforded the products in 43% yield, after 18 h. By means of 20 mol% of iodine though product yields were improved to 72%, but the reaction time is almost same as that of 10 mol%. On the other hand, using 30 mol% of iodine as a catalyst afforded the products in 99% yield in 2 h (Scheme 1).

To check the versatility of iodine catalyzed Michael reaction, various substituted β-nitrostyrenes (**3a**–**c**) were reacted with indole **4** using iodine (30 mol%) in ether (0.5 mL) solution and the results were summarized



a: Ar = C_6H_5

Serial no.	amount of I_2(mol%)	solvent	5a[a]
1	30	$CHCl_3$ (0.5ml)	95%
2	30	DMSO (0.5ml)	10%
3	30	DMF (0.5ml)	10%
4	30	CH_2Cl_2 (0.5ml)	82%
5	30	EtOAc (0.5ml)	91%
6	30	ethyl ether (0.5ml)	99%[b]
7	10	ethyl ether (0.5ml)	43%[b]
8	20	ethyl ether (0.5ml)	72%[b]

a) NMR yields of the crude products
b) The reaction of serial no. 6 was 2h but were over 18h in serial no. 7 and 8

Scheme 1.

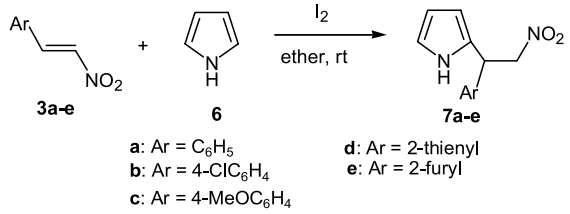
Table 1. Iodine catalyzed Michael addition between nitroolefins and indole

Serial no.	Entry[a]	Time (h)	Product[b]	Yield (%)[c]
1	**3a**	2	**5a**	99
2	**3b**	2.5	**5b**	99
3	**3c**	18	**5c**	99
4	**3d**	2	**5d**	89
5	**3e**	6	**5e**	94

[a] All reactions were performed at 1 mmol scale using 30 mol% of iodine in 0.5 mL of ether.
[b] All products were well characterized by ^1H NMR, ^{13}C NMR, and mass spectroscopy.
[c] NMR yields of the crude products.

(Table 1). Although all Michael adducts were obtained in excellent yields, but the reaction times varied according to the nature of the substitution pattern on the phenyl ring. The electron donating groups substituted in the phenyl ring of the nitrostyrene led to the formation of products with longer reaction times. The heterocyclic nitroolefins (**3d**–**3e**) also afforded the adducts in high yield. The lower yield of **5d** over **5e** can be explained on the basis of the product stability.

In order to extend the scope of this methodology, pyrrole **6** was subjected as a nucleophile in Michael addition with differnet β-nitrostyrenes, to generate 2-pyrrolyl-2-phenyl-1-nitroalkanes **7a**–**e** in good yields (Scheme 2).



Scheme 2.

The reasons for the lower product yields in case of pyrrole, when compared to indole may be ascribed due to polymerization of products in some cases. This is due to high nucleophilicity of pyrroles, which facilitates to react rapidly than indole. Using molecular iodine in catalytic amount to generate 2-alkyl pyrroles in excellent yields is noteworthy (Table 2).

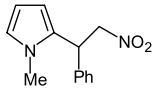
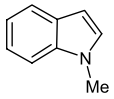
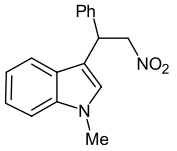
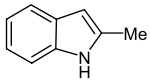
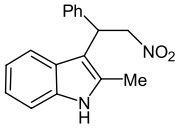
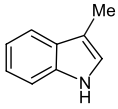
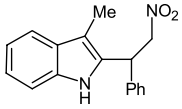
Table 2. Iodine catalyzed Michael addition between nitroolefins and pyrrole

Serial no.	Entry[a]	Time (h)	Product[b]	Yield (%)[c]
1	**3a**	1	**7a**	86
2	**3b**	1	**7b**	76
3	**3c**	1.5	**7c**	79
4	**3d**	1.3	**7d**	85
5	**3e**	1.2	**7e**	81

[a] All reactions were performed at 1 mmol scale using 30 mol% of iodine in 0.5 mL of ether.
[b] All products were well characterized by ^1H NMR, ^{13}C NMR, and mass spectroscopy.
[c] NMR yields of the crude products.

So as to utilize this extensively effective protocol, we examined by taking various other indole and pyrrole derivatives with β-nitrostyrene **3**. *N*-methylpyrrole **8**

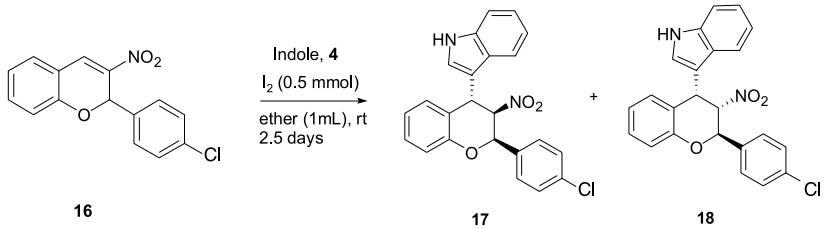
Table 3. Iodine catalyzed Michael addition of β-nitrostyrene with methyl substituted indole or pyrrole

Serial no.	Entry (indole/pyrrole)[a]	Time	Product[b]	Yield (%)[c]
1	 **8**	45 min	 **9**	99
2	 **10**	20 min	 **11**	99
3	 **12**	20 min	 **13**	99
4	 **14**	2 days	 **15**	70

[a] All reactions were performed at 1 mmol scale using 30 mol% of iodine in 0.5 mL of ether.
[b] All products were well characterized by ^{1}H NMR, ^{13}C NMR, and mass spectroscopy.
[c] NMR yields of the crude products.

and methyl substituted indoles such as *N*-methylindole **10**, 2-methylindole **12**, and 3-methylindole **14** afforded the products in good to excellent yields under similar reaction conditions (Table 3). Besides, we have not observed any byproducts, which are iodated either on indole or pyrrole nucleus. The substituted derivatives of both indole and pyrrole gave the products in excellent yield during less time, when compared to their unsubstituted counterparts. A possible explanation may be due to the presence of the methyl group, which not only increases the electron density of the aromatic ring to accelerate the reaction, but also prevents the unwanted side reactions such as polymerization. In case of 3-methylindole **14**, which undergoes conjugate addition at the 2-position may involve more complicated mechanism. It has been reported in the literature, that the mechanism involving addition at 2-position proceeds through the initial attack of the electrophile at C-3, followed by a 1,2-shift in the intermediate cation leads to the formation of final product.[3] The longer reaction times and lower product yields in the addition of β-nitrostyrene to 3-methylindole clearly supports the above proposed mechanism.

In addition to this, we have also examined the fate of a different nitroolefin like 2-(4-chlorophenyl)-3-nitro-2*H*-chromene **16**, since the resultant 2*H*-benzopyran derivatives such as flavonols[13] and amines[14] belongs to medicinally important compounds. In our previous report, we have provided an easy and efficient method to prepare **16** using DABCO in catalytic amount.[15] Using 50 mol% of iodine, 2-(4-chlorophenyl)-3-nitro-2*H*-chromene in a reaction with indole gave 73% of **17** and 26% of **18** in ether solution at room temperature after 2.5 days (Scheme 3). The two stereoisomers were separated through column chromatography and the ratio was determined by the crude NMR analysis. In addition to this, the two stereoisomers were also characterized by the single X-ray crystallography (Figs. 2 and 3) and suggested a trans configuration between the aryl substituent at 2-position and the indolyl group at 4-position for the compound **17**. The formation of major product as



Scheme 3.

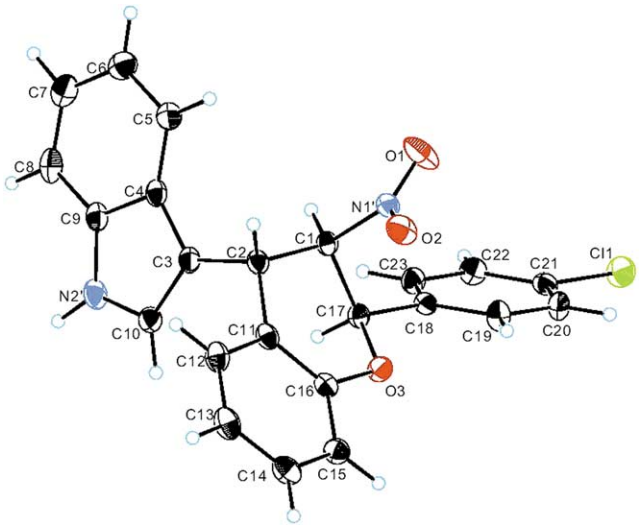


Figure 2. X-ray crystal structure of **17**.

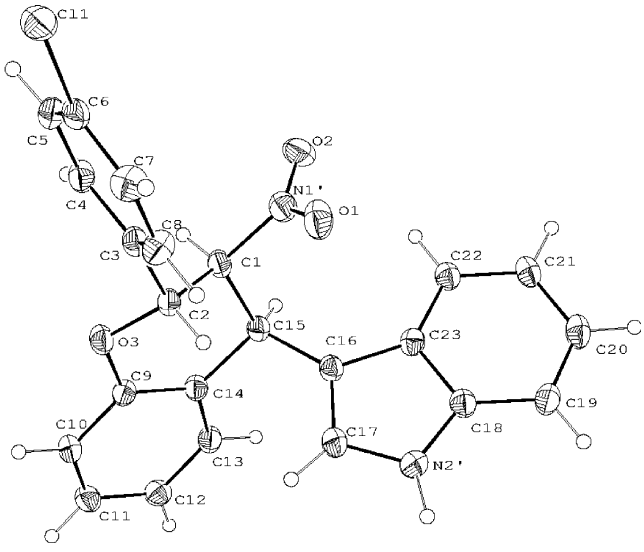
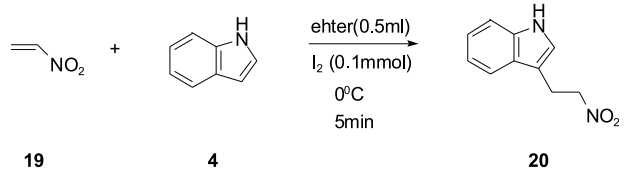


Figure 3. X-ray crystal structure of **18**.

trans isomer can be explained on the basis of steric hindrance in which the two bulky groups are trans to each other separated by a nitro group.

In order to apply the remarkable catalytic activity of iodine, for the synthesis of biologically important compound, a less hindered nitroolefin like nitroethylene **19**[16] was used as Michael acceptor. Using 10 mol% of iodine, nitroethylene **19** reacts readily with indole at 0 °C, to afford the product **20**[17] in 98% yield (Scheme 4). The formation of 2-indolyl-1-nitroethane **20** in less than 5 min, and the formation of 2-(4-chlorophenyl)-4-indolyl-3-nitrochroman **17** in 2.5 days,



Scheme 4.

clearly indicates the steric hindrance of the nitroolefin plays a significant role in determining the reaction time. Moreover, the product **20** is an important intermediate and can be reduced to tryptamine **1**, a neurotransmitter.[17a–b]

3. Conclusions

In summary, we have achieved a simple, efficient, and practical Michael addition process for the synthesis of various 2-indolyl-2-aryl-1-nitroalkanes **5** and 2-pyrrolyl-2-aryl-1-nitroalkanes **7** from β-nitrostyrene using catalytic amount of iodine. The significant advantage of this catalytic reaction lies in its usage under mild and ambient conditions. The yields are generally excellent (up to 98%) without any byproducts and the reaction times are also short. Further investigations are in progress on the application of this methodology to the synthesis of natural product molecules with indole or pyrrole moiety and other heterocyclic ring systems.

4. Experimental

4.1. General

All reactions were performed in room temperature and all chemicals including solvent used for reactions without drying. Analytical thin-layer chromatography was performed with E. Merck silica gel 60F glass plates and flash chromatography by use of E. Merck silica gel 60 (230–400 mesh). MS or HRMS were measured by JEOL JMS-D300 or JEOL JMS-HX110 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded with Bruker Aavance EX 400.

4.2. Material

β-Nitrostyrene **1**, indole, pyrrole, and iodine were purchased from Aldrich Chemical Co. and other commercially available reagents were used without further purification. 2-(4-Chlorophenyl)-3-nitro-2*H*-chromene **16**, nitroethylene **19** were prepared according to the literature procedures and spectral data was consistent with the literature report.

4.3. Typical experimental procedure for the synthesis of adducts 5 or 7 (a: Ar=Ph, b: Ar=4-ClC$_6$H$_4$, c: Ar=4-MeOC$_6$H$_4$, d: Ar=thienyl, e: Ar=furyl)

Indole **4** (4.0 mmol) or pyrrole **6** (4.0 mmol) was added to a suspension of β-nitrostyrene **3** (1.0 mmol) in diethyl ether (0.5 mL) along with iodine (0.3 mmol) at room temperature for several minutes to hours. After completion the reaction (monitored by TLC), it was quenched with water and washed with (2×10 mL) aq $Na_2S_2O_3$ and extracted into CH_2Cl_2 (3×20 mL). The combined organic phases were washed sequentially with brine and water and dried over anhyd Na_2SO_4. Evaporation of the organic solvent afforded the crude products **5a–e** or **7a–e**.

4.3.1. 2-Phenyl-2-indolyl-1-nitroethane (5a). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (s, 1H), 7.40 (d, J=8.00 Hz, 1H), 7.27–7.10 (m, 8H), 6.82 (d, J=2.36 Hz, 1H), 5.12 (dd, J=8.36, 7.68 Hz, 1H), 4.95 (dd, J=12.52, 7.68 Hz, 1H), 4.84

(dd, J=12.52, 8.36 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.13, 136.26, 128.72, 127.58, 127.36, 125.88, 122.38, 121.56, 119.67, 118.66, 113.86, 111.37, 79.33, 41.35. MS *m/z* (relative intensity) 266 (M^+, 8), 219 (100), 204 (44), 178 (19), 115 (11), 108 (17). HRMS calcd for $C_{16}H_{14}N_2O_2$ (M^+) 266.1055, found 266.1051.

4.3.2. 2-(4-Chlorophenyl)-2-indolyl-1-nitroethane (5b). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (s, 1H), 7.39 (d, J= 7.96 Hz, 1H), 7.33 (d, J=8.20 Hz, 1H), 7.29–7.07 (m, 6H), 6.98 (s, 1H), 5.14 (dd, J=8.56, 7.40 Hz, 1H), 5.02 (dd, J= 12.52, 7.40 Hz, 1H), 4.88 (dd, J=12.52, 8.56 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.64, 136.25, 133.04, 128.96, 128.83, 125.67, 122.55, 121.46, 119.80, 118.55, 113.38, 111.43, 79.03, 40.70. MS *m/z* (relative intensity) 300 (M^+, 33), 254 (41), 253 (100), 240 (64), 218 (37), 115 (14), 108 (25). HRMS calcd for $C_{16}H_{13}ClN_2O_2$ (M^+) 300.0666, found 300.0671.

4.3.3. 2-(4-Methoxylphenyl)-2-indolyl-1-nitroethane (5c). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (s, 1H), 7.43 (d, J=7.92 Hz, 1H), 7.36–7.16 (m, 4H), 7.09–7.00 (m, 2H), 6.87–6.23 (m, 2H), 5.13 (dd, J=8.44, 7.48 Hz, 1H), 5.03 (dd, J=12.24, 7.48 Hz, 1H), 4.89 (dd, J=12.24, 8.44 Hz, 1H), 3.77 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.95, 136.57, 131.24, 128.84, 126.15, 122.72, 121.47, 119.97, 119.03, 114.86, 114.33, 111.38, 79.79, 55.28, 40.90. MS *m/z* (relative intensity) 296 (M^+, 38), 250 (32), 249 (88), 236 (100), 218 (20), 115 (12). HRMS calcd for $C_{17}H_{16}N_2O_3$ (M^+) 296.1161, found 296.1161.

4.3.4. 2-Thienyl-2-indolyl-1-nitroethane (5d). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (s, 1H), 7.52–7.50 (m, 1H), 7.38–7.36 (m, 1H), 7.24–7.09 (m, 4H), 6.99–6.93 (m, 2H), 5.44 (dd, J=8.16, 7.56 Hz, 1H), 5.05 (dd, J=12.48, 7.56 Hz, 1H), 4.98 (dd, J=12.48, 8.16 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.85, 136.16, 126.81, 125.48, 125.08, 124.73, 122.45, 121.94, 119.79, 118.57, 113.49, 111.49, 79.78, 36.70. MS *m/z* (relative intensity) 272 (M^+, 33), 226 (26), 225 (100), 212 (74), 210 (20), 167 (9), 115 (15). HRMS calcd for $C_{14}H_{12}N_2O_2S$ (M^+) 272.0619, found 272.0620.

4.3.5. 2-Furyl-2-indolyl-1-nitroethane (5e). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (s, 1H), 7.44 (d, J=7.92 Hz, 1H), 7.21–7.00 (m, 4H), 6.73–6.71 (m, 1H), 6.15–6.13 (m, 1H), 6.01–5.99 (m, 1H), 5.12 (dd, J=8.20, 7.40 Hz, 1H), 4.85 (dd, J=12.56, 8.20 Hz, 1H), 4.69 (dd, J=12.56, 7.40 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.03, 141.96, 135.98, 125.37, 122.68, 122.20, 119.66, 118.34, 111.48, 110.80, 110.25, 107.03, 77.57, 35.39. MS *m/z* (relative intensity) 256 (M^+, 32), 210 (22), 209 (100), 196 (84), 167 (23), 117 (16), 115 (12). HRMS calcd for $C_{14}H_{12}N_2O_3$ (M^+) 256.0848, found 256.0846.

4.3.6. 2-Phenyl-2-pyrrolyl-1-nitroethane (7a). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (s, 1H), 7.35–7.19 (m, 5H), 6.40 (dd, J=4.04, 2.60 Hz, 1H), 6.14 (dd, J=6.04, 2.84 Hz, 1H), 6.05–6.07 (m, 1H), 4.94 (dd, J=11.88, 7.28 Hz, 1H), 4.86 (dd, J=7.52, 7.28 Hz, 1H), 4.76 (dd, J=11.88, 7.52 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.95, 129.13, 128.85, 128.04, 127.83, 118.14, 108.57, 105.73, 79.12, 42.84. MS *m/z* (relative intensity) 216 (M^+, 10), 170 (22), 169 (100), 156 (40), 154 (39), 77 (12). HRMS calcd for $C_{12}H_{12}N_2O_2$ (M^+) 216.0899, found 216.0900.

4.3.7. 2-(4-Chlorophenyl)-2-pyrrolyl-1-nitroethane (7b). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (s, 1H), 7.30 (d, J= 8.40 Hz, 2H), 7.15 (d, J=8.40 Hz, 2H), 6.69–6.67 (m, 1H), 6.16 (dd, J=5.76, 2.76 Hz, 1H), 6.07–6.05 (m, 1H), 4.94 (dd, J=12.12, 7.12 Hz, 1H), 4.84 (dd, J=7.88, 7.12 Hz, 1H), 4.75 (dd, J=12.12, 7.88 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 136.53, 133.99, 129.32, 129.22, 128.28, 118.43, 108.72, 105.95, 78.94, 42.27.

4.3.8. 2-(4-Methoxylphenyl)-2-pyrrolyl-1-nitroethane (7c). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (s, 1H), 7.14–7.11 (m, 2H), 6.88–6.84 (m, 2H), 6.67–6.65 (dd, J=5.96, 2.76 Hz, 1H), 6.16–6.14 (m, 1H), 6.06–6.04 (m, 1H), 4.94 (dd, J=11.92, 6.96 Hz, 1H), 4.82 (dd, J=8.04, 6.96 Hz, 1H), 4.74 (dd, J=11.92, 8.04 Hz, 1H), 3.78 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.30, 129.84, 129.24, 128.98, 118.03, 114.53, 108.58, 105.54, 79.37, 55.26, 42.18. MS *m/z* (relative intensity) 246 (M^+, 13), 199 (100), 186 (66), 171 (14), 168 (20), 77 (9). HRMS calcd for $C_{13}H_{14}N_2O_3$ (M^+) 246.1004, found 246.1006.

4.3.9. 2-Thienyl-2-pyrrolyl-1-nitroethane (7d). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (s, 1H), 7.25–7.23 (m, 1H), 6.93–6.92 (m, 2H), 6.68–6.70 (m, 1H), 6.18–6.15 (m, 1H), 6.11–6.09 (m, 1H), 5.19 (dd, J=7.96, 7.52 Hz, 1H), 4.92 (dd, J= 12.88, 7.52 Hz, 1H), 4.82 (dd, J=12.88, 7.96 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.99, 128.29, 127.17, 125.86, 125.55, 118.25, 108.81, 105.92, 79.66, 38.19. MS *m/z* (relative intensity) 222 (M^+, 1), 206 (2), 168 (11), 88 (37), 73 (40), 79 (78), 61 (100). HRMS calcd for $C_{10}H_{10}N_2OS$ (M^+-16) 206.0463, found 206.0540.

4.3.10. 2-Furyl-2-pyrrolyl-1-nitroethane(7e). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (s, 1H), 7.39–7.38 (m, 1H), 6.72–6.60 (m, 1H), 6.33–6.31 (m, 1H), 6.18–6.13 (m, 2H), 6.09–6.07 (m, 1H), 5.00 (dd, J=7.76, 7.56 Hz, 1H), 4.88 (dd, J=12.76, 7.76 Hz, 1H), 4.79 (dd, J=12.64, 7.56 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.71, 142.70, 126.19, 118.27, 110.56, 108.79, 107.76, 106.65, 77.74, 36.94. MS *m/z* (relative intensity) 206 (M^+, 10), 159 (100), 158 (9), 146 (52), 80 (6). HRMS calcd for $C_{10}H_{10}N_2O_3$ (M^+) 206.0691, found 206.0687.

4.4. Typical experimental procedure for the synthesis of adducts 9, 11, 13 and 15

Methyl substituted indole **10**, **12**, **14** (4.0 mmol) or methyl substituted pyrrole **8** (4.0 mmol) was added to a suspension of β-nitrostyrene **3** (1.0 mmol) in diethyl ether (0.5 mL) along with iodine (0.3 mmol) at room temperature for several minutes to hours. After completion the reaction (monitored by TLC), it was quenched with water and washed with (2×10 mL) aq $Na_2S_2O_3$ and extracted into CH_2Cl_2 (3×20 mL). The combined organic phases were washed sequentially with brine and water and dried over anhyd Na_2SO_4. Evaporation of the organic solvent afforded the crude products **9**, **11**, **13**, **15**.

4.4.1. 2-(*N*-methylpyrroyl)-2-phenyl-1-nitroethane (9). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.20–7.07 (m, 5H), 6.43

(s, 1H), 6.04–6.01 (m, 2H), 4.80–4.72 (m, 2H), 4.60–4.52 (m, 1H), 3.05 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.82, 129.00, 128.59, 127.53, 127.30, 122.47, 106.45, 105.42, 78.79, 41.18, 33.06. MS *m/z* (relative intensity) 230 (M^+, 41), 184 (40), 183 (66), 170 (100), 128 (17), 96 (35), 77 (13). HRMS calcd for $C_{12}H_{12}N_2O_2$ (M^+) 230.1055, found 230.1053.

4.4.2. 2-(*N*-methylindolyl)-2-phenyl-1-nitroethane (11). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39–6.93 (m, 9H), 6.67 (s, 1H), 5.06 (dd, J=8.72, 7.92 Hz, 1H), 4.83 (dd, J=12.32, 7.92 Hz, 1H), 4.76 (dd, J=12.32, 8.72 Hz, 1H), 3.14 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.31, 136.99, 128.59, 127.49, 127.19, 126.32, 126.07, 121.89, 119.15, 118.71, 112.43, 109.33, 79.19, 41.23, 32.24. MS *m/z* (relative intensity) 280 (M^+, 44), 234 (50), 233 (74), 220 (100), 217 (17), 146 (17), 115 (13). HRMS calcd for $C_{17}H_{16}N_2O_2$ (M^+) 280.1212, found 280.1213.

4.4.3. 2-(2-Methylindolyl)-2-phenyl-1-nitroethane (13). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (s, 1H), 7.62 (d, J=7.8 Hz, 1H), 7.50–7.25 (m, 8H), 5.40–5.28 (m, 2H), 5.23 (dd, J=11.76, 8.84 Hz, 1H), 2.35 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.30, 135.11, 132.85, 128.46, 127.00, 126.76, 126.51, 120.87, 119.33, 118.21, 110.65, 108.22, 78.24, 40.21, 11.28. MS *m/z* (relative intensity) 280 (M^+, 62), 234 (49), 220 (100), 146 (49), 115 (8), 77 (8). HRMS calcd for $C_{17}H_{16}N_2O_2$ (M^+) 280.1212, found 280.1218.

4.4.4. 2-(3-Methylpyrroyl)-2-phenyl-1-nitroethane (15). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (s, 1H), 7.52 (d, J=7.68 Hz, 1H), 7.41–7.08 (m, 8H), 5.25 (dd, J=8.28, 7.64 Hz, 1H), 5.08 (dd, J=12.88, 8.28 Hz, 1H), 4.95 (dd, J=12.88, 7.64 Hz, 1H), 2.34 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.00, 135.66, 130.55, 129.23, 128.98, 127.88, 127.16, 122.16, 119.49, 118.56, 110.75, 109.25, 77.41, 40.96, 8.51. MS *m/z* (relative intensity) 280 (M^+, 45), 234 (72), 233 (69), 218 (100), 217 (80), 204 (42), 146 (52), 128 (40), 105 (45), 77 (68). HRMS calcd for $C_{17}H_{16}N_2O_2$ (M^+) 280.1212, found 280.1212.

4.5. Typical experimental procedure for the synthesis of adducts 17 and 18

Indole **4** (4.0 mmol) was added to a suspension of 2-(4-chlorophenyl)-3-nitro-2*H*-chromene **16** (1.0 mmol) in diethyl ether (1.0 mL) along with iodine (0.5 mmol) at room temperature for several minutes to hours. The reaction was monitored by TLC. After completion the reaction mixture was quenched and washed with (2×10 mL) aqueous $Na_2S_2O_3$ solution and extracted with CH_2Cl_2 (3×20 mL). The combined organic phases were washed sequentially with brine and water and dried (Na_2SO_4). The solvent was removed to obtain the crude product **17** and **18**.

4.5.1. (2*R*,3*R*,4*S*)-2-(4-chlorophenyl)-4-indolyl-3-nitrochroman (17). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (s, 1H), 7.64 (d, J=7.80 Hz, 1H), 7.45 (d, J=8.12 Hz, 1H), 7.32–7.00 (m, 10H), 6.71 (d, J=2.28, 1H), 5.34 (d, J=2.20 Hz, 1H), 5.29 (dd, J=2.28, 2.24 Hz, 1H), 5.03 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.76, 136.66, 134.58, 134.28, 130.47, 128.82, 128.55, 127.21, 125.16, 125.13, 123.21, 122.03, 120.66, 119.85, 118.04, 117.66, 117.02, 111.84, 87.20, 72.01, 37.05. MS *m/z* (relative intensity) 406 (M^++2, 8), 404 (M^+, 23), 359 (18), 358 (22), 357 (53), 243 (100), 241 (30), 220 (44), 130 (18). HRMS calcd for $C_{23}H_{17}ClN_2O_3$ (M^+) 404.0928, found 404.0928. Anal. Calcd for $C_{23}H_{17}ClN_2O_3$: C, 68.23; H, 4.23; N, 6.92. Found: C, 68.99; H, 5.02; N, 6.97.

4.5.2. (2*R*,3*S*,4*S*)-2-(4-chlorophenyl)-4-indolyl-3-nitrochroman (18). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (s, 1H), 7.39–6.93 (m, 12H), 6.88 (d, J=2.56 Hz, 1H), 5.54 (d, J=9.32 Hz, 1H), 5.37 (dd, J=9.32, 5.56 Hz, 1H), 5.16 (d, J=5.56 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.06, 136.07, 135.22, 135.03, 130.26, 129.24, 128.96, 126.93, 124.79, 122.67, 121.96, 121.64, 120.37, 118.20, 116.70, 113.43, 111.48, 87.20, 73.51, 37.90. MS *m/z* (relative intensity) 406 (M^++2, 14), 404 (M^+, 45), 359 (13), 358 (17), 357 (36), 243 (27), 241 (89), 220 (100), 132 (37). HRMS calcd for $C_{23}H_{17}ClN_2O_3$ (M^+) 404.0928, found 404.0932. Anal. Calcd for $C_{23}H_{17}ClN_2O_3$: C, 68.23; H, 4.23; N, 6.92. Found: C, 68.54; H, 3.98; N, 6.80.

4.6. Typical experimental procedure for the synthesis of adduct 20

Indole **4** (2.0 mmol) was added to a suspension of nitroethylene **19** (1.0 mmol) in diethyl ether (0.5 mL) along with iodine (0.1 mmol) at 0 °C less than 5 min. The reaction was monitored by TLC. After completion the reaction mixture was quenched and washed with (2× 10 mL) aqueous $Na_2S_2O_3$ solution and extracted with CH_2Cl_2 (3×20 mL). The combined organic phases were washed sequentially with brine and water and dried (Na_2SO_4). The solvent was removed to obtain the crude product **20**.

4.6.1. 2-Indolyl-1-nitroethane 20. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.87 (s, 1H), 7.47–6.75 (m, 4H), 6.75 (s, 1H), 4.47 (t, J=7.12 Hz, 2H), 3.31 (t, J=7.08 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 135.92, 126.36, 122.57, 122.08, 119.47, 117.86, 111.34, 109.40, 75.50, 23.22.

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The microbiological transformation of steroidal saponins by *Curvularia lunata*

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Abstract—The microbiological transformation of polyphyllin I (compound **I**), polyphyllin III (compound **II**), polyphyllin V (compound **III**) and polyphyllin VI (compound **IV**) by *Curvularia lunata* into their corresponding subsaponins, for example, diosgenin-3-*O*-α-L-arabinofuranosyl (1→4)-β-D-glucopyranoside (compound **V**), diosgenin-3-*O*-α-L-rhamnopyranosyl (1→4)-β-D-glucopyranoside (compound **VI**), diosgenin-3-*O*-β-D-glucopyranoside (compound **VII**) and pennogenin-3-*O*-β-D-glucopyranoside (compound **VIII**), were studied in this paper. *Curvularia lunata* is able to hydrolyze terminal rhamnosyls that are linked by 1→2 C– bond to sugar residues of steroidal saponins at C-3 position with high activity and regioselectivity.
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1. Introduction

Rhizoma Paridis refers to the roots and rhizomes of *Paris polyphylla* var. *yunnanensis* (Franch.) *Hand. -Mazz.* or *Paris polyphylla Smith* var. *chinensis* (Franch.) *Hara* of *Lilaceae* family. It is widely used in traditional Chinese medicine for antifebrile, alexipharmic, detumescent, demulcent, hemostatic and the treatment of hepatopathy, etc;[1] also it is the main component of 'Yun-nan-bai-yao' and 'Ji-de-sheng-she-yao-pian', which are famous traditional Chinese medicine preparations. A recent literature survey showed that *Rhizoma Paridis* exhibits a variety of biological activities in heart and vascular malady, anti-tumor, antifertility, spermicidal, immunological enhancement, etc.[2] Compounds **I**, **II**, **III** and **IV**, that is, diosgenin-3-*O*-α-L-arabinofuranosyl(1→4)-(α-L-rhamnopyranosyl (1→2))-β-D-glucopyranoside, diosgenin-3-*O*-α-L-rhamnopyranosyl (1→4)-(α-L-rhamnopyranosyl (1→2))-β-D-glucopyranoside, diosgenin-3-*O*-α-L-rhamnopyranosyl (1→2)-β-D-glucopyranoside and pennogenin-3-*O*-α-L-rhamnopyranosyl (1→2)-β-D-glucopyranoside, are the steroidal saponins in *Rhizoma Paridis*.

Curvularia lunata is a deuteromycete best known in chemistry for 11β-hydroxylation of steroids, and production of hydrocortisone[X1].[3,4,†] We are currently studying a series of chemical-microbiological routes for biotransformation of the compounds **I**, **II**, **III** and **IV** by *Curvularia lunata* 3.4381, and have extracted four main microbiological transformed products, which were characterized with MS and NMR. In this study, we first find that the terminal rhamnosyls with 1→2 linkage to sugar residues of steroidal saponins at C-3 position of compounds **I**, **II**, **III** and **IV** could be hydrolyzed selectively by *Curvularia lunata*, with high activity and regioselectivity.

L-Rhamnose is widely distributed in plants and bacteria as a component of the cell wall and of various natural products, such as ginsenoside, steroidal saponins, and etc. Some rhamnosides are important bioactive compounds, for example, cytotoxic saponins, antifungal plant glycoalkaloids and bacterial virulence factors. In plants, L-rhamnose is also bound to several volatile compounds, for example, aroma terpenol glycosides of wine having a possible protective role against the toxicity of the free lipophilic aglycons.

The production of α-L-rhamnosidases by a number of mammalian tissues, plants, bacteria, and fungi has been described. More recently, α-L-rhamnosidases of fungal origin have been identified in different strains of *Penicillium*, *Aspergillus*, *Fusarium*, *Absidia* species, and etc.[5–10] Several technological applications of fungal α-L-rhamnosidases for the structural modification of natural products, such as the ginsenoside-α-L-rhamnosidase hydrolyzing ginsenoside Rg2 to ginsenoside Rh1, has been investigated.[6]

The aim of the present work was to search the stereoselectivity and regioselectivity of rhamnosidase of *Curvularia lunata* in the biotransformation of steroidal saponins, to understand its reaction rule and characteristic, and to better demonstrate its reaction mechanism.

Keywords: Biotransformation; *Curvularia lunata*; Polyphyllin I; Polyphyllin III; Polyphyllin V; Polyphyllin VI.
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† Hydrocortisone was produced from cortexolone (steroid) by Curvularia lunata by β-hydroxylation at C-11.

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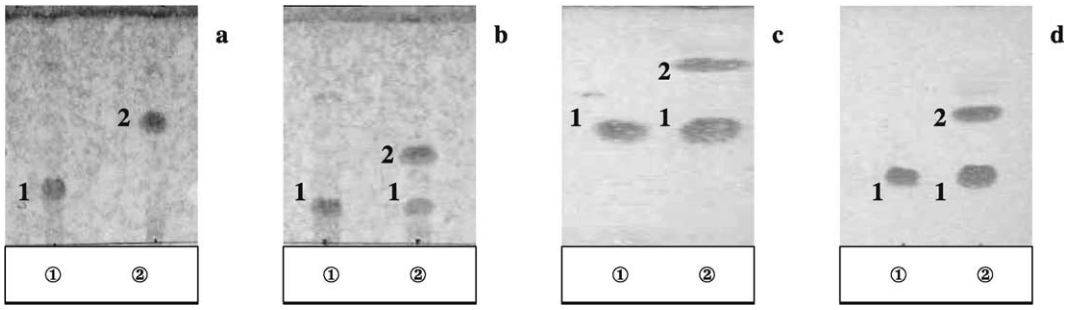

Figure 1. TLC of the biotransformation of compounds **I**, **II**, **III** and **IV** ① substrates; ② transformed products (1. substrates, 2. products); (a) compound **I** and biotransformed product **V**; (b) compound **II** and biotransformed product **VI**; (c) compound **III** and biotransformed product **VII**; (d) compound **IV** and biotransformed product **VIII**.

2. Results

2.1. TLC analysis

Analysis of extracts by TLC indicated the presence of products bioconverted (Fig. 1). New spots ② appear with a higher R_f value than that of substrates; it suggested that these new spots be partially hydrolyzed substrates ①; these new spots were not formed in the substrate control without *Curvularia lunata*.

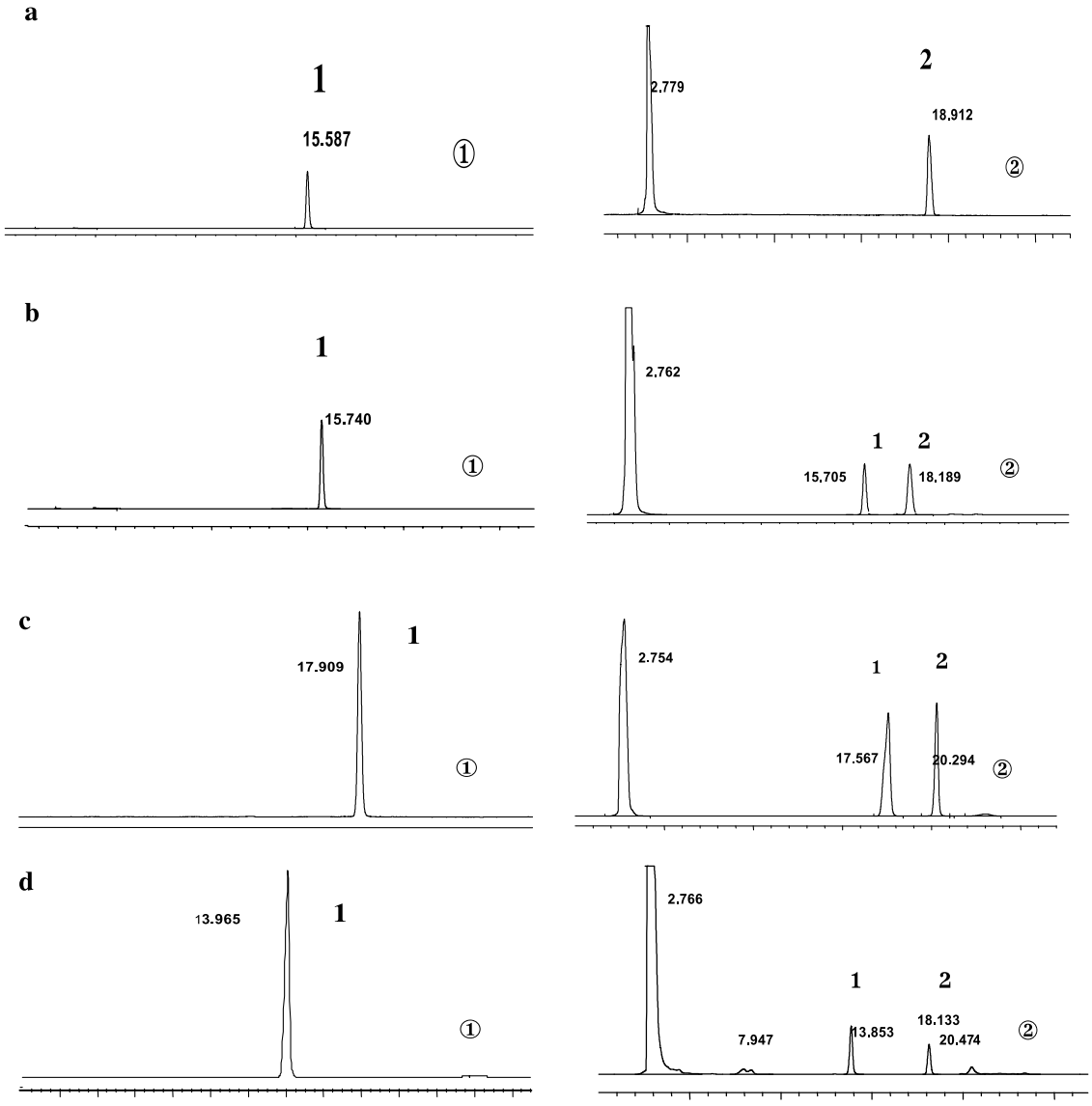

Figure 2. HPLC of the biotransformation of compounds **I**, **II**, **III** and **IV** ① substrates; ② transformed products (1. substrates, 2. products); (a) compound **I** and transformed product **V**; (b) compound **II** and transformed product **VI**; (c) compound **III** and transformed product **VII**; (d) compound **IV** and transformed product **VIII**.

2.2. HPLC analysis

Analysis of extracts by HPLC indicated the presence of bioconverted products (Fig. 2). New peaks, which were considered to be the hydrolyzed products, appeared to have a less polarity than that of substrates, were observed in HPLC chromatographs. The results agree with that of TLC.

2.3. Isolation and purification

The reaction mixture was extracted with *n*-butanol four times. The *n*-butanol layer was chromatographed on Silica gel C_{18} column [acetone–water, 60, 70, 80%] to afford products, compound **V** (15 mg), **VI** (20 mg), **VII** (12 mg) and **VIII** (16 mg), respectively. 95% (compound **V**), 50% (compound **VI**), 55% (compound **VII**) and 30% (compound **VIII**) conversion were achieved from each substrates.

2.4. Structural characterization of products

2.4.1. Biotransformation of compound I. Compound **V** was obtained as a white needle crystal (EtOH), which was soluble in pyridine, ethanol and methanol. It gave a positive Liebermann-Burchard, Molish test, and a negative Ehrlich test. The results imply that the compound is a spirostan with steroidal type skeleton. Mp 239–242 °C. IR (KBr) ν_{max} cm^{-1}: 3600–3100 (OH), 1632 (double bond). FAB-MS (*m/z*): 709.3 $(M+H)^+$, 577.3 (M+H−132), 415.2 (M+H−132–146) implies that the compound has a terminal pentose and a hexose, and the latter is attached to the C-3 hydroxyl. The ^1H NMR spectra display the following representative signals: four steroid methyl protons at δ 0.68 (3H, d, $J=4.8$ Hz, CH_3-27), 0.82 (3H, s, CH_3-18), 0.90 (3H, s, CH_3-19), 1.13 (3H, d, $J=7.2$ Hz, CH_3-21); the anomeric proton signals δ 4.96 (1H, d, $J=7.2$ Hz) and 6.04 (1H, s) attributable to H-1 of glucose (β-linkage) and H-1 of arabinose, respectively; one olefinic proton δ 5.30 (1H, br s, H-6) ascribable to the double bond between 4 and 5. The ^{13}C NMR spectral data (Table 1) were comparable to that of literature.[11]

2.4.2. Biotransformation of compound II. Compound **VI** was obtained as a white needle crystal (EtOH), which was soluble in pyridine, ethanol and methanol; it gave a positive Liebermann-Burchard, Molish test, and a negative Ehrlich test. The results imply that the compound is a spirostan with steroidal type skeleton. Mp 242–246 °C. IR (KBr) ν_{max} cm^{-1}: 3600–3200 (OH), 1628 (double bond). FAB-MS (*m/z*): 815.4 $(M+H+C_3H_8O_3)^+$, 723.4 (M+H), 577.3 (M+H−146), 415.3 (M+H−146–162) indicates that the compound has two hexoses; the ^1H NMR spectra display the following representative signals: four steroid methyl protons at δ 0.68 (3H, d, $J=5.4$ Hz, CH_3-27), 0.82 (3H, s, CH_3-18), 0.91 (3H, s, CH_3-19), 1.13 (3H, d, $J=7.20$ Hz, CH_3-21); δ 4.95 (1H, d, $J=7.8$ Hz) and 5.84 (1H, s) attributable to H-1 of glucose (β-linkage) and H-1 of rhamnose; one olefinic proton δ 5.30 (1H, br s, H-6) attribute to the double bond between 4 and 5. The ^{13}C NMR spectrum data (Table 1) were comparable to that of literature.[12]

2.4.3. Biotransformation of compound III. Compound **VII** was obtained as a white needle crystal (EtOH), which was soluble in pyridine, ethanol and methanol; it gave a positive Liebermann-Burchard, Molish test, and a negative Ehrlich test. The results imply that the compound is a spirostan with steroidal type skeleton. Mp 262–263 °C. IR (KBr) ν_{max} cm^{-1}: 3600–3200 (OH), 1630 (double bond). FAB-MS (*m/z*): 577.3 $(M+H)^+$, 415.2 (M+H−162), 397.2 (M+H−H_2O−162) implies that the compound has a hexose. The ^1H NMR spectra display the following representative signals: four steroid methyl protons at δ 0.68 (3H, d, $J=5.7$ Hz, CH_3-27), 0.82 (3H, s, CH_3-18), 1.05 (3H, s, CH_3-19), 1.13 (3H, d, $J=7.0$ Hz, CH_3-21); δ 5.23 (1H, d, $J=7.7$ Hz) attributable to H-1 of glucose and indicating a β-linkage; one olefinic proton δ 5.42 (1H, br s, H-6) ascribable to the double bond between 4 and 5. The ^{13}C NMR spectrum data (Table 1) were comparable to that of literature.[13,14]

2.4.4. Biotransformation of compound IV. Compound **VIII** was obtained as a white needle crystal (EtOH), which was soluble in pyridine, ethanol and methanol; it gave a positive Liebermann-Burchard, Molish test, and a negative Ehrlich test. The results imply that the compound is a spirostan with steroidal type skeleton. Mp 276–279 °C. IR (KBr) ν_{max} cm^{-1}: 3600–3100 (OH), 1633 (double bond). FAB-MS (*m/z*): 593.2 $(M+H)^+$, 575.2 (M+H–$_2$O), 431.2

Table 1. ^{13}C NMR chemical shifts of transformed products

C	Compound **V**	Compound **VI**	Compound **VII**	Compound **VIII**
1	37.5	37.5	37.5	37.7
2	30.2	30.1	30.4	30.3
3	78.2	78.4	78.2	78.4
4	39.3	39.3	39.0	39.1
5	140.8	140.7	140.8	141.0
6	121.8	121.9	121.8	121.8
7	32.2	32.2	32.3	32.5
8	31.8	31.7	31.7	31.9
9	50.3	50.4	50.3	50.4
10	37.1	37.1	37.2	37.3
11	21.1	21.1	21.1	21.1
12	39.9	39.8	39.9	32.1
13	40.5	40.5	40.5	45.1
14	56.6	56.6	56.7	53.2
15	32.3	32.3	32.2	32.5
16	81.1	81.1	81.1	90.3
17	62.9	62.9	62.9	90.2
18	16.4	16.3	16.3	17.2
19	19.4	19.4	19.4	19.5
20	42.0	41.9	42.0	44.9
21	15.0	15.1	15.0	9.5
22	109.3	109.3	109.3	109.9
23	31.7	31.7	30.6	32.1
24	29.3	29.2	29.3	28.9
25	30.6	30.5	30.6	30.5
26	66.9	66.8	66.9	66.9
27	17.3	17.2	17.3	17.3
Inner-Glc				
1	102.5	102.7	102.5	102.6
2	75.3	75.5	75.5	75.4
3	76.7	76.7	78.2	78.4
4	78.4	78.4	71.8	71.7
5	77.1	77.1	78.2	78.1
6	62.6	61.6	62.7	62.9
		4-Ara	4-Rha	
1		109.3	102.7	
2		82.7	72.7	
3		78.2.	72.9	
4		87.1	74.0	
5		61.7	70.4	
6			18.6	

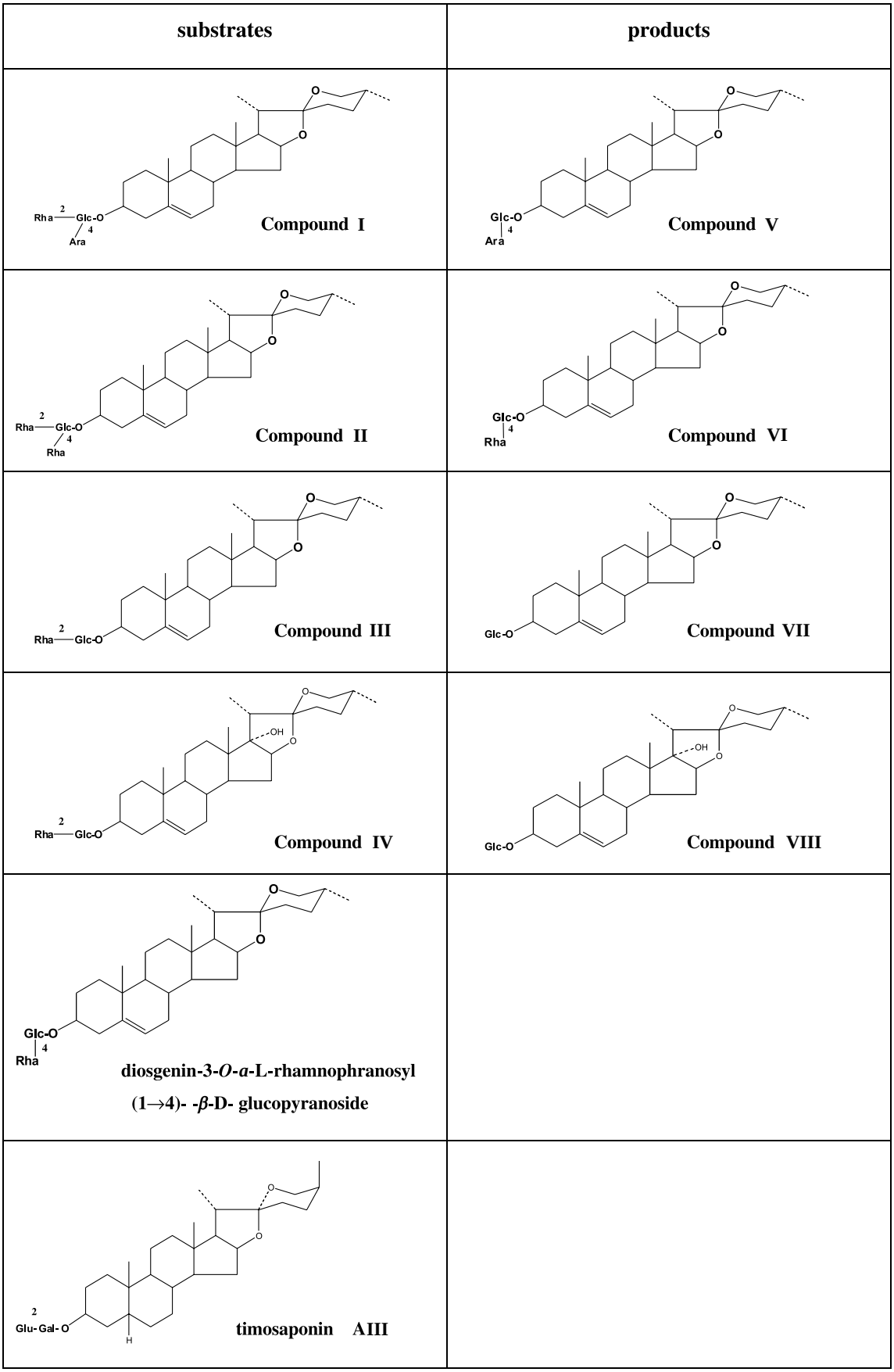

Figure 3. Structures of products bioconverted and substrates.

(M+H−162), 413.2 (M+H–$_2$O−162) implies that the compound has a hexose, and a active hydroxyl. The ^{1}H NMR spectra display the following representative signals: four steroid methyl protons at δ 0.67 (3H, d, J=5.7 Hz, CH_3-27), 0.93 (3H, s, CH_3-18), 0.95 (3H, s, CH_3-19), 1.22 (3H, d, J=7.14 Hz, CH_3-21); δ 5.03 (1H, d, J=7.69 Hz) attributable to H-1 of glucose and indicating a β-linkage; One olefinic proton δ 5.29 (1H, br s, H-6) ascribable to the double bond between 4 and 5. The ^{13}C NMR spectrum data (Table 1) were comparable to that of literature.[13,14]

Thus, the four products were confirmed as diosgenin-3-*O*-α-L-arabinofuranosyl (1→4)-β-D-glucopyranoside (compound **V**), diosgenin-3-*O*-α-L-rhamnopyranosyl (1→4)-β-D-glucopyranoside (compound **VI**), diosgenin-3-*O*-β-D-glucopyranoside (compound **VII**) and pennogenin-3-*O*-β-D-glucopyranoside (compound **VIII**), respectively. All the terminal rhamnosyls of these compounds linked by 1→2 C–bond to sugar residues of saponins at C-3 position were hydrolyzed selectively.

3. Discussion

Curvularia lunata 3.4381 is able to selectively hydrolyze the terminal rhamnosyls with 1→2 linkage to sugar residues of substrates at C-3 position to produce corresponding subsaponins, confirmed by TLC, HPLC and spectral analysis. The microbiological transformation of compounds **I**, **II**, **III** and **IV** is showed as Figure 3.

In order to find the rule and characteristics of biotransformation by *Curvularia lunata*, we choose a variety of steroidal saponins with various terminal glycosyl ,for example, diosgenin-3-*O*-α-L-rhamnophranosyl (1→4)-β-D-glucopyranoside, timosaponin A**III**, polyphyllin I (compound **I**), polyphyllin III (compound **II**), polyphyllin V (compound **III**) and polyphyllin VI (compound **IV**), to study their biotransformation and find that the enzyme, which secreted by *Curvularia lunata*, is capable to hydrolyze terminal rhamnosyls, which are 1→2 linked to sugar residues of steroidal saponins at C-3 position, with high activity and regioselectivity; however, it is unable to hydrolyze the terminal rhamnosyl with 1→ 4 linkage to sugar residues (diosgenin-3-*O*-α-L-rhamnophranosyl (1→4)-β-D-glucopyranoside), the terminal glucosyl (timosaponin A**III**) or the terminal arabinosyl (polyphyllin I) of steroidal saponins at C-3 position (Fig. 3).

In short, we first find that *Curvularia lunata* 3.4381 is capable to selectively hydrolyze the terminal rhamnosyls with 1→2 linkage to sugar residues of steroidal saponins at C-3 position; it has the high selectivity for the type and connective modality of glycosyl; but has low selectivity for the type of aglycone, one or two glycosyl and the glycosyl with side chain or linear chain.

In addition, the α-L-rhamnosidase, which selectively hydrolyzed the terminal rhamnosyls with 1→2 linkage to sugar residues of steroidal saponins at C-3 position has been purified and characterized. The results will soon be reported.

4. Experimental

4.1. General

^{1}H NMR spectra were recorded in pyridine-d_5 solutions at 599.68 Hz with Varian^{UNITY} INOVA 600 spectrometer. ^{13}C NMR spectrophotometry was run in pyridine-d_5 at 150.79 Hz with Varian^{UNITY} INOVA 600 spectrometer, respectively. Chemical shifts are given in ppm (δ). Mass spectra were taken on a Micromass Zabspec EFAB, 1000 spectrophotometer. HPLC analysis were carried out on Agilent 1100 unit with an Alltech Evaporative Light Scattering Detector 2000 using an Alltech-Apollo-C_{18} column (5 μm, 250×4.6 mm). TLC analyses were carried out on pre-coated silica gel GF_{254} plates (0.25 mm thick, Qingdao Haiyang Chemical Group Co., China). Visualization of the TLC plates was performed by 10% H_2SO_4–EtOH spray reagent, followed by heating. All chemicals used were of analytical reagent grade. The isolation and purification of products were carried out on C_{18} column (ODS-A 12 mm S-50; 5409; YMC Co., Japan). Other equipment includes Constant temperature incubator (9080, Shang-hai-yi-heng Technique Co., China); HZS-H shake incubator (Donglian Electric Technique Co., China); 3K18 centrifugation (SIGMA Co.).

4.2. Organism

The fungal strain, *Curvularia lunata* 3.4381, was obtained from the Institute of Microbiology, Chinese Academy of Sciences (AS), Beijing, China

4.3. Incubation experiments

The fungus *Curvularia lunata* was rejuvenated on a potato agar (PDA) slant before being used in transformation experiments and then was grown in 300 mL shake flasks at 29±1 °C, each containing 100 mL sterile medium comprising **1**. Potato dextrose broth (2.0 g); **2**. Corn-brei (1.2 g, North China Pharmaceutical Group corporation, China), glucose (1.0 g), yeast extract (0.2 g), $(NH_4)_2SO_4$ (0.5 g) in water. The substrates dissolved in water were evenly distributed into flasks after 1 day of growth. After another 3–8 days, the mycelium was filtered out, and the fermentation broth and mycelium were extracted with *n*-BuOH. The *n*-BuOH layer was evaporated to give a residue that is ready for analysis.

4.4. Methods

4.4.1. Preparation of compounds I, II, III and IV.

4.4.1.1. Extraction and isolation of compound I. The dried roots of *Rhizoma Paridis* were sliced and then extracted stepwise with 60, 95% EtOH. The 95% EtOH extract was concentrated and further extracted with *n*-BuOH. The *n*-BuOH layer was evaporated under vacuum to give crude extract. The crude extract was passed through normal-phase silica gel column and was eluted with gradient $CHCl_3$–MeOH–$_2$HO (100/1/0.5→10/1/0.1→8/2/0.1→65/30/10) to give compound **I**.

4.4.1.2. Extraction and isolation of compound II. The dried roots of *Dioscorea nipponica* Makino were sliced and

then extracted stepwise with 60% EtOH. The EtOH extract was concentrated and further extracted with *n*-BuOH. The *n*-BuOH layer was evaporated under vacuum to give the crude extract. The crude extract was passed through normal-phase silica gel column and was eluted with gradient $CHCl_3$–MeOH–$_2$HO (50/1/0.1→10/1/0.1→5/1/0.1→60/35/10→5/5/2) to give compound **II**.

4.4.1.3. Extraction and isolation of compounds III and IV. The dried roots of *Rhizoma Paridis* were sliced and then extracted stepwise with 60% EtOH. The EtOH extract was concentrated and extracted with *n*-BuOH. The *n*-BuOH layer was evaporated under vacuum to give the crude extract. The crude extract was passed through normal-phase silica gel column and was eluted with gradient $CHCl_3$–MeOH–$_2$HO (50/1/0.5→10/1/0.2→20/5/1→65/30/10→29/29/10) to give mixture of compound **III** and **IV**. The mixture was separated by HPLC using 90% methanol to give compound **III** and compound **IV**.

4.4.2. Conversion experiments. One 7-day-old slant was used to inoculate four 500 mL conical flasks each containing 300 mL of sterilized liquid medium. The flasks were incubated on a rotary shaker at 150–180 rpm at 29±1 °C. Each substrate (100 mg in water) was added separately to each growing culture for transformation that will continue for another 3–8 days.

Parallel controls, which received no substrate and no organism, were similarly maintained.

4.4.3. Isolation and purification of converted products. After the transformation was over, mycelia were separated by centrifugation at 12,000 × g for 30 min; the supernatant and mycelia were extracted with *n*-BuOH, respectively; the combined *n*-BuOH layers were evaporated under reduced pressure to give the crude converted products. The controls were similarly processed.

The crude converted products were subjected to a Silica gel C_{18} column chromatography, gradiently eluted with 60, 70, 80% aq acetone. Fractions of same compound characterized by TLC and HPLC were collected and evaporated to produce compounds **V**, **VI**, **VII** and **VIII**.

4.4.4. TLC and HPLC analysis.

4.4.4.1. TLC analysis. The homogeneity of the converted products was ascertained by thin-layer chromatography (TLC) on silica gel GF_{254} plate using chloroform–methanol–water (70/15/2) as developing solvent.

4.4.4.2. HPLC analysis. The transformed products were redissolved in analytical methanol and filtered. The solvent system is as follows: temperature: 100 °C; flow rate: 1.0 mL/min, gas-flow rate: 2.4 L/min.

Eluting time (min)	Water (%)	Methanol (%)
0.01	20.0	80.0
8.00	20.0	80.0
9.00	10.0	90.0
14.00	10.0	90.0
16.00	5.0	95.0
21.00	5.0	95.0
22.00	20.0	80.0
27.00	20.0	80.0

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