New Methods for the Synthesis of Biologically Active Natural Products and Related Compounds

A thesis submitted for the Degree of Doctor of Philosophy of The Australian National University

by

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Declaration

I declare that, to the best of my knowledge, the material presented in this thesis represents the result of original work carried out, unless otherwise stated, by myself during the period 2013-2017. It has not been presented for examination for any other degree. This thesis by publication is comprised of six journal articles. Wherever possible, established methodologies have been acknowledged by citation of the relevant original publications.

Xiang Ma
November, 2017
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I would first like to thank my supervisor Prof. Martin Banwell. Throughout my four years of Ph.D. study I have benefited profoundly from his encouragement, assistance, knowledge and patience. The positivity that stems from his kindness and generosity has made the last four years a very enjoyable experience.

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To both my parents and my wife Erpei Wang, thank you for love and support over the past four years. Without you this would not be possible.
Publications and Presentations

The following list details the publications and presentations that have resulted from the author’s research work performed during his candidature for the Degree of Doctor of Philosophy.

Publications:


Presentations:


Commentary on the Contributions of Mr Xiang Ma to the Six Papers Included in this Thesis by Publication

Publication 1
This is a digest article written by Prof. Banwell. It incorporates descriptions of research conducted by the co-authors including Mr Ma. Mr Ma carried out relevant literature surveys as part of his contributions to the preparation of this article.

Publication 2
This is a letter detailing extensive experimental work directed towards total syntheses of the resorcylic acid lactone type natural products paecilomycin F and cochliomycin C. Mr Ma carried out the entirety of the laboratory work reported in this article. In addition, he collated and formatted all of the reported spectral data presented in the Supporting Information document. Mr Ma also wrote the whole of the Experimental Section and conducted relevant literature surveys. Prof. Banwell wrote the body of the paper.

Publication 3
The total synthesis of the tazettine-type alkaloid 3-O-demethylmacronine is reported. Mr Ma carried out most of the laboratory work reported in this article and most particularly that
culminating in the completion of the reported synthesis. In addition, he collated and formatted all of the reported spectral data presented in the Supporting Information document. Mr Ma also wrote the whole of the Experimental Section and conducted relevant literature surveys. Prof. Banwell wrote the body of the paper.

**Publication 4**

This is a full paper detailing extensive experimental work directed towards total syntheses of the *Amaryllidaceae* alkaloids zephycandidine III and lycosinine A. Mr Ma was especially involved in the synthesis of zephycandidine III. He assisted Mr Xingjun Xu in completing the total synthesis of this compound including by recrystallizing two intermediates so as to be able to confirm their structures by single-crystal X-ray analysis. He, Mr Ma, also advised Mr Xu in detail about the best means for effecting certain crucial functional group interconversion and the preparation the Experimental Section. Prof. Martin Banwell wrote the body of the paper.

**Publication 5**

This is a full paper detailing extensive experimental work directed towards the total synthesis of the marine alkaloid discoipyrrole C. Mr Ma assisted Dr Qiao Yan in early steps of the synthesis, especially
the bromination of the pyrrole and the subsequent two-fold Suzuki-Miyaura cross coupling of the product of this first step. Mr Ma conducted parts of relevant literature surveys. Prof. Martin Banwell wrote the body of the paper.

**Publication 6**

This is a letter detailing extensive experimental work directed towards the total synthesis of the antifungal deoxyaminocyclitol nabscessin B from L-(+)-tartaric acid. Mr Ma carried out the entirety of the laboratory work reported in this article. In addition, he collated and formatted all of the reported spectral data presented in the Supporting Information document. Mr Ma also wrote the whole of the Experimental Section and conducted relevant literature surveys. Prof. Banwell wrote the body of the paper.
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Abstract

The body of this thesis is comprised of six scientific articles and is preceded by an overview that contextualises all of this published/submitted work.

The first major part of this thesis is comprised of Publication 1 which is an invited digest article on total syntheses of the cochliomycins and certain related resorcylic acid lactones (RALs). The author’s work on the syntheses of paecilomycin F and cochliomycin C is highlighted in this article.

Publication 2 comprises the second major part of this thesis. This describes total syntheses of the RAL type natural products paecilomycin F (A) and cochliomycin C (B). The key step, a macrocyclisation, was realized by subjecting substrate C to Loh-type α-allylation conditions using indium metal and so affording the required 14-membered macrolide framework. In contrast, when the same substrate was treated under Nozaki–Hiyama–Kishi conditions the 12-membered lactone D was formed through a γ-allylation process. A single-crystal X-ray analysis served to confirm the structure of cochliomycin C and, therefore, the effectiveness of Loh-type α-allylation conditions in producing large macrolides.
Publication 3 details a total synthesis of the racemic modification of the tazettine-type alkaloid 3-O-demethylmacronine (E). A key intermediate was compound F embodying the haemanthidine alkaloid core which was assembled via a reaction sequence including an intramolecular Alder-ene reaction. The lactam-to-lactone rearrangement F → G was achieved under acidic conditions and thereby affording the polycyclic framework associated with target E.

Publication 4 describes total syntheses of the Amaryllidaceae alkaloids zephycandidine III (H) and lycosinine A (I). Their inhibitory effects on acetylcholinesterase were also investigated. A palladium-catalyzed Ullmann cross-coupling reaction was used to link two aryl halides in forming the biaryl scaffold of target H while a Suzuki–
Miyaura cross coupling reaction was used to construct the alkaloid I. Although compound H has been reported to act as a significant inhibitor of acetylcholinesterase, biological testing of the synthetically-derived sample suggests otherwise.

The total synthesis of marine alkaloid discoipyrrole C (J) is reported in Publication 5. In the key step of the reaction sequence, the 2,3,5-trisubstituted pyrrole K was treated with MoOPH in the presence of methanol to afford the methoxylated 1,2-dihydro-3H-pyrrol-3-one L. Exposure of the latter compound to potassium carbonate then aqueous trifluoroacetic acid resulted in hydrolysis of the acetate and aminol ether moieties and formation of natural product J.
The last paper, **Publication 6**, details the total synthesis of the aminocyclitol derivative and antifungal agent nabscessin B (M) from the homochiral γ-hydroxycyclohexenone N which was, in turn, prepared from L-(+)-tartaric acid over six steps. The rigidifying effect of the 1,2-diacetal protecting group associated with compound N and its derivatives affords high levels of regiochemical control in the construction of the aminocyclitol framework. The structure of nabscessin B (M), including its absolute stereochemistry, was confirmed by the author’s successful enantiospecific total synthesis of this natural product.

The Appendix to the thesis contains reports on the single-crystal X-ray analyses of key compounds synthesized by the author. Those analyses were carried out by Dr Anthony Willis, Dr Paul Carr, or Dr Jas Ward.
Thesis Overview

The overarching theme associated with this thesis by publication is the deployment of methodologies developed within the Banwell Group, together with new ones identified by the author, for the purpose of establishing total syntheses of biologically active natural products. The diversity of the target compounds that have been obtained stands as testimony to the utility of these methodologies as well as the broad training that the author has received in the techniques and theory of chemical synthesis.

Publication 1: Chemical Syntheses of the Cochliomycins and Certain Related Resorcylic Acid Lactones

The cochliomycins are a group of six resorcylic acid lactones (RALs) that have been isolated from culture broths of marine fungi found in the South China Sea. Because of their novel structural features and their potential antifouling activities, these RALs have attracted attention as synthetic targets from synthetic chemists. Publication 1 reviews published syntheses of the title compounds, as well as certain related RALs (including L-783,277, paecilomycin F, zeanol and neocosmosin A) that have been described by the Banwell Group and others currently active in this area.
Publication 2: Total Syntheses of the Resorcylic Acid Lactones

Paecilomycin F and Cochliomycin C Using an Intramolecular Loh-Type α-Allylation Reaction for Macrolide Formation

Paecilomycin F and cochliomycin C belong to the RAL class of natural product and process a 14-membered macrolactone ring. Cochliomycin C is a marine-derived RAL that was isolated from the fungus *Cochliobolus lunatus* found in the South China Sea. Paecilomycin F is its unchlorinated derivative.\(^1\)\(^,\)\(^2\) While cochliomycin C displays notable antifouling and/or antifungal properties, paecilomycin F processes modest antimalarial activity.\(^3\)\(^,\)\(^4\) Given their reported biological properties, and with the intention of exploiting aspects of the Banwell Group’s previously reported total syntheses of cochliomycins A and B, paecilomycin F and cochliomycin C became
the author’s target compounds. **Publication 2** details the author’s successful efforts in this regard.

The key step involved a macrolide ring forming event. Thus, an indium-mediated intramolecular Loh-type α-allylation reaction involving substrate 1 led to compound 2, an immediate precursor for the final target. Interestingly, when the same substrate was subjected to a Nozaki-Hiyama-Kishi reaction then lactone 3, incorporating a 12-membered ring, was obtained.

![Chemical Structures](image)
Publication 3: A Total Synthesis of (±)-3-O-Demethylmacronine through Rearrangement of a Precursor Embodying the Haemanthidine Alkaloid Framework

Macronine and 3-O-demethylmacronine are two tazettine-type Amaryllidaceae alkaloids that were isolated from the plant Crinum macrantherum Engl.\textsuperscript{5} and a Galanthus species of Turkish origin,\textsuperscript{6} respectively. It was determined that 3-O-demethylmacronine does not arise from the demethylation of macronine during the isolation process and so both of these compounds are regarded as “true” natural products. Wildman and co-workers\textsuperscript{7} were able to establish that the $N$-demethylmacronine framework results from the lactam-to-lactone rearrangement of the haemanthidine alkaloid framework in
buffer at pH 6.80. The only total synthesis of (±)-macronine was reported by Tsuda et al. in 1976 who exploited a late-stage variant of the lactam-to-lactone rearrangement proposed by Wildman. No other relevant work on macronine and 3-O-demethylmacronine has been reported in the intervening period. Accordingly, a synthesis of (±)-3-O-demethylmacronine was pursued by the author and his colleagues and the successful outcome of such studies are reported in Publication 3.

The first key step involves subjection of alkyne 4 to an intramolecular Alder-ene (IMAE) reaction and thereby affording the C3a-arylated hexahydroindole 5. Cleavage of the nosylate moiety in this late compound triggered a lactamisation reaction and so establishing the haemanthidine alkaloid framework. A lactam-to-lactone rearrangement served as the other key step of the synthesis and this involved the acid-catalysed conversion of compound 6 into isomer 7. The application of an Eschweiler–Clarke reaction to lactone 7 then gave (±)-3-O-demethylmacronine.
In 2016, Yao and co-workers reported\(^9\) the isolation of new *Amaryllidaceae* alkaloid, zephycandidine III, from the plant *Zephyranthes candida* collected at Shiyan, Hubei, China. Lycosinines A and B, which bear strong structural resemblance to zephycandidine III, were isolated from the ornamental plant *Lycoris aurea* collected in Kunming, Yunnan, China.\(^{10,11}\) Zephycandidine III was described as a potent inhibitor of acetylcholinesterase (AChE) (IC\(_{50}\) 8.82 μM). On this basis, lycosinines A and B might also be expected to display AChE-
inhibiting activities. Accordingly, zephycandidine III and lycosinine A became synthetic targets and their attainment is described in Publication 4. The evaluation of the title alkaloids as well as certain derivatives as inhibitors of AChE are also reported in this paper.

Two different cross coupling reactions, namely, a palladium-catalysed Ullmann cross-coupling and a Suzuki–Miyaura cross-coupling, were employed in constructing the biaryl cores, 8 and 9 respectively, of zephycandidine III and lycosinine A. Each of these core compounds was then elaborated by conventional methods to the corresponding natural product. However, testing of them as AChE inhibitors revealed that neither of them was active.
Publication 5: Total Synthesis of the Marine Alkaloid Discoipyrrole C via the MoOPH-mediated Oxidation of a 2,3,5-Trisubstituted Pyrrole

In 2013 the MacMillan Group reported the isolation of four new and structurally novel alkaloids, named discoipyrroles A-D, from the marine bacterium *Bacillus hunanensis*. Significantly, each of these shows selective cytotoxicity toward DDR2-mutant lung cancer cell lines with IC$_{50}$ values in the range from 120 to 400 nM.$^{12}$ Total syntheses of discoipyrroles A, B and D have been reported recently by our group,$^{13,14}$ and key intermediates associated with these were 1,2,3,5-tetrasubstituted pyrroles. Treatment of these polysubstituted pyrroles with oxodiperoxymolybdenum (pyridine)-(hexamethylphosphoric triamide) (MoOPH)$^{15}$ resulted in an oxidative cyclisation reaction leading to the distinctive heterocyclic ring system associated with discoipyrroles A, B and D. Given its intriguing biological properties and the absence of any relevant prior studies, a total synthesis of discoipyrrole C was pursued and the successful outcomes of the relevant studies are described in Publication 5.
The pivotal step was the treatment of the 2,3,5-trisubstituted pyrrole 11 with MoOPH in CH$_2$Cl$_2$/MeOH and thus affording the methoxylated 1,2-dihydro-3$H$-pyrrol-3-one 12. Compound 12 itself was subjected to reaction with potassium carbonate in methanol and then, in a separate step, with aqueous trifluoroacetic acid and so affording discoipyrrole C. All the spectral data acquired on this compound matched those reported for the natural product by MacMillan and co-workers.
In early 2017 two novel aminocyclitol derivatives, named nabscessins A and B, were reported by Ishibashi and co-workers. These were isolated from the culture broth of the pathogenic actinomycete species *Nocardia abscessus* IFM 10029T and display notable activity against *Cryptococcus neoformans* with IC₅₀ values of 32 and 16 µg/mL, respectively. Their structures were elucidated by standard means including 2D NMR spectroscopic techniques. Both compounds incorporate a 2-deoxy-scyllo-inosamine core. With the intention of developing new routes to aminocyclitols and related anti-infective agents, a synthesis of nabscessin B was pursued. The
successful synthesis of this natural product is described in Publication 6.

Thus, the homochiral $\gamma$-hydroxycyclohexenone 13, which was prepared from L-$(+)$-tartaric acid, served as the key intermediate for the synthesis of nabscessin B. The 1,2-diacetal protecting group associated with compound 13 and its derivatives rigidifies the annulated cyclohexane ring and thus allowing for regio- and stereo-selective manipulation of the developing nabscessin B target. The data derived from the synthetic sample of nabscessin B compared favourably with those reported for the natural product. Given this and the fact that single-crystal X-ray analyses were undertaken on two intermediates associated with this synthesis, this work serves to
confirm the structure, including absolute stereochemistry, originally assigned to the natural product.
References


Publication One

Chemical Syntheses of the Cochliomycins and Certain Related
Resorcylic Acid Lactones

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Chemical syntheses of the cochlomiycins and certain related resorcylic acid lactones

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Abstract

The cochlomiycins (7-12) are a group of six resorcylic acid lactones that have recently been isolated from culture broth of marine fungi found in the South China Sea. These natural products have attracted attention as synthetic targets because of their novel structural features and their capacity to suppress histamin release. This short review summarizes the synthesis of these and some related compounds that have been reported to date, including those developed in the authors’ laboratories.

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Introduction

The value of small molecule natural products (SMNPs) as therapeutic agents, as precursors to such agents or as the inspiration for them is well known. Indeed, there are now indications that SMNPs, perhaps especially ones derived from marine environments, are enjoying something of a renaissance not least because of their enormous structural diversity and their occupation of unique parts of chemical space. Among the plethora of different natural product classes, the resorcylic acid lactones (RALs) are notable for the frequency with which they are isolated from fungal sources, their distinctive structural features and their breadth of biological activities. In the following section an overview of the structural variations within the RAL class is provided along with a brief commentary on the source organisms and certain of their biological properties. As a recently discovered and interesting subset of RALs that has not been the subject of any previous reviews, the cochlomiycins are then described and a summary of the synthetic work carried out on them follows.

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Resorcyclic acids lactones (RALs) as a natural product class

The RALs are mycotoxins and the products of a distinctive polyketide biosynthesis that exploits an acetyl CoA starter unit together with malonyl-CoA extenders and involves two fungal polyketide synthases (PKS) that work co-operatively.10 Specifically, a non-reducing PKS is coupled with a highly reducing one that enables the assembly of the relevant resorcyclic acid core annulated to a 14-membered macrolactone (and wherein most of the structural variation resides). Unsurprisingly perhaps, the final step in the biosynthesis is the macrolactonization event that releases the lactamate from the enzyme complex. Post-PKS-mediated processes such as epoxidation, halogenation and alkylation may then follow so as to provide the fully ‘decorated’ (isolated) metabolites.11

Radicicol (1) was the first RAL to be isolated (from Monotormus nordstot) and characterised in the 1950s12 and it has since been obtained from various other fungal strains. In the intervening period numerous other RALs have been identified and these vary in the nature of the substitution pattern on the aromatic ring as well as the location and degree of unsaturation and/or oxygenation within the macrolactone ring. The structures of the RALs hypothe- mycin (2), zearelanone (3), pectochrin (4), L-775,277 (5) and aigialomycin D (6) shown in Fig. 1 serve to highlight such degrees of variation.

Fig. 1. Examples of the structural variations possible within the RAL class.

Initial biological evaluation of radicicol (1) showed it to possess anti-bacterial properties and to act as a mild sedative. However, the later revelation that it acts as a powerful inhibitor of heat shock protein 90 (HSP90) – and thus representing an important lead in the development of oncolytic agents – caused much greater attention to be given to the RALs. In contrast to radicicol (1), the enone-containing hypothemycin (2) has been shown to strongly inhibit the kinase MEK1, while zearelanone (3) acts as an estrogen agonist and its hormone-like properties have been shown to promote growth in cattle and sheep. A closely related RAL is known commercially available and employed to alleviate post-menopausal stress in women and as an anabolic cattle-growth stimulant. Pectochrin (4), on the other hand, inhibits herpes simplex virus (HSV) replication in a potentially therapeutically useful way while the cis-enone L-775,277 (5), like congener 2, inhibits MEK1. Aigialomycin D (6), despite the absence of a cis-enone moiety, also acts as a kinase inhibitor as well as an anti-malarial agent (the latter property seemingly being unrelated to the former).

The discovery of cochliomycins A–F

In papers published in 201111 and 201412 Wang and co-workers from the Ocean University of China in Qingdao reported the isolation of cochliomycins A–F (7–12) (Fig. 2) from the culture broth of Cochliobolus lunatus (M351) or C. lunatus (TA26–40, fang) associated with the gorgonian Dendrothoe gommersi or the sea anemone Polythoa auditiva, respectively. Both the host organisms were collected in the South China Sea. The structures of these RALs were established through the application of the usual battery of spectroscopic methods and the absolute stereochemistries of the last three determined using the CD excitation circularity method in conjunction with TDDFT ECD calculations.13

The most striking features of this subset of RALs are the presence of acetate units within the structures of congeners A and B (7 and 8, respectively). Since acetate was not used in the isolation, purification or spectroscopic characterisation of these compounds they must be considered as natural products rather than artefacts. Wang and co-workers also noted that on standing in CDCl3 at ambient temperatures cochliomycin B (8) slowly isomerised to congener 7 and so suggesting the latter is the thermodynamically more stable compound. Cochliomycin C (9) is the only member of the series lacking a second double bond within the macrocyclic ring. Cochliomycins D (10) and E (11) are isomeric while congener F (12) is not simply a chlorinated derivative of one or other of the first two because of the differing configuration at one or other of the hydroxyl-bearing mechain carbons. Nor, for the same reasons, can cochliomycin F (12) simply be the product of the twofold oxidation of congener 9.

Related, co-occurring natural products

In the course of structurally characterising the cochliomycins, it was noted12 that congener C (9) is the chlorinated derivative of co-isolated pectochrin F (11) (Fig. 3), a previously reported RAL that displays anti-malarial properties. Other RALs also isolated alongside compounds 7–9 were zeesmol (14), LL-Z1640–1 (15) and LL-Z1640–2 (16). During the course of isolating cochliomycins D, E and F (10, 11 and 12, respectively), cochliomycin A (7), zeesmol (14), LL-Z1640–1 (15), LL-Z1640–2 (16), its E-isomer 17 (7’E)-6’-azeadzeesmol, deoxyaigialomycin C (18) and aigialomycin B (19) were also observed in the mixture of isolates. Clearly certain of these co-isolates are isomeric with the cochliomycins or otherwise
closely related. For example, zeaxinol (14) is the acetamide "deprotected" analogue of cochlidiomycins A (7) and B (8).

**Biological properties of the cochlidiomycins**

The most notable biological properties of at least some of the cochlidiomycins are their anti-fouling properties. So, for example, on evaluating the effects of cochlidiomycins A–C (7–9) on the larval settlement of the barnacle Balanus amphitrite, the first of these completely inhibited this process at concentrations of 20.0 µg/mL and still displayed significant effects at 5.0 µg/mL. Zeaxinol (14) and compound 7 as well as two acetate derivatives of the latter displayed potent anti-fouling activities at non-toxic concentrations with EC50 values of 5.0, 1.2, 15.4 and 12.5 µg/mL, respectively. These values are well below the threshold requirement.
(EC_{50} 25 μg/mL) set by the US Navy program as an efficacy level for the development of natural anti-fouling agents. Given the structural relationship between compounds 7 and 14, the presence of the acetonide moiety in the former compound clearly has a beneficial effect on anti-fouling properties. Furthermore, since these same compounds display high therapeutic ratios they might well be useful as environmentally benign anti-fouling agents. Cochliomyacin A's anti-fouling effects are now thought to arise through stimulation of the NO/GMP pathway in the ciliated larval phase of the barnacle’s lifecycle. The subsequent evaluation of cochliomyacins D, E and F revealed that the first and third of these also displayed potent anti-fouling effects at non-toxic concentrations (EC_{50} values of 17.3 and 6.67 μg/mL, respectively). Significantly, the most active compound among the isolates from the culture broth of C. lunatus (TA26-49) was the carboxylic-containing U-216440-2 (16). The EC_{50} value of this compound (1.82 μg/mL) is close to that of the commercially employed anti-fouling agent SeaNine 211® (1.23 μg/mL) but has a significantly more favourable therapeutic ratio (EC_{50}/EC_{50} >50 for 16 vs 20.3). The differing anti-fouling behaviours of cochliomyacins D, E and F suggest that variations in stereochemistry can have a notable impact on activity.

Interestingly, cochliomyacin A (7) displayed moderate anti-bacterial activity against Staphylococcus aureus while, unlike cochliomyacins D, E and F, U-216440-2 (16) displayed potent inhibitory effects against various pathogenic fungi.}

Scheme 1. The Du Group synthesis of cochliomyacin A (7).
Synthetic studies on the cochliomyccins

As with other RAs, the cochliomicyns have been the subject of various synthetic studies, both for the purposes of confirming their structures and as a means of providing more material (as well as analogues). Almost invariably, a major consideration in such work is the manner in which the 14-membered lactone ring is closed. A range of methods has been successfully employed for this purpose and these are presented within the individual descriptions given below of the various syntheses reported to date.

(a) The Du Group syntheses

The Du group’s synthesis of cochliomycin A (7) was reported in 2014 and employed L-arabinose as the chiron for assembling the three contiguous stereogenic centres within the macrocyclic lactone. The detailed reaction sequence is shown in Scheme 1 and started with the conversion of L-arabinose (20) into the corresponding bis-acetonide (21) under standard conditions and the latter compound subjected to a Wittig olefination (to give 22) and then selective acetonide hydrolysis using aqueous acetic acid. Diol 23 so-formed (77% from 21) was selectively tosylated and ester 24 then treated with base so as to form epoxide 25 (78% from 23). Olesin cross-metathesis of compound 25 with the commercially available and 5-configured alcohol 26 gave the F-Silike (31, 85%) and the associated epoxide ring then opened using the anion derived from trimethylsilylacetylene and thus producing the homoallyl alcohol 28 (78%).

Over three steps, including a Pd-catalysed hydrostannylation reaction, the acetylenic unit associated with compound 28 was converted into the alkynylstannane 29 (71%) that was itself engaged in a Siles cross-coupling with the well-known aryl triflate 30 and thus producing compound 31 (81%), the immediate precursor to target 7. Indeed, on treatment with sodium hydride in DMF the conversion 31 → 7 was effected in >93% yield.

The Du Group’s synthesis of cochliomycin B (8) (Scheme 2) also started with L-arabinose but a ring-closing metathesis reaction was now used to construct the associated macrocyclic ring. Thus, compound 20 was converted, under conventional conditions, into the corresponding 3,4-mono-acetonide and this itself subjected to a Wittig olefination reaction and so affording compound 32 (72%). Over three steps this diol was manipulated so as to generate aldehyde 33 (46%) and a Wittig-based homologation of this last.
compound afforded, via enol ether 34 (77%), congener 35 (75%). Takai-type olefination of this last compound then gave the L-configuration enol ether 36 (33%) that was engaged in a Suzuki–Miyaura cross-coupling with the readily obtained allylborane 37 and so affording the mono-styrene 38 (66%). Reaction of this last compound with the acetal derived from homocatalal alcohol 26 then gave ester 39 (75%) that upon reaction with Crabbé's second-generation catalyst afforded, via ring-closing metathesis (RCM), the required macrocycle 40 (57%) and treatment of this with tetra-n-butyllithium ether to give ether 41 (85%). Oxidation of compound 42 under Swern conditions gave the corresponding aldehyde 43 (79%) that was subjected to a highly diastereoselective Knoe asymmetric allylation reaction and so affording, after protection of the resulting homobifurcated alcohol, cleavage of the 1βS ether and oxidation of the resulting alcohol, aldehyde 44 (59%) A Julia–Kocienski olefination reaction was then carried out on compound 44 using the readily prepared sulfone 45. RCM33 and 18-crown-6 and so affording, in

(b) The Nanda Group syntheses

Jana and Nanda reported a synthesis of echiolomycins A in 201211 and this started (Scheme 3) with the conversion, by well established methods, of (+)-lactic acid (40) into 2,3-dihydroxypropan-2-ylacrylic acid (43) and mono-protection of the latter to give ether 42 (85%). Oxidation of compound 42 under Swern conditions gave the corresponding aldehyde 43 (79%) that was subjected to a highly diastereoselective Knoe asymmetric allylation reaction and so affording, after protection of the resulting homobifurcated alcohol, cleavage of the 1βS ether and oxidation of the resulting alcohol, aldehyde 44 (59%). A Julia–Kocienski olefination reaction was then carried out on compound 44 using the readily prepared sulfone 45. RCM33 and 18-crown-6 and so affording, in

Scheme 3. The Nanda Group synthesis of echiolomycins A (7).
a highly selective manner and after silyl ether cleavage, the target
E-alkene 46 in 73% yield. Mitsunobu coupling of this last com-
 pound with acid 47 then gave, after cleavage of the PMB ether resi-
 due, ester 48 (73%). Upon exposure to Grubbs’ second-generation
catalyst compound 48 was converted into cochinomycin A (7)
(72%).

The Nanda Group synthesis of cochinomycin C12 (Scheme 4) also
started with ε-tartaric acid (40) and exploited a Mitsunobu-medi-
 ated lactonization reaction to form the macrolide ring. Specifically,
then, di-acid 40 was, once again, converted into the diol-acetide
41 and the latter mono-protected as the corresponding p-
methoxybenzoyl (PMB) ether 49 (83%). Upon Swern oxidation this

![Scheme 4. The Nanda Group synthesis of cochinomycin C (9).](image-url)
last compound gave the aldehyde 58 (90%) which afforded the terminal olefin 51 (70-75%) that was subjected to an olefin cross-metathesis (COM) reaction with the unsaturated and homochiral ether 52 using the Grubbs' second-generation catalyst. The primary product of this process was then hydrogenated under conventional conditions so as to give compound 53 (76%). Oxidative cleavage of the PMB-ether residue associated with the ether 53 then gave the corresponding alcohol that was oxidized to aldehyde 54 (88%) using the Dess-Martin periodinane. Reaction of compound 54 with the propargyl anion proceeded stereoselectively and Lindlar hydrogenation of the product alkyne gave the corresponding homochiral aldehyde that was protected as the MOM-ether 55 (78%). Heck coupling of the last compound with the iodinated benzaldehyde 56 afforded spireme 57 (84-90%) and oxidation of the associated aldehyde residue gave the corresponding benzoic acid. Cleavage of the TBDPS-ether within product 57 then afforded the substrate 58 (63-79%) used in the macrolactonization reaction. So, compound 58 was subjected to an intramolecular Mitsunobu reaction that provided macroclide 59 (P = MOM) (79%), the MOM-group of which was cleaved and the product RAL viz. paclitaxel C (13), was then chlorinated using sulfuryl chloride and thus affording cochliomycin C (9) in 71% yield.

Nanda and his colleagues have also reported related syntheses of the CS- and CE'-epimers of cochliomycin C.

(c) The Sethi Group approach

The Sethi Group synthesis of cochliomycin C (9) (Scheme 5) is a formal one in that it delivers paclitaxel C (13) as starting material and uses a RCM reaction to construct the macrocyclic ring. The synthesis started with the conversion of compound 60 into the previously reported monoaconitine 61 (95%) and this was subjected to an iminobenzaldehyde alkyne forming reaction that delivered, with accompanying
epimerisation, compound 62 (49%) as a mixture of diastereoisomers. Conversion of this last pair of compounds into the corresponding limonene derivatives and chromatographic separation of the major product 63 (45%) was followed by the regiospecific reaction of the derived diazo with the commercially available and homochiral epoxide 64 and so affording the 2'-alcohol 65 (82%). Exhaustive reduction of the alkyne moiety associated with this last compound and reaction of the oxanyon derived from product 66 (68%) with the readily prepared acrolein 67 gave, after acid treatment, the vinylated salicylate 68 (63%). This was subjected to oxidative cleavage and the ensuing aldehyde alkylated in a diastereoselective manner to give diene 69 (63%). Compound 69 was then engaged in a RCM reaction using the Haywood–Grubbs second generation catalyst and by such means, and after cleavage of the associated acetonide residue, pecilinomycin C (13) was obtained in 68% yield. Since Nanda et al. has previously converted compound 13 into cochloromycin C (9) through electrophilic aromatic chlorination using sulfonyl chloride a formal total synthesis of the latter natural product was realised in this instance.

By related means 4S-epi-cochloromycin C was obtained.13

(i) Background to the Banwell Group studies on the synthesis of RALs

Our group's original efforts in the area arose through an interest in exploiting enzymatically-derived and homochiral cis-1,2-dihydroxycyclohexene54 such as 70 (Fig. 4) in the assembly of various RALs. The pivotal building block employed for this purpose was Weishe
amide 71 obtained through, inter alia, reduction of the non-halogenated double bond associated with the acetoacetic derivative of diol 70 and ozonolytic cleavage of the remaining (halogenated) one. Compound 71 served as a precursor to L-783,250 (72) and its cis-isomer 5, the latter being, as noted above, a potent inhibitor of MEK1. While the macrocyclic ring and the E-configured C=C bond associated with target 72 was constructed using a RCM reaction, a more novel means of assembling the analogous (Z-configured) motif within congener 5 was developed. Details are provided immediately below.

Our synthesis of the cis-enone-containing L-783,277 (5) is shown in Scheme 6 and, like the pathway leading to congener 72, involved, in the early stages, the Heck coupling of aryl iodide 56 with the unsaturated Weinreb amide 71. The immediate product of this process was oxidised to the corresponding acid (under classical conditions) and this then hydrogenated to give compound 73 (413) that, in turn, treated with the organocatalysed derived from the homochiral propargyl alcohol 74 (itself available through enzymatic resolution of the corresponding racemate). The ester 75 (708) so formed was treated with potassium hexamethyldisilazide so as to generate the corresponding acetylide anion that itself engaged in an intramolecular acylation reaction and so producing the cyclic alkyne 76 (645) and for which a single-crystal X-ray analysis was undertaken. This analysis revealed an essentially linear geometry about the internal triple bond and thus highlighting the capacity of the 14-membered macrocyclic ring of RALs to accommodate a range of structural motifs. The completion of the synthesis of target 5 involved Lindlar-type hydrogenation of cyclisation product 76 and twofold deprotection of the enone oxime gave L-783,277 (5) (40%) without compromising the integrity of the Z-configured double bond.

(c) The Banwell Group syntheses

Our syntheses of RALs 5 and 72 were completed just prior to the report of the isolation and structural characterisation of cochliomycins A–C (7–9, respectively). Given this, the presence of the (unusual) acetoacetic residues within congeners A and B and the novel biological properties they display we were attracted to developing syntheses of them. Our route to the first two of these (i.e. the acetoacetic-containing ones) exploited a late-stage and highly stereoselective Noyori–Hiyama–Kishi (NHK) reaction to effect the necessary macrocyclisation process, a relatively unusual one in terms of its application in the synthesis of RALs.

The pivotal elements of the synthetic sequence used are shown in Scheme 7 and involved an OCM of the readily available ester 67 with the D-2-deoxyribose-derived and previously reported chiron 77 to give compound 78 (86%). The p-substituted styrene 76 was then reacted with the readily prepared homoleptic alcohol 79 in the presence of base and so affording, after protection of the phenolic OH group, the ester 80 (89%). Treatment of ester 80 with TFA
resulted in selective cleavage of the TBS-ether moiety and oxidation of the resulting and rather sensitive 1'-alcohol with the Dess-Martin periodinane then gave the corresponding aldehyde. This was immediately engaged in an intramolecular NHK reaction to afford, with high levels of diastereoselectivity, the SEM ether of cochliomycin B (8) (77%). When this ether was treated with TBAF in refluxing THF then cochliomycin B (8) itself was obtained in 73% yield. In contrast, on treating the SEM ether with HCl in methanol at 22 °C for 1 h then compound A (7) (91%) was obtained while extended exposure of the same substrate to the same conditions resulted in acetonide group cleavage and formation of the previously reported RAL zeenol (14) which was obtained in 84% yield.

The end game associated with our approach to cochliomycin C (9) was rather different and resulted in the identification of a new means for forming the macrocyclic ring of RALs. The reaction
sequence started (Scheme 8) with an OCM reaction between the readily available alkenes 81 and 82 (the former compound being obtained from trans-stilic acid) and conventional hydrogenation of the product olefin 83 (88%) to give alkane 84 (98%). The alkene derived from the last compound was reacted with amine 30 and thus affording ester 85 (91%), the phenolic group of which was protected as the corresponding MOM-ether 86 (94%). A Stille cross-coupling reaction between aryli fluoride 87 and the arylnitramine 88 then gave the cinnamyl alcohol 89 (76%) that was converted, over three standard steps, into the rather unstable aldehyde 90 (66%).

Given our previous positive experiences with the NHK reaction we sought to apply this in the macrocyclization of compound 89. However, even exposing this to a mixture of chromous chloride and nickel(II) chloride in DMF only the vinylated 12-member lactone 90 was obtained (as a single diastereoisomer in 73% yield).

In stark contrast, when the same substrate was treated with sodium in a mixture of water and dichloromethane then a Lewis-type n-alkylation reaction took place and so affording, in a highly diastereoselective manner, the 14-membered macrocycle 91 (51%). Removal of the acetate and SEM protecting groups associated with this last compound using aqueous acid then gave paromycin F (13) that was chromanized with sulfuric chloride and so affording echinomycin C (9) in 82% yield.

During the course of our work detailed above Cutler and colleagues reported18 the isolation of three new RALs from a fungus Neocosmospora sp. (LMU 6115099). They were named neocosmosins A–C and structures 92–94 (Fig. 5) respectively, assigned to them. These RALs were found to co-occur with three previously reported ones, namely radicid I (95), monomycin II (96) and monomycin IV (97).

Unlike any of the RALs we had previously targeted for synthesis, all of the Neocosmospora-derived compounds embody a C10 keto residue and three of them (1, 94 and 95) show good binding affinity for the human opioid receptors. Accordingly, we sought to develop a synthesis of the first of these, namely compound 92, embodying the structure assigned to neocosmosin A.

Our synthesis of RAL 92 is shown in Scheme 9 and began with the OCM of styrrene 67 and the unsaturated aldehyde 97. The product di-alkene 98 (72%) was treated with dimethyl disulfide and the resulting epoxide 99 (quant.) engaged in a Meisenheimer-type rearrangement on exposure to Pd(OAc)2 and 8-BaP and thus affording ketone 100 (81%) embodying the pivotal C10 car-benzylic unit (RAL numbering) associated with the target 92. Acid-catalyzed hydrolysis of the acetal moiety within compound 100 afforded the corresponding keto-aldehyde 101 (89%) that could be selectively methylation using the Wittig reagent and so giv-ing the terminal aldehyde 102 (74%). Compound 102 was particularly prone to cyclisation on treatment with either acid or base. So, for example, when it was heated with p-Toluenesulfonic acid in toluene in the presence of ethylene glycol (in an effort to prepare the corresponding tetral), then the unsaturated lactone 103 (82%) was formed but this could be cleaved with potassium hydroxide in aqueous THF and thus gave, after careful acidic work up keto-acid 104 (86%). Compound 104 then served as the nucleophile in a Mitsunobu reaction with the homochiral 2'-alcohol 26 and thus affording the ester 110 (78%) that was itself engaged in an BCM reaction using Grubb's second generation catalyst and so producing the target RAL 92 (83%). All of the NMR, IR and MS spectral data acquired on this product matched those reported for neocosmosin A. However, while the specific rotation of compound 92 was of a similar magnitude to that reported for the natural product it was of the opposite sign. As such we concluded that the absolute configuration of neocosmosin A had been incorrectly assigned and is, in fact, represented by structure ent-92.

The synthesis of compound ent-92 (Scheme 10) involved a trivial adaptation of the process just discussed. Thus, Mitsunobu coupling of keto-acid 104 with the homochiral 2'-alcohol ent-26 gave ester ent-110 (92%) and this underwent an BCM reaction to give neocosmosin A (ent-92; 67%), the structure of which was confirmed by single-crystal X-ray analysis.

During the course of these studies Das and co-workers reported19 a distinctly different synthesis of compound ent-94.

Future Prospects/Conclusion

New RALs, including ones isolated from marine sources, that display intriguing biological properties continue to be reported.20 Studies on the synthesis of such compounds have resulted, over the decades, in the identification of a raft of new methods for their construction and these have now provided chemists with the capacity to prepare new RALs in a predictable manner. As such, completions of total syntheses of RALs no longer elicit the excite-
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Scheme 10. The Bamberli Group synthesis of the true structure of neurocinin A (ent-92).

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Total Syntheses of the Resorcylic Acid Lactones Paecilomycin F and Cochliomycin C Using an Intramolecular Loh-Type α-Allylation Reaction for Macrolide Formation

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Total Syntheses of the Resorcylic Acid Lactones Paecilomycin F and Cochliomycin C Using an Intramolecular Loh-Type α-Allylation Reaction for Macrolide Formation

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Supporting Information

**ABSTRACT:*** Subjection of the resorcylic ester 16 to a Noyori–Hiyama–Kishi reaction afforded the 12-membered lactone 17, while treatment of it under the Loh-type α-allylation conditions using indium metal gave the isomeric, 14-membered macrolide 18. Compound 18 was readily elaborated to the resorcylic acid lactone type natural products paecilomycin F and cochliomycin C.

The resorcylic acid lactones (RALs) are a large and ever-growing group of mycotoxins that embody a β-resorcylic acid residue annulated to a 14-membered macrolactone. Many of these natural products display a range of potent biological properties, perhaps most notably powerful and selective inhibitory activities against ATPases and kinases as well as, inter alia, antifungal, antiparasitic, antimarial, and/or antifungal properties. As a consequence, considerable effort has been devoted to their synthesis. Arguably the most challenging aspect of these endeavors has been the assembly of the macrolide ring, and a significant range of techniques has been developed for this purpose. Macrolactonization, ring-closing metathesis (RCM), intramolecular nucleophilic addition reactions (including NHK and HWE eliminations), intramolecular nucleophilic substitution, and cross-coupling processes as well as radical cyclization and ring contraction reactions represent just some of the numerous techniques used. Herein, we detail a versatile new means for constructing the macrolactone ring of RALs and its exploitation in the synthesis of the title natural products, namely paecilomycin F and cochliomycin C.

The cochliomycins are a series of six RALs isolated from the marine-derived fungus Cochliobolus lunatus that display notable antifungal and/or fungalial properties. The structures of the first three members of the family, namely cochliomycins A–C (1–3, respectively), are shown in Figure 1. The isomeric natural products 1 and 2 each incorporate acetamide residues, while congeners 3 is a chlorine derivative of paecilomycin F (4), another RAL that has been isolated from a filamentous fungus collected in Southern China and shown to display antimalarial activity.

In seeking to extend our recently reported preparations of compounds 1 and 2 to congeners 3 and 4, we have now identified a hitherto unexploited but highly effective means for assembling the macrolactone ring associated with the RALs. The pivotal bond-forming events associated with the route reported herein are shown in Figure 2 with the most important being an intramolecular α-allylation reaction that was used to close the 14-membered ring. To the best of our knowledge, this approach to the construction of RALs has not been examined previously, perhaps in part because of potential competition from formation of the presumably kinetically more favorable 12-

![Figure 1. Cochliomycins A–C and paecilomycin F (1–4, respectively).](image)

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Figure 2. Key bond-forming reactions associated with the assembly of echinomycin B (3) and the key building blocks, 5–8, used.

membered ring lactone. The building blocks required for the purposes of investigating this approach were compounds 5–8.

The synthetic sequence used to assemble these building blocks in readiness for exploring the intramolecular alkylation reaction is shown in Scheme 1 and started with the olefin cross metathesis (OCM) of compounds 7 and 8 using Grubbs' second-generation catalyst,2 to form the diene 9 (88%). This was reacted with dichloromethane in the presence of palladium on carbon so as to effect concurrent reduction of the C–C double bond and hydrogenolytic cleavage of the benzyl ether, thus forming alcohol 10 in 98% yield. Reaction of this last compound with building block 5 in the presence of sodium hexamethyldisilazide (NaHMDS) resulted in the anticipated trans-esterification reaction to produce compound 11 (91%) that was immediately protected as the corresponding 2-(trimethylsilyl)ethoxymethyl or SEM ether 12 (94%) under standard conditions. A Stille cross-coupling reaction8 between this last compound and building block 6 gave the trimethylsilylated alcohol 13 (72%) that was readily converted into the corresponding chloride 14 (84%) on reaction with N-chlorosuccinimide (NCS) and triphenylphosphine. In the final steps used to assemble the substrate required for examining the foreshadowed ring-closing (intramolecular) alkylation reaction, the TBDMS ether 14 was cleared with tetra-n-butylammonium fluoride (TBAF) and the resulting alcohol 15 (92%) oxidized with the Dess–Martin periodinane (DMP)9 to give the target aldehyde 16 in 85% yield. All of the spectral data acquired on compound 16 were in complete accord with the assigned structure. Most notably, the presence of an aldehyde residue was evident in the IR and NMR spectra while the mass spectrum established that the compound contained one chlorine atom.

That variant of the intramolecular NRK reaction wherein the organochromium(III) reagent derived from an allyl iodide is added to a pendant aldehyde has been successfully exploited in the assembly of the 14-membered lactone rings of RALs.11 In contrast, the allyl variant has not, perhaps because of the potential for competing formation of the corresponding 12-membered macrocyclic. Consistent with such possibilities, when compound 16 was subjected (Scheme 2) to reaction with an excess of chromium(II) chloride in the presence of 6 mol % nickel(II) chloride in X/N-dimethylformamide (DMF), the only isolable product of reaction was compound 17. This was obtained as a single diastereoisomer in 33% yield. Product 17 results from a so-called γ- rather than α-allylation reaction.12
transo relationship between the adjacent vinyl and hydroxyl groups is textually assigned on the basis of a consideration of the transition state likely to be operative in the cyclization reactions.  

Loh and co-workers have described a method wherein allylic indium reagents can be regioselectively engaged in α- rather than γ-allylation reactions (through careful control of the amount of water added to the reaction medium), although this protocol does not appear to have been applied to macrocyclization processes. Accordingly, it was pleasing to observe that when compound 16 was treated with a suspension of indium metal in dichloromethane containing 11 mol equiv of water then the desired mode of cyclization took place, thereby producing macrocycle 18 in an unoptimized yield of 61%. No evidence was obtained for the formation of the previously observed compound 17, the Z-isomer of product 18, or its C4-epimer. Confirmation of the illustrated structure of product 18 follows from an X-ray analysis of a derivative as detailed below. A Fell–Arndt-type transition state would account for the observed diastereoselectivity of this macrocyclization reaction.  

The conversion of macrocycle 18 into the title natural products 4 and 3 was effected using the straightforward procedures shown in Scheme 3. Specifically, both the SEM and acetamido protecting groups associated with compound 18 could be cleaved on treatment with HCl in aqueous methanol and paeocamycin F (4) (34) thus obtained in 95% yield. All of the NMR spectral data acquired on compound 4 matched those reported for the natural product (see Table S1 for some relevant comparisons) as did the specific rotation [α]D 20 −103.3 (c 0.6, methanol) vs lit 35 [α] D 20 −106.4 (c 0.28, methanol). The regioselective aromatic methylation of RAL 4 was readily effected using nitryl chloride in dichloromethane 36 and coeholycin C (3) 37 obtained in 96% yield.

Not only did all of the spectral data acquired on this synthetically derived compound (viz. 3) compare favorably with those reported 34 for the natural product (see the Supporting Information), but a single-crystal X-ray analysis served to confirm its structure and, therefore, that of the natural product.

The work reported here highlights the capacity of Loh's indium-mediated α-allylation protocol to effect macrocyclization reactions in a predictable fashion and to introduce functionality relevant to the synthesis of RALs and related natural products. As such, this procedure, which proceeds under mild conditions in an aqueous environment, warrants further attention in macrocycle synthesis.

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SUPPORTING INFORMATION FOR:

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General Experimental Protocols

Unless otherwise specified, proton (\(^1\)H) and carbon (\(^{13}\)C) NMR spectra were recorded at room temperature in base-filtered CDCl\(_3\) on a spectrometer operating at 400 MHz for proton and 100 MHz for carbon nuclei. For \(^1\)H NMR spectra, signals arising from the residual protioforms of the solvent were used as the internal standards. \(^1\)H NMR data are recorded as follows: chemical shift (\(\delta\)) [multiplicity, coupling constant(s) \(J\) (Hz), relative integral] where multiplicity is defined as: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet or combinations of the above. The signal due to residual CHCl\(_3\), appearing at \(\delta_\text{H} = 7.26\) and the central resonance of the CDCl\(_3\), "triplet" appearing at \(\delta_\text{H} = 7.00\) were used to reference \(^1\)H and \(^{13}\)C NMR spectra, respectively. Infrared spectra (\(\nu_{\text{IR}}\)) were recorded on a FTIR Spectrometer. Samples were analyzed as thin films on KBr plates. Low-resolution ESI mass spectra were recorded on a single quadrupole liquid chromatograph-mass spectrometer while high-resolution measurements were conducted on a time-of-flight instrument. Low- and high-resolution ESI mass spectra were recorded on a magnetic-sector machine. Melting points were measured on an automated melting point system and are uncorrected. Analytical thin layer chromatography (TLC) was performed on aluminum-backed 0.2 mm thick silica gel 60 F\(_{254}\) plates. Eluted plates were visualized using a 254 nm UV lamp and/or by treatment with a suitable dip followed by heating. These dips included phosphomolybdic acid: ceric sulfate: sulfanilic acid (conc.): water (37.5 g: 7.5 g: 37.5 g: 720 mL) or potassium permanganate: potassium carbonate: 5% sodium hydrosulfide aqueous solution: water (3 g: 20 g: 5 mL: 300 mL). Flash chromatographic separations were carried out following protocols defined by Stille et al.\(^1\) with silica gel 60 (40–63 \(\mu\)m) as the stationary phase and using the AR- or HPLC-grade solvents indicated. Starting materials, reagents and drying agents as well as other inorganic salts were generally available from commercial sources and used as supplied. Tetrahydrofuran (THF), diethyl ether, methanol and dichloromethane were dried using a solvent purification system that is based upon a technology originally described by Grubbs et al.\(^1\) Where necessary, reactions were performed under an nitrogen atmosphere.
A magnetically stirred solution of (S)-(+) 4-penten-2-ol (172 mg, 2 mmol) in dry DMF (4 mL) maintained at 0 °C under a nitrogen atmosphere was treated, in portions, with NaH (60 wt % dispersion in mineral oil, 120 mg, 3 mmol). After 0.5 h at 0 °C the reaction mixture was treated, dropwise, with benzyl bromide (513 mg, 2.4 mmol). The ensuing mixture was allowed to warm to room temperature then stirred overnight before being cooled to 0 °C then quenched with water (30 mL) (CAUTION: evolution of hydrogen gas) and extracted with diethyl ether (3 x 15 mL). The combined organic phases were washed with LiCl (1 x 50 mL of a 5% w/v aqueous solution) then brine (1 x 50 mL) before being dried (Na₂SO₄), filtered and concentrated under reduced pressure. The residue thus obtained was subjected to flash chromatography (silica, 100:0 → 85:15 v/v hexane/ethyl acetate) afforded compound 8 (350 g, 59%) as a light-green oil, [α]D²⁰ = +8.1 (c 3.3, CHCl₃) [lit.² (for ent-8) [α]D²⁰ = +45.6 (c 0.5, CHCl₃)].

¹H NMR (400 MHz, CDCl₃, δ 7.40–7.52 (complex m, 4H), 7.27 (m, 1H), 5.66 (m, 1H), 5.10 (m, 2H), 4.55 (q, J = 11.8 Hz, 2H), 3.60 (m, 1H), 2.40 (m, 1H), 2.26 (m, 1H), 1.22 (d, J = 6.2 Hz, 3H)

¹³C NMR [100 MHz, CDCl₃] δ 139.0, 135.1, 128.3, 127.6, 116.8, 74.5, 70.4, 40.9, 19.5

IR (KBr) νmax 3030, 2974, 2863, 1642, 1453, 1375, 1341, 1129, 1091, 912, 733, 696 cm⁻¹

MS (ESI, +ve) m/z 199 [(M+Na)⁺, 5%), 102 (100)

HRMS (ESI, +ve) Found (M+Na)⁺, 199.1099. C₁₂H₁₅NO requires (M+Na)⁺, 199.1099.
A magnetically stirred solution of silyl ether 7 (2.64 g, 9.7 mmol) and benzyl ether 8 (5.81 g, 33 mmol) in dichloromethane (4 mL) maintained at ca. 22 °C under a moderate flow of nitrogen was subject to sonication for 0.5 h (for the purpose of removing oxygen). Grubbs' II catalyst (224 mg, 0.26 mmol) was then added to the reaction mixture that was sonicated for a further 0.17 h. The resulting solution was heated at 40 °C for 2 h then cooled and concentrated under reduced pressure. The residue thus obtained was subjected to flash chromatography (silica, 100:0:0 → 90:5:5 v/v/v 40–60 petroleum spirit/diethyl ether/dichloromethane gradient elution) and concentration of the relevant fractions (Rf = 0.5 in 7:3 v/v hexane/ethyl acetate) afforded compound 9 (3.58 g, 88%) as a light-brown oil, [α]_D^22 = −31.7 (c 2.3, CHCl₃).

^1H NMR (400 MHz, CDCl₃) δ 7.36-7.30 (complex m, 4H), 7.27 (m, 1H), 5.80 (m, 1H), 5.55 (m, 1H), 4.53 (m, 2H), 4.31 (t, J = 7.5 Hz, 1H), 3.80-3.70 (complex m, 3H), 3.55 (m, 1H), 2.40 (m, 1H), 2.20 (m, 1H), 1.44 (s, 3H), 1.40 (s, 3H), 1.20 (d, J = 6.1 Hz, 3H), 0.90 (s, 9H), 0.06 (d, J = 2.6 Hz, 6H)

^13C NMR (100 MHz, CDCl₃) δ 138.8, 131.6, 129.8, 128.3, 127.6, 127.4, 108.8, 81.5, 78.8, 74.4, 70.4, 62.4, 39.3, 27.2, 26.9, 25.9, 19.5, 18.4, 5.3, 5.4

IR (KBr) \nu_max 2929, 2958, 1454, 1377, 1252, 1140, 1092, 970, 836, 777 cm⁻¹

MS (ESI, +ve) m/z 443 [(M+Na)^+], 102 (100)

HRMS (ESI, +ve) Found (M+Na)^+ 443.2605. CsH₂₀NaO₃S₁ requires (M+Na)^+ 443.2594.
A magnetically stirred solution of alkene 9 (2.00 g, 4.76 mmol) in ethyl acetate (250 mL) maintained under a nitrogen atmosphere at ca. 22 °C was treated with palladium on carbon (800 mg of 10 wt. % loading of Pd). The nitrogen atmosphere was displaced with hydrogen and the ensuing mixture stirred for 3 h then filtered through a pad of diatomaceous earth. The solids thus retained were washed with ethyl acetate (5 x 20 mL) and the combined filtrates concentrated under reduced pressure. The ensuing residue was subjected to flash chromatography (silica, 4:1 v/v 40–60 petroleum spirit/ethyl acetate elution) and concentration of the relevant fractions (Rf = 0.4 in 7:3 v/v hexane/ethyl acetate) afforded alcohol 10 (1.58 g, 99%) as a clear, brown oil, [α]D^20 = -20.0 (c 1.6, CHCl₃).

H NMR (400 MHz, CDCl₃) δ 3.92-3.3 (complex m, 5H), 1.70-1.53 (complex m, 3H), 1.52-1.41 (complex m, 4H), 1.39 (s, 3H), 1.36 (s, 3H), 1.19 (d, J = 6.1 Hz, 3H), 0.90 (s, 9H), 0.06 (s, 6H)

C NMR (100 MHz, CDCl₃) δ 108.4, 81.0, 78.7, 67.9, 63.7, 39.2, 33.3, 27.4, 27.0, 25.9, 23.5, 22.3, 18.3, 5.3(7), 5.4(3)

IR (KBr) v max 3432, 2953, 2931, 2852, 1469, 1460, 1376, 1368, 1250, 1132, 1090, 838, 776 cm⁻¹

MS (ESI, +ve) m/z 355 ([M+Na]^+), 333 ([M+H]^+), 8

HRMS (ESI, +ve) Found (M+Na)^+ 355.2285. C₁₇H₂₀NaO₆Si requires (M+Na)^+ 355.2281.
A magnetically stirred solution of alcohol 10 (870 mg, 2.64 mmol) in dry THF (11 mL) maintained under nitrogen was cooled to 0 °C then treated, dropwise, with sodium hexamethyldisilazane (NaHMDS, 3.50 mL of a 1.0 M solution in THF, 3.50 mmol). The resulting mixture was stirred at 0 °C for 0.5 h then a solution of compound 5 (1.12 g, 3.26 mmol) in dry THF (6 mL) was added dropwise. The ensuing mixture was warmed to room temperature and stirred for 1 h before being quenched with pH 7 aqueous buffer and extracted with ethyl acetate (2 x 20 mL). The combined organic phases were washed with brine (1 x 100 mL), then dried (Na2SO4) filtered and concentrated under reduced pressure. The residue thus obtained was subjected to flash chromatography (silica, 10:0 → 9:1 v/v 40-60 petroleum spirit/ethyl acetate gradient elution) and concentration of the relevant fractions (Rt = 0.3 in 9:1 v/v hexane/ethyl acetate) afforded compound 11 (1.51 g, 91%) as a clear, colorless oil, [α]D20 = +110.5 (c 3.6, CHCl3).

1H NMR (400 MHz, CDCl3) δ 12.03 (s, 1H), 6.48 (d, J = 2.5 Hz, 1H), 6.32 (dd, J = 2.5 and 0.9 Hz, 1H), 5.30 (m, 1H), 3.88 (m, 1H), 3.83 (s, 3H), 3.77-3.63 (complex m, 3H), 1.91 (m, 1H), 1.75-1.51 (complex m, 5H), 1.41 (d, J = 6.3 Hz, 3H), 1.38 (s, 3H), 1.36 (s, 3H), 0.88 (s, 9H), 0.05 (s, 3H), 0.04 (s, 3H)

13C NMR (100 MHz, CDCl3) δ 168.4, 165.7, 164.2, 149.6, 118.7 (q, JCF = 319 Hz), 108.5, 103.0, 100.9(4), 100.9(1), 81.1, 78.5, 74.5, 63.7, 55.9, 35.4, 33.1, 27.4, 27.0, 25.9, 22.1, 19.7, 18.3, −5.3, −5.5

IR (KBr) νmax 2952, 2933, 2859, 1663, 1628, 1427, 1260, 1211, 1158, 1142, 1091, 1042, 1025, 838, 817, 778, 605 cm−1

MS (ESI, +ve) m/z 653 [(M+Na)+, 100%], 631 [(M+H)+, 6%]

HRMS (ESI, +ve) Found (M+Na)+, 653.2045. C32H43O16Si requires (M+Na)+, 653.2040.
2-(Trimethylsilyl)ethoxymethyl chloride (SEM-Cl, 640 µL, 3.6 mmol) and Hüning's base (450 µL, 3.2 mmol) were added sequentially and dropwise to a magnetically stirred solution of compound 11 (1.20 g, 1.8 mmol) and tetra-n-butylammonium iodide (TBAI, 660 mg, 0.2 mmol) in dichloromethane (10 mL) maintained under nitrogen at room temperature. After 2 h the reaction mixture was quenched with brine (20 mL) then water (20 mL) before being extracted with dichloromethane (3 x 50 mL). The combined organic phases were washed with brine (1 x 100 mL) before being filtered and dried (Na₂SO₄) then concentrated under reduced pressure. The resulting light-yellow oil was subjected to flash chromatography (silica, 9:1 v/v 40–60 petrolæan spirit/ethyl acetate elution) and thus affording, after concentration of the appropriate fractions (RF = 0.3 in 9:1 v/v hexane/ethyl acetate), compound 12 (1.29 mg, 94%) as a clear, colorless oil, [α]D° = +8.0 (c 1.0, CHCl₃).

1H NMR (400 MHz, CDCl₃) δ 6.77 (d, J = 2.2 Hz, 1H), 6.49 (d, J = 2.2 Hz, 1H), 5.25 (ABq, J = 1.3 Hz, 2H), 5.16 (m, 1H), 3.86 (m, 1H), 3.82 (s, 3H), 3.75 (m, 3H), 3.66 (m, 2H), 1.79–1.51 (complex m, 5H), 1.45 (m, 1H), 1.39 (s, 3H), 1.36 (s, 3H), 1.34 (d, J = 6.3 Hz, 3H), 0.90 (m, 2H), 0.88 (s, 9H), 0.06 (s, 3H), 0.05 (s, 3H) 0.00 (s, 9H).

13C NMR (100 MHz, CDCl₃) δ 164.2, 163.3, 158.6, 149.0, 119.9 (q, J_C = 318 Hz), 113.1, 109.8, 102.6, 101.9, 94.8, 82.6, 80.0, 74.4, 68.12, 65.0, 57.3, 37.3, 34.7, 28.8, 28.4, 27.3, 23.6, 21.1, 19.7, 19.4, 0.0, 4.00, -2.1

IR (KBr) v_max 2953, 1730, 1621, 1580, 1428, 1248, 1213, 1144, 1090, 1026, 836, 779, 606 cm⁻¹

MS (ESI, +ve) m/z 783 [(M+Na)⁺, 35%], 102 (100)

HRMS (ESI, +ve) Found (M+Na)⁺, 783.2851. C₃₂H₅₅O₇PF₃NaO₁S₂ requires (M+Na)⁺, 783.2853.

S7
$N,N$-Dimethylformamide (DMF, 7 mL) was added to a Schlenk flask containing triflate 12 (241 mg, 0.32 mmol), PdCl$_2$(PPh)$_3$ (22 mg, 0.032 mmol), and lithium chloride (95 mg, 2.24 mmol). The resulting and magnetically stirred mixture was maintained under a gentle flow of nitrogen while being sonicated for 0.5 h. Then compound 6 (125 mg, 0.36 mmol) was added and the ensuing mixture heated at 80 °C for 1 h. The cooled reaction mixture was diluted with ethyl acetate (10 mL) and water (100 mL) and the separated aqueous phase extracted with ethyl acetate (2 x 10 mL). The combined organic phases were washed with brine (2 x 10 mL) then dried (Na$_2$SO$_4$), filtered and concentrated under reduced pressure. The resulting light-yellow oil was subjected to flash chromatography (silica, 8:2 v/v hexane/ethyl acetate elution) and concentration of the relevant fractions ($R_f = 0.2$ in 8:2 v/v hexane/ethyl acetate) afforded the compound 13 (168 mg, 76%) as a clear, colorless oil, $[a]_D^{10} = +53.0$ (c 2.4, CHCl$_3$).

$^1$H NMR [400 MHz, (CD$_2$)$_2$CO] δ 6.79 (d, $J = 2.5$ Hz, 1H), 6.66 (d, $J = 2.5$ Hz, 1H), 6.62 (m, 1H), 6.41 (m, 1H), 5.26 (m, 2H), 5.15 (m, 1H), 4.22 (m, 2H), 3.90 (m, 2H), 3.83 (s, 3H), 3.76 (m, 4H), 3.64 (m, 4H), 1.75–1.61 (complex m, 4H), 1.59–1.44 (complex m, 2H), 1.31 (m, 9H), 0.98–0.91 (complex m, 2H), 0.90 (m, 9H), 0.07 (s, 6H), 0.00 (s, 9H)

$^{13}$C NMR [100 MHz, (CD$_2$)$_2$CO] δ 166.8, 161.0, 155.4, 136.5, 133.3, 125.3, 117.9, 108.0, 102.6, 100.5, 92.8, 81.4, 78.3, 71.1, 66.0, 63.6, 62.1, 54.9, 36.0, 33.3, 26.9, 25.4, 22.4, 19.7, 18.0, 17.7, –2.1, –6.0(7), –6.1(1)

IR (KBr) $\nu_{max}$ 3469, 2953, 2931, 2858, 1723, 1601, 1579, 1463, 1379, 1251, 1162, 1095, 1049, 975, 836, 779 cm$^{-1}$

MS (ESI, +ve) $m/z$ 691 [(M+Na)$^+$, 15%], 102 (100)

HRMS (ESI, +ve) Found (M+Na)$^+$, 691.3674. $C_{29}H_{30}NaO_4Si_2$ requires (M+Na)$^+$, 691.3674.
N-Chlorosuccinimide (NCS, 43 mg, 0.34 mmol) was added to a magnetically stirred solution of compound 13 (168 mg, 0.24 mmol) and Ph₃P (92 mg, 0.34 mmol) in THF (5 mL) maintained under nitrogen at room temperature. After 0.5 h the reaction mixture was diluted with ethyl acetate (10 mL) then washed with water (1 x 20 mL) and brine (1 x 20 mL) before being dried (Na₂SO₄), filtered and concentrated under reduced pressure. The resulting light-yellow oil was subjected to flash chromatography (silica, 8:2 v/v 40–60 petroleum spirit/ethyl acetate elution) to afford, after concentration of the appropriate fractions (Rf = 0.3 in 7:3 v/v hexane/ethyl acetate), compound 14 (140 mg, 84%) as a clear, colorless oil, [α]D²⁰ = −34.0 (c 1.0, CHCl₃).

¹H NMR (400 MHz, CDCl₃) δ 6.78–6.56 (complex m, 3H), 6.25 (m, 1H), 5.20 (m, 3H), 4.18 (m, 2H), 3.87 (m, 1H), 3.82 (s, 3H), 3.78–3.71 (complex m, 3H), 3.71–3.62 (complex m, 2H), 1.77–1.53 (complex m, 5H), 1.48 (m, 1H), 1.39 (s, 3H), 1.36 (s, 3H), 1.34 (d, J = 6.3 Hz, 3H), 0.94 (m, 2H), 0.89 (s, 9H), 0.06 (s, 6H), 0.00 (s, 9H)

¹³C NMR (100 MHz, CDCl₃) δ 168.5, 162.5, 157.2, 137.0, 132.1, 129.3, 118.9, 109.8, 105.2, 102.9, 94.6, 82.5, 80.0, 73.4, 67.8, 65.1, 56.9, 46.3, 37.5, 34.8, 28.8, 28.4, 27.3, 23.8, 21.5, 19.7, 19.4, 0.0, −3.9(9), −4.0(4)

IR (KBr) νmax 2952, 2930, 2858, 1723, 1601, 1579, 1463, 1378, 1251, 1163, 1098, 1049, 971, 938, 836, 779 cm⁻¹

MS (ESI, +ve) m/z 711 and 709 [(M+Na)⁺, 8 and 16%, respectively], 102 (100)

HRMS (ESI, +ve) Found (M+Na)+, 709.3336. C₃₆H₅₅ClNaO₃S requires (M+Na)+, 709.3335.
Compound 15

Tetra-n-butylammonium fluoride (TBAF, 210 µL of a 1.0 M solution in THF, 0.21 mmol) was added, dropwise, to a magnetically stirred solution of compound 14 (140 mg, 0.2 mmol) in THF (1.5 mL) maintained at 0°C under a nitrogen atmosphere. The ensuing mixture was stirred for 3 h then diluted with ethyl acetate (5 mL) and washed with water (1 × 20 mL) and brine (1 × 20 mL) before being dried (Na₂SO₄), filtered, and concentrated under reduced pressure. The residue thus obtained was subjected to flash chromatography (silica, 8:1 v/v hexane/ethyl acetate) to afford, after concentration of the relevant fractions (Rᵢ = 0.2 in 4:1 v/v hexane/ethyl acetate), compound 15 (140 mg, 92%) as a clear, colorless oil, [α]₂⁰° = +190 (c 1.1, CH₂Cl₂).

¹H NMR [400 MHz, (CD₃)₂CO] δ 6.84 (d, J = 2.2 Hz, 1H), 6.75–6.69 (complex m, 2H), 6.45 (dt, J = 15.5 and 6.9 Hz, 1H), 5.29 (m, 2H), 5.18 (m, 1H), 4.33 (dd, J = 6.9 and 1.2 Hz, 2H), 3.87 (m, 1H), 3.84 (t, 3H), 3.78 (m, 3H), 3.65 (m, 3H), 1.76–1.59 (complex m, 4H), 1.58–1.43 (complex m, 2H), 1.34–1.29 (complex m, 9H), 0.90 (m, 2H), 0.00 (s, 9H)

¹³C NMR [100 MHz, (CD₃)₂CO] δ 167.5, 162.0, 156.4, 136.2, 131.1, 129.2, 119.0, 108.8, 103.8, 102.3, 93.7, 82.8, 78.9, 72.3, 66.9, 63.1, 55.9, 45.8, 36.9, 34.1, 27.8, 27.5, 23.3, 20.6, 18.6, –1.2

IR (KBr) νmax 3479, 2982, 2950, 2874, 1721, 1601, 1579, 1459, 1444, 1379, 1322, 1266, 1250, 1198, 1163, 1102, 1048, 969, 936, 858, 836 cm⁻¹

MS (ESI, +ve) m/z 597 and 595 [(M+Na)⁺, 46 and 100%, respectively]

HRMS (ESI, +ve) Found (M+Na)⁺, 595.2471. C₂₃H₂₅ClNaO₂Si requires (M+Na)⁺, 595.2470.
Compound 16

The Dess-Martin periodinane (DMP) (118 mg, 0.28 mmol) was added to a magnetically stirred mixture of alcohol 15 (106 mg, 0.185 mmol), pyridine (100 \(\mu\)L) and NaHCO\(_3\) (44 mg, 0.53 mmol) in dichloromethane (14 mL) maintained at 0 °C. After 0.5 h the reaction mixture was warmed to room temperature and after a further 6 h it was filtered through a pad of TLC-grade silica gel (5 g). The solids thus retained were washed with diethyl ether (50 mL) and the combined filtrates concentrated under reduced pressure. The ensuing light-yellow was subjected to flash chromatography (silica, 8:1 v/v 40–60 petroleum spirit/ethyl acetate elution) to afford, after concentration of the relevant fractions \(R_f = 0.2\) in 4:1 hexane/ethyl acetate, compound 16 (90 mg, 85%) as a clear, colorless oil, \([\alpha]_{D}^{20} = +13.0 (c 1.0, \text{CHCl}_3)\).

\(^1\)H NMR [400 MHz, (CD\(_2\))\(_2\)CO] \(\delta\) 9.70 (d, \(J = 2.3\) Hz, 1H), 6.84 (d, \(J = 2.2\) Hz, 1H), 6.76–6.67 (complex m, 3H), 6.45 (m, 1H), 5.28 (m, 2H), 5.15 (m, 1H), 4.32 (m, 2H), 4.14 (m, 1H), 3.97 (m, 1H), 3.84 (s, 3H), 3.77 (m, 2H), 1.76–1.61 (complex m, 4H), 1.50 (m, 1H), 1.43 (s, 3H), 1.39 (s, 3H), 1.32 (d, \(J = 6.2\) Hz, 3H), 0.94 (complex m, 2H), 0.00 (s, 9H)

\(^13\)C NMR [100 MHz, (CD\(_2\))\(_2\)CO] \(\delta\) 201.7, 167.5, 162.0, 156.4, 136.2, 131.1, 129.2, 119.0, 111.4, 103.8, 102.3, 93.7, 85.7, 77.5, 72.2, 66.9, 55.9, 45.8, 36.7, 34.1, 27.5, 26.5, 22.7, 20.5, 18.6, –1.2

IR (KBr) \(\nu_{\text{max}}\) 2953, 2926, 2853, 1724, 1601, 1579, 1460, 1444, 1379, 1265, 1198, 1163, 1099, 1050, 970, 936, 859, 836 cm\(^{-1}\)

MS (El, +ve) \(m/z\) 572 and 570 (M\(^+\), 2 and 5%, respectively), 477 (15), 329 and 327 (15 and 8, respectively), 279 (100), 189 (58), 73 (85)

HRMS (El, +ve) Found M\(^+\) 570.2418. \(C_{23}H_{33}ClO_3Si\) requires M\(^+\) 570.2416.
Compound 17

CrCl$_2$ (170 mg, 1.35 mmol) then NiCl$_2$ (1 mg, ca. 0.01 mmol) were each added, in a single portion, to a solution of aldehyde 16 (77 mg, 0.14 mmol) in dry DMF (30 mL, freshly distilled from CaH$_2$) maintained under a nitrogen atmosphere in a dry box. The ensuing reaction was sonicated for 0.5 h while being maintained under gentle stream of nitrogen then the reaction vessel was sealed and the contents stirred magnetically at room temperature for 72 h. The resulting mixture was quenched with water (50 mL) then extracted with diethyl ether (2 x 50 mL). The combined organic phases were washed with LiCl (1 x 50 mL of a 5% w/v aqueous solution) then brine (1 x 50 mL) before being dried (Na$_2$SO$_4$), filtered and concentrated under reduced pressure. The resulting light-yellow oil was subjected to flash chromatography (silica, 1:1 v/v 40–60 petroleum spirit/diethyl ether gradient elution) to afford, after concentration of the appropriate fractions ($R_f = 0.3$ in 3:2 v/v hexane/ethyl acetate), compound 17 (24 mg, 33%) as a single diastereoisomer and light-yellow oil. Subjecting this oil to further flash chromatography (silica, 1:1 → 1:4 v/v 40–60 petroleum spirit/diethyl ether gradient elution) then preparative TLC (silica, 8:2 v/v 40–60 petroleum spirit/ethyl acetate) afforded a spectroscopically pure sample of macroclide 17, [α]$_D^{25}$ = +67.0 (c 1, CHCl$_3$).

$^1$H NMR [400 MHz, (CD$_3$)$_2$CO] δ 6.65 (d, J = 2.3 Hz, 1H), 6.60 (d, J = 2.3 Hz, 1H), 6.44 (m, 1H), 5.25 (s, 2H), 5.11–5.00 (complex m, 2H), 4.91 (m, 1H), 4.13 (m, 1H), 4.09–3.97 (complex m, 2H), 3.90 (d, J = 5.7 Hz, 1H), 3.79 (m, 6H), 1.83–1.61 (complex m, 4H), 1.48 (m, 4H), 1.32 (s, 3H), 1.26 (s, 3H), 1.01–0.91 (complex m, 2H), 0.01 (s, 9H) (signal due to hydroxyl group proton not observed).

$^{13}$C NMR [100 MHz, (CD$_3$)$_2$CO] δ 166.4, 160.9, 156.1, 143.4, 138.8, 117.2, 115.5, 107.9, 107.3, 99.4, 93.2, 84.1, 74.6, 74.4, 72.2, 66.1, 54.8, 47.6, 33.5, 31.3, 26.7, 26.3, 21.6, 20.3, 17.6, −2.2.
Compound 18

Water (100 μL) then indium powder (84 mg, 0.73 g atom) were added to a magnetically stirred solution of aldehyde 16 (279 mg, 0.49 mmol) in dichloromethane (25 ml) maintained under a nitrogen atmosphere. The resulting mixture was stirred in a sealed reaction vessel at room temperature for 48 h before being quenched with water (25 mL) and then extracted with ethyl acetate (2 x 50 mL). The combined organic phases were washed with brine (1 x 100 mL) before being dried (Na2SO4), filtered and concentrated under reduced pressure. The residue thus obtained was subjected to flash chromatography (silica, 8:2 v/v 40-60 petroleum spirit/ethyl acetate elution) to afford, after concentration of the appropriate fractions (RF = 0.4 in 7:3 v/v hexane/ethyl acetate), compound 18 (158 mg, 61%) as a clear, brown oil, [α]D 20 +31.8 (c 1.2, CHCl3).

$^1$H NMR (400 MHz, CDCl3) δ 6.70–6.50 (complex m, 3H), 6.07 (m, 1H), 5.20 (d, J = 2.3 Hz, 2H), 5.12 (m, 1H), 4.17 (m, 1H), 4.00 (m, 1H), 3.83 (m, 1H), 3.81 (s, 3H), 3.76 (m, 2H), 2.55 (m, 2H), 2.38 (broad s, 1H), 1.88 (m, 1H), 1.72–1.48 (complex m, 5H), 1.43–1.34 (complex m, 9H), 0.94 (m, 2H), 0.00 (s, 9H)

$^{13}$C NMR (100 MHz, CDCl3) δ 168.7, 162.6, 157.2, 139.0, 131.8, 129.9, 118.1, 109.1, 106.1, 102.5, 94.8, 83.3, 76.2, 72.5, 70.0, 67.8, 56.9, 36.9, 36.0, 34.2, 28.6(1), 28.5(7), 22.3, 21.4, 19.4, 0.0

IR (KBr) νmax 3481, 2932, 1721, 1601, 1579, 1456, 1379, 1262, 1198, 1161, 1104, 1048, 982, 934, 859, 836, 756 cm⁻¹

MS (ESI, +ve) m/z 559 [(M+Na)+, 100%], 537 (81)

HRMS (ESI, +ve) Found (M+Na)+, 559.2703. C29H43NaO3Si requires (M+Na)+, 559.2703.
Compound 4

Compound 23 (158 mg, 0.30 mmol) was treated with HCl (24 mL of a 1 M solution in 9:1 v/v CH₂OH/water) and the resulting mixture was stirred magnetically at room temperature for 3 h then quenched with anhydrous K₂CO₃ (3.0 g). The mixture thus obtained was filtered through a pad of TLC-grade silica gel (1.0 g) and the solids thus retained were washed with ethyl acetate (50 mL). The combined filtrates were concentrated under reduced pressure and the resulting light-yellow oil was subjected to flash chromatography (silica, 6:4 → 2:8 v/v 40–60 petroleum spirit/ethyl acetate gradient elution). Concentration of the relevant fractions (Rf = 0.4 in ethyl acetate) gave compound 4 (99 mg, 91%) as an amorphous, white solid, [α]D²⁰⁰ = −103.3 (c 0.6, methanol) [lit. [α]D²⁰⁰ = −106.4 (c 0.28, methanol)].

¹H NMR (400 MHz, CDCl₃) δ 12.28 (s, 1H), 7.14 (dd, J = 15.4 and 2.3 Hz, 1H), 6.40 (s, 2H), 5.70 (m, 1H), 4.98 (m, 1H), 4.19 (m, 2H), 3.82 (s, 3H), 3.54 (m, 1H), 3.05 (s, 1H), 2.95 (broad s, 2H), 2.69 (m, 1H), 2.45 (m, 1H), 1.90–1.75 (complex m, 2H), 1.55 (partially obscured m, 1H), 1.50–1.15 (complex m, 3H), 1.41 (d, J = 6.1 Hz, 3H)

¹³C NMR (100 MHz, CDCl₃) δ see Table S1

IR (KBr) νₐ₅ 3397, 2940, 2865, 1640, 1605, 1574, 1428, 1351, 1313, 1254, 1202, 1156, 1044, 974, 831, 803, 724 cm⁻¹

MS (ESI, +ve) m/z 389 [(M+Na)⁺, 100], 367 [(M+H)⁺, 8]  

HRMS (ESI, +ve) Found (M+Na)⁺, 389.1576. C₁₆H₁₅NaO₅ requires (M+Na)⁺, 389.1576.
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*spectra recorded in CDCl₃ at 100 MHz, *data obtained from reference 8, spectrum recorded in CDCl₃ at 100 MHz.
Sulfuryl chloride (14 µL, 0.174 mmol) was added, dropwise, to a magnetically stirred solution of compound 4 (20 mg, 0.055 mmol) in dichloromethane (10 mL) maintained at 0 °C under a nitrogen atmosphere. The ensuing mixture was allowed to warm to room temperature and after 3 h it was treated with NH₄Cl (20 mL of 5% aqueous solution) then dichloromethane (30 mL). The separated organic phase was washed with brine (1 x 50 mL) before being dried (Na₂SO₄), filtered and concentrated under reduced pressure. The residue thus obtained was subjected to flash chromatography (silica, 7:3 v/v 40-60 petroleum spirit/ethyl acetate gradient elution) and concentration of the relevant fractions (Rf = 0.4 in ethyl acetate) afforded a white solid, recrystallization (methanol/water) of which gave compound 3 (19 mg, 91%) as a white solid, mp = 161.5–164 °C, [α]D.5° = –17.5 (c 0.6, methanol) [lit. 7 [α]D.5° = –18.0 (c 0.04, methanol)].

**1H NMR** (400 MHz, CDCl₃) δ 12.18 (s, 1H), 6.63 (m, 1H), 6.48 (s, 1H), 5.55 (m, 1H), 5.15 (m, 1H), 4.17 (m, 2H), 3.91 (s, 3H), 3.44 (m, 1H), 2.97 (broad s, 2H), 2.85 (d, J = 15.2 Hz, 1H), 2.71 (broad s, 1H), 2.52 (d, J = 15.2 and 11.3 Hz, 1H), 1.83 (m, 2H), 1.68–1.39 (complex m, 3H), 1.34 (d, J = 6.1 Hz, 3H), 1.30 (m, 1H)

**13C NMR** (100 MHz, CDCl₃) δ 159.5, 133.9, 130.4, 129.0, 127.8, 126.0, 123.3, 122.6, 121.2, 110.9, 108.1, 107.8, 105.9, 105.6, 104.7, 101.7, 97.8, 83.3, 81.3, 73.1

**IR (KBr) νmax cm⁻¹: 3411, 2942, 2874, 1639, 1594, 1430, 1389, 1353, 1314, 1243, 1209, 1110, 1080, 1047, 978, 833, 813, 736**

**MS (ESI, +ve) m/z: 425 and 423 [(M+Na)⁺, 35 and 100%, respectively]**

**HRMS (ESI, +ve) Found [(M+Na)⁺, 423.1177, C₁₉H₂₅NaO₅ requires [(M+Na)⁺, 423.1187.**
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¹: Spectrum recorded in CDCl₃ at 100 MHz. ²: Data obtained from reference ². ³: Spectrum recorded in CDCl₃ at 150 MHz. ²¹: This difference is attributed to variations in the pH of the medium in which each spectrum was recorded. ²²: Nardo et al. also report a chemical shift of 86.5 ppm for the methoxy methyl carbon of synthetically-derived cochlaurine.
Crystallographic Studies

Crystallographic Data for Compound 3

3(C17H27ClO2)H2O, M = 1218.57, T = 150 K, monoclinic, space group P21/c, Z = 2, a = 20.6774(2) Å, b = 5.6019(1) Å, c = 25.4783(2) Å; β = 90.3838(8)°; V = 2951.16(6) Å³, Dm = 1.371 g cm⁻³, 10967 unique data (2θmax = 144.6°), R = 0.041 [for 10503 reflections with I > 2σ(I)]; Rw = 0.117 (all data), S = 1.02.

Structure Determination

Images were measured on a CCD diffractometer (CuKα, mirror monochromator, λ = 1.54184 Å) and data extracted using the CrysAlis PRO package. Structure solution was by direct methods (SIR92). The structure of compounds 3 was refined using the CRYSTALS program package. Atomic coordinates, bond lengths and angles, and displacement parameters for compound 3 have been deposited at the Cambridge Crystallographic Data Centre (CCDC no. 1451755). These data can be obtained free-of-charge via www.ccdc.cam.ac.uk/data_request/cif by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.
Figure S1. Structure of compound 3 (C14(D17SS)) with labeling of selected atoms. Anisotropic displacement ellipsoids show 30% probability levels. Hydrogen atoms are drawn as circles with small radii.
References

100 MHz $^{13}$C NMR Spectrum of Compound 9
(recorded in CDCl$_3$)

$\text{BnO}$

$\text{P = TBS}$
400 MHz $^1$H NMR Spectrum of Compound 10
(recorded in CDCl$_3$)
100 MHz $^{13}$C NMR Spectrum of Compound 10
(recorded in CDCl$_3$)
400 MHz $^1$H NMR Spectrum of Compound 11
(recorded in CDCl$_3$)
100 MHz $^{13}$C NMR Spectrum of Compound 11
(recorded in CDCl$_3$)

$P = TBS$
400 MHz $^1$H NMR Spectrum of Compound 12
(recorded in CDCl$_3$)

P = TBS

CHCl$_3$

water
100 MHz $^{13}$C NMR Spectrum of Compound 12 (recorded in CDCl$_3$)

SEM O
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O

P = TBS
400 MHz $^1$H NMR Spectrum of Compound 13
(recorded in (CD$_3$)$_2$CO)

SEMO
MeO
P = TBS

water

partial protio-forms of
(CD$_3$)$_2$CO
400 MHz $^1$H NMR Spectrum of Compound 14 (recorded in CDCl$_3$)

$P = $ TBS

$\text{CHCl}_3$

water
400 MHz $^1$H NMR Spectrum of Compound 15
(recorded in (CD$_3$)$_2$CO)
400 MHz $^1$H NMR Spectrum of Compound 16
(recorded in (CD$_3$)$_2$CO)

partial protio-forms
of
(CD$_3$)$_2$CO

water
400 MHz $^1$H NMR Spectrum of Compound 17
(recorded in (CD$_3$)$_2$CO)
$^{13}$C NMR Spectrum of Compound 17
(recorded in (CD$_3$)$_2$CO)
400 MHz $^1$H NMR Spectrum of Compound 18
(recorded in CDCl$_3$)
400 MHz $^1$H NMR Spectrum of Compound 4
(recorded in CDCl$_3$)
100 MHz $^{13}$C NMR Spectrum of Compound 3
(recorded in CDCl$_3$)
A Total Synthesis of (±)-3-O-Demethylmacronine through Rearrangement of a Precursor Embodying the Haemanthidine Alkaloid Framework

Xiang Ma, Nadia Gao, Martin G. Banwell, Paul D. Carr, and Anthony C. Willis

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A Total Synthesis of (±)-3-O-Demethylmacroine through Rearrangement of a Precursor Embodying the Haemanthidine Alkaloid Framework

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Research School of Chemistry, Institute of Advanced Studies, The Australian National University, Canberra, ACT 2601, Australia

ABSTRACT: A total synthesis of the racemic modification, (±)-2, of the tetrazine-type alkaloid 3-O-demethylmacroine is described. The key steps are an intramolecular Alder-ene (IMAE) reaction and a lactam-to-lactone rearrangement of tetrahydro-13, a compound that embodies the haemanthidine alkaloid framework.

INTRODUCTION

In 1984, Hauth and Staabfacher reported the isolation of the alkaloid macroine (1) (Figure 1) from the plant Combretum macrophorum Engl. (Combretaceae), and the assignment of its full structure, by Wildman and co-workers,3 followed shortly thereafter. The latter group noted that compound 1 represents the first example of a lactonic Combretaceae alkaloid possessing the tetrazine ring system. They also revealed that a strained lactam incorporated within the haemanthidine alkaloid framework rearranged to give 3-O-demethylmacroine in buffer at pH 6.8. Whether or not such a rearrangement has biosynthetic relevance remains unclear. In 1999, Hose and co-workers described the isolation of 3-O-demethylmacroine (2) from a Galiiusus species of Turkish origin and the illustrated structure was established using conventional NMR spectroscopic methods. The same group also determined that the compound does not arise through demethylation of the parent 1 during the isolation process. Accordingly, 3-O-demethylmacroine (2) is considered to be a naturally occurring alkaloid.

Thus far, no biological evaluation of compound 2 has been reported. Furthermore, while macroine (1) has been isolated from a range of plant sources since 1984, studies of its potential therapeutic properties appear to have been confined to ones utilizing crude extracts of the producing plants and thus suggesting that it may possess, at a minimum, useful antibacterial and/or antifungal properties.4–7

In 1976, Tsuda et al. reported a 14-step synthesis of (±)-macroine that exploited, as a late-stage transformation, a rearrangement reaction of the type described by Wildman. No other relevant work on alkaloids 1 or 2 has been reported since then and not does there appear to have been any studies on the stereochemical requirements (if any) of this protocol and potentially versatile rearrangement process. It is against this background that we now report a 10-step synthesis of (±)-3-O-demethylmacroine (±-2) from readily available materials and also detail a synthesis of its C3a-epimer through an analogous, even more facile, rearrangement reaction.

RESULTS AND DISCUSSION

The synthetic route used to obtain the requisite, strained lactam embedded within the haemanthidine alkaloid framework is shown in Scheme 1. Thus, Suzuki–Miyaura cross-coupling of the known benzoate ester 3 with the previously reported cyclohexyl bromide 4 gave the arylated cyclohexene 5 (83%), and this was readily propargylated at nitrogen using 1-bromo-2-butyne in the presence of sodium hydride to give derivative 6 in 91% yield. This last compound participated in an intramolecular Alder-ene (IMAE) reaction,8 using Pd(OAc)₂, and the strong σ-donating ligand N,N′-bis(benzyliden)ethylene-diamine (BBEDA) in refluxing toluene, thereby affording the C3a-arylated hexahydroazepine 7 in 73% yield. This IMAE product was accompanied by small amounts of uncharacterized materials, one of which is likely to the isomeric cyclopropyl-containing system analogous to that observed9 when the tosylate

Figure 1. Structures the alkaloid macroine (1) and its 3-O-demethyl congenere (2).
Scheme 1. Synthesis of the Strained Keten-lactam 9 Embodying the Harmothione Alkaloid Framework

Scheme 2. Chemical Manipulation of Compound 9 Leading to Lactone 12 and Lactam 13

analogue of substrate 6 was engaged in the same type of reaction.

Subjection of compound 7 to reaction with p-chlorobenzonitriyl and cesium carbonate in dimethylformamide (DMF) at ambient temperature, conditions defined by Fukuyama for the cleavage of nitriles, 7 not only resulted in removal of the sulfonylimidate residue but also effected a lactamisation reaction involving the pendant ester residue. As a result compound 8 (79%) was obtained, the structure of which was secured by single-crystal X-ray analysis (see the Experimental Section and Supporting Information for details). The enecyclic double bond associated with lactam 8 could be oxidatively cleaved through its initial and selective diyldovinyl oxidation under conditions defined by Blackwell 8 and then submitting the vinylcyclo diene so-formed to treatment with iodosobenzene diacetate. 9 By such means, the ketene 9 was obtained in 51% yield over the two steps involved. Compound 9 displays a lactam carbonyl absorption band at 1700 cm⁻¹ in the infrared spectrum, while in the ¹³C NMR spectrum of this same material, the associated carbon resonates at δ 179.5 ppm. These values stand as testimony to the strained nature of this nitrogen-containing ring system (the equivalent values for di-vinylketate are ca. 1672 cm⁻¹ and δ 169.1, respectively). 10

When a methanolic solution of keten-lactam 9 maintained at 0°C was treated with sodium borohydride, nonselective reduction of the associated ketone residue took place to afford a chromatographically separable mixture of compounds 10 and 11 (Scheme 3). Since each of these reduction products was obtained as an interconverting mixture of epimers/anomers, they were subjected, as a mixture and without extensive spectroscopic characterization, to oxidation with manganese dioxide and thereby affording the chromatographically separable lactone 12 (45% from 9) and lactam 13 (26% from 9), respectively. The structure of compound 13 was confirmed by single-crystal X-ray analysis (see the Experimental Section and Supporting Information for details). Presumably, compound 10 arises through initial reduction of the ketone carbonyl residue within precursor 9 such that the hydroxyl group within the resulting alcohol sits, as is evident from inspection of molecular models, directly above the lactam carbonyl moiety and thus approach the latter along a Birch-Pinetti trajectory 11 and so facilitating conversion into the isomeric lactone that is itself reduced to the observed mixture of lactones 10. In contrast, the epimeric alcohol arising from reduction of the ketone residue within compound 9 cannot so readily engage in a lactone-to-lactam isomerization process, and thus the residual (and strained) lactam carbonyl group is reduced directly to give compound 11.

The simple synthetic pathway used to convert lactone 12 into compound 13, the Cα-epimer of (±)-3-(3-dimethylamino)-pyrrolidinone, is shown in Scheme 3. Thus, treatment of ester ether 12 with H₂O₂/acetone in THF at ambient temperature for 1 h gave the expected aliphatic alcohol 14 in 99% yield, and when this was treated with potassium hydroxide and methyl iodide in THF at 0°C for 0.5 h, then the anticipated 3°-amine 15 was obtained in near quantitative yield. Interestingly, in the second step of this reaction sequence, no product arising from α- methylation of the aliphatic alcohol moiety was observed. The H and ¹³C NMR spectra of compound 15 were in complete accord with the assigned structure and quite distinct from those recorded for the natural product 2.

The route used in completing the total synthesis of target (±)-2 is shown in Scheme 4. This involved, as a pivotal step, the p-toluenesulfonyl chloride-promoted conversion of the harmothione-based hydroxylactam 13 into the lactone 16 (85%), and as part of this process, the ester ether associated with the starting material was cleaved. The precise pathway by which this rearrangement takes place remains unclear. However, given the likely abnormaly basic nature of the nitrogen associated with the bridged lactam 12 in substrate 13, protonation at this...
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Scheme 3. Conversion of Lactone 12 into the C6a-Epimer, 15, of (±)-5-O-Demethylmaacrine: ([±]-2)

Scheme 4. Completion of the Total Synthesis of (±)-5-O-Demethylmaacrine: ([±]-2)

center, followed by cleavage of the N–C=O single bond and reaction of the resulting acylim ion with the pendant hydroxyl group would afford the observed lactone 16. Reductive N-methylation of compound 16 using sodium cyanoborohydride and formamide in acetic acid at ambient temperatures then gave (±)-5-O-demethylmaacrine ([±]-2) in 80% yield. Interestingly, attempts to effect the O-methylation of the last compound under a range of conditions1 failed to generate (±)-maacrine ([±]-1). While the origins of this situation are not clear, the likely close spatial arrangement of the hydroxyl and amine groups within compound ([±]-2) could be responsible.

All the spectral data acquired on compound ([±]-2) were in complete accord with the assigned structure, while the 'H and 13C NMR spectra recorded on the synthetic material matched those reported by Itose5 for the natural product (see the SI for tabulated comparisons).

**CONCLUSION**

The results reported here demonstrate that strained lactam units embedded within a C12-hydroxylated haematinidine framework can be engaged in rearrangement reactions that generate the triterpenoid skeletons of the maacrine alkaloids. These rearrangements appear to proceed regardless of the stereocchemistry at C12, although the reaction pathways involved are quite different in each instance. While the biosynthetic relevance (or otherwise) of such processes remains to be determined, they are likely to enable the preparation of a range of frameworks of biological interest. Work directed toward examining such possibilities is now underway.

**EXPERIMENTAL SECTION**

**General Protocols.** Unless otherwise specified, proton (1H) and carbon (13C) NMR spectra were recorded at 18 °C in deuterated CDC13 on a spectrometer operating at 400 MHz for proton and 100 MHz for carbon nuclei. If NMR data are recorded as follows: chemical shift (δ, multiplicity, coupling constants), relative integral, where multiplicity is defined as s = singlet; d = doublet; t = triplet, q = quartet, m = multiplet, or combinations of the above. In relevant cases, the signal due to residual CHCl3 appearing at δ 7.26 and the central resonance of the CHCl3 "triplet" appearing at δ 7.55 were used to reference 1H and 13C NMR spectra, respectively. Samples were analyzed by infrared spectroscopy (KBr) as a thin film on KBr plates or as neat material using the following spectrometers: low and high-resolution electron impact (EI) mass spectra were recorded on a double-focusing, triple-sector machine. Low- and high-resolution FAB mass spectra were recorded on a triple-quadrupole mass spectrometer operating in either positive or negative ion mode. Melting points are uncorrected. Analytical thin-layer chromatography (TLC) was performed on aluminum-backed 0.2 mm thick silica gel 60 F254 plates. Eluted plates were visualized using a 254 nm UV lamp and/or by treatment with a suitable dip, followed by heating. These dips included phosphomolybdic acid/conc. sulfuric acid (conc.)/water (3:7:7 g/g/g), potassium permanganate/potassium carbonate 3% sodium hydride aqueous solution/water (3:10 g/g), and p-anisaldehyde or vanillin/sulfuric acid (conc.)/ ethanol (15 g/2.5 mL/250 mL). Flash chromatographic separations were carried out following protocols defined by Schöllhorn17 with silica gel 60 (40–63 μm) as the stationary phase and using the AR- or HTELC-grade solvents indicated. The melting points of solids purified by such means were normally recorded directly (i.e., after they had crystallized from the concentrated chromatographic fractions). Starting materials, reagents, drying agents, and other inorganic salts were generally commercially available and were used as supplied.

Fumaraldehyde (TFA), methanol, and dichloromethane were dried using a solid purification system that is based upon a technology originally described by Giubbi et al.8 Where necessary, reactions were performed under a nitrogen atmosphere.

**Specific Chemical Transformations.** Methyl 6-[(4S,6S)-6-4-[(tert-butyl(dimethyl)silyl)oxy]-6-[(1,1,1,3,3-pentamethyldisiloxanyl) cyclohexyl]-1-yl]-furan-2-carboxylate (5). A magnetically stirred solution of compound 3 (1.96 g, 4.0 mmol, 1.0 equiv), compound 4 (1.75 g, 6.0 mmol, 1.5 equiv), B(OTf)3 (400 mg, 1.02 mmol, 0.17 mol equiv), and cesium carbonate (7.50 g, 23.0 mmol, 3.8 mol equiv) in THF/water (30 mL of a 9:1 v/v mixture) was degassed for 30 min. After degassing, the mixture was heated under reflux for 3 h and then cooled, quenched with water (100 mL), and extracted with ethyl acetate (3 × 100 mL). The combined organic layers were washed with brine (1 × 100 mL) before being dried (MgSO4) filtered, and concentrated under reduced pressure. The light yellow oil thus obtained was subjected to flash chromatography (silica gel, 10.0–7.5 v/v CH2Cl2/MeOH) followed by concentrated under reduced pressure. The light yellow oil thus obtained was subjected to flash chromatography (silica gel, 10.0–7.5 v/v CH2Cl2/MeOH) followed by concentrated under reduced pressure. The light yellow oil thus obtained was subjected to flash chromatography (silica gel, 10.0–7.5 v/v CH2Cl2/MeOH) followed by concentrated under reduced pressure. The light yellow oil thus obtained was subjected to flash chromatography (silica gel, 10.0–7.5 v/v CH2Cl2/MeOH) followed by concentrated under reduced pressure. The light yellow oil thus obtained was subjected to flash chromatography (silica gel, 10.0–7.5 v/v CH2Cl2/MeOH) followed by concentrated under reduced pressure. The light yellow oil thus obtained was subjected to flash chromatography (silica gel, 10.0–7.5 v/v CH2Cl2/MeOH) followed by concentrated under reduced pressure. The light yellow oil thus obtained was subjected to flash chromatography (silica gel, 10.0–7.5 v/v CH2Cl2/MeOH) followed by concentrated under reduced pressure. The light yellow oil thus obtained was subjected to flash chromatography (silica gel, 10.0–7.5 v/v CH2Cl2/MeOH) followed by concentrated under reduced pressure.
cylindrical solid, mp = 175–177°C. 71% NBR (400 MHz, CDCl₃), δ 7.86 (Ar, J = 7.3 Hz, 20H), 7.32 (J = 7.6 Hz, 21H), 7.45 (s, 1H), 7.39 (s, 1H), 6.85 (m, 15H), 6.31 (s, 1H), 5.88 (s, 1H), 5.40 (s, 1H), 4.70 (s, 2H), 4.63 (s, 2H), 3.40 (s, 3H). 2.56 (m, 1H), 2.27 (s, 1H), 2.19 (m, 1H), 0.95 (s, 1H), 0.16 (s, 3H), 0.15 (s, 3H). ¹³C NMR (100 MHz, CDCl₃), δ 166.0, 154.7, 149.7, 146.3, 138.3, 135.8, 135.2, 127.5, 124.8, 124.5, 121.7, 110.8, 109.7, 101.7, 59.5, 53.5, 51.8, 54.7, 38.1, 26.3, 18.3, –47 (two signals overlapping). IR (KBr) 3374, 2928, 2858, 1520, 1541, 1489, 1437, 1407, 1308, 1223, 1166, 1248, 1038, 883, 850, 780 cm⁻¹. 1H NMR (400 MHz, 1H NMR: 6.25 (m, 1H), 0.43 (s, 3H) requires (M + Na⁺) 613.859. C₂₃H₂₃N₂O₄S requires (M + Na⁺) 616.853.

2-Methyl-6-(isoflav-1-en-3-yl)-N-(6-nitroresorufin)- 
4H-pyron-1-one (6). A magnetically stirred solution of compound 5 (3.29 g, 5.57 mmol), 10 equiv, in DMF (50 mL) maintained under a nitrogen atmosphere at 50°C was treated with sodium hydroxide (276 mg of a 68% suspension in oil, 6.61 mmol, 1.2 equiv). After 0.3 h the reaction mixture was treated with bromomethane (2-yr 0.6 nu., 11.1 mmol, 2.0 equiv) and the mixture thus obtained was allowed to warm to 22°C and then treated to 60°C. It was then stirred at this temperature for 6 h before being cooled, quenched with ice-water (40 mL of 1M Reported Conditions of hydrogen gas evolution), and diluted with ethyl acetate (50 mL). The separated aqueous layer was extracted with ethyl acetate (3 x 50 mL), and the combined organic layers were washed with 1L (approximately 100 mL of a 3% aqueous sodium solution) and then brine (1 x 100 mL) before being dried (MgSO₄). filtered, and concentrated under reduced pressure. The resulting light yellow oil was subjected to flash chromatography (silica gel 100 – 230 mesh) and purified by preparative thin layer chromatography (silica gel 100 – 230 mesh) and purified by preparative thin layer chromatography (20 × 20 cm) with 100% ethyl acetate (3 x 50 mL) and concentrated under reduced pressure at 40°C for 10 min.

Step 1: A magnetically stirred solution of compound 8 (300 mg, 1.66 mmol, 1.0 equiv) in acetonitrile/water (100 mL of a 3 M with H₂O) maintained at 22°C was treated with cesium carbonate (18.45 g, 114 mmol, 1.1 equiv) and p-chloroaniline (41.4 mg, 0.34 mmol, 0.24 equiv). The resulting mixture was stirred at room temperature for 3 h and then quenched with I2C (5 mL of a saturated aqueous solution) and extracted with dichloromethane (3 x 50 mL). The combined organic layers were washed with brine (1 x 100 mL) before being dried (MgSO₄), filtered, and concentrated under reduced pressure. The resulting light yellow oil was immediately in the next step as described above.

Step 2: A solution of the oil obtained as described above (step 1) in dichloromethane (10 mL) was treated with tetrasubstituted ethylene chloride (363 mg, 2.05 mmol, 1.2 equiv). The ensuing mixture was stirred vigorously at 22°C for 3 h. The reaction mixture (10 mL) was treated with potassium hexamethyldisilazane (363 mg, 2.05 mmol, 1.2 equiv) and concentrated under reduced pressure. The resulting light yellow oil was immediately in the next step as described above.

Step 3: A solution of the oil obtained as described above (step 1) in dichloromethane (10 mL) was treated with nitrobenzene (540 mg, 3.0 equiv). The ensuing mixture was stirred vigorously at 22°C for 3 h. The reaction mixture was treated with sodium hydroxide (276 mg of a 68% suspension in oil, 6.61 mmol, 1.2 equiv) and concentrated under reduced pressure. The resulting light yellow oil was immediately in the next step as described above.
3-[3-(tert-Butyldimethylsilyloxy)-12-hydroxy-4,4a,5,6-tetrahydro-2H-3,1-benzoxazine-4-ol]-5,10-dihydro-3H-1,4-dioxy-3,6-naphthoquinone-6-one (11). Step 1: A magnetically stirred solution of compound 9 (248 mg, 0.66 mmol) in THF (5 mL) was stirred at 23 °C and then treated with chilled methanol (0 mL, 0.0 mol) followed by sodium borohydride (24 mL, 3.4 mmol, 0.0 mol equiv). The resulting mixture was heated to 22 °C stirred at this temperature for 0.5 h, then quenched with NaHCO₃ (5 mL of a saturated aqueous solution), and then filtered with ethyl acetate (10 mL and water (30 mL). The separated aqueous phase was extracted with ethyl acetate (2 × 30 mL) and the combined organic phases were then washed with brine (100 mL). The organic layer was then dried (MgSO₄), filtered, and concentrated under reduced pressure. The resulting light-brown oil was subjected to flash chromatography (silica gel, 10% to 95% v/v dichloromethane/ammonium-saturated methanol gradient elution) to afford the fractions A, A and B.

Concentration of fraction B (Rf = 0.5 to 0.5 in 95% v/v dichloromethane/ammonium-saturated methanol gradient elution) afforded compound 12 (389 mg, 40%) as a colorless, white powder, crystallized from water (91% yield). The melting point was not determined. The white powder was subjected to flash chromatography (silica gel, 10% to 95% v/v dichloromethane/ammonium-saturated methanol gradient elution) to afford two fractions, A and B.

Concentration of the fraction A (Rf = 0.1 to 0.4 in 95% v/v dichloromethane/ammonium-saturated methanol gradient elution) afforded compound 13 (109 mg, 9%) as a colorless, white powder, crystallized from water (51% yield). The melting point was not determined. The white powder was subjected to flash chromatography (silica gel, 10% to 95% v/v dichloromethane/ammonium-saturated methanol gradient elution) to afford two fractions, A and B.

Concentration of the fraction B (Rf = 0.5 to 0.5 in 95% v/v dichloromethane/ammonium-saturated methanol gradient elution) afforded compound 14 (14 mg, 99%) as a colorless, white powder. The melting point was not determined. The white powder was subjected to flash chromatography (silica gel, 10% to 95% v/v dichloromethane/ammonium-saturated methanol gradient elution) to afford two fractions, A and B.

Concentration of the fraction A (Rf = 0.0 to 0.5 in 95% v/v dichloromethane/ammonium-saturated methanol gradient elution) afforded compound 15 (10 mg, 99%) as a colorless, white powder. The melting point was not determined. The white powder was subjected to flash chromatography (silica gel, 10% to 95% v/v dichloromethane/ammonium-saturated methanol gradient elution) to afford two fractions, A and B.

Concentration of the fraction B (Rf = 0.5 to 0.5 in 95% v/v dichloromethane/ammonium-saturated methanol gradient elution) afforded compound 16 (10 mg, 99%) as a colorless, white powder. The melting point was not determined. The white powder was subjected to flash chromatography (silica gel, 10% to 95% v/v dichloromethane/ammonium-saturated methanol gradient elution) to afford two fractions, A and B.

Concentration of the fraction B (Rf = 0.5 to 0.5 in 95% v/v dichloromethane/ammonium-saturated methanol gradient elution) afforded compound 17 (10 mg, 99%) as a colorless, white powder. The melting point was not determined. The white powder was subjected to flash chromatography (silica gel, 10% to 95% v/v dichloromethane/ammonium-saturated methanol gradient elution) to afford two fractions, A and B.

Concentration of the fraction B (Rf = 0.5 to 0.5 in 95% v/v dichloromethane/ammonium-saturated methanol gradient elution) afforded compound 18 (10 mg, 99%) as a colorless, white powder. The melting point was not determined. The white powder was subjected to flash chromatography (silica gel, 10% to 95% v/v dichloromethane/ammonium-saturated methanol gradient elution) to afford two fractions, A and B.

Concentration of the fraction B (Rf = 0.5 to 0.5 in 95% v/v dichloromethane/ammonium-saturated methanol gradient elution) afforded compound 19 (10 mg, 99%) as a colorless, white powder. The melting point was not determined. The white powder was subjected to flash chromatography (silica gel, 10% to 95% v/v dichloromethane/ammonium-saturated methanol gradient elution) to afford two fractions, A and B.

Concentration of the fraction B (Rf = 0.5 to 0.5 in 95% v/v dichloromethane/ammonium-saturated methanol gradient elution) afforded compound 20 (10 mg, 99%) as a colorless, white powder. The melting point was not determined. The white powder was subjected to flash chromatography (silica gel, 10% to 95% v/v dichloromethane/ammonium-saturated methanol gradient elution) to afford two fractions, A and B.
(3 × 15 mm2). The combined organic phases were washed with brine (1 × 50 mL) and then dried (Na2SO4) and filtered before being concentrated under reduced pressure. The residue thus obtained was subjected to flash column chromatography (silica gel, 10:1) to afford a powder that was recrystallized (methanol/water) to give compounds 1-16 (mg 89%) as a white, crystalline solid, m.p. = 199-202°C. 1H NMR (400 MHz, CD3OD) δ 7.45 (s, 1H), 7.04 (d, J = 10 Hz, 1H), 6.08 (d, J = 10 Hz, 1H), 7.17 (d, J = 16.2 and 14 Hz, 1H), 6.71 (m, 1H), 4.14 (m, 2H), 3.71 (m, 1H), 2.25 (m, 1H), 2.90 (m, 1H), 2.58 (m, 2H), 2.44 (m, 1H), 2.23 (m, 1H) (signal due to O-H proton not observed). 13C NMR (100 MHz, CD3OD) δ 167.2, 154.1, 140.9, 143.3, 132.7, 121.6, 114.4, 108.5, 103.9, 82.6, 65.6, 46.5, 42.7, 62.8, 31.4. (ESI) m/z 427.2905, 2877, 2622, 1723, 1613, 1481, 1276, 1247, 1036 amu. MS (ESI) + /− m/z 434 ([M + H]+), 428 ([M + Na]+). HRMS (ESI) [M + H]+: Found (M + H)+ 316.1812; Calcd 316.1818.

Crystallographic Details: Crystallographic Data for Compound 8. C8H8NO2S, M = 145.16. T = 150 K, orthorhombic, space group P212121 (Z = 8, a = 43.8101 (4) Å, b = 27.198 (4) Å, c = 9.0073 (1) Å, V = 9530.111 (19) Å3, α = 90°, β = 90°, γ = 90°, D = 0.7815 g cm−3, 2717 unique reflections (2F0 − Fc) = 14.68%, R = 0.0313; for 3632 reflections with F > 2σ(F).

Crystallographic Details: Crystallographic Data for Compound 13. C13H17NO2S, M = 200.30. T = 150 K, monoclinic, space group P21/c (Z = 4, a = 62.409 (3) Å, b = 58.6140 (3) Å, c = 82.5600 (6) Å, β = 103.2704 (77)°, V = 5225.83 (3) Å3, Dcalcd = 1.316 g cm−3, 3319 unique reflections (2F0 − Fc) = 14.70%, R = 0.0516; for 4330 reflections with F > 2σ(F); R = 0.1297 (all data), S = 1.0.

Structure Determination. Images were collected on a CCD diffractometer (Cu Kα, monochromator, μ = 1.54184 A), and data were extracted using the CRYSTALS program package.59 Atomic coordinates, bond lengths and angles, and displacement parameters for compounds 8 and 13 have been deposited in the Cambridge Crystallographic Data Centre (CCDC nos. 1512145 and 1512146, respectively). These data can be obtained free-of-charge via www.ccdc.cam.ac.uk/data_request or by contacting the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK, fax +44 1223 336033.

Associated Content

Supporting Information

Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.6b00340.

Crystallographic Details for 8 (CIF)

Crystallographic Details for 13 (CIF)

Crystallographic and anisotropic displacement ellipsoid plots derived from the single-crystal X-ray analyses of compounds 8 and 13. Tabular comparison of the 1H NMR data reported for S-Demethylthiacromine with those on the synthetically derived compound (δ = 3.14 H and 4.03 13C NMR spectra of compounds 8-9, 12-16, and δ = 5.2 TDF)

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Notes

The authors declare no competing financial interest.

Acknowledgments

We thank the Australian Research Council for financial support. X.M. is the grateful recipient of a PhD Scholarship provided by the Guangzhou Elite Project of the Guangzhou Municipal Government, People’s Republic of China.

References

16. The following reagent/substrate combinations were used in attempts to effect this decomposition: (1) MeI, NaI, TFD; (2) Mel, NaI, TFD; (3) MeI, AgOAc, THF; (4) (MeO)3Si, SOCl2, THF. Either no reaction or decomposition of the substrate was observed in each instance.
20. Crystallographic Data for 8 (CIF) (PDF)

Crystallographic Data for 13 (CIF) (PDF)

Crystallographic Data for 8 (CIF) (PDF)

Crystallographic Data for 13 (CIF) (PDF)
SUPPORTING INFORMATION FOR:

A Total Synthesis of (±)-3-O-Demethylmacrone Through Rearrangement of a Precursor Embodying the Haemannidine Alkaloid Framework

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Research School of Chemistry, Institute of Advanced Studies, The Australian National University, Canberra, ACT 2601, Australia

CONTENTS

(i) Tabular Comparison of the $^{13}$C NMR Chemical Shifts Recorded for Compound (±)-2 with those Reported for 3-O-Demethylmacrone. S2

(ii) Anisotropic Displacement Ellipsoid Plots from the Single-crystal X-ray Analyses of Compounds 8 and 13. S3

(iii) $^1$H and $^{13}$C NMR Spectra of Compounds 5-9, 12-16 and (±)-2. S5
Table S1: Comparison of the $^{13}$C NMR Chemical Shifts Recorded for Compound (±)-2 with those Reported$^\text{a}$ for the Natural Product 3-O-Demethylmacronine

<table>
<thead>
<tr>
<th>$^{13}$C NMR Data for Compound (±)-2 ($\delta_c$)$^\text{a}$</th>
<th>$^{13}$C NMR Data for 3-O-Demethylmacronine ($\delta_c$)$^\text{b}$</th>
<th>$\Delta\delta$</th>
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<td>154.4</td>
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<td>104.0</td>
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</tr>
<tr>
<td>82.0</td>
<td>82.2</td>
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</tr>
<tr>
<td>65.6</td>
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<tr>
<td>31.1</td>
<td>31.3</td>
<td>−0.2</td>
</tr>
</tbody>
</table>

$^a$ Spectrum recorded in CD$_3$OD at 100 MHz;

$^b$ Data obtained from Hesse; spectrum recorded in CD$_3$OD at 150 MHz

Figure S1: Structure of compound 8 (CCDC 1531843) with labeling of selected atoms. Anisotropic displacement ellipsoids show 30% probability levels. Hydrogen atoms are drawn as circles with small radii.
Figure S2: Structure of compound 13 (CCDC 1531844) with labeling of selected atoms. Anisotropic displacement ellipsoids show 30% probability levels. Hydrogen atoms are drawn as circles with small radii.
400 MHz $^1$H NMR Spectrum of Compound S
(recorded in CDCl$_3$)
400 MHz $^1$H NMR Spectrum of Compound 6 (recorded in CDCl$_3$)
100 MHz $^{13}$C NMR Spectrum of Compound 9
(recorded in CDCl$_3$)
400 MHz $^1$H NMR Spectrum of Compound 9
(recorded in CDCl$_3$)

CH$_3$
100 MHz $^{13}$C NMR Spectrum of Compound 12
(recorded in CDCl$_3$)
400 MHz $^1$H NMR Spectrum of Compound 15
(recorded in CDCl$_3$)

- CHCl$_3$
- dichloromethane
- grease
100 MHz $^{13}$C NMR Spectrum of Compound 15
(recorded in CDCl$_3$)
100 MHz $^{13}$C NMR Spectrum of Compound (±)-2
(recorded in CD$_3$OD)
Publication Four

Total Syntheses of the Amaryllidaceae Alkaloids
Zephycandidine III and Lycosinine A and Their Evaluation as
Inhibitors of Acetylcholinesterase

Xingjun Xu, Hye-Sun Kim, Wei-Min Chen, Xiang Ma, Galen J.
Correy, Martin G. Banwell, Colin J. Jackson, Anthony C. Willis, and
Paul D. Carr

Alkaloid Synthesis

Total Syntheses of the Amaryllidaceae Alkaloids
Zephyrcandidine III and Lycosinine A and Their Evaluation as
Inhibitors of Acetylcholinesterase

Xingjun Xu, a,b Hye-Sun Kim, b Wei-Min Chen, b] Xiang Ma, b] Galen J. Correy, b]
Martin G. Banwell, a,b Colin J. Jackson, b] Anthony C. Willis, b] and Paul D. Carr b]

Abstract: The title alkaloids, 1 and 2, have been prepared using cross-coupling chemistry and together with various analogues they have been evaluated for their capacity to inhibit acetylcholinesterase. Contrary to an earlier report, it was found that biaryl 1 is not a significant inhibitor of this enzyme, and neither are any of its congeners, including alkaloid 2.

Introduction

Very recently Yao and co-workers reported the isolation of three new Amaryllidaceae alkaloids through extraction of the dried whole plant Zephyranthes candida collected at Shiyuan, Huangpi, in China. Their structures were established by conventional spectroscopic methods and each was evaluated as a possible inhibitor of acetylcholinesterase (AChE). The most active of these (with an IC50 of 8.82 μM) was reported to be the novel biaryl zephyrcandidine III (1), (Figure 1). This compound bears a strong structural resemblance to the Amaryllidaceae alkaloid lycosinine A (2) that was isolated by Zhao and co-workers from the ornamental plant Lycoris aurea collected in Kunming, Yunnan Province, China. The corresponding aldehyde, viz. lycosinine B (3), was also obtained from the same source. Lycosinine B (3) has since been isolated from the bulbs of Lycoris sprengeri collected from Taizhou City, Zhejiang Province, China and from the bulbs of Hippeastrum bivellatum Herb. Amaryllidaceae (flowering sago) collected in São Francisco de Paula in the Brazilian state of Rio Grande do Sul. No biological activities have been ascribed to the lycosinines thus far, but given the structural resemblance of compound 2 to zephyrcandidine III, it might also be expected to display AChE-inhibiting activities.

To date there have been no reports on the synthesis of zephyrcandidine III (1) and just one dealing with the preparation of the lycosinines. Specifically, Hsieh and co-workers reported the preparation of compounds 2 and 3 (using as the key step, the Suzuki-Miyaura cross-coupling of a boronate derivative of 3,4-dimethoxybenzaldehyde with 3-bromo-1-pentanol). It is against this background that we now report our own studies in the area that have culminated in the syntheses of the title alkaloids (viz. 1 and 2) and their evaluation as inhibitors of AChE derived from Electrophorus electricus.

Results and Discussion

1. Total Synthesis of Zephyrcandidine III (1)

Our initial approach to the biaryl core of zephyrcandidine III (1) is shown in Scheme 1 to exploit Suzuki-Miyaura cross-coupling chemistry. The known nitroarene 4 was reduced to the corresponding and previously reported aldehyde 5 (57%) using iron filings in mildly acidic aqueous ethanol, and the latter compound was subjected to electrophilic aromatic iodonation with molecular iodine. Product 6 (75%) was then converted into the corresponding p-toluenesulfonamide 7 (92%) under standard conditions. Disappointingly, and despite extensive experimentation involving a range of reaction conditions, attempts to engage amine 6 in a Suzuki-Miyaura cross-coupling reaction with the known and readily available arylobromate 8 failed to deliver the biaryl 9. Related attempts to cross-couple sulfonamide 7 with ester 8 and thereby generate compound 10 were equally unsuccessful.

In a second approach (Scheme 2) to the biaryl core of target 1 that sought to exploit the often beneficial effects of electron-
Scheme 1. Initial attempts to assemble the biaryl core of zephyrandimidine III (11) using Suzuki-Miyaura cross-coupling protocols. Reagents and conditions: (a) (i) Fe, HClO₄, ethanol/petrol, reflux, 3 h, 67 % (ii) NaNO₃, DCI, DCM, 22 °C, 18 h, 73 % (iii) PtCl₂, pyridine, 22 °C, 1 h, 92 %.

withdrawing groups in Suzuki-Miyaura cross-coupling reactions, attempts were made to link boronate ester 8 and 3-bromo-4-nitrobenzaldehyde (11) (96) under a range of seemingly relevant reaction conditions. Unfortunately, none of these led to the formation of the hoped-for biaryl 12.

The palladium-catalyzed Ullmann cross-coupling reaction has proven to be a useful but under-utilized method for cross-coupling various aryl halides with other species, most notably n-iodoanilines (111). Given our familiarity with this process we sought to apply it in the present context. To such ends (Scheme 3) the commercially available alcohol 13 was treated with a combination of molecular iodine and silver trifluoroacetate and thereby affording compound 14 (83 %) that was oxidized to the corresponding aldehyde 15 (95 %) using Attenborrow manganese dioxide (112) under selenium-catalyzed Perkin oxidation of compound 15 to the corresponding acid and esterification of this using methyl iodide in the presence of potassium carbonate to give ester 16 (97) in 85 % overall yield. Gratifyingly, when a DMSO solution of this last compound was treated with bromoanarene 11 in the presence of copper metal as well as small amounts of CuI and Ph₃P(dppf) at 90 °C for 5 h, biaryl 17 was obtained in 60 % yield. Treatment of compound 17 with sodium borohydride in methanol gave the benzyl alcohol 18, and O-methylation of this using methyl iodide in DMSO containing potassium hydroxide then afforded methyl ether 19 (63 %). The structure of compound 18 was confirmed by single-crystal X-ray analysis (see the Exp. Sect. and Supporting Information for details). Unfortunately, all attempts to effect the reduction of the nitro- and ester-groups associated with this last compound, and thereby generate zephyrandimidine III (11) directly, were unsuccessful. In every instance complex product mixtures were obtained.

Scheme 2. An alternate attempt to effect a relevant Suzuki-Miyaura cross-coupling reaction.

Scheme 3. Successful synthesis of the biaryl core of target 1. Reagents and conditions: (a) (i) Ag(ococ)₂, MeOH, −5 °C; 8 h, 95 % (ii) Ph₃P(dppf), DCM, 22 °C, 18 h, 95 % (c) Perkin oxidation then Me₃SiCl, acetone, 22 °C, 18 h, 90 % (d) CuI, CuBr, CuCl, PPh₃, dppf, DMSO, 50 °C, 5 h, 60 % (e) NaAlH₄, MeOH, 0 to 22 °C, 0.5 h; (f) Me₂I, KOH, DMSO, 22 °C, 2 h, 43 % (from 17)
The ultimately successful route to zephrandidine III (1) is displayed in Scheme 4 and exploits various chemistries defined in Scheme 3. Thus, the reaction sequence started with the conversion of aldehyde 11, through a reduction/C-methylation sequence, into the methyl ether 20 (85%). Like its precursor 11, compound 20 could be engaged in a palladium-catalyzed Ullmann cross-coupling, this time with aldehyde 15 and thereby affording bilirubin 21, the structure of which was confirmed by single-crystal X-ray analysis. The aldehyde residue within compound 21 was reduced with sodium borohydride and the nitro-group associated with product 22 (90%) subjected to hydrogenation using chymohydrazine in the presence of 10% palladium on carbon. By such means, aniline 23 was obtained in 95% yield. Reductive monomethylation of compound 23 using one molar equivalent of formaldehyde in the presence of sodium cyanoborohydride then gave compound 1 as a clear, colorless oil in 95% yield. All of the spectroscopic data acquired on this product were in complete accordance with the assigned structure. Furthermore, relevant comparisons with the analogous data reported by Yao and co-workers for zephrandidine I revealed a good match (tabular comparisons of the 13C NMR spectroscopic data sets are provided in the Supporting Information) and thus leaving no doubt about the structure of the natural product.

The twofold reduction/N-methylation of aniline 23 was readily effected (Scheme 5) using 9 molar equivalents of formaldehyde in the presence of sodium cyanoborohydride, and compound 24 was thereby obtained in 95% yield. This product is the N-methyl derivative of zephrandidine III (1) and arguably, therefore, more closely resembles lysocin A (2).

Scheme 5. Reductive methylation of aniline 23 leading to the N-methyl derivative 24 of zephrandidine III (1). Reagents and conditions (a) HCHO, MeOH, 0 to 22°C, 15 h, 95%.

2. Total Synthesis of Lysocin A (2) and an Examination of Its Behavior under Oxidative Conditions

A two-step synthesis of lysocin A (2) from known materials is shown in Scheme 6. This simply involved Suzuki–Miyaura cross-coupling of the known C7-bocylated indole 25 (prepared in a

Scheme 4. Successful total synthesis of zephrandidine III (1). Reagents and conditions: (a) NaBH₄, MeOH, 0 to 22°C, 8.5 h; then Mel, HCl, DMSO, 22°C, 2 h, 85%; (b) OsO₄, H₂O, Ph₃P, H₂O, Ph₃P, DMSO, 50°C, 3 h, 70%; (c) NaBH₄, MeOH, 0 to 22°C, 8.5 h, 90%; (d) HCHO, 10% w/v in EtOH, MeOH, 22°C, 15 h, 95%; (e) HClO₂ (1.0 equiv), NaBH₄, MeOH, 22°C, 16 h, 95%.

Scheme 6. Two-step synthesis of lysocin A (2). Reagents and conditions: (a) Pd(dppf)Cl₂, Et₃N, THF/water, 85°C, 19 h, 91%; (b) (CH₃)₂CO, NaBH₄, MeOH, 0 to 22°C, 24 h, quant.
one-pot process and 80% yield from indole itself using a procedure
described by Hartwig(10) with the readily available aryl iodide 20(11)
under relatively standard conditions, affording the 7-
arylindole 27 (91%), the structure of which was confirmed by
single-crystal X-ray analysis. Reductive N-methylation of the last
compound using parformaldehyde in the presence of sodium
cyanoborohydride was accompanied by conversion of the
indole residue into the corresponding indoline and so affording
lyscine A (2) directly and in quantitative yield. All of the
spectroscopic data acquired on this compound were in accord
with the assigned structure and matched those reported(12)
for the natural product (tabular comparisons of the 1H NMR spec-
troscopic data sets are provided in the Supporting Information).
In addition, a single-crystal X-ray analysis of the synthetically
derived material was carried out and served to confirm the illus-
trated structure.

With compound 2 in hand, various efforts were made to con-
vert it into lycosine B (3). However, despite numerous efforts
this conversion failed due to the oxidative sensitivity of the
indoline unit associated with the substrate. For example, on
treating lycosine A (2) (Scheme 7) with Attenborough man-
aganese dioxide(13) in dichlormethane indole 28 (76%) was ob-
tained. On the other hand, when the same substrate (viz. 2)
was treated with pyridinium chlorochromate (PCC) in the pres-
ence of sodium acetate then indole 28 (5%) was again ob-
tained but now the twofold oxidation product 29 (15%) was
the predominant one. It is interesting to note that in Hishi’s
synthesis of lycosine B (3)(14) the associated aldehyde residue
was installed directly through a cross-coupling reaction involv-
ing a C3-borylated benzaldehyde and this natural product was
then reduced to lycosine A (2).

Scheme 7. Outcomes of the oxidation of lycosine A (2). Reagents and condi-
tions: a) MnO2, CH2Cl2, 22 °C, 72 h; b) PCC, NaAc, DCM, 22 °C, 17 h.

3. Evaluation of Compounds 1, 2, 17–19, 21–24, and 27 as
Inhibitors of AChE

Alkaloids 1 and 2, as well as congeners 17–19, 21–24, and 27
were each evaluated for their ability to inhibit AChE derived
from eleutherospermum electricus(15). A summary of the inhibi-
tion data thus obtained is shown in Table 1.

Table 1. Inhibition of AChE by compounds 1, 2, 17–19, 21–24, and 27.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
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<tr>
<td>2</td>
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<td>&gt; 500</td>
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<tr>
<td>3</td>
<td>17</td>
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<td>24</td>
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</tr>
<tr>
<td>10</td>
<td>27</td>
<td>&gt; 500</td>
</tr>
<tr>
<td>11</td>
<td>Galanthamine (positive control)</td>
<td>1.1 (0.9–1.2)</td>
</tr>
</tbody>
</table>

(a) Values in brackets represent the 95% confidence interval in the IC50. The
IC50 was calculated from a dose-response curve with three repeat measure-
ments of enzyme activity at each concentration. (b) A lower limit is given for
compounds where the IC50 was greater than the maximum possible solution
concentration of the compound.

Compounds 17 and 24 (viz. the N-methyl derivative of
zebranoidine III) were the most effective inhibitors exhibiting
IC50 values of 1.20 and 1.40 μM, respectively; however, this is two
orders of magnitude weaker than that of the alkaloid galanth-
amine, an established AChE inhibitor used to treat Alzheimer’s
disease. The assay results are also at odds with those of Yao
and co-workers, who have suggested(16) that zebranoidine III
(compound 1) is a notable inhibitor of the enzyme. When as-
sayed against AChE, compound 1 reduced activity by only 25%
at the maximum concentration tested (500 μM). The origins of
the discrepancies between the work of Yao and co-workers
and our own reported herein are unclear but could be because
the natural product was contaminated with a potent but as yet
unidentified inhibitor of AChE.

Conclusions

The studies reported herein serve to confirm the structures
assigned to the title natural products but cast serious doubt on
the merits of trying to develop related polyfunctionalized bi-
aryls as new, effective inhibitors of AChE.

Experimental Section

General Experimental Procedures: Unless otherwise specified,
proton (1H) and carbon (13C) NMR spectra were recorded at room
temperature in base-filtered CDC13 with a Varian spectrometer oper-
ating at 400 MHz for proton and 100 MHz for carbon nuclei. The
signal due to residual CHCl3 appearing at δC = 7.26 ppm and the
central resonance of the CDCl3 triplet appearing at δH = 77.6 ppm
were used to reference 1H and 13C NMR spectra, respectively.
1H NMR spectroscopic data are recorded as follows: chemical shift (δ)
(multiplicity, coupling constants (J) / Hz), relative integral) where
multiplicity is defined as s = singlet, d = doublet, t = triplet; q =
quartet; m = multiplet, or combinations of the above. Infrared spec-
tra were recorded with a Perkin-Elmer 1800 Series FTIR Spectrom-
tator. Samples were analyzed as thin films on KBr plates. Low-
resolution (EI) mass spectra were recorded with a single-quadrupole liquid
chromatograph mass spectrometer, while high-resolution measure-
ments were conducted with a time-of-flight instrument. Low- and
high-resolution EI mass spectra were recorded with a magnetic-
sector machine. Melting points were measured with an Optimel automated melting point system and are uncorrected. Analytical thin layer chromatography (TLC) was performed on aluminum-backed 0.2 mm thick silica gel 60 F254 plates as supplied by Merck. Eluted plates were visualized using a 254 nm UV lamp and/or by treatment with a suitable dip followed by heating. These dips included phosphomolybdic acid in acetic anhydride conc/water (57:43) or potassium permanganate/potassium carbonate to precipitate the sodium hydrogen sulfide aqueous solution/water (3:2:5:5:1 mL:300 mL). Flash chromatographic separations were carried out following protocols defined by Still et al. with silica gel 60 (40-63 μm) as the stationary phase and using the AE- or HFL-grade solvents indicated. The melting points of solids purified by such means were recorded directly (i.e., after they had crystallized) or dried in a vacuum oven (r.t.) and used as supplied. Dehydrating agents and other inorganic salts were purchased from the AIAX, BOM, or Unisl Chemical Companies. Tetrahydrofuran (THF), methanol, and dichloromethane were dried using a Glass Contour solvent purification system that is based upon a technology originally described by Grubbs et al. Where necessary, reactions were performed under a nitrogen atmosphere.

Specific Chemical Transformations

4-Methoxyaniline (5): A magnetically stirred solution of compound 5 (66.9 mg, 0.30 mmol) in MeOH (20 mL) maintained at 45 °C was treated with lithium acetylide (7.15 mg, 110 μmol) in dry THF (3 mL) and the mixture was stirred for 18 h. The reaction mixture was filtered through a basic column using CHCl3 (25 mL). The combined organic layers were washed with brine (1 x 25 mL) and dried (Na2SO4) filtered and concentrated under reduced pressure. The residue was purified by flash chromatography to give compound 6 (53.5 mg, 90% yield) as a clear brown oil.

1H NMR (400 MHz, CDCl3, δ = 7.62 (d, J = 8.0 Hz, 1H), 7.51 (d, J = 8.1 Hz, 1H), 7.22 (d, J = 8.1 Hz, 1H), 4.43 (q, J = 2.2 Hz, 2H), 4.30 (2H, 3H), 3.34 (2H, 3H). 1H NMR (100 MHz, CDCl3): δ = 146.3, 136.8, 132.5, 131.5, 127.8, 57.8 ppm. 13C NMR (100 MHz, CDCl3): δ = 216.1, 153.1, 134.3, 129.2, 127.8, 126.0, 115.8, 54.0 ppm. IR (KBr): 3299, 1596, 1480, 1386, 1335, 1164, 1091, 1037, 816, 846, 545 cm−1. MS (ESI: m/z): [M+H] + 340 (100) [M+Na]+. HRMS: calculated for C12H13NO2 (M+H) + 340.0982, found 340.0976.

N-(2-bromo-4-methylphenyl)phthalimido-4-fluoronitrobenzenesulfonyl amide (7): A solution of compound 6 (522 mg, 2.1 mmol) and p-toluenesulfonyl chloride (477 mg, 2.5 mmol) in pyridine (10 mL) was stirred at 22 °C for 4 h. Water (30 mL) was then added, and the resulting aqueous phase was washed with ethyl acetate (3 x 30 mL). The combined organic fractions were washed with CHCl3 (1 x 50 mL of a 10% v/v aqueous solution) and water (1 x 50 mL) before being dried (Na2SO4) filtered, and concentrated under reduced pressure. The resulting light yellow oil was subjected to flash chromatography (silica gel; 5 v/v 40:60 petroleum spirit/ethyl acetate elution). Concentration of the relevant fractions afforded compound 7 (606 mg, 92%) as a white, crystalline solid. mp: 121.5-123 °C. 1H NMR (400 MHz, DMSO): δ = 8.95 (s, 1H), 7.25 (d, J = 8.1 Hz, 1H), 7.59 (d, J = 8.1 Hz, 2H), 7.36 (d, J = 8.1 Hz, 2H), 3.35 (2H, 3H). 13C NMR (100 MHz, DMSO): δ = 145.1, 138.7, 138.3, 137.8, 137.2, 127.6, 127.8, 98.6, 71.9, 73.7, 71.2, 21.0 (one signal obscured or overlapping) ppm. HRMS: calculated for C12H12NO6SNa (M+Na)+ 343.0976, found 343.0979.

3-Bromo-4-nitrotoluene (10): Compound 11 was prepared according to the method of Klotz[10] and obtained as crystalline yellow color, mp: 99-101 °C (ref.10 m.p. 101-102 °C). 1H NMR (400 MHz, CDCl3): δ = 10.56 (s, 1H), 8.23 (d, J = 6.1 Hz, 1H, 7.97 (dd, J = 8.2 and 1.6 Hz, 1H), 7.93 (d, J = 8.2 Hz, 1H) ppm. 13C NMR (100 MHz, CDCl3): δ = 168.9, 153.2, 136.0, 138.2, 132.1, 126.1, 115.6 ppm. IR (KBr): 3299, 1094, 1054, 1239, 1337, 1369, 1308, 1191, 1037, 800, 831, 748, 699, 662 cm−1. MS (EI, 70 eV): m/z (%) = 231 and 229 (38 and 60) ([M]), 75, 100, HRMS: calculated for C7H4BrNO4 (M+H) + 229.0375, found 229.0376.

2-bromo-4-(3,5-difluorophenyl)toluene-1-carboxaldehyde (14): A flame-dried 200 mL round-bottom flask was covered with aluminum foil and then charged with pyridine alcohol (50 g, 49.7 mmol) Ag2CO3 (4.80 g, 26.2 mmol) a magnetic stirring bar, and CH2Cl2 (20 mL). The ensuing mixture was cooled to −5 °C (ice/salt bath) then treated, dropwise over ca. 0.5 h, with a solution of molecular iodine (1.50 g, 18.0 mmol) in CH2Cl2 (25 mL). Additional quantities of solid iodide were added to the reaction mixture until TLC analysis revealed that all of the starting alcohol had been consumed. The reaction mixture was then filtered through a pad of tightly packed diatomaceous earth and the solids thus retained rinsed with CH2Cl2 (150 mL). The combined organic layers were washed with brine (1 x 25 mL) and dried (Na2SO4) filtered and concentrated under reduced pressure. The resulting oil was subjected to flash chromatography (silica gel; 5 v/v 40:60 petroleum spirit/ethyl acetate elution) and concentration of the relevant fractions (δ = 6.2-6.4 ppm, 1H, δ = 7.4-7.6 ppm, 2H) afforded compound 14 (23 g) as a white, crystalline solid. mp: 109-111 °C (ref.[11] m.p. 109.4-109.6 °C). 1H NMR (400 MHz, CDCl3): δ = 7.24 (s, 1H), 6.59 (1H, 5.98 (d, J = 2.8 Hz, 2H), 4.59 (s, 2H), 1.88 (s, 1H) ppm. 13C NMR (100 MHz, CDCl3): δ = 146.8, 148.1, 134.6, 118.7, 192.8, 101.3, 85.5, 69.4 ppm.

15.) 2-bromo-4-(3,5-difluorophenyl)toluene-1-carboxaldehyde (15): A magnetically stirred solution of compound 14 (4.94 g, 14.4 mmol) in CH2Cl2 (75 mL) was warmed in one portion with K2CO3 (5.60 g, 413.9 mmol) and the resulting dark suspension was stirred at 22 °C for 18 h then filtered through a pad of diatomaceous earth. The solids thus retained were washed with CH2Cl2 (2 x 40 mL) and the combined filtrates were concentrated under reduced pressure. The resulting brown oil was subjected to flash chromatography (silica gel; 5 v/v 40:60 petroleum spirit/ethyl acetate elution) and concentration
of the relevant fractions (R = 0.4 in 4:1 190 40–60 petroleum spirit/ethyl acetate) then give aldehydes 18 19 20 (3.65 g, 32% yield) as a white, crystalline solid, m.p. 112–113 °C (lit. 19 21 m.p. 112–113 °C). 1H NMR (200 MHz, CDCl3) δ = 7.71 (s, 1 H), 7.37 (s, 1 H), 7.05–7.08 (m, 7 H), 6.88 (s, 2 H) ppm. 13C NMR (100 MHz, CDCl3) δ = 146.4, 137.3, 149.3, 129.0, 119.5, 109.3, 102.2, 98.9 ppm. IR: νmax = 1664, 1618, 1505, 1253, 1113, 1035, 725 cm⁻¹. MS (EI, re: m/z (%) = 344 [100] (M⁺ + Na⁺), 352 [5] (M⁺ + H⁺), 310 [< 1]). HRMS: calc. for C₃₀H₂₇NO₃ [M⁺ + Na⁺] 532.0433; found 532.0437.

Methyl 6-(5-Hydroxymethyl)-2-nitrophenyl[benzimidazol-1(3H)]-1,3-disoxo-5-carboxylate (18): A suspension of NaN₃ (21 mg, 0.61 mmol) in methanol (30 mL) was added to a magnetically stirred solution of compound 17 (200 mg, 0.61 mmol) in methanol (5 mL) maintained at 0 °C. The ensuing mixture was stirred for 22 h then stirred at this temperature for 0.5 h before being quenched with NH₄Cl (5 mL of a 15% v/v aqueous solution). The ensuing mixture was stirred until it became homogenised and then it was concentrated under reduced pressure. The residue was extracted with CH₂Cl₂ (3 × 10 mL) and the combined organic phases were washed with NaHCO₃ (2 × 10 mL of a saturated aqueous solution) then H₂O (2 × 10 mL) before being dried (Na₂SO₄) filtered, and concentrated under reduced pressure to give a yellow light oil. Subjecting this material to flash chromatography (silica gel, SiO₂ → 1:1 v/v 45–60 petroleum ether/ethyl acetate elution) gave, after concentration of the appropriate fractions (R = 0.25 in 2:1 v/v petroleum ether/ethyl acetate) a yellow solid. Recrystallisation (CH₂Cl₂/Hexane) of this solid then gave compound 18 (182 mg, 50%) as yellow crystals, m.p. 114.5–116.2 °C. δ = 7.98 (s, 1 H), 7.64 (d, J = 8.4 Hz, 1 H), 5.71 (s, 1 H), 5.67 (d, J = 8.4 and 1.8 Hz, 1 H), 4.72 (s, 2 H), 3.62 (s, 3 H) ppm. δ = 131.0, 129.3, 125.9, 120.7, 113.4, 113.9, 110.4, 103.2, 86.1, 52.5 ppm. IR: νmax = 2971, 2961, 2927, 1764, 1115, 1520, 1505, 1343, 1246, 1132, 1035, 938, 819 cm⁻¹. MS (ESI, re: m/z (%) = 356 (169) (M⁺ + Na⁺); HRMS: calc. for C₂₉H₂₂N₂O₄ [M⁺ + Na⁺] 544.0293; found 544.0291.

Methyl 6-(5-Methyl-2-nitrophenyl)[benzimidazol-1(3H)]-1,3-disoxo-5-carboxylate (19): A solution of compound 18 (160 mg, 0.3 mmol) and methyl isodide (75 µL, 1.2 mmol) in DMF (2 mL) was added in portions over 1.5 h, to a magnetically stirred suspension of potassium hydroxide (58 mg, 1.2 mmol) in DMF (3 mL) maintained at 22 °C under an atmosphere of nitrogen. The ensuing mixture was stirred for a further 1.0 h then poured into water (25 mL) and extracted with CH₂Cl₂ (3 × 26 mL). The combined organic phases were washed with water (1 × 50 mL) and brine (1 × 50 mL) before being dried (Na₂SO₄) filtered, and then concentrated under reduced pressure. The oily residue thus obtained was subjected to flash chromatography (silica gel, 1:1 v/v 45–60 petroleum spirit/ethyl acetate elution), and concentration of the relevant fractions (R = 0.4 in 4:1 v/v hexane/ethyl acetate) afforded compound 19 (80 mg, 77%) as a yellow, crystalline solid, m.p. 150.7–153.4 °C. δ = 8.07 (d, J = 8.4 Hz, 1 H), 7.95 (s, 1 H), 7.71 (s, 1 H), 7.13 (d, J = 1.8 Hz, 1 H), 6.68 (s, 1 H), 6.09 (s, 1 H), 6.71 (d, J = 4.5 Hz, 1 H), 4.53 (s, 2 H), 3.61 (s, 3 H), 3.43 (s, 3 H) ppm. 1H NMR (400 MHz, CDCl₃) δ = 166.0, 150.8, 143.0, 134.7, 134.5, 137.5, 131.6, 129.3, 126.3, 134.3, 124.9, 124.9, 119.3, 106.7, 842, 766 cm⁻¹. MS (ESI, re: m/z (%) = 368 (100) (M⁺ + Na⁺); HRMS: calc. for C₂₉H₂₂N₂O₄ [M⁺ + Na⁺] 560.0749; found 560.0741. 2-Bromo-4-methoxyphenyl-1-nitrobenzene (20): Step A: A suspension of NaN₃ (165 mg, 4.36 mmol) in methanol (15 mL) was added in portions over 0.17 h to a magnetically stirred solution of compound 11 (1.50 g, 6.50 mmol) in methanol (30 mL) maintained at 22 °C. The ensuing mixture was stirred for a further
0.5 h then quenched with NaOH (40 mL of a 10 % w/v aqueous solution). Stirring was continued until a clear solution was obtained. This was then concentrated under reduced pressure, and the aqueous residue was extracted with CH₂Cl₂ (3 × 50 mL). The combined organic phases were washed with NaHCO₃ (2 × 50 mL of saturated aqueous solution) and water (2 × 34 mL) before being dried (Na₂SO₄), filtered, and concentrated under reduced pressure to give a yellow solid presumed to be 3-[(bromo-4-nitrophenyl)methanol]. This material was used without purification in step 2 as detailed immediately below.

Step 2: A solution of the product from step 1 and methyl iodide (1.60 mL, 26 mmol) in DMSO (10 mL) was added over 1.5 h to a magnetically stirred suspension of potassium hydroxide (1.44 g, 26 mmol) in DMSO maintained at 22 °C. The resulting mixture was stirred for 1 h then poured into water (150 mL) and extracted with CH₂Cl₂ (3 × 100 mL). The combined organic phases were washed with water (1 × 150 mL) and brine (1 × 50 mL) before being dried (Na₂SO₄), filtered, and concentrated under reduced pressure. The residue thus obtained was subjected to flash chromatography (silica gel, 4:1 v/v 40:60 petroleum spirit/ethyle acetate elution), and concentration of the relevant fractions (Rf = 0.1 in 4:1 v/v 40:60 petroleum spirit/ethyle acetate eluent) afforded compound 1 (1.5 g, 72 %) as a clear, light-brown oil. ’H NMR (400 MHz, CDCl₃): δ = 7.84 (d, J = 8.3 Hz, 4 H), 7.73 (s, J = 0.8 Hz, 1 H), 7.40 (t, J = 8.3 and 0.8 Hz, 1 H), 4.56 (s, 2 H), 3.84 (s, 3 H) ppm. 13C NMR (100 MHz, CDCl₃): δ = 144.6, 133.2, 126.9, 125.1, 114.7, 100.2, 72.6, 58.7 ppm. IR (v, cm⁻¹): 3492, 2932, 2823, 1584, 1528, 1490, 1345, 1179, 1109, 1039, 825, 747 cm⁻¹. MS (ESI+): m/z: [M+Na]⁺ = 270, 268 (both 10 %). HRMS: calc. for C₂₇H₂₆Na₂O₇S (M + Na)⁺: M + Na⁺ = 477.0585; found 477.0581.

6-[3-Methoxyphenyl]-2-ethynylphenyl[benzimidazol-2(1H),3-dioxole-5-carboxamide (3a): A magnetic stirrer solution of compound 2 (125 mg, 0.40 mmol) in methanol (10 mL) containing 10 % Pd on carbon (20 mg) was placed under a hydrogen atmosphere at 22 °C for 12 h and then filtered through a short pad of diatomaceous earth that was washed with ethyl acetate (20 mL). The combined filtrates were concentrated under reduced pressure to afford a light-yellow oil, and this was subjected to flash chromatography (silica gel, 9:1 v/v CH₂Cl₂/Et₂O/diethyl ether) elution. Concentration of the appropriate fractions (Rf = 0.3 in 1:1 v/v CH₂Cl₂/Et₂O/diethyl ether) then provided the title compound 3a (102 mg, 73 %) as a clear, colorless oil. ’H NMR (400 MHz, CDCl₃): δ = 7.17 (d, J = 8.1 and 2.0 Hz, 2 H), 7.01 (d, J = 2.0 Hz, 1 H), 7.01 (s, 1 H), 6.83 (d, J = 8.1 Hz, 1 H), 6.69 (s, 1 H), 5.99 (ABq, J = 14.6 Hz, 2 H), 4.18 (s, 2 H), 3.30 (s, 3 H), 3.16 (s, 2 H) ppm. 13C NMR (100 MHz, CDCl₃): δ = 142.7, 147.6, 145.7, 142.3, 133.2, 131.0, 130.5, 127.8, 128.4, 116.7, 115.2, 110.9, 101.6, 74.6, 62.8, 58.1 ppm. IR (v, cm⁻¹): 3430, 2930, 2899, 1627, 1502, 1482, 1365, 1224, 1086, 1038, 981, 872, 766 cm⁻¹. MS (ESI+): m/z: [M+Na]⁺ = 310 (100 %). HRMS: calc. for C₂₉H₂₇NO₂S (M + Na)⁺: M + Na⁺ = 466.0938; found 466.0935.

6-[3-Methylthio-2-ethyl-2-methylamino]phenyl[benzimidazol-2(1H),3-dioxo-5-methyl]ethanol (3b): A magnetically stirred solution of compound 2 (30 mg, 0.11 mmol) in methanol (10 mL) maintained at 0 °C was treated with formic acid (1 mL of a 37 % aqueous solution, 0.11 mmol). The ensuing mixture was stirred for 1 h at 0 °C then treated with NaNat-CN (7 mg, 0.11 mmol) before being warmed to 22 °C and stirred at this temperature for 6 h; it was then concentrated under reduced pressure to afford a light-yellow oil. This was partitioned between ethyl acetate (10 mL) and NaOH (12 mL of a 10 % aqueous solution), and the separated aqueous phase was extracted with ethyl acetate (1 × 10 mL). The combined organic extracts were washed with brine (1 × 20 mL) then dried (Na₂SO₄), filtered, and concentrated under reduced pressure. The light-yellow oil thus obtained was subjected to flash chromatography (silica gel, 1:1 v/v CH₂Cl₂/Et₂O/diethyl ether, and concentration of the relevant fractions (Rf = 0.3 in 9:1 v/v CH₂Cl₂/Et₂O/diethyl ether) afforded compound 2 (150 mg, 46 %) in methanol (2 mL) maintained at 0 °C. The ensuing mixture was warmed to 22 °C, stirred at this temperature for 0.5 h then quenched with a sodium hydroxide (5 mL of a 10 % w/v aqueous solution). Stirring was continued until a clear solution was obtained, and this was then concentrated under reduced pressure. The resulting aqueous residue was subjected to flash chromatography (silica gel, 1:1 v/v CH₂Cl₂/Et₂O/diethyl ether, 3:2:1 v/v 40:60 petroleum spirit/ethyl acetate elution) to afford a mixture of the appropriate fractions (Rf = 0.4 in 2:1 v/v 40:60 petroleum spirit/ethyl acetate, compound 2 (137 mg, 90 %) as a light-yellow oil. ’H NMR (400 MHz, CDCl₃): δ = 7.05 (d, J = 8.4 Hz, 1 H), 7.47 (d, J = 8.4 and 1.8 Hz, 1 H), 7.31 (d, J = 1.8 Hz, 1 H), 7.01 (s, 1 H), 6.58 (s, 1 H), 6.01 (ABq, J = 1.4 Hz, 2 H), 4.53 (s, 2 H), 4.34 (s, 3 H) ppm due to the hydroxyl group proton not observed. 13C NMR (100 MHz, CDCl₃): δ = 148.6, 148.1, 144.2, 138.1, 132.3, 131.8, 130.3, 127.2, 124.5, 101.6, 76.3, 63.1, 58.9 ppm (signal due to one carbon obscured or overlapping). IR (v, cm⁻¹): 3420, 2992, 2523, 1502, 1476, 1347, 1217, 1105, 1038, 931, 841 cm⁻¹. MS (ESI+): m/z: [M+Na]⁺ = 340 (100 %). HRMS: calc. for C₂₈H₂₃N₇O₂S (M + Na)⁺: M + Na⁺ = 514.0979; found 514.0876.
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(sila, diethyl ether elution) followed by concentration of the appropriate fractions (6% 0.2 ml gave compound 28 (15 mg, 76%) as a colorless, colorless oil. "H NMR (CDCl3, 400 MHz) δ = 7.87 (d, J = 7.5 Hz, 2 H), 7.50 (s, 1 H), 7.22 (d, J = 7.5 Hz, 2 H), 7.13 (d, J = 7.5 Hz, 1 H), 6.98 (s, J = 3.1 Hz, 1 H), 6.93 (s, J = 11 Hz, 2 H), 3.94 (s, 3 H), 3.35 (t, J = 10 Hz, 2 H). 13C NMR (CDCl3, 100 MHz) δ = 153.0, 151.3, 149.5, 138.4, 131.1, 129.6, 128.8, 125.6, 124.3, 121.2, 120.9, 118.6, 113.8, 107.1, 104.1, 56.3, 56.1, 36.1 ppm. MS (EI, 70 eV) m/z (%) = 295 (100), 280 (1), 266 (2), 252 (15), 236 (17), 186 (12), 128 (19). HREIMS calculated for C31H25NO3: 456.1809. Found: 456.1791."

**Compound 27**: C27H26NO2, M = 283.33. T = 150 K, monocrystalline, space group P21/a, Z = 4, a = 17.4272(2) Å, b = 6.8511(1) Å, c = 18.9278(2) Å, β = 96.6805(8) Å2, V = 2483.936(8) Å3, D2 = 1.325 g cm−3, 5017 unique reflections (2θmax = 144.5°, R = 0.038 for 2987 reflections with I > 2σ(I). Re = 0.197 (all data), S = 1.00.

**Structure Determinations**: The images for compound 21 were measured with a diffractometer (Mo Kα, mirror monochromator, λ = 0.71073 Å) fitted with an area detector, and the data was extracted using Crysalis PRO.19 Images for compounds 2, 18, and 27 were measured with a diffractometer (Cu Kα, mirror monochromator, λ = 0.18498 Å) fitted with an area detector, and the data was extracted using Crysalis PRO.19 The structures for all four compounds were solved by direct methods (SIR92)19 and then refined using the Overture program.19

**CCDC 1543361 (for 2); 1543362 (for 18); 1543363 (for 21); and 1543364 (for 27)** contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre.

**Acknowledgments**

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**Keywords**: ACHE Inhibition — Alkaloids — Barylides — Cross-coupling — Lycsinone A — Zephycandine III
SUPPORTING INFORMATION

Title: Total Syntheses of the Anuraylidae Alkaloids Zephycandinone III and Lyconamine A and Their Evaluation as Inhibitors of Acetylcholinesterase

Authors: Xingjun Xu, Hye-Sun Kim, Wei-Min Chen, Xiang Mu, Gleen J. Corney, Martin G. Banwell, Colin J. Jackson, Anthony C. Willis, Paul D. Carr
1. Comparison of the $^{13}$C NMR Data Derived from Zephyrcandidine III and Lycosinine A with those Recorded on Compounds 1 and 2 Obtained by Synthesis

2. ORTEPs Derived from Single-crystal X-ray Analyses of Compounds 2, 18, 21 and 27

3. AChE Inhibition Curves for Compounds 1, 2, 17-19, 21-24 and 27.

4. $^1$H and $^{13}$C NMR Spectra of Compounds 1, 2, 5-7, 11, 14-25 and 27-29.
Table S1: Comparison of the $^{13}$C NMR Chemical Shifts Recorded for Compound I with those Reported$^1$ for the Natural Product Zephyrrrhizidine III

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$^a$ spectrum recorded in CD$_3$OD at 100 MHz;
$^b$ data obtained from Yao$^1$, spectrum recorded in CD$_3$OD at 100 MHz

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<td>+0.8</td>
</tr>
<tr>
<td>55.9</td>
<td>56.6</td>
<td>+0.7</td>
</tr>
<tr>
<td>40.9</td>
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<td>0.0</td>
</tr>
<tr>
<td>28.9</td>
<td>29.6</td>
<td>+0.7</td>
</tr>
</tbody>
</table>

$^a$ spectrum recorded in CDCl$_3$ at 150 MHz;  
$^b$ data obtained from Yang, spectrum recorded in CDCl$_3$ at 125 MHz.

Figure S2: Structure of compound 18 (CCDC 1543362) with labeling of selected atoms. Anisotropic displacement ellipsoids show 30% probability levels. Hydrogen atoms are drawn as circles with small radii.
Figure S3: Structure of compound 21 (CCDC 1543363) with labeling of selected atoms. Anisotropic displacement ellipsoids show 30% probability levels. Hydrogen atoms are drawn as circles with small radii.
Figure S4: Structure of compound 27 (CCDC 1543363) with labeling of selected atoms. Anisotropic displacement ellipsoids show 30% probability levels. Hydrogen atoms are drawn as circles with small radii.
Figure S5. Dose-response curves for the inhibition of *Electrophorus electricus* acetylcholinesterase by compounds 1, 2, 17-19, 21-24, 27 and galanthamine (galanthamine). Three repeat measurements of enzyme activity were conducted at each concentration of compound. The IC<sub>50</sub> values were determined by fitting a sigmoidal dose-response curve to percentage activity using GraphPad Prism. The curve was constrained to 0 (bottom) and 100% (top), and the Hill slope was constrained to -1.
400 MHz $^1$H NMR Spectrum of Compound 1
(recorded in CD$_3$OD)
400 MHz $^1$H NMR Spectrum of Compound 5
(recorded in CDCl₃)
100 MHz $^{13}$C NMR Spectrum of Compound 6
(recovered in CDCl$_3$)
100 MHz $^{13}$C NMR Spectrum of Compound 7
(recording in DMSO-$d_6$)
400 MHz $^1$H NMR Spectrum of Compound 11
(recorded in CDCl$_3$)
100 MHz $^{13}$C NMR Spectrum of Compound 11
(recorded in CDCl$_3$)
$400 \text{ MHz } ^1H \text{ NMR Spectrum of Compound } 14$

(Recorded in CDCl$_3$)

$^{14} \text{ CHCl}_3$
100 MHz $^1$C NMR Spectrum of Compound 15
(recorded in CDCl$_3$)
400 MHz $^1$H NMR Spectrum of Compound 16 (recorded in CDCl$_3$)
100 MHz $^{13}$C NMR Spectrum of Compound 16
(recorded in CDCl$_3$)
400 MHz $^1$H NMR Spectrum of Compound 17
(recorded in CDCl$_3$)

17

CHCl$_3$

acetone
100 MHz $^{13}$C NMR Spectrum of Compound 17
(recording in CDCl$_3$)
400 MHz $^1$H NMR Spectrum of Compound 18
(recorded in CDCl$_3$)
100 MHz ²³NMR Spectrum of Compound 18
(recorded in CDCl₃)
400 MHz $^1$H NMR Spectrum of Compound 19
(recorded in CDCl$_3$)
100 MHz $^{13}$C NMR Spectrum of Compound 19
(recorded in CDCl$_3$)
400 MHz $^1$H NMR Spectrum of Compound 20
(recorded in CDCl$_3$)
100 MHz $^{13}$C NMR Spectrum of Compound 20 (recorded in CDCl$_3$)
100 MHz $^{13}$C NMR Spectrum of Compound 21
(recorded in CDCl$_3$)
400 MHz 1H NMR Spectrum of Compound 22 (recorded in CDCl₃)
$^{13}$C NMR Spectrum of Compound 22 (recorded in CDCl$_3$)
400 MHz $^1$H NMR Spectrum of Compound 23
(recorded in CDCl$_3$)
400 MHz 1H NMR Spectrum of Compound 24
(recorded in CD3OD)
100 MHz $^{13}$C NMR Spectrum of Compound 24
(recorded in CD$_3$OD)
Publication Five

Total Synthesis of the Marine Alkaloid Discoipyrrole C via the MoOPH-mediated Oxidation of a 2,3,5-Trisubstituted Pyrrole

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Total Synthesis of the Marine Alkaloid Discoipyrole C via the MoOPH-Mediated Oxidation of a 2,3,5-Trisubstituted Pyrrole

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Supporting Information

Abstract: A total synthesis of the marine alkaloid discoipyrole C (3) is described. In the pivotal step, the 2,3,5-trisubstituted pyrrole 19 was treated with MoOPH in the presence of MeOH, and the resulting methoxylated 1,2-dihydropyrrole-3-one 20 subjected to reaction with potassium carbonate in MeOH then trifluoroacetic acid and H2O. This gave a mixture of target 3 and its hydrolysis product, and the structure of the former compound was confirmed by single-crystal X-ray analysis.

The production of discoipyroles A–D by the marine bacterium Bacillus subtilis was reported by the MacMillan group in 2013 and, on the basis of a range of spectroscopic studies, these alkaloids were assigned structures 1–4, respectively. In addition to congeners 1, 2, and 4 embodying the previously unobserved 3H-benzo[4]pyrrole-1,2(1H)-oxazine-5,6-dione heterocyclic framework, all four compounds showed intriguing biological properties. Specifically, each of them inhibited the discoidin domain receptor 2 or DDR2-dependent migration of B16 melanoma. They also showed additive cytotoxicity toward DDR2 mutant lung cancer cell lines with IC50 values in the 120 to 400 nM range.

The racemic nature of the first three of these alkaloids and the isolation of the fourth as a ca. 1:1 mixture of diastereoisomers led to the proposal that the core structures of the discoipyroles are produced in vivo by nonenzymatic pathways. Support for this proposal followed from the in vitro assembly, under close to physiological conditions, of congeners 1 and 2. However, the racemic state of 4 also suggests that the stereochemical diversity displayed by discoipyroles A–D and another structurally simpler metabolite, discodermolide, is derived from a common precursor.

We have recently described modular total syntheses of discoipyroles A, B, and D that involve, as key intermediates, tetrahydrofuran pyrroles wherein a benzoic acid moiety is attached to the ring nitrogen through the ortho-position. On treatment of such systems with oxo(oxomethyl)phosphoridene (pyridine)(heptamethylenophosphonic triamide) (MoOPH), they undergo oxidative cyclization with the carboxylic acid residue acting as an internal nucleophile, thus producing the central heterocyclic ring system characteristic of compounds 1, 2, and 4.

While discoipyrole C (3) is the structurally simplest member of this small family of natural products, it is distinct because it lacks the benzylated 1,2-oxazoline-5,6-dione ring system associated with congeners 1, 2, and 4. Rather it embodies a 2-alkyl-2-hydroxy-1,2-dihydropyrrole-3-one core and thus bears some structural resemblance to the biologically active compound 4.

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active natural products myceliothermophiles A and B, oteromycin, pyrocicline A, and PL-090 (each of which embodies a 5-alkyl-5-hydroxy-1,5-dihydro-2H-pyrroly-2-one moiety). The table nature of the core of discopryrole C, its intriguing biological properties, and the lack of any published work on its synthesis prompted us to begin exploring methods for doing so. Herein we report the outcomes of our studies on this topic that have culminated in the total synthesis of compound 3 and its characterization by single-crystal X-ray analysis.

RESULTS AND DISCUSSION

Converting the 2,2,2-Trisubstituted Pyrrole 10 into the Corresponding 2-Alkyl-2-hydroxy-1,2-dihydro-2H-pyrrol-3-one. Our initial studies (Scheme 1) focused on establishing whether or not a 2,2,2-trisubstituted pyrrole (and, therefore, lacking a substituent on the ring nitrogen) could be oxidized in the presence of a nucleophilic solvent such as MeOH (and in an analogous way to that observed for certain indoles) so as to generate a 2-alkyl-2-methoxy-1,2-dihydro-2H-pyrrol-3-one that it was expected could be hydrolyzed to form the heterocyclic core of discopryrole C. So, following our earlier work, the readily available pyrrole-2-carboxaldehyde (5) was brominated using N-bromosuccinimide (NBS) at low temperature, thereby generating the known dibromoderivative 6 (83%). Compound 6 was readily engaged in a 2-fold Suzuki–Miyaura cross-coupling reaction with an excess of the commercially available aryboronic acid 7 under standard conditions, thus affording the previously reported11 diarylated pyrrole 8 (85%), and this was itself treated with isopropylglycine...
Scheme 2. Outcome of the Oxidation of Pyrrole 10 with MoOPH in the Presence of MeOH

Scheme 3. Outcomes of Treating Compound 12 with Aqueous Acid

Nenium bromide to afford the secondary alcohol in 95% yield. Treatment of compound 9 with lithium aluminium hydride resulted in its reductive deoxygenation to afford the required 2,3,5-trisubstituted pyrrole 10 in 75% yield. In an initial attempt to effect the desired oxidation of compound 10 it was treated with the Ley-Goffeir reagent. A very slow and clean reaction ensued, but this involved an oxidative dimerization process that afforded the bis-pyrrole 11 (98% yield), the structure of which was determined by single-crystal X-ray analysis. No evidence for the formation of the desired 2-alkyl-2-methoxy-1,2-dihydro-3H-pyrrole 3-one was obtained.

In contrast to the foregoing, when a 1:1 v/v CH2Cl2/MeOH solution of substrate 10 was treated with MoOPH at ambient temperature, a quite distinct oxidation process (Scheme 2) took place, thus affording a mixture of compounds 12 (34%), 13 and 14 (15% combined yield), and the known diketone 15 (51%). Subjection of this mixture to flash column chromatography allowed for the isolation of the first and last of these products in pure form, while amides 13 and 14 were obtained as a ca. 1:1 mixture. The spectroscopic data recorded on the 2-alkyl-2-methoxy-1,2-dihydro-3H-pyrrole 3-one 12 were in complete accord with the assigned structure, but final confirmation of this was followed from a single-crystal X-ray analysis. On the other hand, the spectroscopic data recorded on 4,4'-dimethoxybenzil (15) matched those reported in the literature. The mixture of compounds 13 and 14 was obtained as a crystalline conglomerate, and an individual crystal of each was subjected to X-ray analysis, thereby establishing the illustrated structures for them. Clearly compounds 13, 14, and 15 result from oxidative cleavage of the pyrrole ring associated with the starting material 10, but the details of the pathways involved have yet to be fully investigated.

Despite the dominance of the oxidative cleavage processes described immediately above, sufficient quantities of compound 12 could be obtained so as to test whether or not it could be successfully hydrolysed to give the corresponding 2-hydroxy-1,2-dihydro-3H-pyrrole 3-one. Disappointingly, on treating this...
substrate with trifluoroacetic acid (TFA) in a mixture of CH$_3$Cl and H$_2$O under conditions similar to those employed by Uchiro and co-workers (Scheme 3). Only traces of the target compound 16 were observed, the major one formed being the elimination product 17 (87%) embodying an exocyclic olefin. The illustrated Z-configuration about this double bond is assigned by analogy with the work of Uchiro and co-workers. Fortunately, when the same substrate was treated with TFA in the same solvent mixture but now at lower temperatures and for shorter periods of time, compound 16 was produced in 93% yield. All of the spectroscopic data acquired on product 16, which represents the bis-O-methyl ether of discopyrrole C, were completely in accord with the assigned structure. The strong resemblance of these data to those reported for natural product 3 was notable.

Various attempts were made to convert, through 2-fold demethylation, bis-ether 16 into discopyrrole C including by treating the former compound with boron tribromide. Unsurprisingly, though, only decomposition of the substrate was observed under such conditions. Accordingly, a pathway to compound 3 was pursued wherein such a demethylation reaction was effected prior to the pyrolyzation oxidation step. The successful outcome of this approach is detailed immediately below.

**Completing a Total Synthesis of Discopyrrole C.** A synthesis of discopyrrole C (3) that exploited the results of the
above-mentioned studies is outlined in Scheme 4. This started with the 2-fold demethylation of the diarylated pyrrole 10 using boron trichloride. The resulting bis-pyrrole 18 (87%) was acetylated under conventional conditions, and the bis-ester 19 (96%) so formed was treated with MoOPH in CH_2Cl_2/McOH, thus starting a main product 20 and 21 in ca. 80% combined yield. Traces of the isomeric acid or amide-imidocarbamins 22 and 23 were also evident in the 1H NMR spectrum of the crude reaction mixture obtained from the oxidation of pyrrole 19, but these could not be obtained in quantities sufficient for rigorous characterisation. In contrast, each of the oxidation products 20 and 21 could be purified by flash column chromatography and fully characterised. When compound 20 was treated with potassium carbonate in MeOH, the bis-acetate 21 gave 23 (75%) yield. Exposure of compound to TFA in CH_2Cl_2/H_2O mixture at 0 °C for less than an hour followed by workup in the cold then gave dipeptide C (3) in 89% yield. Traces of the dehydro product 25 were also produced under these conditions, and most of this (41%) was formed when extended reaction times and higher temperatures were employed in the hydrolysis step (see Experimental Section). Once again, the illustrated Z-configuration about the double bond is assigned by analogy with the work of Uchino and co-workers.

All of the spectroscopic data acquired on the synthetically derived compounds 23 and 25 were in full accord with the illustrated structures, and those derived from the former product proved an excellent match with these reported 1H and 13C NMR data sets. A single-crystal X-ray analysis was undertaken on compound 23, and this served to confirm its structure and, therefore, that of the natural product. It is interesting to note that the heterocyclic core associated with the dipeptide products 23 and 25 is similar to that seen in the natural product mycetotheinol E. Furthermore, there were indications that, on standing, compound 23 began to react with itself to give the 25. A related isomerisation was observed during the synthesis of mycetotheinol E.

**CONCLUSIONS**

The MoOPH-mediated oxidation of N-unsaturated pyrroles provides a hitherto unrecognised capacity to generate functionalized 1,2,3-triazole-4-pyrrole-3-amines such as dipeptide C. The opportunities to extend the types of processes reported here to related systems seem significant, although there is an attractor need to understand the mechanistic detail of these reactions and thereby optimise them. Work directed to such ends is now underway in our laboratories, and the outcomes of relevant studies will be reported in due course.

**EXPERIMENTAL SECTION**

General Experimental Procedures. Melting points were measured on an Optimel automated melting point system and are uncorrected. Infra-red spectra (κ_M) were recorded on a Perkin-Elmer 1000 Series FTIR spectrometer. Samples were analysed as thin films on KBr plates. Proton (1H) and carbon (13C) NMR spectra were recorded at room temperature in deuterated CDCl_3, CD_3OD, or (CD_3)CO on a Varian spectrometer operating at 400 MHz for proton and 100 MHz for carbon signals. The signal due to residual CDCl_3, appearing at δ_0.37 and the central resonance of the CD_3OD triplet appearing at δ_0.72, was used to reference 1H and 13C NMR spectra, respectively. Mass spectra recorded in CI-ESI mode there were referenced to the signals at m/z 331 and m/z 490, respectively, while the equivalent resources employed for spectra recorded in (CD_3)CO 2.85 and 29.30/26.43 ppm. Low-resolution EI mass spectra were recorded on a simple quadruple liquid chromatograph mass spectrometer, while high-resolution measurements were conducted on a time-of-flight FAB mass spectrometer. Mass spectra were recorded on a magnetic-sector machine. Analytical thin-layer chromatography (TLC) was performed on aluminum-backed 62 mm thick silica gel 60 F_254 plates as supplied by Merck. Eluted plates were visualised using a 254 nm UV lamp and/or by treatment with a suitable step followed by heating. The TLC plates included phosphoric acid or cation exchanger silica gel 60 (37.5 g/75 g/75 g/700 mL) or potassium permanganate/potassium carbonate/3% sodium hydroxide aqueous solution/H_2O (3 g/20 g/5 mL/100 mL). Flash chromatographic separations were carried out following protocols defined by Still et al. with silica gel 60 (60–230 μm) as the stationary phase and using the AR- or HPLC-grade solvents indicated. The mobile phases used for purification by these means were recorded directly (i.e., after they had crystallised from the concentrated chromatographic fractions). Starting materials and reagents were generally available from Sigma–Merck, Merck, TLC, or Lancaster Chemical Compounds and were used as supplied. Drying agents and other inorganic salts were purchased from the AAE, Noble, or Unilab Chemical Companies. Tetrahydrofuran (THF), MeOH, and CH_2Cl_2 were dried using a Glass Contour solvent purification system that is based upon a technology originally described by Grubbs et al. Where necessary, reactions were performed under a nitrogen atmosphere.

**Synthesis of 4,5-Dibromo-1H-pyrrole-2-carboxylic acid (6).** In a modification of a published procedure, a magnetically stirred solution of commercially available 1H-pyrrole-2-carboxylic acid (3) (5.00 g, 5.26 mmol) in anhydrous THF (200 mL) and protected from light using aluminum foil was cooled to −78 °C, then treated with NBS (20.60 g, 117.5 mmol). The cooling bath was then removed, and the reaction mixture allowed to warm to 22 °C, stirred at this temperature for 20 min, then cooled to 0 °C and treated with NaHCO_3 (14.00 g, 111.08 mmol). The resulting mixture was stirred at 0 °C for 0.5 h, then filtered through a pad of diatomaceous earth. The filtrate was concentrated under reduced pressure to give a brown oil, and subjecting this material to flash chromatography (silica, 3:1 196/65 v/v EtOAc/40–60 petroleum ether gradient elution) afforded, after concentration of the appropriate fractions (R_t = 0.4 in 1:4 v/v EtOAc/40–60 petroleum ether), 4,5-dibromo-1H-pyrrole-2-carboxylic acid (6) (11.0, 83%). Purity, ascertained by thin layer chromatography (silica, 3:1 196/65 v/v EtOAc/40–60 petroleum ether elution), and purity, ascertained by thin layer chromatography (silica, 3:1 196/65 v/v EtOAc/40–60 petroleum ether elution), indicated that the crude product (6) was 99% pure. The 1H and 13C NMR spectroscopic data acquired on this material matched those reported previously.

**Synthesis of 4-(3-Methoxyphenyl)yl-1H-pyrrole-2-carboxaldehyde (8).** In a modification of a published procedure, a magnetically stirred solution of 4,5-dibromo-1H-pyrrole-2-carboxaldehyde (6) (~5.0 g, 18.36 mmol), (4-methoxyphenyl)benzoic acid (7) (8.85 g, 58.13 mmol), (4-Ph)PhCH_2, (820 mg, 0.71 mmol), and NaOH (13.3 g, 170 mmol) in deionized 1:10 dimethylformamide/H_2O (196 mL of a 6:1 v/v mixture) was treated at 85 °C under a nitrogen atmosphere for 20 h, then cooled to 22 °C and treated with H_2O (200 mL) and extracted with EtOAc (3 × 160 mL). The combined organic phases were then dried (NaSO_4), filtered, and concentrated under reduced pressure. The residue thus obtained was subjected to flash column chromatography (silica, 3:1 196/65 v/v EtOAc/40–60 petroleum ether elution), and purity, ascertained by thin layer chromatography (silica, 3:1 196/65 v/v EtOAc/40–60 petroleum ether elution), of the appropriate fractions (R_t = 0.4 in 1:4 v/v EtOAc/40–60 petroleum ether). Thereby, a pure 4-(3-methoxyphenyl)yl-1H-pyrrole-2-carboxaldehyde (8) (3.00 g, 85%) yellow, crystalline solid, the 1H and 13C NMR spectroscopic data acquired on this material matched those reported previously.

**Synthesis of 4-(5-Lys(4-Methoxyphenyl)yl)-1H-pyrrole-2-carbolin-1-yl N-acetyl-L-lysine (9).** In a modification of a published procedure, a magnetically stirred solution of 4-(5-Lys(4-methoxyphenyl)yl)-1H-pyrrole-2-carboxaldehyde (8) (922 mg, 3.00 mmol) in anhydrous THF (3 mL) of a 6:1 v/v mixture was treated with 2 M sodium carbonate solution (3.5 g) of a 2.4 M solution in dioxane (9.00 mL). The ensuing mixture was stirred at 0 °C for 1 h, and the mixture was diluted with 100 mL of 1:1 chloroform/methanol and filtered through a pad of diatomaceous earth. The filtrate was concentrated under reduced pressure to give a brown oil, and purity, ascertained by thin layer chromatography (silica, 3:1 196/65 v/v EtOAc/40–60 petroleum ether elution), indicated that the crude product (9) was 99% pure. The 1H and 13C NMR spectroscopic data acquired on this material matched those reported previously.
then quenched by the slow addition of ice (CAUTION: EXOTHERMIC REACTION). The ensuing mixture was poured into 100 ml. of water, and extracted with 3 portions of chloroform.

The combined organic phases were then distilled (NaSO₄ filtered, and concentrated under reduced pressure. The residue thus obtained was subjected to flash column chromatography (silica, 1/9 v/v EtOAc/40–60 petroleum ether) to give, after concentration of the appropriate fractions (Rₚ 0.45), 301 mg (69% yield) of the pure product (m.p. 129–130°C).

Synthesis of 3-tert-butyl-2,3-bis(4-methoxyphenyl)-1H-pyrrrole (10). In 1,4-dioxane (100 ml., 37.3 mmol) was added to a magnetically stirred solution of 2-chloro-1,3-cyclopentadiene (150 mg, 2 ml) in EtOAc (10 ml, 37.3 mmol) maintained at 23°C under an atmosphere of nitrogen. The resulting mixture was stirred magnetically while being heated under reflux for 16 h. After this time the reaction mixture was cooled to 0°C, then quenched by the slow addition of ice (CAUTION: EXOTHERMIC REACTION AND THE POSSIBILITY OF EXPLOSION). The ensuing mixture was poured into H₂O (200 ml.) and extracted with EtOAc (3 x 100 ml.), and the combined organic phases were then dried (Na₂SO₄, filtered, and concentrated under reduced pressure. The residue thus obtained was subjected to flash column chromatography (silica, 1/9 v/v EtOAc/40–60 petroleum ether) to give, after concentration of the appropriate fractions (Rₚ 0.5), 475 mg (62% yield) of the pure product (m.p. 129–130°C).

Synthesis of 2,3-dibromo-4,4'-tert-butyl-4,4'-5-tris(4-methoxyphenyl)-1,1'-bipyrrole (11). A magnetically stirred solution of 3-tert-butyl-2,3-bis(4-methoxyphenyl)-1H-pyrrrole (100 mg, 37.3 mmol) in 1,4-dioxane (100 ml, 37.3 mmol) was treated with 4-methylmorpholine N-oxide (37.3 mmol, 74.6 mg), Na(Ot-Bu)₃ (37.3 mmol, 1.06 g) and CuCl (37.3 mmol, 1.01 g). The mixture was stirred at 50°C under an atmosphere of nitrogen for 17 h. The mixture was then cooled to 0°C, then filtered through a pad of diatomaceous earth, and the separated aqueous phase was discarded. The remaining solid was dried with nitrogen, and the crude product was subjected to flash column chromatography (silica, 1/9 v/v EtOAc/40–60 petroleum ether) to give, after concentration of the appropriate fractions (Rₚ 0.9), 160 mg (65% yield) of the pure product (m.p. 129–130°C).

Concentration of a mixture (Rₚ 0.1) of 37.3% EtOAc/40–60 petroleum ether gave 120 mg (24%) of the pure product (m.p. 129–130°C).

Concentration of a mixture (Rₚ 0.1) of 37.3% EtOAc/40–60 petroleum ether gave 120 mg (24%) of the pure product (m.p. 129–130°C).
proteins not observed. 1C NMR (100 MHz, CDCl3) δ 142.3, 174.1, 165.5, 159.6, 153.8, 125.6, 114.8, 114.7, 108.8, 80.8, 56.5, 58.7, 26.7, 24.5, 24.9, 13.8. IR (KBr) 2926, 2866, 1611, 1601, 1544, 1527, 1515, 1010, 980, 853 cm⁻¹; MS (ESI) +/− m/z 273 [M + Na]⁺ (100%), 187 [M−Na]⁻ (86%), 185 (M−HOAc)−, 179 (M−HOAc−H2O)−, 161 (M−3HOAc)−, 139 (M−4HOAc)−, 121 (M−5HOAc)−, 86 (M−6HOAc)−, 83 (M+H)⁺ (97%), 85 (2-Methylpyridine)−. [M + H]+ (for C₂H₅NO₃, 288.1356). 12-2). Methyl-3-(4-methyl-3-phenylpyridyl)-1,2-dihydropyridine-3-one (17). A magnetically stirred solution of 2-isobutyl-1-methyl-4,6-bis(4-methylphenyl)-1,2-dihydropyridine-3-one (4 mg, 0.02 mmol) in CH₂Cl₂ (750 μL of a 6:1 v/v mixture) maintained at 0 °C under a nitrogen atmosphere for 5 min, treated with trichloroacetic acid (300 μL, 3.9 mmol). The ensuing mixture was stirred at 0 °C for 1 h, then warmed to 22 °C, and stirred at this temperature for another 2.5 h. The mixture thus obtained was then treated with HCl (1 × 10 mL) before being extracted with EtOAc (1 × 10 mL). The combined organic phases were washed with H₂O (1 × 20 mL) and NaHCO₃ (2 × 10 mL of a saturated aqueous solution) before being dried (Na₂SO₄, filtered, and concentrated under reduced pressure. The residue thus obtained was subjected to flash column chromatography (silica, 1:1 → 3:1 v/v EtOAc/60–80 petroleum ether gradient elution) to give, after concentration of the appropriate fractions (Rf = 0.5 in 1:1 v/v EtOAc/60–80 petroleum ether solvent), compound 17 (34 mg, 87%). Light green oil. 1H NMR (400 MHz, CDCl₃) δ 7.67 (d, 1J = 8.9 Hz, 2H), 7.09 (d, 1J = 9.0 Hz, 2H), 6.93 (d, 1J = 9.0 Hz, 2H), 6.84 (d, 1J = 9.0 Hz, 2H), 6.08 (d, 1J = 10.1 Hz, 1H), 3.83 (s, 3H), 3.76 (s, 3H), 3.68 (s, 3H), 2.98 (s, 3H), 1.18 (s, 6H, HOAc) (signal due to HCHM proton not observed). 13C NMR (100 MHz, CDCl₃) δ 166.5, 166.3, 159.8, 157.8, 132.0, 131.8, 125.0, 135.9, 124.1, 118.0, 114.8, 113.8, 85.5, 8.7, 24.1, 24.9, 24.8, 39.9. mp 290 °C. IR (KBr) ν 3436, 1422, 1273, 2101, 1735 (C = N). [M+H]+ (for C₁₉H₁₈N₂O₂, 350.1751) (calc for C₁₉H₁₈N₂O₂, 350.1754). 4.4'-Disobutyl-1,2-biphenyl-3,3'-diphenylamine (14). Synthesis of 4.4'-Disobutyl-1,2-biphenyl-3,3'-diphenylamine (14). A magnetically stirred solution of compound 10 (100 mg, 0.29 mmol) in anhydrous CH₂Cl₂ (200 mL) maintained at 78 °C under an atmosphere of nitrogen, reacted for 0.37 h, before being quenched with HCl (1 × 10 mL) before being extracted with EtOAc (1 × 10 mL). The combined organic phases were washed with H₂O (1 × 20 mL) and NaHCO₃ (2 × 10 mL of a saturated aqueous solution) before being dried (Na₂SO₄, filtered, and concentrated under reduced pressure. The residue thus obtained was subjected to flash column chromatography (silica, 1:1 → 3:1 v/v EtOAc/60–80 petroleum ether elution) to give, after concentration of the appropriate fractions (Rf = 0.5 in 1:1 v/v EtOAc/60–80 petroleum ether solvent), compound 14 (30 mg, 89%). Light yellow oil. 1H NMR (400 MHz, CDCl₃) δ 7.39 (d, 1J = 7.8 Hz, 2H), 7.29 (d, 1J = 7.8 Hz, 2H), 7.30 (d, 1J = 7.8 Hz, 2H), 2.32 (s, 3H), 2.29 (s, 3H), 2.25 (s, 3H), 1.47 (s, 18H), 7.27 (s, 1H), 0.86 (s, 1H), (6H) (signal due to He group proton not observed). 13C NMR (100 MHz, CDCl₃) δ 141.7, 135.2, 135.0, 128.2, 128.4, 128.3, 128.0, 125.7, 122.0, 121.1, 91.3, 22.0, 48.4, 48.5, 24.7, 24.3, 21.0, 20.8, 18.4, mp 379 °C, 379 °C. IR (KBr) ν 3436, 1422, 1273, 2101, 1735 (C = N). [M+H]+ (for C₂₁H₂₃N₂O₂, 359.1712) (calc for C₂₁H₂₃N₂O₂, 359.1743). Concentration of fraction 14 (Rf = 0.3, eluted with 3:1 v/v EtOAc/60–80 petroleum ether elution) gave compound 14 (30 mg, 89%). Yellow oil. 1H NMR (400 MHz, CDCl₃) δ 7.39 (d, 1J = 7.8 Hz, 2H), 7.29 (d, 1J = 7.8 Hz, 2H), 7.30 (d, 1J = 7.8 Hz, 2H), 2.32 (s, 3H), 2.29 (s, 3H), 2.25 (s, 3H), 1.47 (s, 18H), 7.27 (s, 1H), 0.86 (s, 1H), (6H) (signal due to He group proton not observed). 13C NMR (100 MHz, CDCl₃) δ 141.7, 135.2, 135.0, 128.2, 128.4, 128.3, 128.0, 125.7, 122.0, 121.1, 91.3, 22.0, 48.4, 48.5, 24.7, 24.3, 21.0, 20.8, 18.4, mp 379 °C, 379 °C. IR (KBr) ν 3436, 1422, 1273, 2101, 1735 (C = N). [M+H]+ (for C₂₁H₂₃N₂O₂, 359.1712) (calc for C₂₁H₂₃N₂O₂, 359.1743).
was treated, dropwise over 5 min, with trifluoroacetic acid (870 mL, 8.75 mol). The ensuing mixture was stirred at 0 °C for 25 min, and then 0.0 (10 mL) solution of EtOH (20 mL) was added at the same temperature. The separated organic phase was washed, at 0 °C, with 0.1 N (10 mL) solution of NaOH (2), 10 mL of a saturated aqueous solution, until the pH of the aqueous washings was between 5 and 7. The separated organic layer was dried (Na2SO4, filtered, and concentrated under reduced pressure, and the residue thus obtained was subjected to flash column chromatography (silica, 1.2 v/v %/EtOAc

\textbf{Synthesis} of 2-((5R,6S)-5-bromo-6-methylpentyl)-1-(2-methylpropyl)pyrroliidine-1,2-dihydro-3H-pyrrol-3-one (2E). A magnetically stirred solution of compound 2E (57 mg, 0.028 mmol) in CHCl3

\textbf{ASSOCIATED CONTENT}

\textbf{Supporting Information}

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatmed.7b00072.

Data derived from the single-crystal X-ray analyses of compounds 3, 11, 12, 13, and 14. H NMR spectra of compounds 6, 8, and 10. 1H NMR spectra of compounds 3, 9, 11–12, 14, and 25 (PDF)

\textbf{Crystallographic data (CIF)}

\textbf{AUTHOR CORRESPONDING INFORMATION}

\textbf{Martin Burrell, PNNL, Richland, WA 99352, USA.

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Martin G. Barwell: 0000-0002-0562-472X

Notes

The authors declare no competing financial interest.

Atomic coordinates, bond lengths and angles, and displacement parameters have been deposited at the Cambridge Crystallographic Data Centre (CCDC) nos. 1573703, 1573704, 1573705, and 1573706. These data can be obtained free-of-charge via www.ccdc.cam.ac.uk/data_request/cif, by emailing data_request@ccdc.cam.ac.uk, or by contacting the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK, fax: +44 1223 336033.

\textbf{ACKNOWLEDGMENTS}

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SUPPORTING INFORMATION FOR:

Total Synthesis of the Marine Alkaloid Discobipyrole C via the MoOPH-mediated Oxidation of a 2,4,5-Trisubstituted Pyrrole

Qiao Yan, Xiang Ma, Martin G. Banwell* and Jas S. Ward

Research School of Chemistry, Institute of Advanced Studies, The Australian National University, Canberra, ACT 2601, Australia

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Figure S2: Structure of compound 11 (CCDC 1573707) showing co-crystallized iso-propanol. Anisotropic displacement ellipsoids show 30% probability levels. Hydrogen atoms are drawn as circles with small radii.
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Figure S5: Structure of compound 14 (CCDC 1573704). Anisotropic displacement ellipsoids show 30% probability levels. Hydrogen atoms are drawn as circles with small radii.
Table S1: Comparison of the $^{13}$C NMR Chemical Shifts Recorded for Compound 3 with those Reported$^1$ for the Natural Product Discopyrrole C

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<tr>
<th>$^{13}$C NMR Data for Compound 3 (δC)$^2$</th>
<th>$^{13}$C NMR Data Discopyrrole C (δC)$^3$</th>
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<tr>
<td>202.2</td>
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<td>174.4</td>
<td>174.6</td>
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<tr>
<td>162.2</td>
<td>162.3</td>
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<tr>
<td>156.9</td>
<td>157.0</td>
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<tr>
<td>131.9</td>
<td>131.9</td>
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<td>131.8</td>
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<td>116.3</td>
<td>116.4</td>
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<td>24.7</td>
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</tr>
<tr>
<td>24.6</td>
<td>24.7</td>
<td>−0.1</td>
</tr>
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$^a$ Spectrum recorded in CD$_3$OD at 100 MHz.  
$^b$ Data obtained from MacMillan.  
$^c$ Spectrum recorded in CD$_3$OD at 150 MHz.

Publication Six

A Total Synthesis of the Antifungal Deoxyaminocyclitol
Nabscessin B from L-(+)-Tartaric Acid

Xiang Ma, Qiao Yan, Martin G. Banwell and Jas S. Ward

Org. Lett. 2018, 20, 142
A Total Synthesis of the Antifungal Deoxyaminocyclitol Nabscessin B from L-(+)-Tartaric Acid

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Supporting Information

**ABSTRACT:** Aminocyclitol 2, a recently isolated and notable antifungal agent, was prepared from homochiral y-hydroxyoxycoumarone 4, which is itself available in six steps from L-(+)-tartaric acid (3). The well-defined rigidifying effect arising from the 1,2-diacelet protecting group associated with compound 4 and its derivatives allows for high levels of regio- and stereo-chemical control in the manipulation of the cyclitol framework.

Recently, Ishihashi and co-workers reported the isolation of deoxyaminocyclitols 1 and 2 (Figure 1) from the culture broths of the pathogenic actinomycete Noardiopsis albidus IMI 100239, a species derived from an intracutaneous abscess associated with the knee of a human patient. Compounds 1 and 2 were named nabcessins A and B, respectively, and the illustrated and isomeric structures were established through extensive spectroscopic analyses, most notably 2D NMR studies. Both nabcessins embody a 2-deoxy-sylloxy-inosamine core.

A preliminary biological evaluation of compounds 1 and 2 revealed that they act against Cryptococcus neoformans with IC_{50} values of 32 and 15 μg/mL, respectively (C. neoformans is a ubiquitous and encapsulated yeast that has been a significant cause of mortality in immune-compromised patients, especially those suffering from AIDS).

The novel structures associated with natural products 1 and 2 together with the ongoing interest in the development of selective approaches to variably functionalized aminocyclitols and their deployment as anti-infective agents prompted us to develop routes to these compounds. We now report a synthesis of nabcessin B (2) from L-(+)-tartaric acid that serves to confirm its structure, including its absolute stereochemistry.

We have recently described the generation of various homochiral cyclitols from either the (+)- or (−)-form of tartaric acid and deployed these in the preparation of the fungal metabolite aspergillitol B and certain analogues of the alkaloid galanthamine. As revealed here, one of these cyclitols serves as a precursor to the title alkaloid. The synthetic sequence leading to a deoxypimilositol precursor of nabcessin B (2) is shown in Scheme 1. Thus, as previously reported 12, 13 L-(+)-tartaric acid (3) was converted over six steps, including those involving vinylation and ring-closing metathesis reactions, into the 1,2-diacelet-containing y-hydroxyoxycoumarone 4.

Protection of the hydroxyl group within the last compound under standard conditions gave the tert-butyldiphenylsilyl (TBDPS) ether 5 (98%), which was itself subjected to Luche reduction to afford, stereoselectively, the protected conidendrit 6 (95%). Hydroxyl-directed epoxidation of this aliphatic alcohol using 2-chloroperoxycbenzoic acid (m-CPBA) then gave epoxy alcohol 7 (85%). Treatment of compound 7 with sodium borohydride in the presence of boron trifluoride diethyl etherate resulted in preferential trans-Dixial and thus regioselective cleavage of the externe ring to give x chromato-

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**Figure 1.** Structures of the deoxyaminocyclitol nabcessins A (1) and B (2).

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Scheme 1. Synthesis of Deynosiositol 9

The synthetic sequence used to effect the conversion of compound 9 into the 2-deoxy-sydno-insosamine core of target 2 is shown in Scheme 1. The equatorial hydroxyl group within the 2-deoxy-sydno-insosamine core of target 2 is shown in Scheme 2. The equatorial hydroxyl group within the 2-deoxy-sydno-insosamine core of target 2 was selectively protected as the corresponding p-methoxyphenyl (PMB) ether through treatment with PMB-Cl in the presence of 6-Bu3SiO and tetra-n-butylammonium iodide (TBAlI). This was followed by tritiation of the remaining axial alcohol using triflic anhydride in the presence of pyridine, thus giving 10 in 83% yield over the two steps involved. Reaction of a solution of the last compound in DMF with sodium azide resulted in rapid displacement of the newly formed triflate moiety and the formation of azido-substituted cytidine 11 (91%), the structure of which was confirmed by single-crystal X-ray analysis (see the Supporting Information for details). Staudinger reduction of 11 using triphenylphosphine in the presence of water then gave the corresponding amine which was immediately coupled with 1-(3-methoxyxysteryl)heptane-1-carboxylic acid in the presence of N-[(dimethylaminomethyl)-1H,1,2,3-triazole-4,5-F]-pyridine-1-carboxylic acid (HATU) and N,N-dicyclohexylcarbodiimide (DCC) to afford amide 12 (81%). All of the spectral data acquired on compound 12 were in complete accord with the assigned structure, with the most diagnostic feature being the appearance of a resonance due to an amide carbonyl aromatic at δ 166.8 in the 1H NMR spectrum and the presence of N-H and amide carbonyl stretching bands at 3256 and 1640 cm⁻¹, respectively, in the infrared spectrum.

The next stage of the synthesis of nenasosigosane B (2) is shown in Scheme 2 and involved the successive cleavage of the PMB and TBAlI ether moieties associated with compound 12 using 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) followed by tetra-n-butylammonium fluoride (TBAF), thus affording diol 13 in 93% yield. The structure of compound 13 was confirmed by single-crystal X-ray analysis (see the SI for details). Selective esterification of the equatorially oriented and β-configured hydroxyl group over its α-oriented counterpart on the opposite side of the cyclohexene ring was achieved using commercially available 6-methoxyhexyl ester in the presence of N-[(dimethylaminomethyl)propan-2-yl]-N,N-diethylcarbodiimide (EDCI), affording compound 14 (60%). This was accomplished by a ca. 18% yield of the corresponding lactone. The structure of compound 14 follows unambiguously from a range of NMR studies. Most particularly, the resonance due to the proton associated with the oxymethylene moiety carrying the newly introduced ester group appears as a one-proton triplet (J = 9.0 Hz) at δH 5.57 and is vicinal coupled to the multiplet at δH 4.58 arising from the proton attached to the ring carbon carrying the amide moiety. The selectivity observed in this reaction is interesting since the naturally more congested hydroxyl group in precursor 12 is esterified. Hydrogen-bonding and/or π-stacking interactions between the benzamidine residue of substrate 13 and...
Scheme 3. Synthesis of Compound 15, a Protected Form of Nabscessin B (2)

Scheme 4. Deprotection of Compound 15 to Form Nabscessin B (2)

the activated form of 6-methylsalicylic acid may be responsible. Treatment of compound 14 with 2,2,2-trichloroethyl isocyanate in dichloromethane led, after treatment with methanol and silica gel, to give a crude product, which formed a white, amorphous solid (88%).

The final step in the total synthesis of the title aminocyclitol (Scheme 4) involved treating compound 15 with a 9:1 (v/v) mixture of trifluoroacetic acid (TFA) and water at 22 °C, which gave, after concentration of the reaction mixture and gel filtration, a white solid. The 1H and 13C NMR, IR, and mass spectral data obtained on this material were consistent with the assigned structure and matched those reported for the natural product (see Table 1 for a tabular comparison of the two sets of 13C NMR spectroscopic data). The specific rotation of the synthetic material was somewhat higher than that reported for the natural product [α]D +21.3 (c 1.5, MeOH) vs. [α]D +15.5 (c 1.0, MeOH)). The origins of this difference are not entirely clear, but a possible explanation is that the natural product contains a levorotatory impurity. Nevertheless, the work reported here serves to confirm the illustrated structure of nabscessin B, including its absolute stereochemistry.

The protocols described above provide a distinct new route to aminocyclitols that do not start from an isocytol. Instead, the cyclitol framework is constructed de novo. Given that both enantiomeric forms of tartaric acid are readily obtained (each at a modest price), a significant range of aminocyclitols will be available using various chemically applicable modifications of the methods described above.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.or glett.7b03495.

Experimental procedures, spectroscopic data, copies of the NMR spectra of compounds 5–15 and 2, and X-ray data and derived ORTEPs for compounds 11 and 13 (PDF)

Accession Codes

CCDC 1578392 and 1584440 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by e-mailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, U.K.; fax: +44 1223 336033.

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Author Contributions

The manuscript was written through contributions from all of the authors. All of the authors have given approval to the final version of the manuscript.
Organic Letters

Notes

The authors declare no competing financial interest.

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SUPPORTING INFORMATION FOR:

A Total Synthesis of the Antifungal Deoxyaminocyclitol Nabscessin B from L-(−)-Tartaric Acid

Xiang Ma, Qiao Yao, Martin G. Banwell,* and Jas S. Ward

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General Experimental Protocols

Unless otherwise specified, proton (1H) and carbon (13C) NMR spectra were recorded at room temperature in base-filtered CDCl3 on a spectrometer operating at 400 MHz for proton and 100 MHz for carbon nuclei. For 1H NMR spectra, signals arising from the residual proto-forms of the solvent were used as internal standards. 1H NMR data are recorded as follows: chemical shift (δ) [multiplicity, coupling constant(s) J (Hz), relative integral] where multiplicity is defined as: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet or combinations of the above. The signal due to residual CHCl3 appearing at δH 7.26 and the central resonance of the CDCl3 “triplet” appearing at δC 77.0 were used to reference 1H and 13C NMR spectra, respectively. Infrared spectra (νmax) were recorded on a FTIR Spectrometer. Samples were analyzed as thin films on KBr plates. Low-resolution ESI mass spectra were recorded on a single quadrupole liquid chromatograph-mass spectrometer, while high-resolution measurements were conducted on a time-of-flight instrument. Low- and high-resolution EI mass spectra were recorded on a magnetic-sector machine. Melting points were measured on an automated melting point system and are uncorrected. Analytical thin layer chromatography (TLC) was performed on aluminum-backed 0.2 mm thick silica gel 60 F254 plates. Eluted plates were visualized using a 254 nm UV lamp and/or by treatment with a suitable dip followed by heating. These dips included phosphomolybdic acid : ceric sulfate : sulfuric acid (conc.) : water (37.5 g : 7.5 g : 37.5 mL : 720 mL) or potassium permanganate : potassium carbonate : 5% sodium hydroxide aqueous solution : water (3 g : 20 g : 5 mL : 300 mL). Flash chromatographic separations were carried out following protocols defined by Still et al.1 with silica gel 60 (40–63 μm) as the stationary phase and using the AR- or HPLC-grade solvents indicated. Starting materials, reagents and drying agents as well as other inorganic salts were generally available from commercial sources and used as supplied. Tetrahydrofuran (THF), diethyl ether, methanol and dichloromethane were dried using a solvent purification system that is based upon a technology originally described by Grubbs et al.2 Where necessary, reactions were performed under a nitrogen atmosphere.
Specific Chemical Transformations

Compound 5

A magnetically stirred solution of γ-hydroxy-cyclohexenone 4 (1.00 g, 3.87 mmol) and tert-butylidiphenylchlorosilane (1.60 g, 5.81 mmol) in dichloromethane (30 mL) maintained at 22 °C was treated with imidazole (395 mg, 5.81 mmol). After 16 h, the reaction mixture was quenched with water (20 mL) before being extracted with dichloromethane (3 x 30 mL). The combined organic phases were washed with brine (1 x 100 mL) then dried (Na₂SO₄), filtered and concentrated under reduced pressure. The resulting light-yellow oil was subjected to flash chromatography (silica, 95:5 v/v 30–40 petroleum spirit-diethyl ether elution) and thus affording, after concentration of the appropriate fractions (Rf = 0.8 in 9:1 v/v hexane/ethyl acetate), compound 5 (1.88 g, 98%) as a clear, colorless and viscous oil, [α]D³⁰ = -100.0 (c 3.3, CH₂Cl₂).

¹H NMR (400 MHz, CDCl₃) δ 7.83 (dd, J = 8.0, 1.3 Hz, 2H), 7.71 (dd, J = 8.0, 1.3 Hz, 2H), 7.47-7.37 (complex m, 6H), 6.31 (dd, J = 10.5, 1.9 Hz, 1H), 5.82 (dd, J = 10.5, 2.3 Hz, 1H), 4.70 (m, 1H), 4.13 (m, 2H), 3.35 (s, 3H), 3.25 (s, 3H), 1.41 (s, 3H), 1.37 (s, 3H), 1.11 (s, 9H)

¹³C NMR (100 MHz, CDCl₃) δ 193.6, 149.8, 136.2, 135.9, 133.7, 132.6, 130.1, 130.0, 127.9, 127.6, 127.2, 99.9, 99.0, 74.4, 72.0, 71.1, 48.3, 48.0, 26.8, 19.3, 17.5(0), 17.4(8)
IR (KBr) ν_max 2953, 2933, 2858, 1707, 1380, 1106, 1114, 1039, 838, 702 cm⁻¹

MS (ESI, +ve) m/z 535 [(M+K)⁺, 65%], 519 [(M+Na)⁺, 100]

HRMS m/z 519.2176 [M+Na]⁺ (calcd for C₂₉H₅ₐO₈SiNa, 519.2179).

**Compound 6**

A magnetically stirred solution of compound 5 (1.20 g, 2.4 mmol) and cerium(III) chloride heptahydrate (1.80 g, 4.81 mmol) in methanol (30 mL) maintained at 0 °C was treated, in portions, with sodium borohydride (182 mg, 4.81 mmol) (CAUTION: evolution of hydrogen gas). After 0.5 h the reaction mixture was quenched with acetone (10 mL) and the resulting mixture concentrated under reduced pressure. The resulting light-yellow oil was dissolved in 100 mL ethyl acetate and the solution thus obtained washed with water (2 x 50 mL) and brine (1 x 100 mL) before being dried (Na₂SO₄), filtered then concentrated under reduced pressure. The resulting light-yellow oil was subjected to flash chromatography (silica, 8:2 v/v 40–60 petroleum spirit/ethyl acetate elution) and thus affording, after concentration of the appropriate fractions (R_f = 0.2 in 8:2 v/v hexane/ethyl acetate), compound 6 (1.15 g, 95%) as a clear, colorless and viscous oil, [α]_D^20 = -117.1 (c 1.5, CH₂Cl₂).

**¹H NMR** (400 MHz, CDCl₃) δ 7.80 (dd, J = 8.0, 1.3 Hz, 2H), 7.68 (dd, J = 8.0, 1.3 Hz, 2H), 7.50-7.30 (complex m, 6H), 5.42 (dt, J = 10.4, 2.3 Hz, 1H), 5.18 (dt, J = 10.4, 2.3 Hz, 1H), 4.51 (m, 1H), 4.37 (m, 1H), 3.83 (m, 1H), 3.53 (m, 1H), 3.36 (s, 3H), 3.25 (s, 3H), 3.05 (s, 3H).
2.28 (d, J = 3.9 Hz, 1H), 1.36 (s, 3H), 1.34 (s, 3H), 1.09 (s, 9H)

$^{13}$C NMR (100 MHz, CDCl$_3$) δ 136.3, 136.0, 134.5, 133.6, 130.5, 129.8(4), 129.7(5), 128.1, 127.7, 127.6, 99.0, 98.9, 72.7, 72.6, 71.7, 70.0, 48.1, 48.0, 27.0, 19.4, 17.8, 17.7

IR (KBr) $\nu_{max}$ 3479, 2932, 2953, 2858, 1740, 1428, 1374, 1119, 1020, 910, 837, 701 cm$^{-1}$

MS (ESI, +ve) m/z 521 [(M+Na$^+$), 100%].

HRMS m/z 521.2335 [M+Na$^+$] (calcd for C$_2$H$_3$O$_2$SiNa, 521.2335).

**Compound 7**

![Compound 7](image)

A magnetically stirred solution of compound 6 (800 mg, 1.6 mmol) in dichloromethane (30 mL) maintained at 22 °C was treated, in portions, with m-chloroperoxybenzoic acid (1.08 g of ca. 77% peracid-containing material, 4.81 mmol). After 16 h the reaction mixture was diluted with dichloromethane (50 mL) then treated with sodium sulfite (600 mg). The resulting mixture was stirred at 22 °C for 0.25 h then sodium bicarbonate (50 mL of a saturated aqueous solution) was added. The separated organic phase was washed with water (1 x 50 mL) then brine (1 x 50 mL) before being dried (Na$_2$SO$_4$) filtered then concentrated under reduced pressure. The resulting light-yellow oil was subjected to flash chromatography (silica, 7.3 v/v 40-60 petroleum spirit/ethyl acetate elution) and
thus affording, after concentration of the appropriate fractions ($R_f = 0.3$ in 6:4 v/v hexane/ethyl acetate), compound 7 (700 g, 85%) as a clear, colorless and viscous oil, [a]$_D^{20}$ = +95.1 ($c$ 0.5, CH$_2$Cl$_2$).

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.80 (dd, J = 8.0, 1.3 Hz, 2H), 7.68 (dd, J = 8.0, 1.3 Hz, 2H), 7.50-7.30 (complex m, 6H), 4.20 (dd, J = 8.0, 0.9 Hz, 1H), 4.08 (m, 1H), 3.58 (m, 1H), 3.43 (m, 1H), 3.30 (s, 3H), 3.22 (m, 1H), 3.20 (s, 3H), 2.82 (d, J = 3.7 Hz, 1H), 2.21 (d, J = 5.2 Hz, 1H), 1.30 (s, 3H), 1.29 (s, 3H), 1.11 (s, 9H).

$^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 136.2, 135.8, 133.8, 132.9, 130.0, 129.8, 127.5, 127.8, 127.5, 98.9, 98.7, 72.3, 70.3, 70.2, 67.3, 56.4, 55.8, 48.1, 47.9, 26.9, 19.2, 17.6, 17.4.

IR (KBr) $\nu_{max}$ 3469, 2995, 2951, 2896, 2932, 2858, 1428, 1375, 1118, 1086, 909, 838, 735, 703 cm$^{-1}$

MS (ESI, +ve) m/z 537 [M+Na]$^+$, 100%)

HRMS m/z 537.2281 [M+Na]$^+$ (calcd for C$_{26}$H$_{35}$O$_2$SiNa, 537.2285).

**Compounds 8 and 9**

A magnetically stirred solution of compound 7 (1.05 g, 2.04 mmol) in 1,2-dimethoxyethane (50 mL) maintained at 22 °C was treated, in one portion, with NaBH$_4$ (773 mg, 20.3 mmol) and then, dropwise, with BF$_3$·OEt$_2$ (1.08 mL, 8.38 mmol). After 3 h the reaction
mixture was diluted with dichloromethane (50 mL) then quenched with acetone (10 mL). The separated organic phase was washed with water (1 x 50 mL) and brine (1 x 50 mL) before being dried (Na₂SO₄), filtered then concentrated under reduced pressure. The resulting light-yellow oil was subjected to flash chromatography (silica, 10.0 to 5.5 v/v 40–60 petroleum spirit/ethyl acetate elution) and thus affording two fractions, A and B.

Concentration of fraction A ($R_f = 0.3$ in 6:4 v/v hexane/ethyl acetate) gave compound 8 (158 mg, 15%) as a clear, colorless and viscous oil, $[\alpha]_D^{22} = -67.9$ (c 10.2, CH₂Cl₂)

$^1\text{H NMR}$ (400 MHz, CDCl₃) $\delta$ 7.80 (dd, $J = 8.0$, 1.3 Hz, 2H), 7.68 (dd, $J = 8.0$, 1.3 Hz, 2H), 7.50-7.40 (complex m, 6H), 3.70 (m, 2H), 3.50 (m, 2H), 3.35 (m, 1H), 3.30 (s, 3H), 3.20 (s, 3H), 2.25 (s, 1H), 2.10 (m, 1H), 1.52 (s, 1H), 1.30 (s, 3H), 1.29 (s, 3H), 1.25 (m, 1H), 1.11 (s, 9H).

$^{13}\text{C NMR}$ (100 MHz, CDCl₃) $\delta$ 136.6, 135.6, 135.1, 132.7, 130.1, 130.0, 128.1, 127.8, 99.6, 99.2, 77.7, 73.5, 71.3, 70.2, 66.9, 48.4, 48.0, 36.0, 27.1, 19.8, 17.8, 17.5

IR (KBr) $\nu_{max}$ 3567, 3461, 2952, 2933, 2858, 1472, 1462, 1427, 1374, 1132, 1086, 957, 908, 813, 738, 702 cm⁻¹

MS (ESI, +ve) m/z 539 (M+Na)⁺, 100%

HRMS m/z 539.2428 [M+Na]⁺ (calculated for C₃₂H₂₆O₅SiNa, 539.2436).

Concentration of fraction B ($R_f = 0.4$ in 6:4 v/v hexane/ethyl acetate) gave compound 9 (895 mg, 85%) as a clear, colorless and viscous oil, $[\alpha]_D^{22} = -68.2$ (c 1.1, CH₂Cl₂)

$^1\text{H NMR}$ (400 MHz, CDCl₃) $\delta$ 7.82 (dd, $J = 8.0$, 1.3 Hz, 2H), 7.69 (dd, $J = 8.0$, 1.3 Hz, 2H), 7.50-30 (complex m, 6H), 4.18 (m, 1H), 3.87 (s, 1H), 3.76 (t, $J = 10.0$ Hz, 1H), 3.62 (m, 1H), 3.55 (m, 1H), 3.35 (s, 3H), 3.22 (s, 3H), 2.40 (s, 1H), 2.00 (s, 1H), 1.77 (dt, $J =$
14.1, 4.0, 2.9 Hz, 1H), 1.38 (m, 1H), 1.34 (s, 3H), 1.33 (s, 3H), 1.07 (s, 9H)

$^{13}C$ NMR (100 MHz, CDCl$_3$) δ 136.2, 135.9, 134.7, 133.8, 129.6, 129.5, 127.5, 127.3, 99.3, 73.5, 71.9, 69.0, 68.0, 67.9, 47.9, 47.8, 36.5, 26.9, 19.3, 17.7, 17.5 (one signal obscured or overlapping)

IR (KBr) $v_{max}$ 3453, 2951, 2932, 2858, 1428, 1374, 1126, 1108, 1010, 914, 855, 739, 702 cm$^{-1}$

MS (ESI, +ve) m/z 539 [M+Na$^+$, 100%]

HRMS m/z 539.2447 [M+Na$^+$] (calcd for C$_{32}$H$_{49}$O$_5$SiNa, 539.2436).

**Compound 10**

**Step i:** A magnetically stirred solution of compound 9 (516 mg, 1.00 mmol) and p-methoxybenzyl chloride (163 µL, 1.20 mmol) in toluene (30 mL) was heated under reflux then treated with dibutyltin oxide (274 mg, 1.10 mmol). After 3 h the reaction mixture was cooled and concentrated under reduced pressure. The residue thus obtained was dissolved in ethyl acetate (30 mL) and the resulting solution washed with water (1 x 50 mL) and brine (1 x 50 mL) before being dried (Na$_2$SO$_4$), filtered then concentrated under reduced pressure. The resulting light-yellow oil was used, without any further purification in step ii as detailed immediately below.

**Step ii:** A magnetically stirred solution of the light-yellow oil obtained from step i in dichloromethane (30 mL) containing pyridine
(0.5 mL) was cooled to 0 °C then treated, dropwise, with triflic anhydride (185 µL, 1.10 mmol). After 3 h at 0 °C the reaction mixture was treated with sodium bicarbonate (30 mL of a saturated aqueous solution). The separated organic phase was washed with water (1 x 30 mL) and brine (1 x 30 mL) before being dried (NaSO₄), filtered then concentrated under reduced pressure. The resulting light-yellow oil was was subjected to flash chromatography (silica, 9:1 v/v 40–60 petroleum spirit/ethyl acetate elution) and thus affording, after concentration of the appropriate fractions (Rf = 0.4 in 8:2 v/v hexane/ethyl acetate), compound 10 (615 mg, 81%) as a clear, colorless and viscous oil, [α]D²⁸ = −76.9 (c 2.1, CH₂Cl₂).

¹H NMR (400 MHz, CDCl₃) δ 7.79 (dd, J = 8.0, 1.3 Hz, 2H), 7.63 (dd, J = 8.0, 1.3 Hz, 2H), 7.50-7.30 (complex m, 6H), 7.25 (d, J = 8.7 Hz, 2H), 6.84 (d, J = 8.7 Hz, 2H), 4.92 (m, 1H), 4.67 (s, 2H), 4.08 (m, 1H), 3.90 (t, J = 10.1 Hz, 1H), 3.79 (s, 3H), 3.66 (t, J = 10.1 Hz, 1H), 3.49 (dd, J = 10.1, 3.0 Hz, 1H), 3.39 (s, 3H), 3.25 (s, 3H), 1.83 (m, 1H), 1.50 (m, 1H), 1.39 (s, 3H), 1.35 (s, 3H), 1.06 (s, 9H).

¹³C NMR (100 MHz, CDCl₃) δ 159.1, 136.0, 135.6, 134.0, 133.0, 130.0, 129.9, 129.8, 129.2, 127.8, 127.3, 118.1 (q, J = 320 Hz), 113.6, 99.5, 99.4, 85.3, 75.2, 73.7, 72.9, 68.9, 66.9, 55.2, 47.9, 47.8, 35.5, 26.9, 19.2, 17.7, 17.5.

IR (KBr) νmax 2956, 2931, 2860, 1514, 1413, 1246, 1207, 1137, 1106, 911, 852, 701 cm⁻¹.

MS (ESI, ′+′) m/z 791 [(M+Na)⁺, 100%], 521 (85).

HRMS m/z 791.2508 [M+Na]⁺ (calculated for C₂₇H₄₇O₃(S)SSNa, 791.2507).
Compound 11

A magnetically stirred solution of compound 10 (544 mg, 0.68 mmol) in N,N-dimethylformamide (25 mL) maintained at 22 °C was treated, in portions, with sodium azide (91 mg, 1.40 mmol). After 0.5 h the reaction mixture was diluted with ethyl acetate (50 mL) then washed with lithium chloride (2 x 50 mL of a 5% w/v aqueous solution) and brine (1 x 50 mL) before being dried (Na2SO4), filtered then concentrated under reduced pressure. The resulting pink oil was subjected to flash chromatography (silica, 95:5 v/v 40-60 petroleum spirit/ethyl acetate elution) and thus affording, after concentration of the appropriate fractions (Rf = 0.6 in 82 v/v hexane/ethyl acetate), a white solid. Recrystallization (dichloromethane/diethyl ether) of this material gave compound 11 (410 mg, 91%) as a white, crystalline product, mp = 138-140 °C, [α]D20 = -98.5 (c 1.35, CH2Cl2).

1H NMR (400 MHz, CDCl3) δ 7.79 (dd, J = 8.0, 1.3 Hz, 2H), 7.65 (dd, J = 8.0, 1.3 Hz, 2H), 7.50-30 (complex m, 6H), 7.29 (d, J = 8.7 Hz, 2H), 6.84 (d, J = 8.7 Hz, 2H), 4.86 (d, J = 10.3 Hz, 1H), 4.66 (d, J = 10.3 Hz, 1H), 3.78 (s, 3H), 3.71 (m, 1H), 3.61 (t, J = 9.4 Hz, 1H), 3.50 (t, J = 9.4 Hz, 1H), 3.41 (t, J = 9.4 Hz, 1H), 3.38 (s, 3H), 3.22 (s, 3H), 3.02 (m, 1H), 1.61 (dt, J = 13.1, 4.7 Hz, 1H), 1.37 (s, 3H), 1.25 (s, 3H), 1.30 (m, 1H), 1.06 (s, 9H)

13C NMR (100 MHz, CDCl3) δ 159.3, 136.2, 135.8, 134.1, 133.3, 130.4, 129.9(3), 129.8(7), 129.7, 127.7, 127.4, 113.7, 99.3, 99.2, 81.1, 74.9, 73.3, 72.0, 68.5, 59.5 55.3, 47.9, 47.8, 36.2, 26.8, 19.2, 17.8, 17.5

S10
IR (KBr) \( \nu_{\text{max}} \) 2954, 2932, 2905, 2858, 2102, 1613, 1514, 1249, 1112, 1035, 825, 703 cm\(^{-1}\)

MS (ESI, +ve) \( m/z \) 684 [(M+Na\(^+\)], 100%]

HRMS \( m/z \) 684.3090 [M+Na\(^+\)] (calcd for C\(_{30}\)H\(_{31}\)O\(_2\)N\(_2\)SiNa, 684.3075).

**Compound 12**

![Chemical Structure](image)

**Step i:** A magnetically stirred solution of compound 11 (132 mg, 0.2 mmol) in methanol/water (4 mL of a 1:1 v/v mixture) maintained at 50 °C was treated with triphenylphosphine (101 mg, 0.4 mmol). After 3 h the reaction mixture was cooled to 22 °C then treated with Na\(_2\)SO\(_4\) (1.00 g) then filtered and the solids so retained were washed with dichloromethane (5 mL). The combined filtrates were concentrated under reduced pressure to yield a white solid comprised of a mixture of the anticipated amine and triphenylphosphine oxide. This material was used without purification in **step ii** as detailed immediately below.

**Step ii:** A magnetically stirred solution of 3-(methoxymethoxy)benzoic acid (47 mg, 0.26 mmol) in dichloromethane (5 mL) maintained at 22 °C was treated with freshly distilled \( N,N \)-diisopropylethylamine (51 \( \mu \)L, 0.29 mmol) and HATU (107 mg, 0.28 mmol). The resulting mixture was stirred at 22 °C for 0.25 h then treated, dropwise, with a solution of the white solid obtained from

S11
step i in dichloromethane (5 mL). After 16 h the reaction mixture was diluted with dichloromethane (15 mL) then washed with water (1 x 50 mL) and brine (1 x 50 mL) before being dried (Na₂SO₄), filtered then concentrated under reduced pressure. The resulting light-yellow oil was subjected to flash chromatography (silica, 9:1 v/v 40–60 petroleum spirit/ethyl acetate elution) and thus affording, after concentration of the appropriate fractions (Rf = 0.4 in 7:3 v/v hexane/ethyl acetate), compound 12 (129 mg, 81%) as a clear, colorless and viscous oil, [α]D²⁰ = −55.8 (c 3.6, CH₂Cl₂).

¹H NMR (400 MHz, CDCl₃) δ 7.80 (m, 2H), 7.64 (m, 2H), 7.44-7.24 (complex m, 6H), 7.13 (m, 3H), 7.03 (m, 1H), 6.67 (m, 2H), 5.54 (d, J = 7.4 Hz, 1H), 5.18 (s, 2H), 4.79 (d, J = 11.8 Hz, 1H), 4.61 (d, J = 11.8 Hz, 1H), 3.86 (m, 1H), 3.72 (s, 3H), 3.71-3.58 (complex m, 3H), 3.47 (s, 4H), 3.36 (s, 3H), 3.27 (s, 3H), 1.98 (m, 1H), 1.45 (m, 1H), 1.37 (s, 3H), 1.34 (s, 3H), 1.06 (s, 9H)

¹³C NMR (100 MHz, CDCl₃) δ 166.8, 159.2, 157.4, 136.4, 136.3, 135.9, 134.4, 133.6, 130.8, 130.0, 129.8(4), 129.7(6), 129.5, 127.7, 127.5, 120.1, 119.2, 115.1, 113.9, 99.4, 99.2, 94.5, 78.4, 73.8, 73.6, 72.7, 69.2, 56.2, 55.2, 49.0, 48.1, 47.9, 37.0, 27.1, 19.4, 17.9, 17.6

IR (KBr) νmax 3296, 2952, 2857, 1640, 1514, 1428, 1206, 1135, 1021, 825, 704 cm⁻¹

MS (ESI, +ve) m/z 822 [(M+Na)⁺, 100%], 800 [(M+H)⁺, 10%]

HRMS m/z 800.3841 [M+H]⁺ (calcld for C₄₀H₂₄NO₃₃Si, 800.3824).
Compound 13

Step i: A magnetically stirred solution of compound 12 (1.04 g, 1.31 mmol) in dichloromethane/water (27.5 mL of a 10:1 v/v mixture) maintained at 22 °C was treated with DDQ (446 mg, 1.9 mmol). After 16 h NaHCO₃ (2.00 g) was added to the reaction mixture that was then filtered and the solids so retained washed with dichloromethane (25 mL). The combined filtrates were washed with water (1 x 50 mL) and brine (1 x 50 mL) before being dried (Na₂SO₄), filtered then concentrated under reduced pressure to yield a light-yellow oil. This material was used without further purification in step ii as detailed immediately below.

Step ii: A magnetically stirred solution of the crude product from step i in dichloromethane (20 mL) maintained at 22 °C was treated with tetra-a-butyramonium fluoride (3 mL of a 1 M solution in THF, 3 mL, 3 mmol). After 16 h, the reaction mixture was diluted with dichloromethane (20 mL) and the resulting solution washed with water (1 x 50 mL) and brine (1 x 50 mL) before being dried (Na₂SO₄), filtered then concentrated under reduced pressure. The resulting light-yellow oil was subjected to flash chromatography (silica, 6:4 v/v 40–60 petroleum spirit/ethyl acetate elution) and thus affording, after concentration of the appropriate fractions (Rₓ = 0.4 in 1:1 v/v hexane/ethyl acetate) a white solid. Recrystallization (ethyl acetate/n-hexane) of this material afforded compound 13 (538 mg, 93%) as a white, crystalline solid, mp = 198.5-200 °C, [α]D₂⁰ = −98.5 (c 1.35, CH₂Cl₂).

¹H NMR (400 MHz, CD₃OD) δ 7.51 (m, 1H), 7.47 (d, J = 7.8, 1H), 7.37 (t, J = 7.8 Hz, 1H), 7.20 (m, 1H), 5.24 (s, 2H), 4.00 (m, 1H), 3.69 (m, 1H), 3.62 (t, J = 9.5 Hz, 1H), 3.46 (s, 3H), 3.42 (m, 2H), 3.29 (s, 6H), 2.18 (m, 1H), 1.49 (m, 1H), 1.31 (s, 6H) (signals due the OH and NH group protons not observed).
$^{13}$C NMR (100 MHz, CD$_3$OD) δ 168.6, 157.3, 135.9, 129.1, 120.3, 119.1, 114.9, 99.2, 99.0, 94.1, 73.3, 71.7 (1), 71.6 (6), 66.7, 54.9, 50.1, 46.9, 46.8, 35.7, 16.5 (one signal obscured or overlapping)

IR (KBr) $v_{max}$ 3473, 3230, 3089, 2924, 1636, 1567, 1440, 1240, 1130, 973, 903, 825, 741 cm$^{-1}$

MS (ESI, +ve) m/z 464 [(M+Na)$^+$, 100%], 442 [(M+H)$^+$, 5]

HRMS m/z 442.2081 [M+H]$^+$ (calcld for C$_{20}$H$_2$NO$_3$, 442.2071).
Compound 14 and the corresponding bis-ester

A magnetically stirred solution of compound 13 (230 mg, 0.52 mmol) in dichloromethane (25 mL) maintained at 22 °C was treated with EDC (150 mg, 0.78 mmol). A solution of 6-methylsalicylic acid (87 mg, 0.57 mmol) in dichloromethane (5 mL) was then added, via syringe pump, to the reaction mixture over 8 h and immediately thereafter it was concentrated under reduced pressure. The ensuing light-yellow oil was subjected to flash chromatography (silica, 10:0 to 6:4 v/v 40–60 petroleum spirit/ethyl acetate elution) and thus affording three fractions, A, B and C.

Concentration of fraction A (Rf = 0.8 in 6:4 v/v hexane/ethyl acetate) gave the illustrated bis-ester (50 mg, 15%) as an amorphous white solid, [α]D20 = −66.1 (c 7.0, CH2Cl2).

1H NMR (400 MHz, [D6]DMSO) δ 10.50 (s, 1H), 10.42 (s, 1H), 7.84 (d, J = 9.1 Hz, 1H), 7.4–7.21 (complex m, 5H), 7.13 (m, 1H), 6.79 (m, 2H), 6.72 (m, 2H), 5.63 (t, J = 10.1 Hz, 1H), 5.47 (m, 1H), 5.17 (s, 2H), 4.79 (m, 1H), 4.18 (t, J = 10.0 Hz, 1H), 4.03 (t, J = 10.0 Hz, 1H), 3.99 (s, 3H), 3.26 (s, 3H), 2.62 (m, 1H), 2.56 (s, 3H), 2.54 (s, 3H), 2.16 (m, 1H), 1.26 (s, 3H), 1.24 (s, 3H).
$^{13}$C NMR (100 MHz, [(CD$_3$)$_2$CO] $\delta$ 171.2, 170.5, 166.8, 161.8, 161.5, 158.0, 141.4, 140.9, 136.9, 134.3, 134.2, 130.0, 123.2(3), 123.2(1), 123.1(5), 120.9, 119.7, 115.8, 115.7, 115.6, 115.1, 114.6, 100.0(4), 99.9(8), 94.8, 74.6, 74.5, 71.1, 70.9, 69.5, 55.9, 48.4, 48.1, 48.0, 33.7, 23.3, 23.0, 17.7(0), 17.6(6) (the additional signals are attributed to the presence of amide rotamers)

IR (KBr) $\nu_{max}$ 3281, 3065, 2955, 2937, 1733, 1658, 1456, 1250, 1209, 1006, 801, 700 cm$^{-1}$

MS (ESI, +ve) $m/z$ 732 [(M+Na)$^+$, 100%], 710 [(M+Na)$^+$, 5]

HRMS $m/z$ 710.2825 [M+H]$^+$ (caled for C$_9$H$_9$NO$_2$, 710.2807).

Concentration of fraction B ($R_f$ = 0.5 in 6:4 v/v hexane/ethyl acetate) gave compound 14 (177 mg, 60%) as a clear, colorless and viscous oil, [α]$_D^{20}$ = −94.2 (c 0.7, CH$_2$Cl$_2$).

$^1$H NMR (400 MHz, [(CD$_3$)$_2$CO] $\delta$ 10.55 (s, 1H), 7.76 (d, $J$ = 9.3 Hz, 1H), 7.40-7.21 (complex m, 4H), 7.12 (m, 1H), 6.72 (m, 2H), 5.47 (t, $J$ = 10.2 Hz, 1H), 5.17 (s, 2H), 4.58 (m, 1H), 4.34 (d, $J$ = 3.9 Hz, 1H), 3.88 (m, 2H), 3.53 (t, $J$ = 9.7 Hz, 1H), 3.39 (s, 3H), 3.27 (s, 3H), 3.17 (s, 3H), 2.51 (s, 3H), 2.24 (dt, $J$ = 13.0, 4.8 Hz, 1H), 1.88 (m, 1H), 1.26 (s, 3H), 1.21 (s, 3H)

$^{13}$C NMR (100 MHz, [(CD$_3$)$_2$CO] $\delta$ 171.5, 171.4, 166.9, 162.1, 162.0, 158.2, 141.7, 137.3, 137.2, 134.5, 130.2, 123.5, 123.4, 121.1, 119.8, 116.0, 115.9, 115.8, 114.6, 100.1, 99.8, 95.0, 75.6, 74.5(1), 74.4(9), 70.0, 67.5, 67.4, 56.1, 48.7, 48.6, 48.0, 47.9, 37.1(2), 37.0(9), 23.5, 18.0, 17.9 (the additional signals are attributed to the presence of amide rotamers)

IR (KBr) $\nu_{max}$ 3461, 3311, 2942, 1730, 1652, 1541, 1292, 1136, 1116, 906, 801, 693 cm$^{-1}$

MS (ESI, +ve) $m/z$ 598 [(M+Na)$^+$, 100%], 576 [(M+H)$^+$, 7]

HRMS $m/z$ 576.2451 [M+H]$^+$ (caled for C$_9$H$_9$NO$_2$, 576.2439).

Concentration of fraction C ($R_f$ = 0.4 in 1:1 v/v hexane/ethyl acetate) gave compound 13 (41 mg, 18% recovery) as a white, crystalline
solid that was identical in all respects with an authentic sample.

**Compound 15**

![Chemical Structure](image)

A magnetically stirred solution of compound 14 (27 mg, 0.046 mmol) in dichloromethane (5 mL) maintained at 0 °C was treated, dropwise over 0.5 h, with a solution of trichloroacetyl isocyanate (6 μL, 0.05 mmol) in dichloromethane (5 mL). After the addition was complete the reaction mixture was immediately quenched with methanol (1 drop) then concentrated under reduced pressure. The resulting oil was dissolved in methanol (5 mL) and the solution thus obtained treated with silica gel (200 mg). The resulting suspension was stirred at 22 °C for 5 h then concentrated under reduced pressure and the solid thus obtained subjected to flash chromatography (silica, 100:0 to 60:40 v/v 40–60 petroleum spirit/ethyl acetate elution). Concentration of the appropriate fractions (Rf = 0.6 in 6:4 v/v hexane/ethyl acetate) then gave compound 15 (25 mg, 88%) as a white solid, mp (decomposition above 200 °C), [α] D 20 = -69.5 (c 6.7, MeOH).
$^1$H NMR (400 MHz, [CD$_3$]$_2$CO) δ 10.53 (s, 1H), 7.75 (d, $J = 9.1$ Hz, 1H), 7.40-7.21 (complex, m, 4H), 7.12 (m, 1H), 6.71 (dd, $J = 8.0, 3.0$ Hz, 2H), 5.95 (broad s, 2H), 5.51 (t, $J = 10.2$ Hz, 1H), 5.17 (s, 2H), 4.92 (m, 1H), 4.64 (m, 1H), 4.04 (t, $J = 10.0$ Hz, 1H), 3.77 (t, $J = 10.0$ Hz, 1H), 3.39 (s, 3H), 3.25 (s, 3H), 3.18 (s, 3H), 2.53 (s, 3H), 2.40 (m, 1H), 1.93 (m, 1H), 1.24 (s, 3H), 1.21 (s, 3H).

$^{13}$C NMR (100 MHz, [CD$_3$]$_2$CO) δ 171.6, 167.1, 162.2, 158.3, 157.1, 157.0, 141.8, 137.3(3), 137.3(0), 134.7, 130.3, 123.6, 121.2, 119.9, 116.1, 115.9, 114.8, 100.3, 100.1, 95.2, 75.1(1), 75.1(3), 71.7, 70.1, 69.4(4), 69.4(3), 56.2, 48.6, 48.5, 48.2, 48.0, 34.5(8), 34.5(5), 23.6, 18.0(5), 17.9(8) (the additional signals are attributed to the presence of amide and/or carbamate rotamers)

IR (KBr) ν$_{max}$ 3657, 3465, 3461, 3319, 3288, 3185, 2976, 2897, 1709, 1653, 1605, 1586, 1547, 1374, 1251, 1118, 1081, 1037, 803, 702 cm$^{-1}$

MS (ESI, +ve) $m/z$ 641 [(M+Na)$^+$, 100%], 619 [(M+H)$^+$, 5]

HRMS $m/z$ 619.2512 [M+H]$^+$ (calcd for C$_{29}$H$_{37}$N$_2$O$_6$, 619.2498).
Compound 2

A sample of compound 15 (5 mg, 0.008 mmol) maintained at 22 °C was treated with trifluoroacetic acid (2.7 mL) then water (0.35 mL) and the resulting mixture stirred magnetically for 48 h before being diluted with water (20 mL) and then sufficient sodium bicarbonate (saturated aqueous solution) to achieve neutrality. The ensuing mixture was extracted with ethyl acetate (3 x 30 mL) and the combined organic phases were washed with brine (1 x 100 mL) before being dried (Na₂SO₄), filtered then concentrated under reduced pressure. The resulting light-yellow oil was subjected to flash chromatography (silica, 10:0 to 5:5 v/v chloroform/methanol elution) and thus affording, after concentration of the appropriate fractions (Rf = 0.3 in 8:2 v/v chloroform/methanol), compound 2\textsuperscript{1} (3 mg, 81%) as a white solid, no mp (decomposition above 200 °C), [α]\textsubscript{D}\textsuperscript{20} = +21.3 (c 1.5, MeOH).

\textsuperscript{1}H NMR (600 MHz, [D\textsubscript{6}]\textsubscript{2}CO) δ 7.60 (d, J = 9.1 Hz, 1H), 7.27-7.17 (complex m, 4H), 6.92 (m, 1H), 6.71 (m, 2H), 5.85 (broad s, 2H), 5.35 (dd, J = 10.6, 9.4 Hz, 1H), 4.76 (m, 1H), 4.54 (m, 1H), 3.84 (t, J = 9.4 Hz, 1H), 3.59 (t, J = 9.4 Hz, 1H), 2.46 (s, 3H), 2.34 (m, 1H), 1.89 (m, 1H) (signals due to OH protons not observed)

\textsuperscript{13}C NMR (150 MHz, [D\textsubscript{6}]\textsubscript{2}CO) δ 171.4, 167.0, 161.8, 158.3, 157.4, 141.9, 137.3, 134.4, 130.2, 123.4, 119.0, 118.9, 115.8, 115.3, 115.1, 78.4, 76.1, 74.6, 72.8, 48.1, 34.1, 23.2
IR (KBr) $\nu$ max 3349, 1675, 1358, 1194, 1134, 801, 722 cm$^{-1}$

MS (ESI, +ve) m/z 483 [(M+Na)$^+$, 100%], 461 [(M+H)$^+$, 3]

HRMS m/z 461.1564 [M+H]$^+$ (caled for C$_{22}$H$_7$N$_2$O$_6$, 461.1555).
**Table S1: Comparison of the $^{13}$C NMR Spectral Data Reported by Ishibashi and Co-workers for Niacinamide II with the Equivalent Data Recorded for Compound 2 Prepared by the Present Route**

<table>
<thead>
<tr>
<th>$\delta_C$ (ex. Ishibashi)$^a$</th>
<th>$\delta_C$ (ex. Present Route)$^b$</th>
<th>$\Delta \delta$</th>
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<td>171.3</td>
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<tr>
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<tr>
<td>23.1</td>
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</tr>
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</table>

$^a$Spectrum recorded in (CD$_3$)$_2$CO at 150 MHz, data taken from reference 4.

$^b$Spectrum recorded in (CD$_3$)$_2$CO at 150 MHz.
Crystallographic Studies. Crystallographic Data.

Compound 11. Cu$_{2}$H$_{16}$N$_{4}$O$_{6}$Si, $M = 661.85$, $T = 150$ K, orthorhombic, space group $P2_12_12_1$, $Z = 4$, $a = 9.4205(1)$ Å, $b = 17.8471(2)$ Å, $c = 21.3542(2)$ Å; $V = 3590.25(6)$ Å$^3$, $D_a = 1.224$ Mg cm$^{-3}$, 7262 unique data ($2\theta_{\text{max}} = 147.8^\circ$), $R = 0.037$ [for 7034 reflections with $I > 2.0\sigma(I)$]; $R_W = 0.100$ (all data), $S = 1.04$.

Compound 13. C$_{20}$H$_{14}$N$_{4}$O$_{6}$, $M = 441.47$, $T = 150$ K, monoclinic, space group $P2_1$, $Z = 2$, $a = 6.4650(1)$ Å, $b = 12.7607(2)$ Å, $c = 13.6633(2)$ Å; $V = 1127.19(4)$ Å$^3$, $D_a = 1.301$ Mg cm$^{-3}$, 3237 unique data ($2\theta_{\text{max}} = 147^\circ$), $R = 0.029$ [for 3139 reflections with $I > 2.0\sigma(I)$]; $R_W = 0.074$ (all data), $S = 1.06$.

Structure Determinations. Images for compounds 11 and 13 were measured on a diffractometer (Cu K$_\alpha$, mirror monochromator, $\lambda = 1.54184$ Å) fitted with an area detector and the data extracted using the CrystAlis package.$^7$ The structure solution for these compounds were solved by direct methods (SIR92)$^8$ then refined using the CRYSTALS program package.$^7$ Atomic coordinates, bond lengths and angles, and displacement parameters have been deposited at the Cambridge Crystallographic Data Centre (CCDC nos. 1578392 and 1584449). These data can be obtained free-of-charge via www.ccdc.cam.ac.uk/data_request/cif, by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.
Figure S2. Structure of compound 13 (CCDC 1584449). Anisotropic displacement ellipsoids show 30% probability levels. Hydrogen atoms are drawn as circles with small radii.
References

100 MHz $^{13}$C NMR Spectrum of Compound 6
(recorded in CDCl$_3$)
400 MHz $^1$H NMR Spectrum of Compound 7
(recording in CDCl$_3$)
100 MHz $^{13}$C NMR Spectrum of Compound 8
(recorded in CDCl$_3$)
100 MHz $^{13}$C NMR Spectrum of Compound 9
(recorded in CDCl$_3$)
400 MHz $^1$H NMR Spectrum of Compound II
(recorded in CDCl$_3$)
400 MHz $^1$H NMR Spectrum of Compound 12
(recorded in CDCl$_3$)
400 MHz $^1$H NMR Spectrum of Compound 13
(recorded in CD$_3$OD)
400 MHz $^1$H NMR Spectrum of bis-ester
[recorded in (CD$_3$)$_2$CO]
400 MHz $^1$H NMR Spectrum of Compound 15
[recorded in (CD$_3$)$_2$CO]
100 MHz $^{13}$C NMR Spectrum of Compound 15
[recorded in (CD$_3$)$_2$CO]
Structure of compound 4 of Publication 2 (CCDC 1451755) with labeling of selected atoms. Anisotropic displacement ellipsoids show 30% probability levels. Hydrogen atoms are drawn as circles with small radii.
Structure of compound 8 of Publication 2 (CCDC 1531843) with labeling of selected atoms. Anisotropic displacement ellipsoids show 30% probability levels. Hydrogen atoms are drawn as circles with small radii.
Structure of compound 13 of Publication 2 (CCDC 1531844) with labeling of selected atoms. Anisotropic displacement ellipsoids show 30% probability levels. Hydrogen atoms are drawn as circles with small radii.
Structure of compound 11 of Publication 6 (CCDC 1578392) with labeling of selected atoms. Anisotropic displacement ellipsoids show 30% probability levels. Hydrogen atoms are drawn as circles with small radii.
Structure of compound **13** of *Publication 6* (CCDC 1584449) with labeling of selected atoms. Anisotropic displacement ellipsoids show 30% probability levels. Hydrogen atoms are drawn as circles with small radii.