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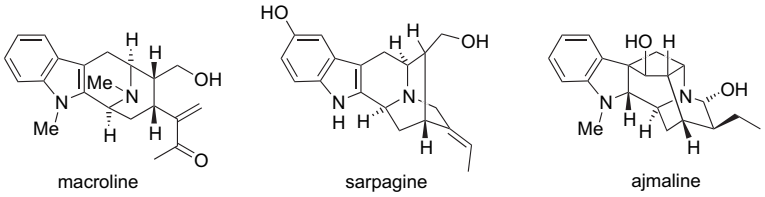
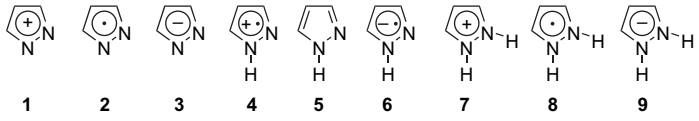
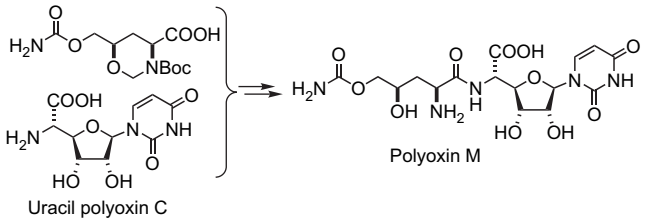
Contents

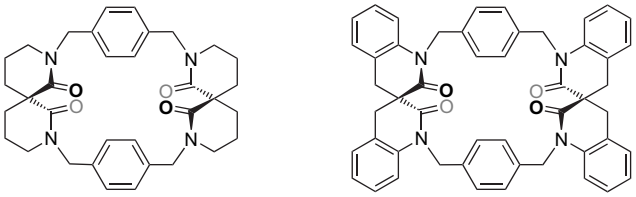
REPORT



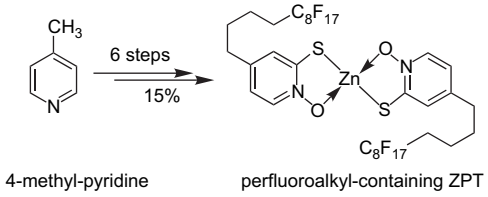
ARTICLES

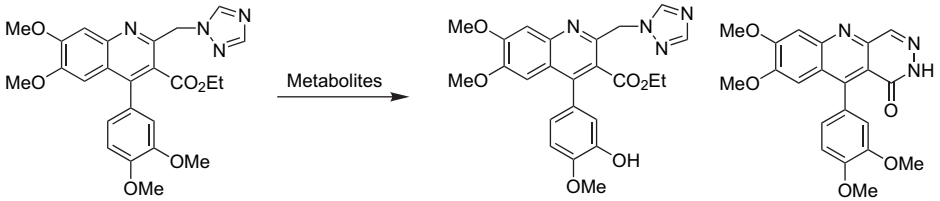


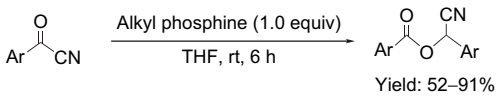




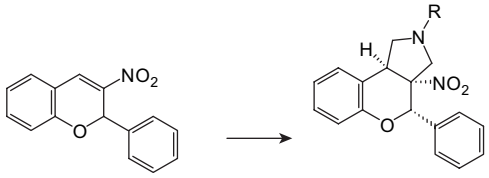
Macrocyclic ionophores *d*,*l*-**3** and *d*,*l*-**4** with four amide carbonyl ligands were synthesized and investigated in lithium ion-selective electrodes.

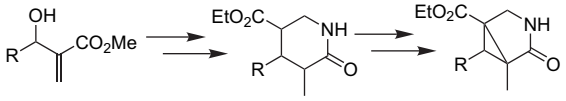


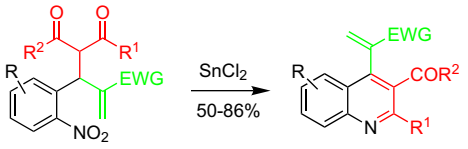


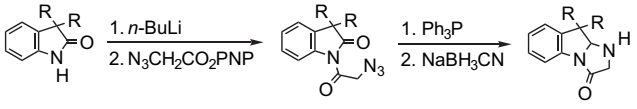


In the presence of alkyl phosphines, reductive coupling of acyl cyanides proceeded smoothly at room temperature in THF to give the corresponding *O*-acyl cyanohydrins in moderate to high yields. The possible mechanism was discussed on the basis of deuterium labeling and control experiments, indicating that one hydride transfer took place from alkyl phosphine to cyanohydrin.

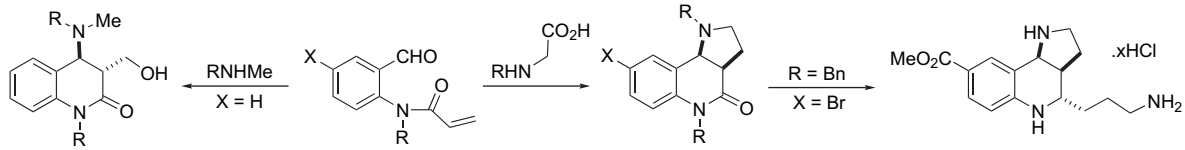


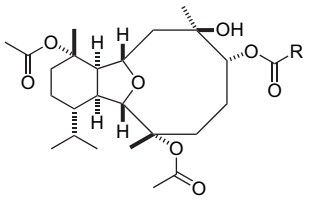




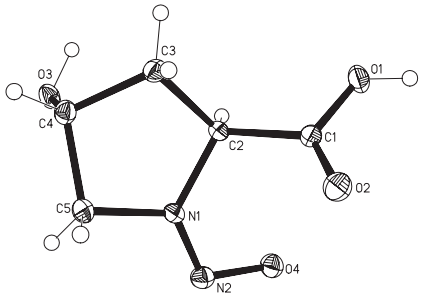


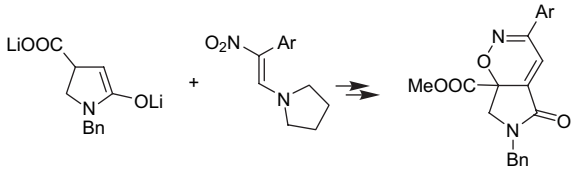
Two methods for annulation of glycine to the 1 and 2 positions of oxindoles are described. One method introduces *p*-nitrophenyl α-azidoacetate as an N-acylation reagent. The other method involves a selective BF_3–Et_3SiH imide reduction.

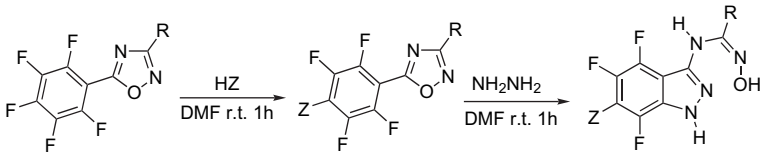


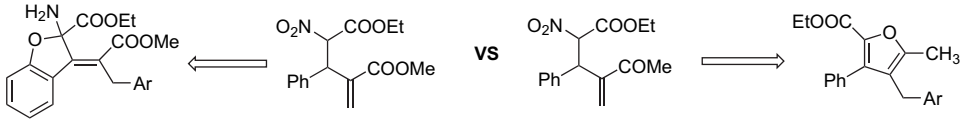


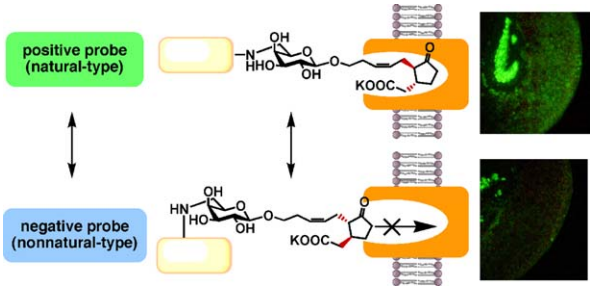
The discovery and structure determination of a homologous series of eunicellin-based diterpenes from the gorgonian *Acalycigorgia* sp. by NMR, MS and MS/MS methods are described.

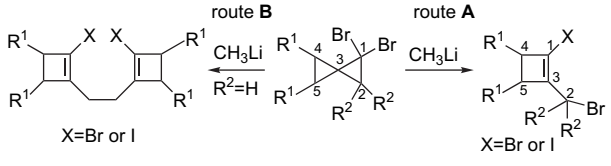


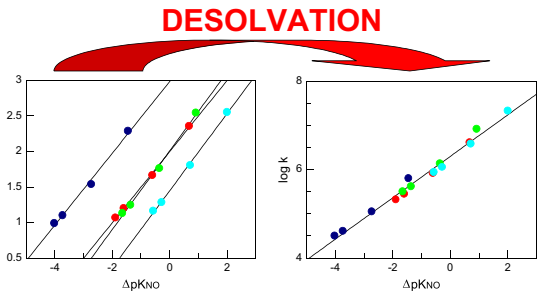


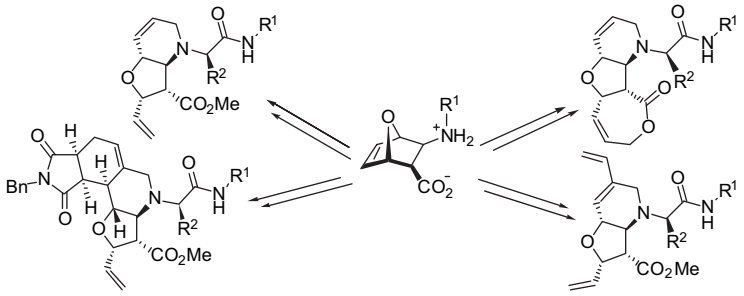


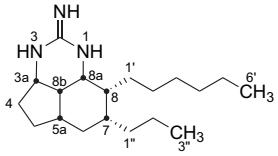


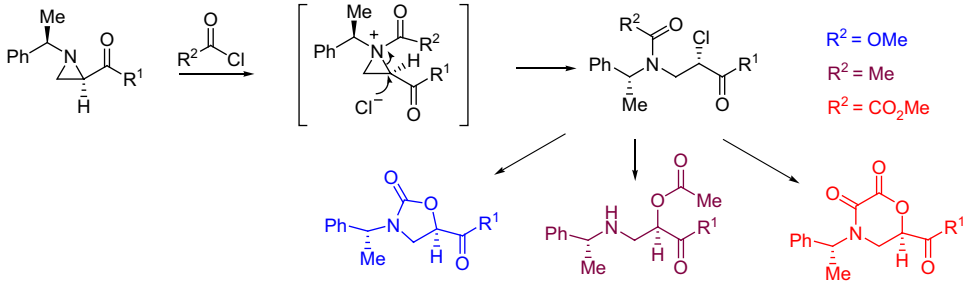


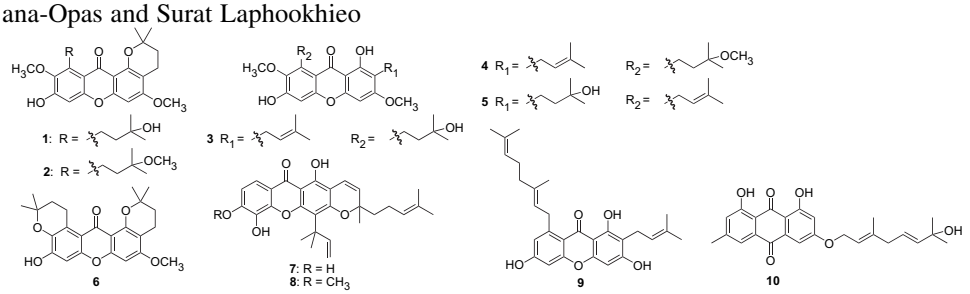












Nine new prenylated xanthones (**1–9**) and a new anthraquinone (**10**) were isolated from the roots and barks of *Cratoxylum formosum* ssp. *pruniflorum*. In addition, antibacterial and cytotoxic activities of the isolates were also evaluated.

*Corresponding author
ⓘ+ Supplementary data available via ScienceDirect

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Recent advances in the chemistry of macroline, sarpagine and ajmaline-related indole alkaloids

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Contents

Abbreviations: Ac, acetyl; AD, asymmetric dihydroxylation; AIBN, 2,2′-azobis*iso*butyronitrile; Ar, aryl; 9-BBN, 9-borabicyclo[3.3.1]nonane; Bn, benzyl; Boc, *tert*-butoxycarbonyl; Bu, butyl; Bz, benzoyl; cat, catalytic; CBz, benzyloxycarbonyl; CLB, chlorobenzoyl; Cy, cyclohexyl; d, days; dba, (*E*,*E*)-dibenzylideneacetone; DBN, 1,5-diazabicyclo[4.3.0]non-5-ene; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; DDQ, 2,3,5,6-dichlorodicyanoquinone; de, diastereoisomeric excess; DHQ, dihydroquinine; DHQD, dihydroquinidine; DIBAL-H, di*iso*butylaluminium hydride; DMAP, 4-(*N*,*N*-dimethylamino)pyridine; DME, 1,2-dimethoxyethane; DMF, *N*,*N*-dimethylformamide; DMP, Dess–Martin periodinane; DMPU, *N*,*N*′-dimethyl-*N*,*N*′-propyleneurea; DMS, dimethyl sulfide; DMSO, dimethylsulfoxide; dr, diastereoisomeric ratio; *E*, entgegen; EDCI, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; ee, enantiomeric excess; Et, ethyl; h, hours; IBX, 2-iodoxybenzoic acid; IMDA, intramolecular Diels–Alder; LDA, lithium di*iso*propylamide; Me, methyl; min, minutes; N, normal; NBS, *N*-bromosuccinimide; NMO, *N*-methylmorpholine-*N*-oxide; Np, naphthalenide; *o*-Ns, *ortho*-nitrophenylsulfonyl; Ph, phenyl; PHAL, phthalazine; *p*-TSA, *para*-toluenesulfonic acid; py, pyridine; rt, room temperature; Sia_2BH, di*iso*amylborane; SM, starting material; TBAF, tetrabutylammonium fluoride; TBDMS, *tert*-butyldimethylsilyl; TES, triethylsilyl; Tf, trifluoromethanesulfonyl; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TIPS, tri*iso*propylsilyl; TMS, trimethylsilyl; TPAP, tetrapropylammonium perruthenate; Ts, *para*-toluenesulfonyl; *Z*, zusammen.

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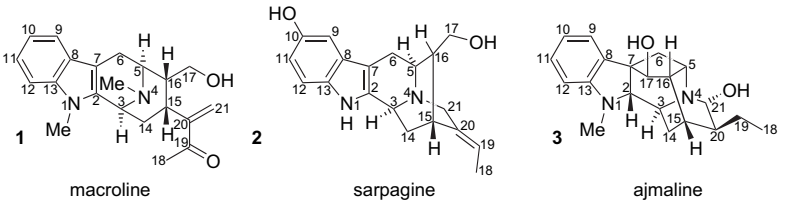
1. Introduction and scope

A huge variety of indole alkaloids are known,[1–7] many of which have been submitted to total synthesis. This review concerns the chemistry of indole alkaloids related to macroline **1**, sarpagine **2** and ajmaline **3**. The structures of these three species are shown in Scheme 1.



Scheme 1.

The skeletal numbering shown is the biogenetic numbering proposed[8] by LeMen and Taylor and is used throughout this review. It may be seen that there is significant structural similarity among the three compounds. All possess an indole-annulated azabicyclo[3.3.1] structure and various efforts towards this structural motif are detailed below. Macroline-related alkaloids are defined as those having the same skeletal connectivity as macroline. They crucially do not possess an N4–C21 linkage. Sarpagine-related alkaloids are defined as those having the same skeletal connectivity as sarpagine, specifically with an N4–C21 linkage and the C16-(*R*) configuration shown. Ajmaline-related alkaloids are defined as those having the same skeletal connectivity as ajmaline, also with an N4–C21 linkage but with the C16-(*S*) configuration epimeric to that of sarpagine as shown. Alkaloids with a quaternary C16 are known and are included herein. There also may or may not be a C7–C17 linkage, the quaternary C7 implied thus rendering the C2–C7 bond saturated. Additionally, the compounds under consideration may or may not be N1- and N4-substituted and may or may not possess indole ring oxygenation. Bis(indole) alkaloids in which one or both of the subunits consist of a macroline/sarpagine/ajmaline indole base are also included in this review.

It must be noted that, unlike ajmaline and sarpagine, macroline has not been isolated from natural sources. Many macroline-related alkaloids have, however, been isolated and it is believed that macroline, or an equivalent, is a likely biosynthetic precursor of various sarpagine alkaloids.

One can envisage the relationship in a synthetic sense, with 1,2- or 1,4-addition of N4 to C19 or C21, respectively, providing access to the sarpagan skeleton. Such a synthetic strategy has been employed in some of the total syntheses detailed herein. The reverse transformation may also be envisaged—quaternisation of N4, followed by Hofmann elimination (provided C20 has an appropriate hydrogen, e.g., in ajmaline) resulting in N4–C bond scission. This strategy has also been adopted in total synthesis, as will be seen, and interconversions of this nature are important in structural elucidation and stereochemical correlation.

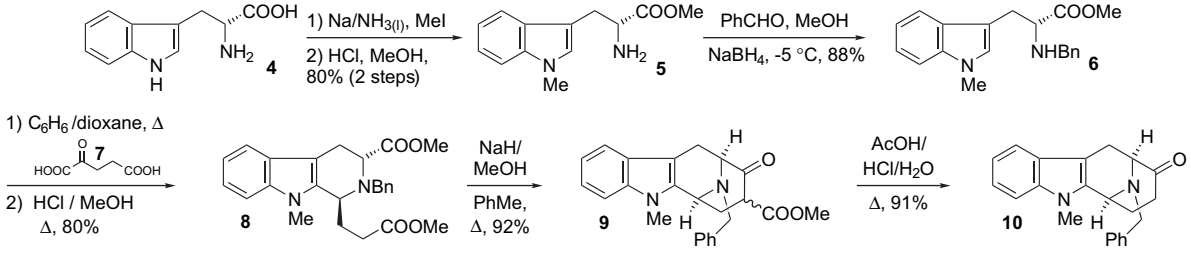
The field of macroline, sarpagine and ajmaline-related alkaloids was reviewed extensively by Cook[9,10] in 1993 and 1994 and again by Lounasmaa[11,12] in 1999 and 2001. As well as detailing reported synthetic endeavours relevant to the field, these excellent reviews give a comprehensive account of the species from which these alkaloids have been isolated (mostly genera *Rauvolfia* and *Alstonia*) and an overview of their biology, pharmacology, spectroscopic characteristics and proposals for their biosyntheses. Only chemistry of particular relevance, as well as that reported subsequent to these prior reviews or that not covered therein, is included here.

2. Cook's syntheses

Cook and co-workers have published extensively in the area of indole alkaloids and, in the last decade, have reported the partial and total syntheses of more than 40 macroline/sarpagine/ajmaline-related alkaloids, as well as bis(indole) alkaloids and related degradation products. These syntheses are detailed in this section and are grouped by the methodology used, as opposed to the final targets in question.

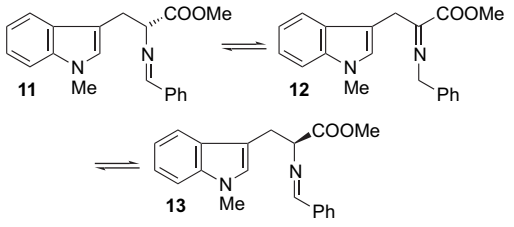
2.1. The tetracyclic ketone

Fundamental to Cook's syntheses is the tetracyclic ketone intermediate **10**. Its synthesis has been reviewed before,[9,11] but will be detailed here also due to its relevance to the following sections. The overview of the synthesis is shown in Scheme 2.



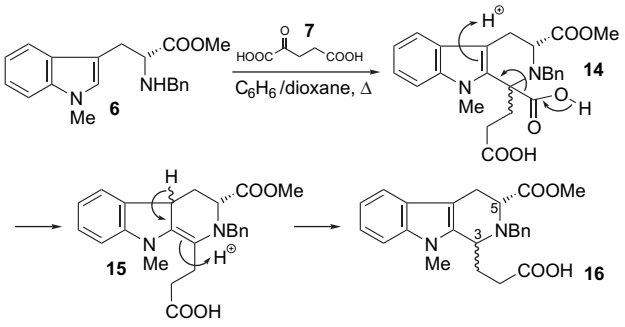
Scheme 2.

The synthesis outlined above, whilst only seven steps, has been the subject of extensive study and optimisation.[13] The individual steps merit consideration in detail. Starting from unnatural D-tryptophan **4**, N1-methylation and esterification were routine. The reductive amination to protect N4, however, required careful control. After stirring **5** with benzaldehyde for 2 h at room temperature to form the imine, sodium borohydride was added at −5 °C and allowed to react for 3 h. Longer reaction times or higher reaction temperatures led to erosion of the ee of **11** by imine isomerisation to **13** via **12** (Scheme 3).



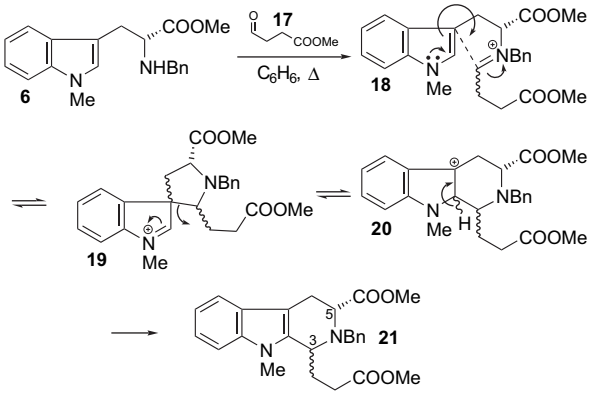
Scheme 3.

The Pictet–Spengler condensation (and subsequent esterification) shown in Scheme 2 is represented as affording solely the C3,C5-*trans* tetrahydro-β-carboline **8**. In fact a more complex series of events was occurring. As shown in Scheme 4, the initial Pictet–Spengler cyclisation proceeded to give a diastereoisomeric mixture of tetrahydro-β-carboline diacids **14**. These underwent decarboxylation as shown and it was therefore the protonation upon rearrangement of intermediate **15** that determined the diastereoisomeric ratio in the product, not the inherent selectivity in the Pictet–Spengler reaction.



Scheme 4.

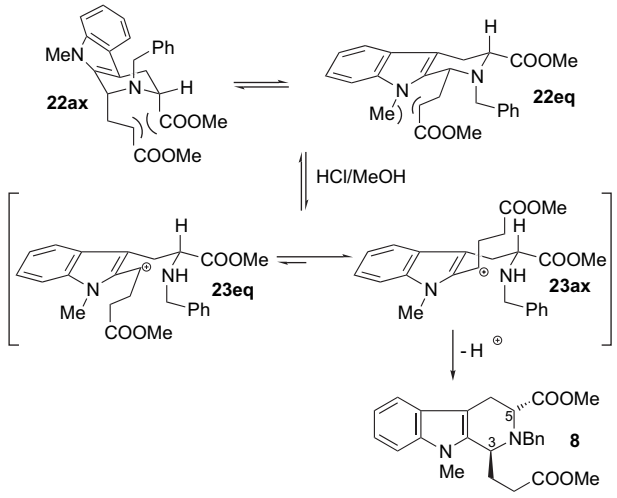
If the tetrahydro-β-carboline monoacid intermediates **16** were isolated, the diastereoisomeric ratio was found to be C3,C5-cis/trans=42:58. Alternatively, if methyl 3-formylpropionate **17** was used in place of 2-ketoglutaric acid **7**, the diastereoisomeric ratio in **21** was found to be C3,C5-cis/trans=28:72 (Scheme 5). This enhanced diastereoisomeric ratio was observed due to the lack of a post-cyclative decarboxylation step; in this instance, the ratio is a true representation of the inherent selectivity of the Pictet–Spengler cyclisation.



Scheme 5.

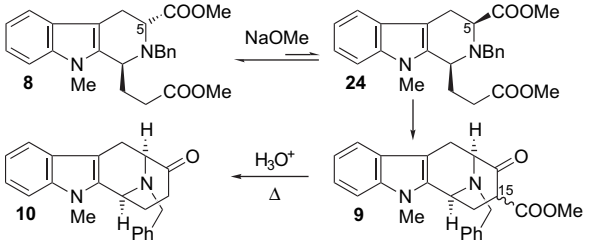
Whilst the reaction of methyl 3-formylpropionate **17** with **6** increased the diastereoselectivity in the formation of **21** via **18–20**, total selectivity was desired in order that tedious chromatography might be avoided and the sequence might be executed on a large scale. This was achieved by acid-catalysed isomerisation of the C3,C5-cis isomer to the more stable C3,C5-trans isomer, simply by treating the diastereoisomeric mixture **16** or **21** with methanolic HCl (for **16**, this also effected esterification). The isomerisation of **22** is

thought to proceed via a C3–N4 bond cleavage and formation of stabilised C3 cation **23** (Scheme 6).



Scheme 6.

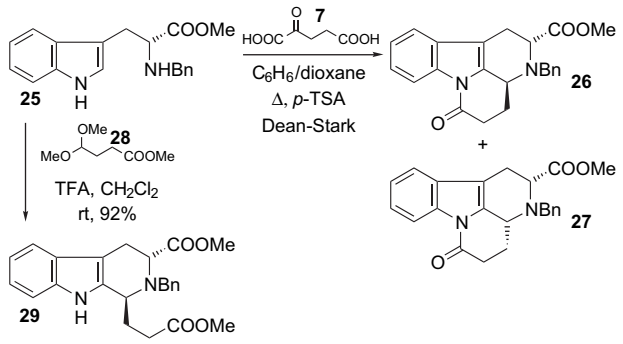
With pure **8** in hand, Dieckmann condensation to the tetracyclic system **9** was effected with sodium methoxide. The C3,C5-*trans*-configured tetrahydro-β-carboline **8** is unable to attain a conformation suitable for cyclisation, and so base-induced epimerisation of C5 must occur prior to cyclisation. Whilst the *cis* tetrahydro-β-carboline **24** is the less stable diastereoisomer (as established in Scheme 6), the small amount formed is irreversibly transformed to the tetracycle, the equilibrium then replenishes the amount of **24** present and all materials are eventually transformed into tetracycle **9** (Scheme 7). The epimerisation prior to Dieckmann cyclisation is the reason Cook's synthesis commences with the unnatural amino acid antipode. This (incorrect) initial C5 configuration induces the correct C3 configuration which, in turn, induces complete epimerisation at C5 to the correct configuration.



Scheme 7.

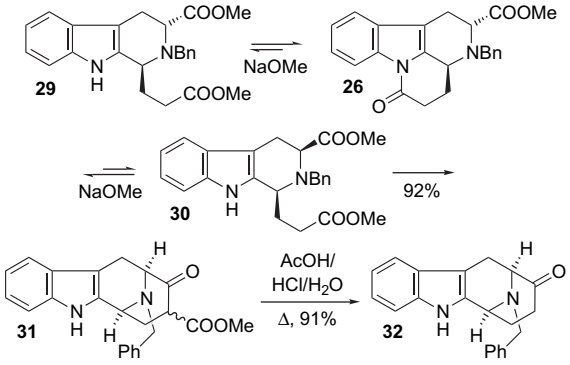
The uncontrolled configuration of C15 in **9** is of no consequence as acid-induced decarboxylation leads to key tetracycle **10** (seven steps from D-tryptophan, 47% overall yield). Cook's group have routinely performed this synthetic sequence on a 100-gram scale. As not all macroline/sarpagine/ajmaline alkaloids are N1-substituted, the tetracyclic ketone **32** has also been prepared[14] from **25** with a free N1–H. The synthesis was complicated by unwanted lactam formation, as shown in Scheme 8.

Acid/methanol-induced transformation of **27** to **29** did not occur, probably because the lactam moiety would destabilise the α-aryl cation intermediate. The reaction occurred as desired in the absence of a free carboxyl group, using **28**



Scheme 8.

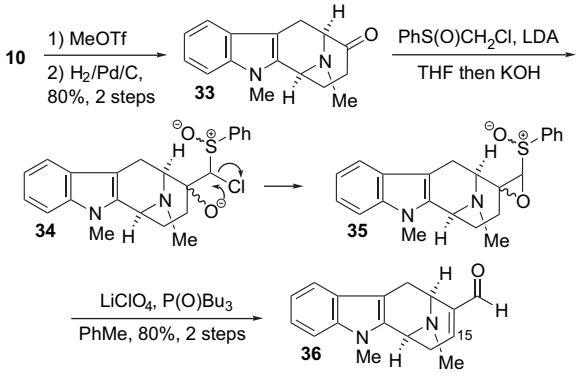
to give **29**. Upon exposure to base, **29** initially formed lactam **26**, but eventually gave the desired Dieckmann product **31** via **30**. Decarboxylation as before gave **32** (Scheme 9).



Scheme 9.

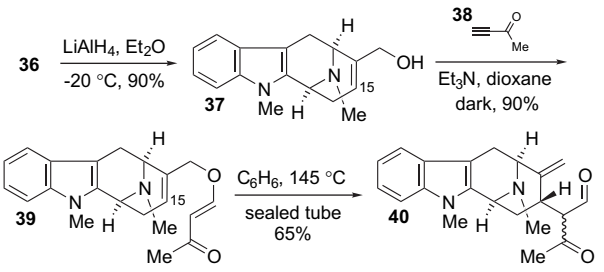
2.2. α,β-Unsaturated aldehyde formation and Claisen rearrangement: alstonerine, anhydromacrosalhinemethine and macrocarpamine

Tetracyclic ketone **10** was elaborated by Cook's group in the first total synthesis of (−)-alstonerine,[15] as shown in Scheme 10. Exchange of the N4-benzyl group for methyl to give **33** and elaboration of the ketone gave α,β-unsaturated aldehyde[16] **36** (via **34** and the intermediate epoxide **35**).



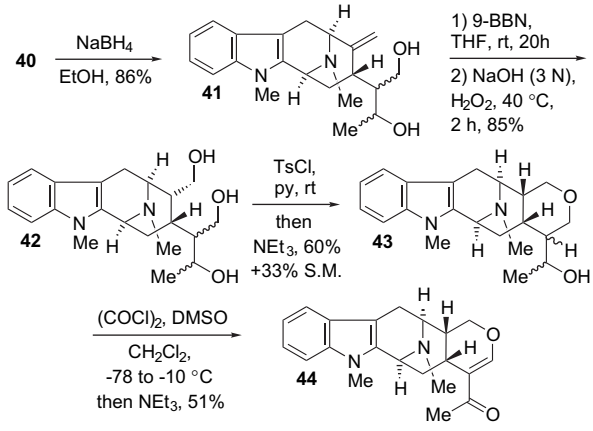
Scheme 10.

Studies had shown that intermolecular addition to the C15 position of **36** was not a facile process, so an intramolecular strategy was used. Reduction of **36** to **37** and formation of vinylogous ester **39** using **38** allowed C15 functionalisation via a Claisen rearrangement to give **40** (Scheme 11).



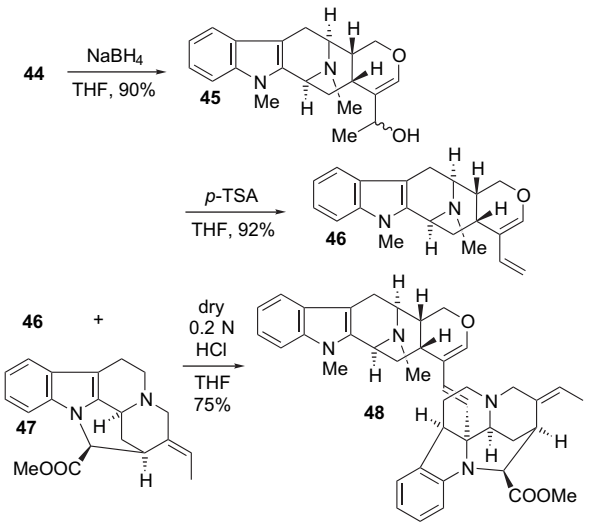
Scheme 11.

Carbonyl reduction and hydroboration gave triol **42** via **41**, and then selective tosylation of a primary alcohol and cyclisation gave **43**. A modified Swern oxidation[17] regenerated the vinylogous ester functionality and so led to (−)-alstonerine **44** (along with 31% dihydroalstonerine) in 8% overall yield from tetracyclic ketone **10** (not considering recycling of material) or 4% overall yield from D-tryptophan (Scheme 12).



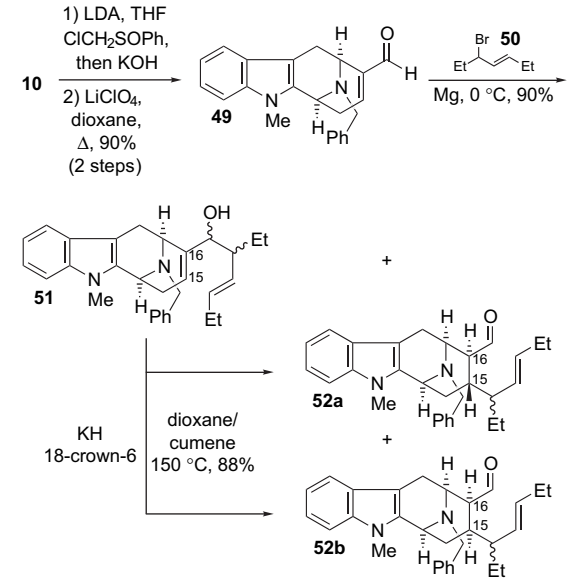
Scheme 12.

The strategy detailed above for the synthesis of (−)-alstonerine **44** was later extended by Cook et al. for the synthesis[18,19] of (−)-anhydromacrosalhine-methine **46**. Whilst not a natural product, this indole base constitutes the indole unit of the macroline-related bis(indole) alkaloid (−)-macrocarpamine **48**. Reduction of (−)-alstonerine **44** gave secondary alcohol **45**, which underwent acid-induced elimination to give (−)-anhydromacrosalhine-methine **46**. Coupling of **46** with a natural sample of pleiocarpamine **47** (Scheme 13) completed the partial synthesis of (−)-macrocarpamine **48** (2% overall yield from D-tryptophan).



Scheme 13.

γ-addition. A mixture of 1,2- and 1,4-addition products resulted, as shown, but, in an elegant resolution to this problem, Cook was able to transform the undesired 1,2-addition product **51** into the 1,4-addition product **52** by means of an oxyanion-Cope rearrangement (Scheme 14).



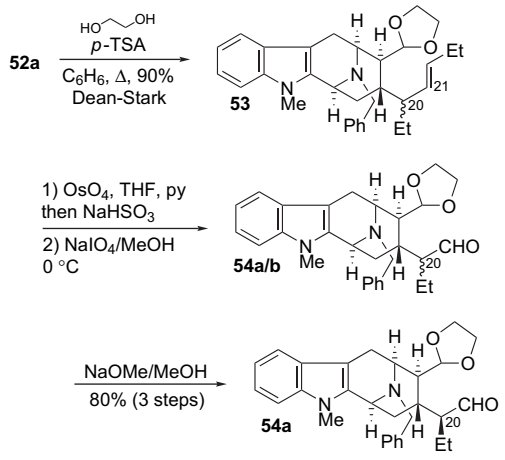
Scheme 14.

2.3. Ajmaline and alkaloid G

2.3.1. First-generation syntheses: 1,4-addition, oxyanion-Cope rearrangement and selective oxidations. Cook and co-workers employed tetracyclic ketone **10** in the first total synthesis of (−)-ajmaline.[20,21] Ketone **10** was elaborated into α,β-unsaturated aldehyde **49** as before, although the reaction was found to proceed in the absence of the phosphine oxide (also the N4-benzyl group was still in place). As mentioned in Section 2.2, intramolecular C15 functionalisation had been found to be difficult, but it transpired that successful organometallic addition was possible by use of a Barbier–Grignard process. A pseudo-symmetric allyl bromide **50** was used to circumvent ambiguity regarding α- versus

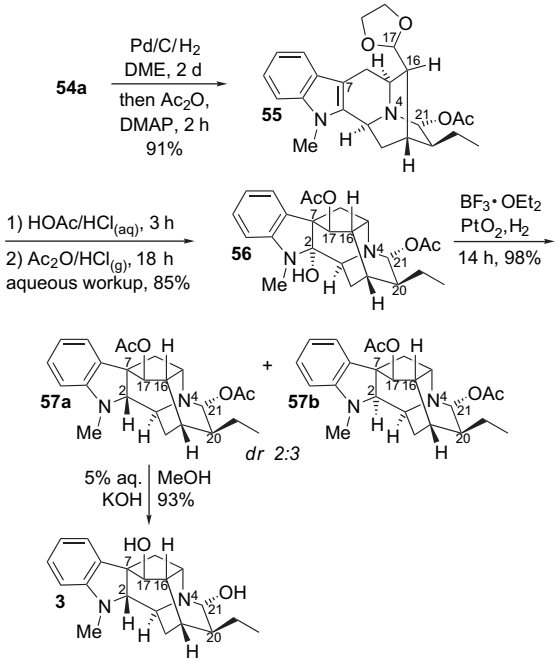
From the initial Barbier–Grignard reaction, **51** and **52** were formed in a ratio of 51:49. Of this, the 1,4-addition product **52** was formed in a ratio of **52a**:**52b** of 3:1, where **52a** was the desired isomer having the (15*S*) configuration. When **51** underwent an oxyanion-Cope rearrangement, **52a** and **52b** were isolated in a ratio of 3:2. Subsequent elaboration of **52a** was by ethylidene acetal protection of the aldehyde (giving **53**) and oxidative cleavage of the olefin. In order to effect chemoselective cleavage in the presence of the oxidatively sensitive indole, a stoichiometric osmylation was required, with subsequent periodate cleavage of the resultant diol. At this point in the sequence it was possible to epimerise C20 via the aldehyde enolate, giving a **54a**:**54b** 1:1 epimeric mixture, separable by chromatography. With

recycling of the undesired epimer **54b**, >80% conversion from **53** was possible (Scheme 15).



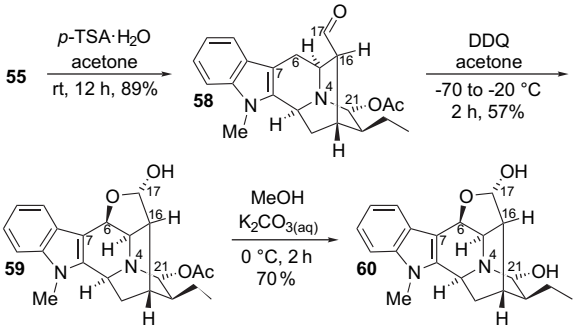
Scheme 15.

N4-deprotection allowed formation of the *O*-acetyl aminal **55**. Treatment with $HCl_{(aq)}$/AcOH, then $Ac_2O/HCl_{(g)}$, effected the final cyclisation to the ajmalan skeleton by electrophilic addition to C7. The resultant C2 hemiaminal **56** was reduced under Lewis acidic conditions to furnish a C2-epimeric mixture, **57a**:**57b** of 2:3. The epimer having the correct C2 configuration, **57a**, underwent base-mediated hydrolysis to afford (−)-ajmaline **3** (Scheme 16) in 11% yield from tetracyclic ketone **10** (5% from D-tryptophan). Whilst the formation of only 40% of the desired C2 epimer in the penultimate step is not ideal, Cook notes that 2-*epi*-diacetyl ajmaline **57b** is the thermodynamic product and many reagent systems provide solely **57b**.



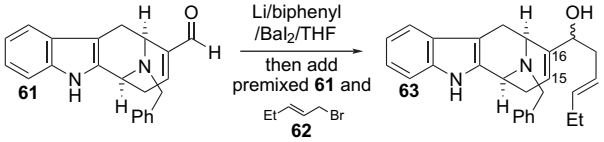
Scheme 16.

Hydrolysis of acetal **55** gave **58**, which had previously been converted via **59** into alkaloid G by Stöckigt and co-workers[22] (Scheme 17), employing a DDQ oxidation to functionalise the C6 position. Cook's report therefore constitutes a formal synthesis of alkaloid G **60** in 10 steps and 12% yield from tetracyclic ketone **10** (6% overall yield from D-tryptophan).



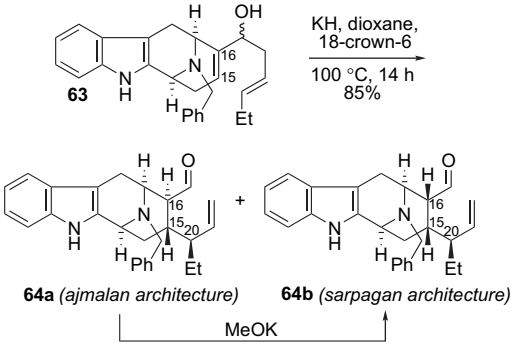
Scheme 17.

2.3.2. Second-generation syntheses: organobarium chemistry and kinetic enolate quenching. Shortly after the reports summarised in Section 2.3.1, Cook's group published improved syntheses of (−)-ajmaline[23] and alkaloid G.[23,24] The improvements address the issue of stereocontrol in the organometallic addition and oxyanion-Cope steps. Using methodology due to Yamamoto,[25] Cook and co-workers treated N1-unsubstituted α,β-unsaturated aldehyde **61** with an organobarium reagent derived from (*E*)-pent-2-enyl bromide **62**. This addition took place solely from the α-position of the metallate, hence the need for a pseudo-symmetric alkenyl halide was removed. Additionally, only 1,2-addition to **61** was observed, giving **63** as the sole product (Scheme 18).



Scheme 18.

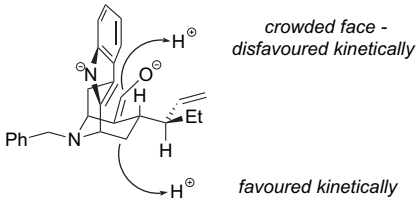
Oxyanion-Cope rearrangement of **63** took place as before; in this instance, however, near total selectivity for the desired configurations was observed at C15 *and* C20 (cf. selectivity of 3:2 in Section 2.3.1). At C16, in the first instance, the selectivity was 1:4 for **64a**:**64b** for the undesired sarpagan (16*R*) configuration. Upon prolonged exposure of (16*S*) **64a** to base, epimerisation to mostly (16*R*) **64b** was observed, implying **64b** was the thermodynamic product (Scheme 19).



Scheme 19.

The 3D structure (Scheme 20) of the enolate resulting from the oxyanion-Cope rearrangement suggested that the α-face

might be less hindered and as such **64a** might be the kinetic product. After optimisation, it was found that quenching the oxyanion-Cope rearrangement with 1 N trifluoroacetic acid at low temperature favoured the formation of **64a**. After the rearrangement had gone to completion, THF was added, allowing the reaction mixture to be cooled below the melting point of dioxane. At −100 °C in dioxane/THF, addition of 1 N trifluoroacetic acid in THF afforded **64a**:**64b** in a ratio of 43:1.

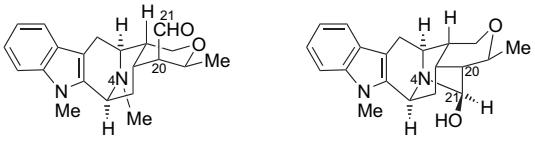


Scheme 20.

The ability to vary reaction conditions to favour either **64a** or **64b** permits stereospecific entry to either the macroline/sarpagine (16*R*) series or the ajmaline (16*S*) series. Aldehyde **64a** was protected as the ethylidene acetal and then N1-methylated to converge on the (−)-ajmaline synthesis detailed in Section 2.3.1. The second-generation synthesis was thus completed in 9% overall yield from D-tryptophan methyl ester, an appreciable improvement. In completing the second-generation synthesis of alkaloid G, Cook's laboratory reports a significant improvement to the DDQ-mediated α-aryl oxidation step—performing the reaction in wet THF leads to a yield of 94% of **42** (one diastereoisomer only). The improved alkaloid G synthesis was therefore completed in 25% overall yield from D-tryptophan methyl ester.

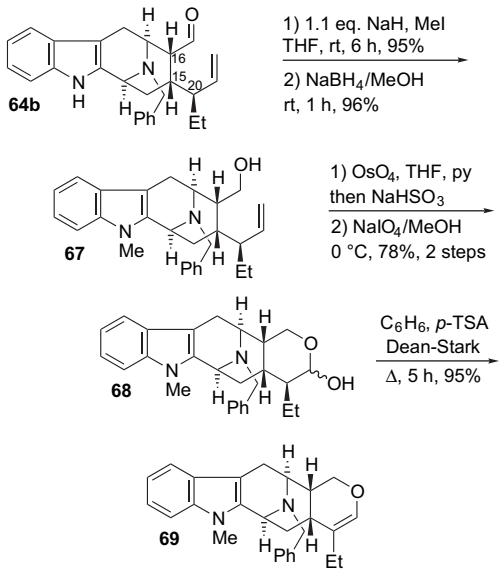
2.4. Selenium chemistry and an unusual pyrolytic rearrangement: talpinine, talcarpine, alstonerine and anhydromacrosalhine-methine

Cook et al. have reported syntheses[26,27] of the two structurally related macroline/sarpagine alkaloids, (−)-talcarpine **65** and (−)-talpinine **66**. They employ much of the methodology used for the synthesis of (−)-ajmaline and alkaloid G. It may be seen (Scheme 21) that **65** and **66** are epimeric at C20 and that **66** lacks the N4-methyl group, but has a hemiaminal moiety containing a C21–N4 linkage.



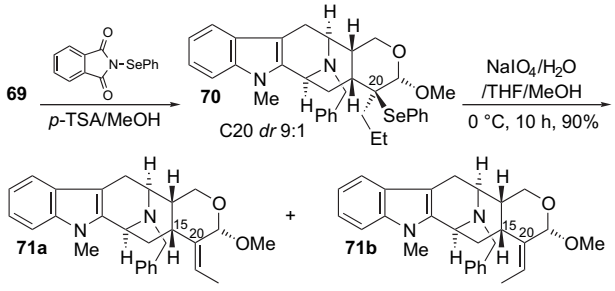
Scheme 21.

The synthetic sequence was executed as per Section 2.3.2, this time from the N1-unsubstituted tetracyclic ketone **32**. As the sarpagan configuration (16*R*) was required in this instance, the enolate deriving from the oxyanion-Cope rearrangement was quenched under thermodynamic conditions, simply by adding MeOH to the reaction mixture and stirring at room temperature for 2 h to give **64b**. After N1-methylation, the aldehyde moiety was reduced and oxidative olefin cleavage (as previously) this time afforded a diastereoisomeric mixture of lactols **68**, which were then dehydrated (Scheme 22).



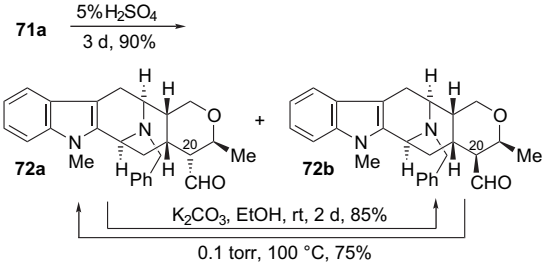
Scheme 22.

A key feature of this synthesis is the use of *N*-(phenylseleno)phthalimide to effect the addition of selenium[28] and a methoxy group across the enol ether, giving **70**, followed by selenium oxidation and elimination with rearrangement to afford a mixture of exocyclic olefin geometries (Scheme 23) in a ratio **71a**:**71b** of 4:1 (where **71a** is the desired isomer).



Scheme 23.

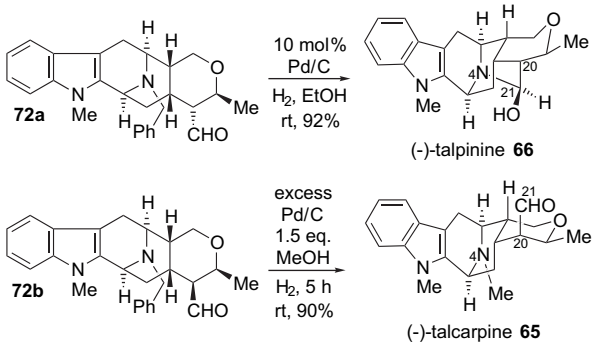
The desired isomer **71a** was treated with 5% H_2SO_4 for 3 days, which induced acetal opening, C15–C20 bond rotation and Michael addition, to generate saturated C20 aldehydes as a C20 epimeric mixture, 3:5 of **72a**:**72b**. Aldehyde **72a** (20*R* configuration) is the precursor of talpinine and, similarly, **72b** (20*S* configuration) is the precursor of talcarpine. The two epimeric precursors may, in fact, be interconverted (Scheme 24).



Scheme 24.

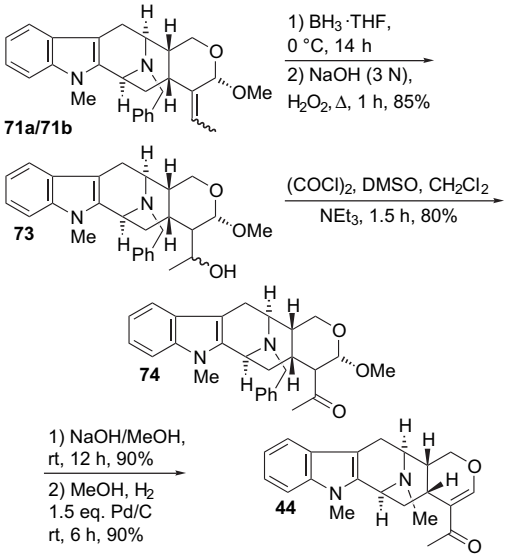
Conversion of **72a** into **72b** is simply base-induced epimerisation to the thermodynamic product. The pyrolytic

conversion[29] of **72b** into **72a** is not fully understood mechanistically. Conversion of **72a** into talpinine (10% from D-tryptophan, Scheme 25) was effected simply by N4-debenzylation (with spontaneous hemiaminal formation). Conversion of **72b** into talcarpine (10% from D-tryptophan, Scheme 25) was effected by N4-debenzylation with concomitant N4-methylation, a transformation speculated to involve in situ formaldehyde formation.



Scheme 25.

The methodology detailed above has also been employed in the second-generation syntheses[27] of anhydromacrosalhine-methine and alstonerine. The geometric mixture of olefins (**71a** and **71b**) was subjected to hydroboration, Swern oxidation, elimination of methanol and N4-debenzylation/methylation to furnish (−)-alstonerine **44** (Scheme 26) via **73** and **74** in an improved 12% overall yield from D-tryptophan (cf. Section 2.2).

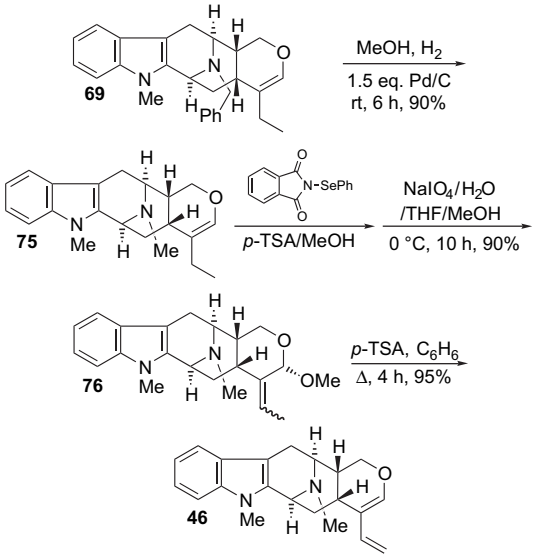


Scheme 26.

Anhydromacrosalhine-methine **46** was synthesised from **69** (Scheme 27), by N4-debenzylation/methylation at an earlier stage, then selenium introduction, oxidation and elimination as before, followed by acid-induced elimination to the vinylogous enol ether product **46** via **75** and **76** (14% from D-tryptophan, cf. Section 2.2).

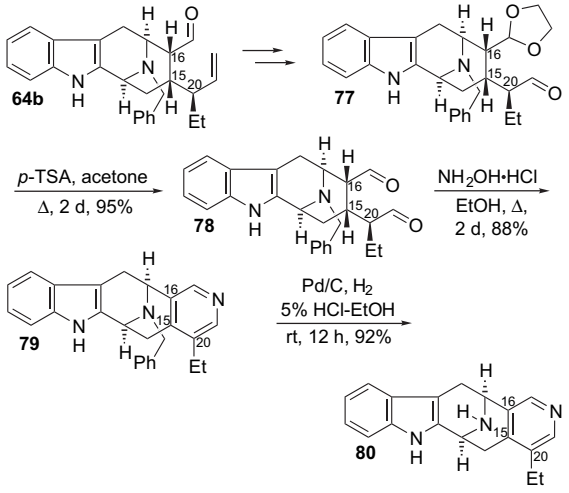
2.5. Pyridine formation: norsuaveoline

Cook's laboratory has also reported the synthesis of the pyridyl macroline alkaloid, norsuaveoline.[21,30] This synthesis



Scheme 27.

has much in common with Cook's earlier synthesis of suaveoline.[31] From the N1-unsubstituted tetracyclic ketone **32**, the synthesis proceeded as per the ajmaline synthesis in Section 2.3.2. Cook and co-workers opted to use the sarpagan C16-configured oxyanion-Cope product, although, in this instance, the configurations of C15, C16 and C20 are of less concern, since all are ultimately incorporated into the pyridine ring. Ethylidene acetal formation and oxidative olefin cleavage were executed as before to give **77**. In this case, however, the acetal was deprotected to furnish a 1,5-dialdehyde **78**. This was treated with ethanolic hydroxylamine hydrochloride to access the pyridine ring directly; N4-debenzylation of **79** afforded norsuaveoline **80** in 28% yield from D-tryptophan methyl ester (Scheme 28).

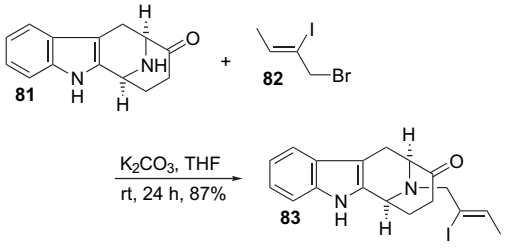


Scheme 28.

2.6. Palladium sarpagan methodology: *ent*-affinisine, 16-*epi*-affinisine, alkaloid Q3, dehydro-16-*epi*-affinisine, koumidine, 16-*epi*-*N*-methylpericyclivine, *N*-methylvellosimine, normacusine B, 16-*epi*-normacusine B, panarine and vellosimine

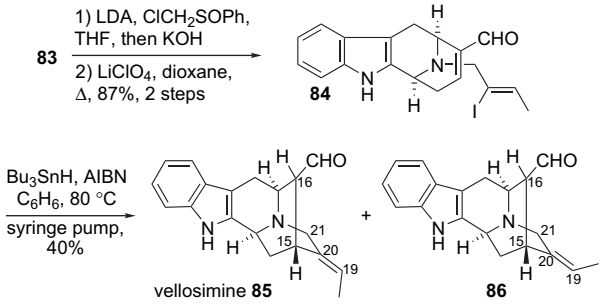
For the synthesis of alkaloids possessing the sarpagan skeleton, a key question is how to construct the skeleton such that

the C19–C20 olefin geometry is controlled. Cook attempted to address this problem in various ways and met with success when he employed a palladium-mediated cyclisation. The key reaction may be illustrated with the example of Cook's total synthesis[32,33] of (+)-vellosimine **85**. Iodoalkene **82** (which has been employed by other workers[34–40]) was reacted with the N1-unsubstituted, N4-debenzylated tetracyclic ketone **81** to give **83** (Scheme 29).



Scheme 29.

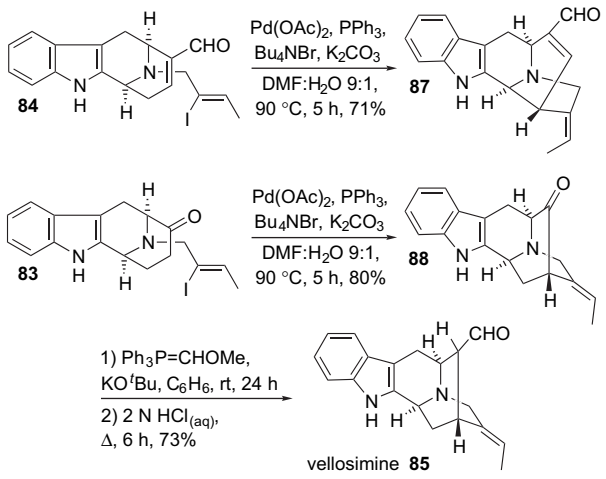
Ketone **83** was elaborated to the corresponding α,β-unsaturated aldehyde **84**, as previously. One can envisage that transmetallation and Michael addition would give access to the sarpagan skeleton, but, in fact, this approach was unsuccessful. Instead, it was found that a radical-mediated coupling could effect C15–C20 bond formation. This occurred with scrambling of the C19–C20 olefin geometry, however, and the desired (+)-vellosimine **85** was the minor product in a ratio **85**:**86** of 1:3 (Scheme 30).



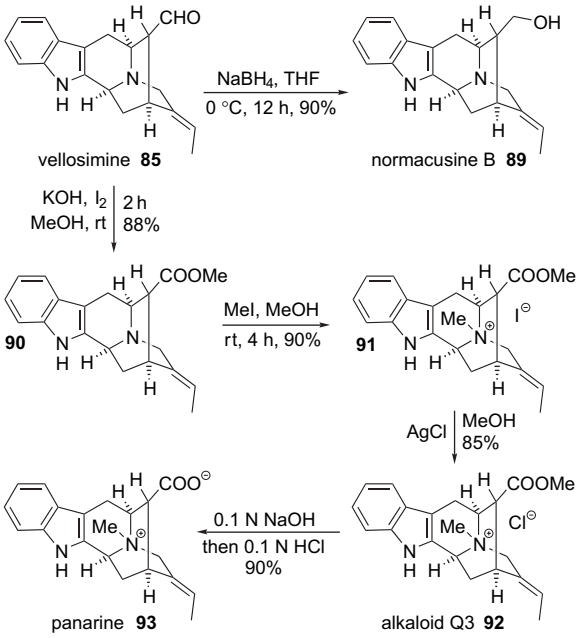
Scheme 30.

In view of the failure of both metallate and radical methods, the desired stereospecific cyclisation of **84** was attempted under Pd^0 catalysis. The unexpected product **87** was isolated (as a single geometric isomer), presumably arising from the enolate of **84**. Such a cyclisation had been previously observed in other systems.[41] By inference from this result, it followed that **83** might undergo cyclisation to the desired vellosimine skeleton. Ketone **83** did, indeed, give **88** stereospecifically under the same conditions. This was transformed into (+)-vellosimine **85** via a masked aldehyde, which was unmasked and epimerised to the more stable C16 sarpagan configuration (Scheme 31). The first total synthesis of this sarpagine alkaloid was therefore completed in 27% overall yield from D-tryptophan methyl ester.

Several more sarpagine alkaloids[33,42] were, in turn, synthesised from (+)-vellosimine **85** (Scheme 32). Reduction of the aldehyde in **85** gave (+)-normacusine B **89** (24% from D-tryptophan methyl ester). Conversely, oxidation of the aldehyde in **85** and esterification gave **90**, quaternisation of which with methyl iodide (to furnish **91**) and subsequent anion exchange gave (−)-alkaloid Q3 **92** (18% from



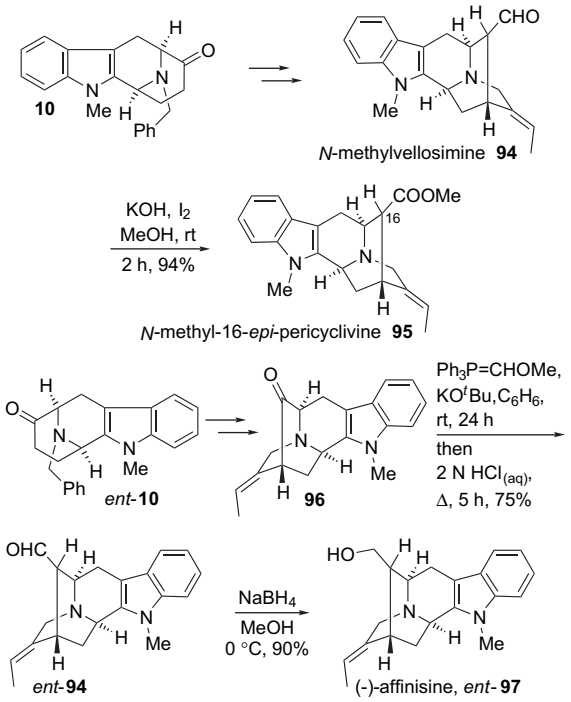
Scheme 31.



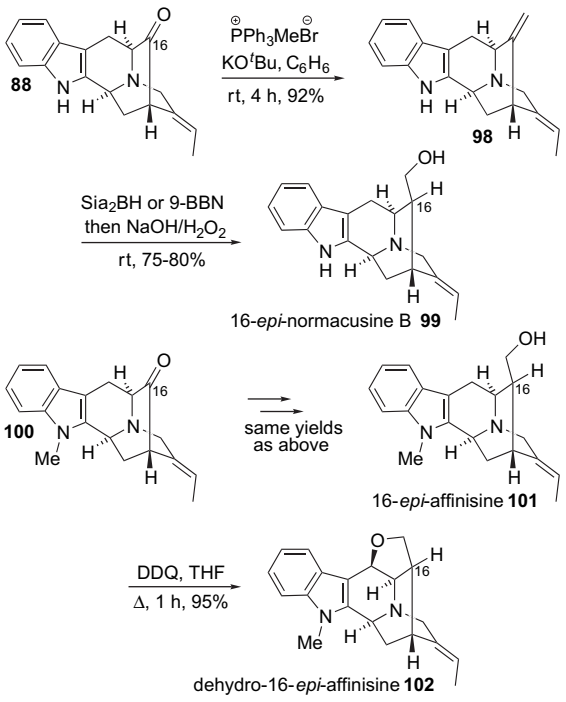
Scheme 32.

D-tryptophan methyl ester). Ester hydrolysis of **92** and neutralisation gave zwitterionic (−)-panarine **93** (16% from D-tryptophan methyl ester).

The same synthetic sequence used to prepare (+)-vellosimine was applied to the N1-methyl tetracyclic ketone **10** to produce (+)-*N*-methylvellosimine[33] **94** (29% overall yield from D-tryptophan, Scheme 33). Oxidation and esterification provided (+)-*N*-methyl-16-*epi*-pericyclivine[33] **95** (27% overall yield from D-tryptophan). Reduction of the aldehyde in **94** provided (+)-affinisine[33] **97** (26% overall yield from D-tryptophan). Cook's group also executed the entire synthetic sequence from L-tryptophan, via **96**, thus providing *ent*-**97** (−)-affinisine,[43] the enantiomer of the natural product (Scheme 33). This *ent*-affinisine was required for the synthesis of 'mismatched' unnatural bis(indole) alkaloids, to probe their biological activities and SAR. As LeQuesne had previously reported[44,45] partial syntheses of macroline **1** and alstonerine **44** from affinisine, Cook's work constitutes formal syntheses of the antipodes of these alkaloids also.



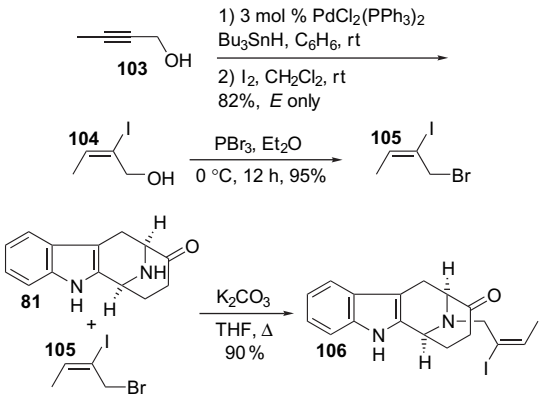
Scheme 33.



Scheme 34.

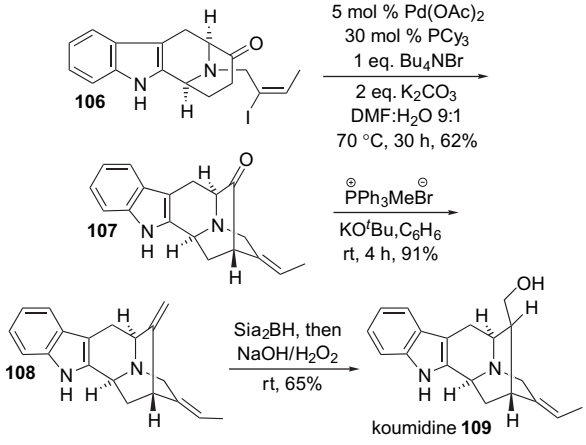
A slightly different approach was used to access sarpagine alkaloids possessing the opposite configuration at C16 (ajmaline configuration). From sarpagan C16 ketone **88**, Wittig methylenation and selective hydroboration of the disubstituted olefin from the less hindered face gave 16-*epi*-normacusine B[24,46] **99** (26% from D-tryptophan methyl ester). In the N1-methyl series, from sarpagan C16 ketone **100**, the same Wittig methylenation and selective hydroboration gave 16-*epi*-affinisine[24,46] **101** (25% from D-tryptophan methyl ester). DDQ-mediated α-aryl oxidation gave dehydro-16-*epi*-affinisine[24,46] **102** (24% from D-tryptophan methyl ester), as shown in Scheme 34.

Cook employed a modified version of the palladium-catalysed coupling in the synthesis[47] of (−)-koumidine **109**, which differs from the various species shown above in that the geometry of the C19–C20 olefin is (*Z*). To access this alternative geometry, the alternate iodoalkene **105** was synthesised from **103** via **104** as shown in Scheme 35 and coupled to N1-unsubstituted tetracyclic ketone **81**.



Scheme 35.

The palladium-mediated cyclisation was less facile than in previous examples with the opposite (*E*) olefin geometry—despite much optimisation, on reaction of **106** significant amounts of dealkylated product **81** were isolated along with the desired **107**. Completion of the synthesis (Scheme 36) was via hydroboration of **108** as for the other C-16-*epi* alkaloids, in 21% yield from D-tryptophan methyl ester.

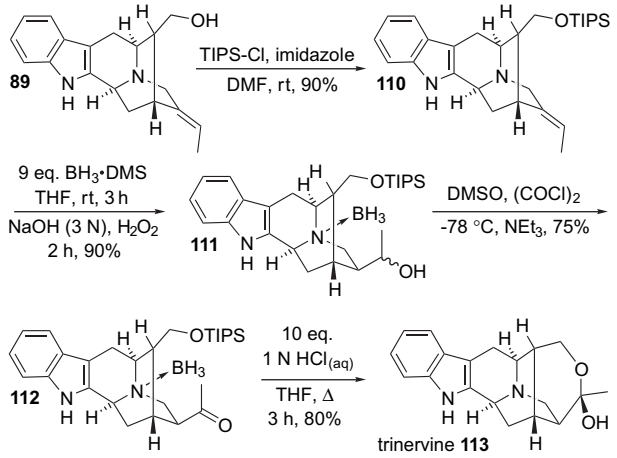


Scheme 36.

2.7. Selective hydroboration: trinervine

The sarpagine alkaloid trinervine **113**, a cyclic hemiacetal, was synthesised from (+)-normacusine B **89**, the synthesis of which is detailed in Section 2.6. Silylation of the alcohol was followed by attempts at selective hydroboration of the trisubstituted C19–C20 olefin (Scheme 37). Surprisingly,

the initial selectivity (at 0 °C) for the secondary hydroxyl product **111** over the tertiary regioisomer was only 7:3. It was postulated that this may be due to complexation of the first equivalent of borane to N4, thus altering the electronic characteristics of the olefin. A detailed optimisation study was carried out[48]—use of bulky hydroborating agents resulted in no reaction, but increased selectivity was observed by using **110** (with R=TIPS) at room temperature, furnishing the desired regioisomer in a ratio of 25:1. This was oxidised, in turn, to the ketone and upon deprotection of the hydroxyl group in **112** (and cleavage of the borane adduct), spontaneous cyclisation gave trinervine **113** (20% from tetracyclic ketone **32**).



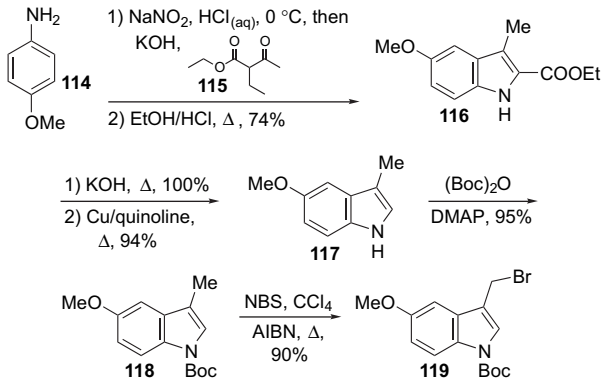
Scheme 37.

2.8. Indole oxygenation

As alluded to in Section 1, many macroline/sarpagine/ajmaline alkaloids possess indole ring oxygenation. Cook has synthesised many of these and the key to these syntheses has been the optimisation of routes to the relevant oxygenated tryptophan derivatives. Cook has successfully introduced oxygenation in the C10-, C11- and C12-positions. In each instance, the Schöllkopf chiral auxiliary[49] was used to introduce the correct amino acid stereochemistry. The precise details vary depending on the ring substitution pattern, however, and so will be discussed individually.

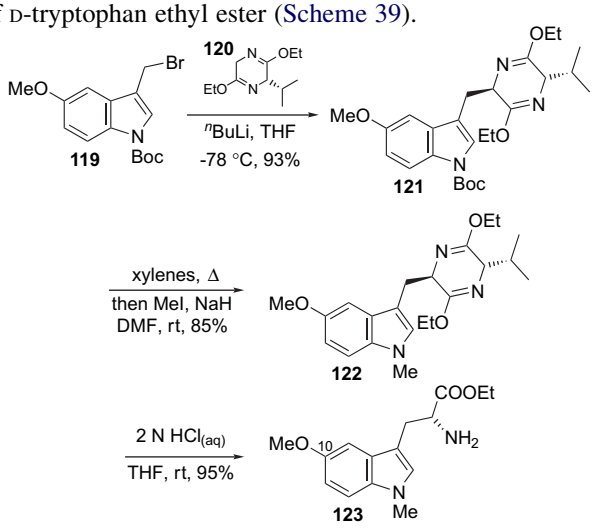
2.8.1. C10 oxygenation: majvinine, 10-methoxyaffinisine, *N*-methylsarpagine and macralstonidine. *p*-Anisidine was employed as a starting material for a synthesis[50,51] that Cook's laboratory has executed on a >600-gram scale (Scheme 38). Fischer indole formation via a Japp–Klingemann azo-ester intermediate[52,53] formed from **114** and **115** gave the trisubstituted indole **116**. C2-Decarboxylation to give **117** was followed by N1-protection, either with a Boc group (giving **118**) or as a sulfonamide (only the Boc series is considered here). Optimisation of the brominating conditions[51] was required to access the desired α-aryl brominated product **119** and avoid indolyl C2-bromination.

Cook has studied the effect of the leaving group and other parameters on the diastereoselectivity of the reaction with Schöllkopf auxiliaries.[54,55] Bromide **119** was coupled with the Schöllkopf auxiliary **120** (derived from L-valine) to give **121** as a single diastereoisomer. The Boc group was cleaved



Scheme 38.

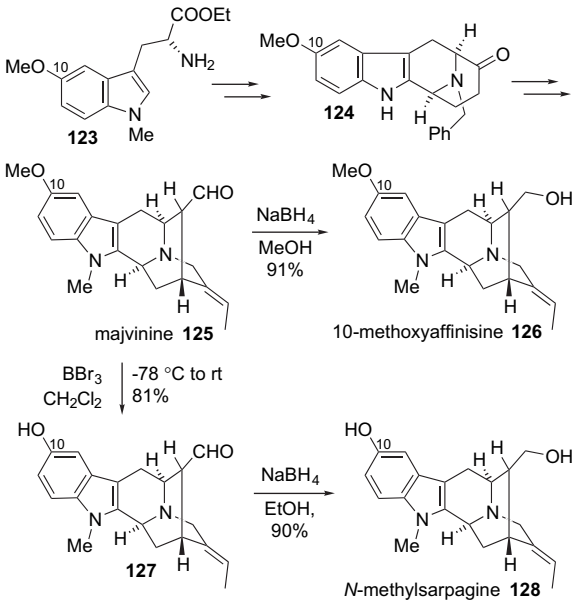
thermolytically, followed by N1-methylation in one pot, giving **122**. The auxiliary was removed under conditions of acidic hydrolysis to furnish **123**, the C10-methoxy analogue of D-tryptophan ethyl ester (Scheme 39).



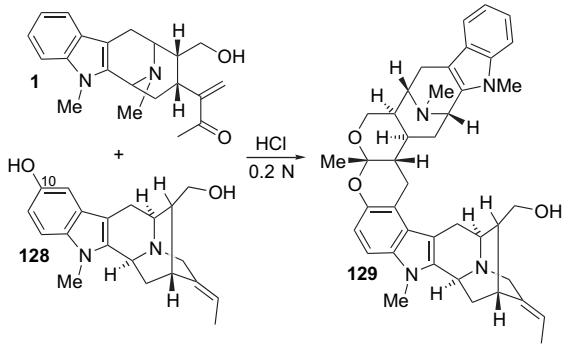
Scheme 39.

The ring-oxygenated amino acid **123** was amenable to the chemistry developed by Cook and co-workers detailed in Sections 2.1–2.7. Thus, the synthesis of C10-methoxy tetracyclic ketone **124** was high yielding (although it was necessary to avoid harshly acidic conditions in the Pictet–Spengler and C3-isomerisation steps, otherwise decomposition of the indole occurred). The conversion of **124** to the sarpagan skeleton via the palladium enolate methodology described previously was similarly high yielding (Scheme 40). Synthesis of (+)-majvinine **125** (28% yield from C10-methoxy D-tryptophan ethyl ester analogue **123**) was executed as per *N*-methylvellosimine **94** (majvinine is simply the C10-methoxy analogue of **94**). Reduction of the aldehyde moiety in **125** gave (+)-10-methoxyaffinisine **126** (25% yield from **123**). For the synthesis of (+)-*N*-methylsarpagine **128**, a C10-hydroxy group was required as opposed to a C10-methoxy group. Therefore, (+)-majvinine **125** was demethylated with boron tribromide (giving **127**) prior to reduction to (+)-*N*-methylsarpagine **128** (20% yield from **123**).

Cook also reported the first total synthesis of the bis(indole) alkaloid, (+)-macralstonidine **129**, from the coupling[45] of synthetic *N*-methylsarpagine **128** with synthetic macroline **1** (Scheme 41).



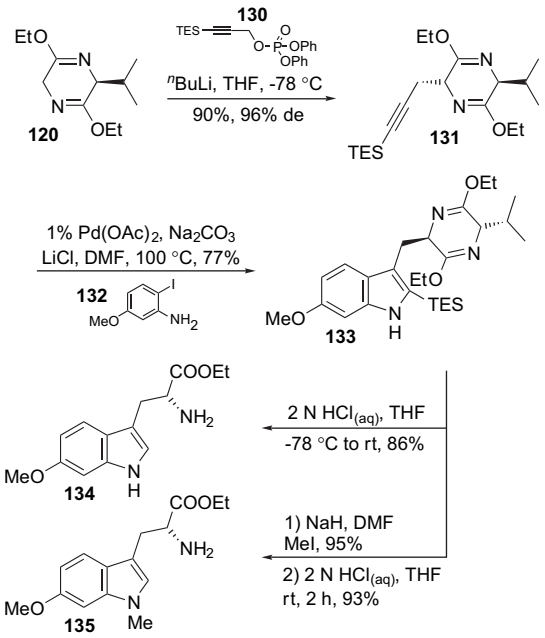
Scheme 40.



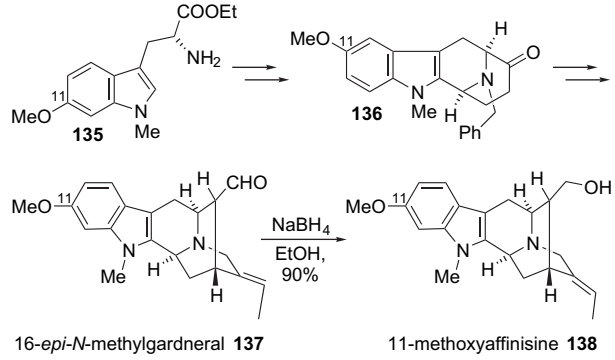
Scheme 41.

2.8.2. C11 oxygenation: gardnerine, gardnutine, 11-methoxyaffinisine and 16-*epi*-*N*-methylgardneral. Synthesis of a C11-oxygenated tryptophan analogue would have been subject to regiochemical ambiguity if attempted via a Fischer indole formation. Cook and co-workers accessed this series[56] by means of a Larock heteroannulation.[57] The order of events is reversed from that in Section 2.8.1, in that reaction of **130** with the Schöllkopf auxiliary occurs prior to indole formation with **132** to give **133** (Scheme 42). The formation of **131** in high de is due in part to the choice of phosphonate leaving group.[54] The Larock heteroannulation has been carried out on a 300-gram scale.

Both N1-methyl and N1-unsubstituted amino acids are easily accessible by this method. Once again, Cook's previously developed methodology was viable with these C11-oxygenated amino acids (Scheme 43): (+)-16-*epi*-*N*-methylgardneral **137** was synthesised via **136** (35% from C11-methoxy, N1-methyl D-tryptophan ethyl ester **135**) as per *N*-methylvellosimine **94** (Section 2.6, **137** is simply the C11-methoxy analogue of **94**). Reduction of **137** gave 11-methoxyaffinisine **138** (32% from **91**). Note that **137** and **138** have not been isolated from a natural source to date; they are precursors of natural products discussed in Sections 2.11 and 2.12.

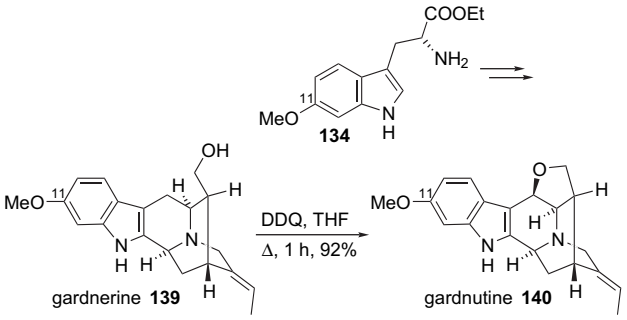


Scheme 42.



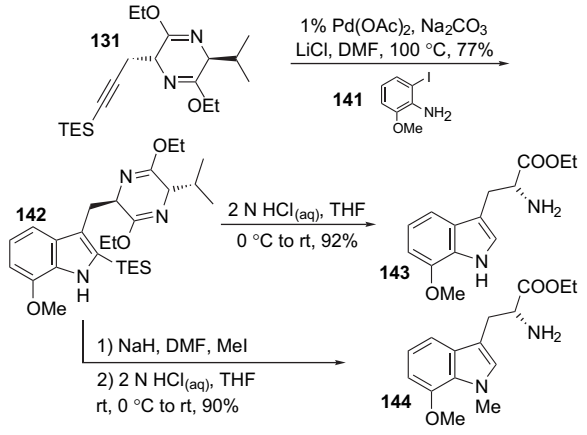
Scheme 43.

(−)-Gardnerine **139** and (+)-gardnutine **140** are N1-unsubstituted C11-methoxy sarpagine alkaloids synthesised from C11-methoxy D-tryptophan ethyl ester **134** by Cook and co-workers[58] in a manner analogous to that for 16-*epi*-normacusine B **99** (Section 2.6, **139** is simply the 11-methoxy analogue of **99**). (−)-Gardnerine **139** was synthesised in 20% overall yield from **134**. (+)-Gardnutine **140** was synthesised from **139** by DDQ-mediated α-aryl oxidation (18% overall yield from **134**, Scheme 44).



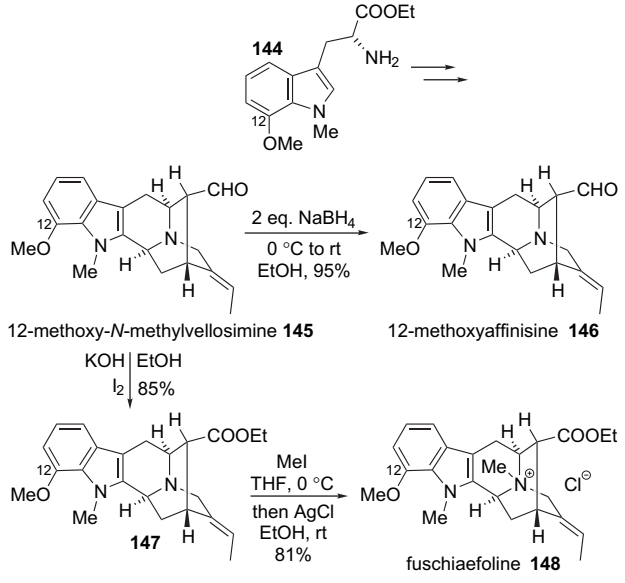
Scheme 44.

2.8.3. C12 oxygenation: fuchsiaefoline, 12-methoxyaffinisine and 12-methoxy-*N*-methylvellosimine. The required C12-methoxy amino acids were prepared by the same process used for the C11-methoxy series (namely a Larock heteroannulation), employing a regioisomeric iodoanisidine **141**, giving **142** as a common intermediate for the synthesis of **143** and **144** (Scheme 45).



Scheme 45.

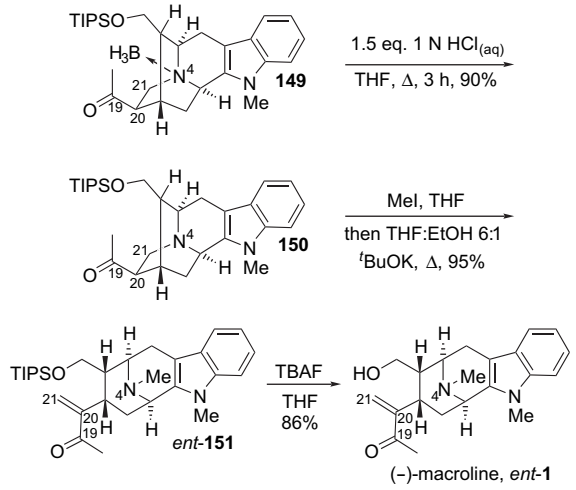
The C12-methoxy amino acids were compatible with Cook's previously developed methodology, thus permitting the synthesis[59,60] of (+)-12-methoxy-*N*-methylvellosimine **145** (overall yield 40% from **144**) and (+)-12-methoxyaffinisine **146** (overall yield 38% from **144**) as per the unsubstituted analogues **85** and **97**. The quaternary alkaloid (−)-fuschiaefoline **148** was synthesised via **147** (27% yield from **144**) in two steps from **145** (Scheme 46).



Scheme 46.

2.9. Hofmann elimination: alstophylline, *ent*-macroline, 11-methoxymacroline, macralstonine

As mentioned in Section 1, the macroline skeleton may be accessed by Hofmann elimination of the sarpagine skeleton, a transformation used by Cook to synthesise many macroline alkaloids. For example,[61] starting from L-tryptophan, Cook et al. synthesised **149**, the enantiomer of the N1-methyl analogue of C19-oxo borane adduct **112** from the synthesis of trinervine (Section 2.7). Whereas in the trinervine synthesis **112** was treated with excess acid to effect both dative bond scission and desilylation, in this instance **149** was treated with a small excess of acid, removing the borane, but leaving the silyl group intact to give **150**. N4 was quaternised with methyl iodide, then under basic conditions Hofmann elimination occurred with regiospecific N4–C21 bond scission to give *O*-silylated macroline derivative *ent*-**151**. This was stable upon storage, or could be deprotected to give reactive (−)-macroline, *ent*-**1** (Scheme 47), in 12% overall yield from L-tryptophan methyl ester (intended for use in the synthesis of mismatched bis(indole) alkaloid analogues).

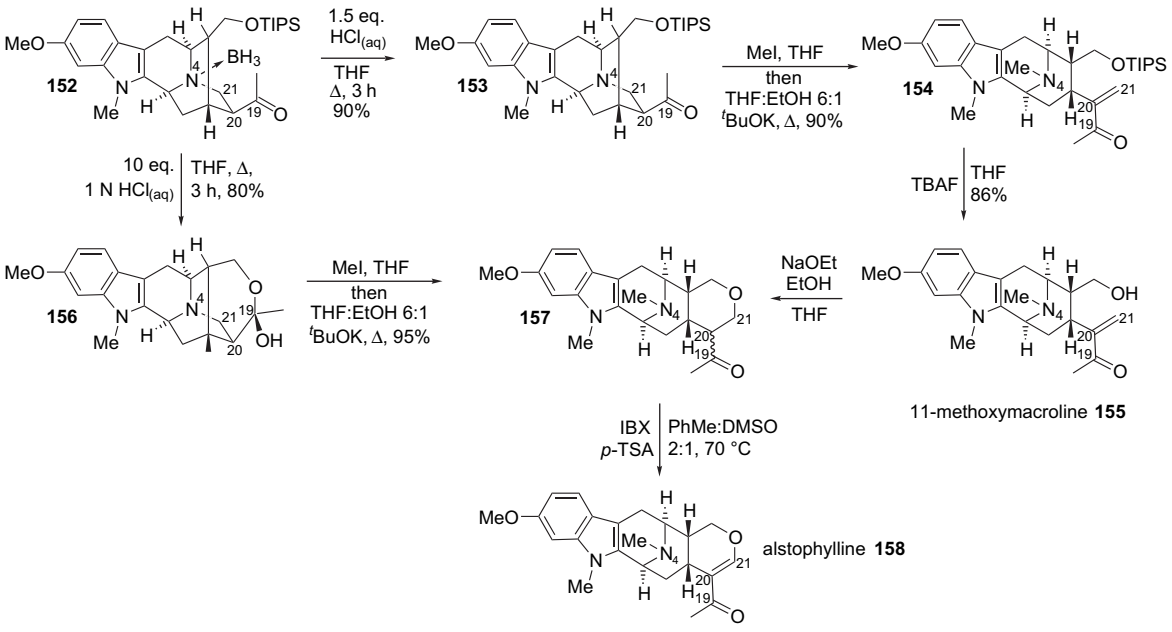


Scheme 47.

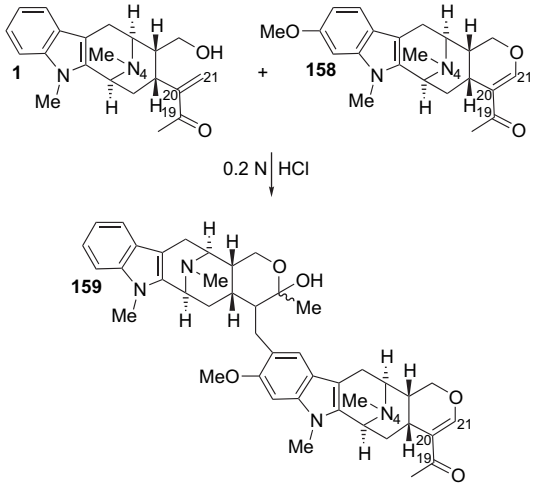
11-Methoxymacroline **155** was synthesised[56] by an entirely analogous route from the (naturally configured) 11-methoxy amino acid ester **134** (detailed in Section 2.8.2) in 14% overall yield. (−)-Alstophylline **158** (the 11-methoxy analogue of alstonerine **44**) was also synthesised by this route[56]—in this case, two possible pathways were available, only one of which utilised 11-methoxymacroline **155** as an intermediate (via **152**, **153** and **154**, Scheme 48), the other being via **156**. The final step in the synthesis of (−)-alstophylline **158** is an IBX-mediated oxidation of common intermediate **157**. Note that the yields are not quoted for all steps (preliminary communication). The bis(indole) alkaloid, macralstonine **159**, was synthesised by the protocol of LeQuesne and Cook[62] from macroline and alstophylline monomer units (Scheme 49).

2.10. Diastereospecific oxindole formation: alstonisine

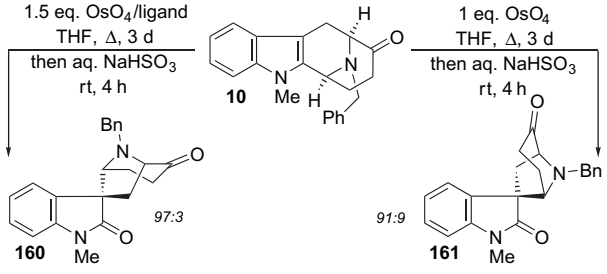
Brief consideration will be given to Cook's synthesis of the macroline-related oxindole (+)-alstonisine **163**. Oxindoles may be formed from the corresponding indoles by C2–C7 oxidation, with rearrangement to the C7-spirocyclic skeleton in the case of tetrahydro-β-carbolines. Model studies performed by Cook[63] on the tetracyclic ketone **10** (Scheme 50) led to the discovery that if osmium tetroxide was used as an oxidant, a particular diastereoisomer (**160** or **161**) could be favoured by the presence or absence of a Sharpless ligand (quinuclidine, DHQ–CLB, DHQD–CLB, $(DHQ)_2PHAL$ and $(DHQD)_2PHAL$ were used).



Scheme 48.

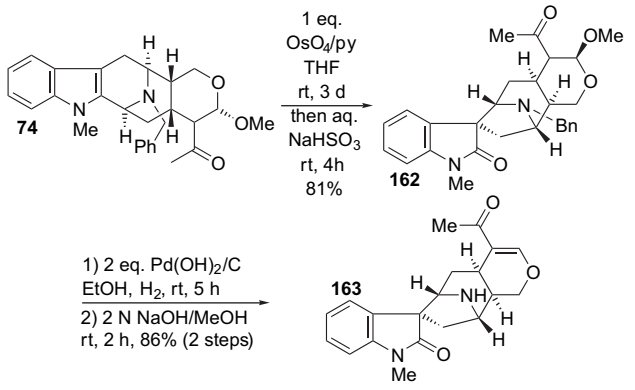


Scheme 49.



Scheme 50.

Cook applied the findings from the model studies to the synthesis[64] of (+)-alstonisine. Acetal **74** (a late-stage intermediate from the second-generation synthesis of (−)-alstonerine **44**, detailed in Section 2.4) was oxidised diastereoselectively to furnish oxindole **162** as the sole diastereoisomer. Cook proposes that coordination of the N4 lone pair to the osmium enhances the selectivity. N4-Debenzylation was followed by elimination to form the vinylogous ester product (+)-alstonisine **163** (12% overall yield from D-tryptophan, Scheme 51).

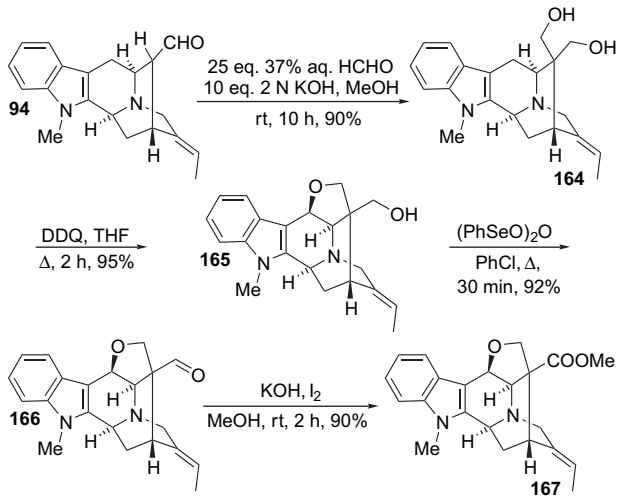


Scheme 51.

2.11. Tollens reaction: dehydrovoachalotine, 11-methoxy-17-*epi*-vincamajine and vincamajinine

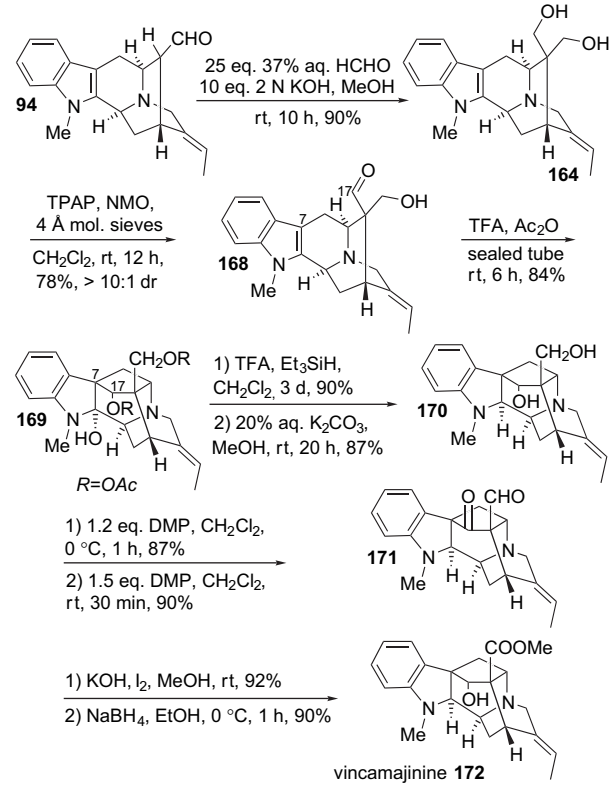
Various sarpagine/ajmaline-related alkaloids are known, which have a quaternary C16 motif. To access this substitution pattern from tertiary C16 species such as those dealt with in Sections 2.6–2.8, Cook et al. employed the Tollens reaction. For example, in the synthesis[65,66] of (+)-dehydrovoachalotine **167**, *N*-methylvellosimine **94** was transformed into 1,3-diol **164** in a yield of up to 90% after optimisation (Scheme 52). DDQ-mediated α-aryl oxidation was high yielding, as before, but oxidation of the neopentyl hydroxyl group in **165** proved problematic; eventually, it was found that a selenium-mediated oxidation furnished aldehyde **166**, which, in turn, could be oxidised to (+)-dehydrovoachalotine **167** (21% overall yield from D-tryptophan).

The Tollens reaction was also used by Cook and co-workers in their syntheses[66,67] of (−)-vincamajinine **172** and (−)-11-methoxy-17-*epi*-vincamajine **176**. The synthesis of **172**



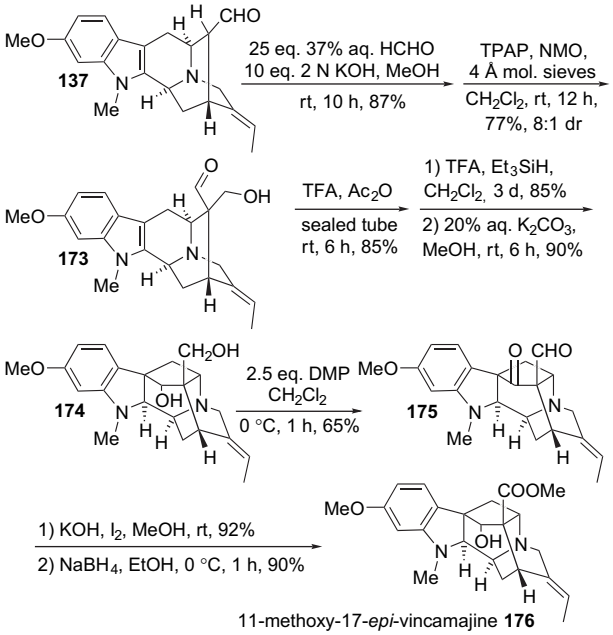
Scheme 52.

(Scheme 53) also commenced with the transformation of *N*-methylvellosimine into 1,3-diol **164**. To enable cyclisation to the ajmaline skeleton, a selective oxidation to a β-hydroxyaldehyde was needed. In the event, TPAP was able to selectively oxidise the less hindered hydroxymethyl group with diastereoselectivity >10:1. Treatment of **168** with trifluoroacetic acid and acetic anhydride in a sealed tube effected the C7–C17 cyclisation, giving **169**, and then the unwanted C2-hydroxyl was reduced to give **170**. Completion of the synthesis of **172** (via **171**) required several sequential oxidations and reductions—all attempts to combine these steps resulted in a dramatic drop in yield. (−)-Vincamajinine **172** was obtained in 12% overall yield from D-tryptophan methyl ester.



Scheme 53.

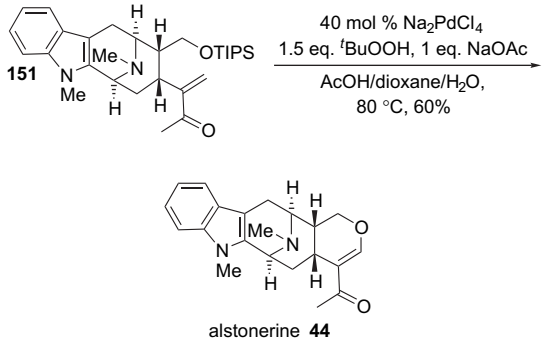
The synthesis of (−)-11-methoxy-17-*epi*-vincamajine **176** (Scheme 54) was broadly similar to that of **172**, except that a ring-oxygenated precursor (*N*-methyl-16-*epi*-gardneral **137**) was employed. The Tollens reaction has been shown to be compatible with both C10 and C11 oxygenation.[65] (−)-11-Methoxy-17-*epi*-vincamajine **176** was obtained via **173**, **174** and **175** in an overall yield of 8% from 10-methoxy D-tryptophan ethyl ester **123**. Cook has also prepared[66] related compounds such as quebranchidine diol, epimeric at C17.



Scheme 54.

2.12. Modified Wacker oxidation: alstophylline, 6-oxoalstophylline, alstonerine and macralstonine

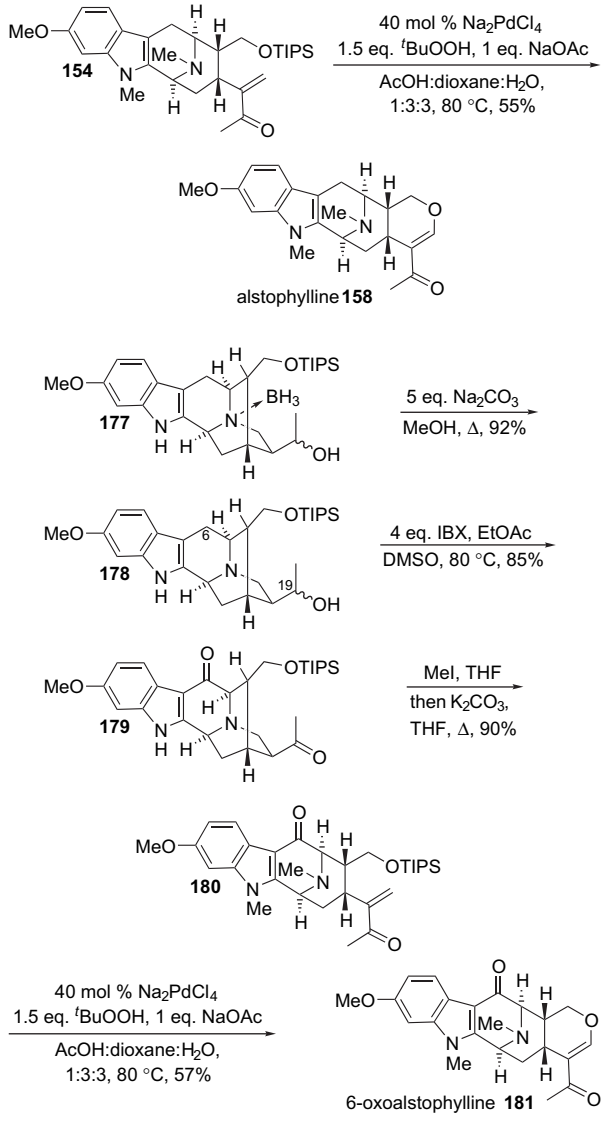
Cook has recently reported[68] the use of a modified Wacker protocol[69] to improve on the previous syntheses of the above-named alkaloids. For example, in the third-generation synthesis of (−)-alstonerine, silylated macroline equivalent **151** (described in Section 2.9) undergoes deprotection and oxidative cyclisation directly to (−)-alstonerine **44** in a palladium-catalysed process employing ^{t}BuOOH as an oxidant (Scheme 55). The yield of 60% is the result of optimisation work.



Scheme 55.

(−)-Alstonerine **44** was synthesised in 9% overall yield from D-tryptophan methyl ester. In a second-generation synthesis of (−)-alstophylline **158** (Scheme 56), the same protocol

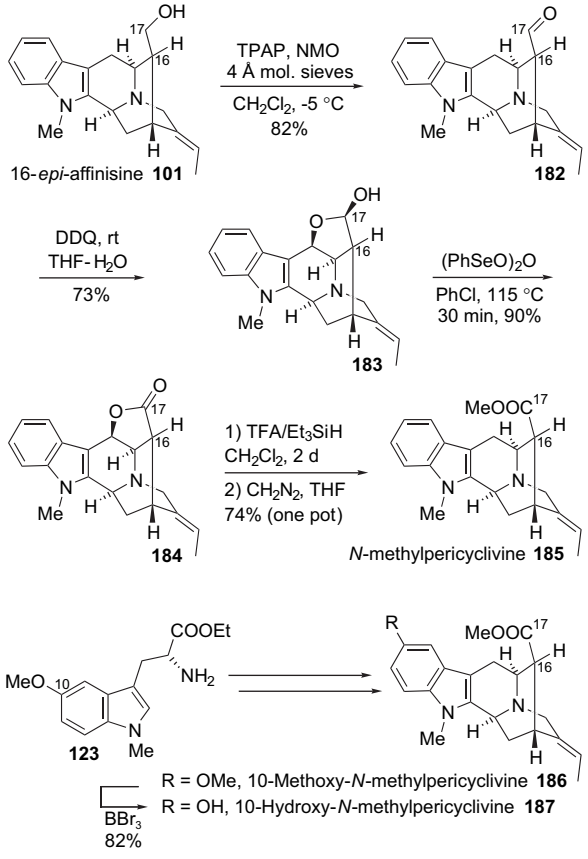
was applied to the corresponding 11-methoxymacroline equivalent **154**, affording **158** directly in 55% yield. (−)-Alstophylline **158** was obtained in 9% overall yield from 11-methoxy amino acid ester **135**. This improved synthesis of (−)-alstophylline also constituted a second-generation synthesis of macralstonine **159** (cf. Section 2.9). Finally, to effect the first total synthesis of (+)-6-oxoalstophylline **181**, silylated sarpagan borane adduct **177** underwent N4–B bond scission to give **178**, and was then oxidised[70] with excess IBX to effect not only C19, but also C6, ketone formation. Tertiary amine **179** underwent Hofmann elimination as expected, giving **180**, and the modified Wacker protocol furnished (+)-6-oxoalstophylline in 10% overall yield from 11-methoxy amino acid ester **135**. The mechanism of the modified Wacker oxidation has not yet been fully elucidated.



Scheme 56.

2.13. Lactol protection: 10-hydroxy-*N*-methylpericyclivine, 10-methoxy-*N*-methylpericyclivine, 12-methoxy-*N*-methylvoachalotine, *N*-methylakuammidine and *N*-methylpericyclivine

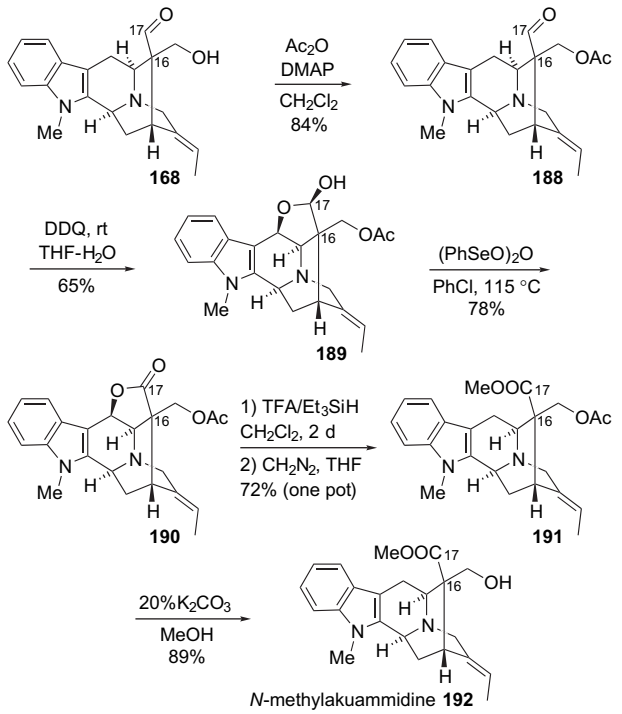
Certain of Cook's syntheses have been of sarpagine-related alkaloids that have required protection of C17. For instance, in the synthesis[71] of *N*-methylpericyclivine **185**, formation of the C17 ester was complicated by the fact that C16 epimerisation gave the more stable isomer, *N*-methyl-16-*epi*-pericyclivine **95**, under many ester-forming conditions. It was ascertained after experimentation that protection of the C17 aldehyde of **182** as a lactol (using the DDQ methodology outlined in Section 2.3.2) permitted oxidation of C17 (in **183**) to the correct oxidation state (in **184**) with retention of the desired C16 configuration. Reductive deprotection of the lactone with Et_3SiH and TFA and in situ esterification gave the desired *N*-methylpericyclivine **185** (10% overall yield from D-tryptophan methyl ester). A similar approach[71] starting from ring-oxygenated tryptophan derivative **123** afforded 10-methoxy-*N*-methylpericyclivine **186** (9% from **123**) and 10-hydroxy-*N*-methylpericyclivine **187** (7% from **123**), Scheme 57.



Scheme 57.

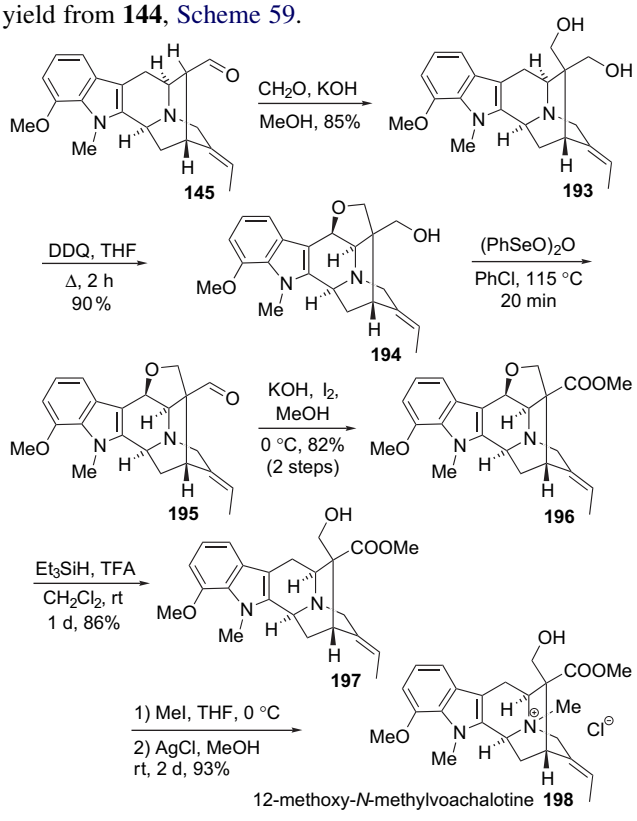
In the case of *N*-methylakuammidine[71] **192**, the configuration at the quaternary C16 was retained by the same protection strategy. In this instance, protection of the hydroxyl moiety in the final product as an acetate was also indicated (via **188**–**191**, Scheme 58). *N*-methylakuammidine **192** was synthesised in 6% yield from D-tryptophan.

A similar protection strategy was adopted in Cook's recent synthesis[60] of 12-methoxy-*N*-methylvoachlotine **198**. In this instance, the protection was at a lower level of oxidation—as a cyclic ether, as opposed to a γ-lactol or lactone. 12-Methoxy-*N*-methylvellosimine **145** was subjected to the Tollens reaction as before to give **193**, and then to the sequence of transformations effecting the protection (**194**), transformation (**195** and **196**) and deprotection (**197**); quaternisation



Scheme 58.

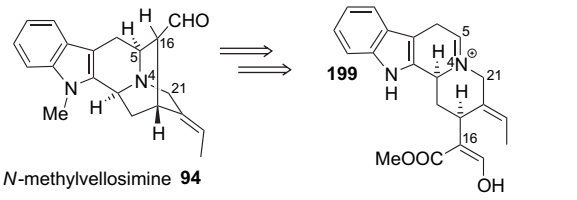
furnished 12-methoxy-*N*-methylvoachlotine **198** in 20% yield from **144**, Scheme 59.



Scheme 59.

3. Martin's biomimetic synthesis of (+)-*N*-methylvellosimine

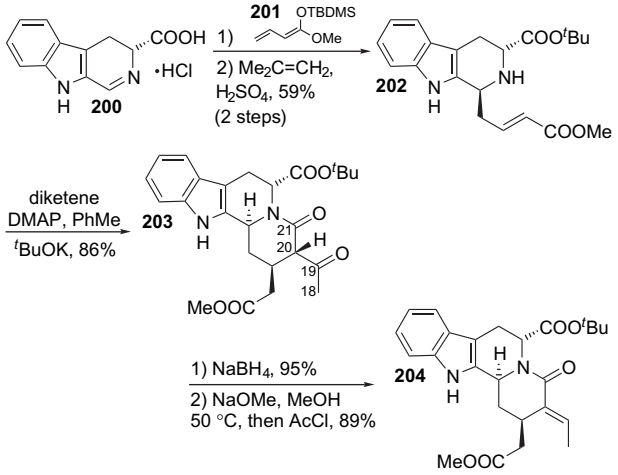
Martin et al. have reported[72] an enantiospecific total synthesis of *N*-methylvellosimine **94**, which differs fundamentally from that of Cook in that formation of the C5–C16 bond is the final C–C bond-forming event (**199**, Scheme 60).



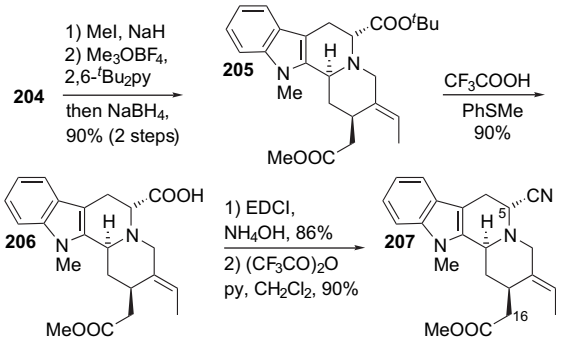
Scheme 60.

That such a reaction might occur in the biosynthesis of **94** was first proposed by van Tamelen,[73,74] a proposition supported by the subsequent report[75,76] of a biogenetic-type synthesis of ajmaline involving just such a transformation. Later, Lounasmaa et al. attempted the cyclisation of similar iminium ions, but with no success.[77] This led them to propose an alternative biosynthesis for the formation of the sarpagan skeleton, with C5–C16 bond formation as the *penultimate* skeletal bond-forming transformation and N4–C21 bond formation as the final cyclisation. Partly to discern which pathway was most likely to operate, Martin and co-workers undertook the synthesis outlined below.

Martin's synthesis (Scheme 61) commenced with the vinylogous Mannich reaction of dihydro-β-carboline **200** (derived from D-tryptophan and formic acid in 60% yield) with silyl ketene acetal **201** to give tetrahydro-β-carboline **202** with total diastereoselectivity. Introduction of the 4-carbon C18–C21 fragment with diketene (and concomitant cyclising Michael addition) gave tetracycle **203**. Stepwise borohydride reduction and elimination gave α,β-unsaturated amide **204** as a single geometric isomer. N1-methylation, amide reduction (giving **205**) and selective ester hydrolysis gave the potential iminium precursor **206**. It was decided to employ an α-aminonitrile as the actual iminium precursor, as these were known to furnish iminium ions under mild conditions. α-Aminonitrile **207** was thus synthesised by introduction of an amide at the C5 position and its subsequent dehydration (Scheme 62).

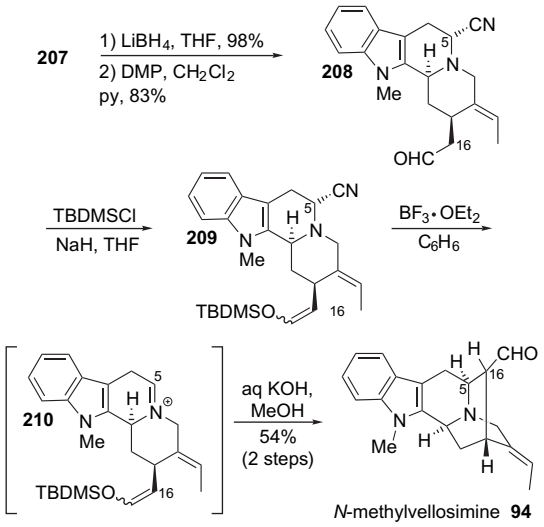


Scheme 61.



Scheme 62.

α-Aminonitrile **207** was subjected to imine-generating conditions, but no C5–C16 cyclisation was observed. This was taken to mean that the ester was insufficiently activating and so it was converted into aldehyde **208**. This also was inert to cyclisation, but, upon formation of the corresponding silyl enol ether **209** and treatment with $BF_3 \cdot OEt_2$, cyclisation to the sarpagan skeleton was observed (Scheme 63).

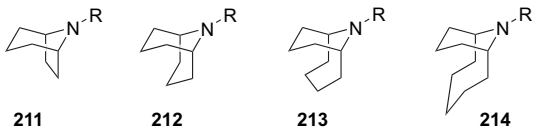


Scheme 63.

The target was obtained as an epimeric mixture (7:3 (+)-*N*-methylvellosimine/(+)-16-*epi*-*N*-methylvellosimine). As the desired natural epimer is the more thermodynamically stable, conversion into pure **94** was achieved by exposure of the mixture to aqueous KOH in MeOH. This elegant synthesis (7% overall yield from D-tryptophan) provides significant evidence for the feasibility of van Tamelen's original biogenetic pathway. Furthermore, it points to the possibility that the total synthesis of other sarpagine/ajmaline alkaloids might be viable via such an iminium-induced cyclisation.

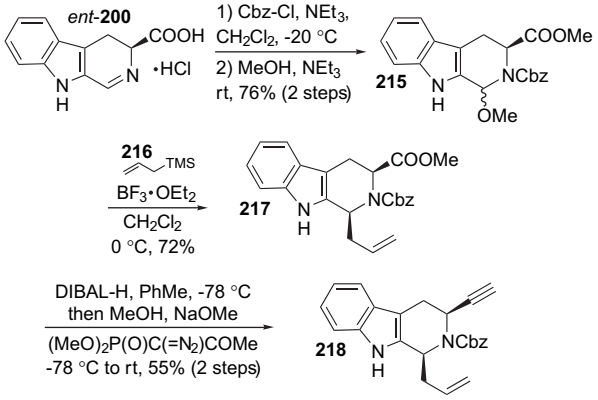
4. Martin's olefin metathesis route to azabicyclo[3.3.1]-nonenes

Martin et al. have conducted an extensive study[78] on olefin metathesis as a method of accessing various azabicyclo[*m*.*n*.1] structures (*m*=3–5, *n*=2–3, with the nitrogen in the 1-atom bridge). Such structural motifs (**211**–**214**) are common in alkaloids (Scheme 64).



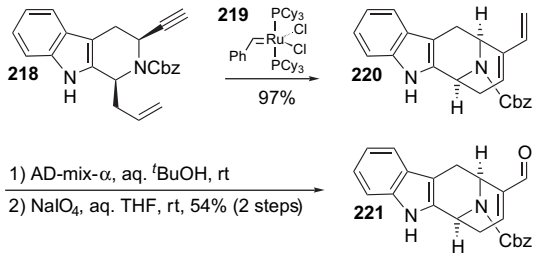
Scheme 64.

An indole-annulated azabicyclo[3.3.1] structure constitutes the tetracyclic skeleton of the macroline/sarpagine/ajmaline alkaloids and Martin and co-workers have been able to access this skeleton, as shown in Scheme 65.



Scheme 65.

Starting this time from L-tryptophan, the dihydro-β-carboline *ent*-**200** (accessed in 63% yield) was N-protected before aminal formation with in situ esterification. The diastereoisomeric mixture **215** was treated with allyltrimethylsilane **216** and boron trifluoride etherate to afford C3,C5-*cis* tetrahydro-β-carboline **217** in a 5.5:1 diastereoisomeric ratio. The ester was then selectively reduced and the aldehyde reacted with the diazophosphonate shown to afford the alkyne in a one-pot procedure. This alkyne **218** underwent enyne metathesis (Scheme 66) with Grubbs' first-generation catalyst **219** to give tetracyclic diene **220** in essentially quantitative yield. The monosubstituted olefin of this diene was then selectively cleaved with AD-mix-α[79] and $NaIO_4$ to give α,β-unsaturated aldehyde **221**.

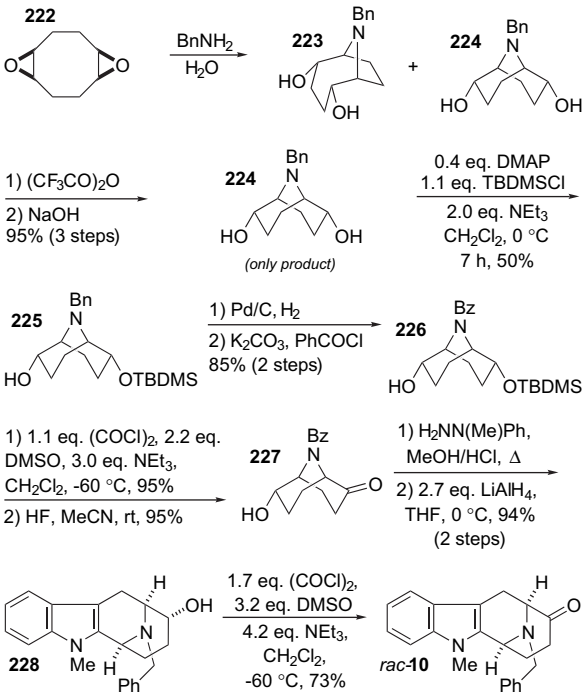


Scheme 66.

The α,β-unsaturated aldehyde **221** (10% yield from L-tryptophan) is a differentially protected form of the advanced intermediate **61** reported by Cook in the enantiospecific syntheses of macroline/sarpagine/ajmaline alkaloids, as detailed in Section 2. As such, this report from Martin constitutes a useful alternative approach to these natural products, starting, as it does, from L-tryptophan.

5. Rassat's synthesis of the tetracyclic ketone

In 2000, Rassat and co-workers reported[80,81] a synthesis of Cook's tetracyclic ketone intermediate **10** (summarised in Scheme 67). The crucial strategic difference in this approach is that formation of the [3.3.1]bicyclic skeleton occurs prior to the introduction of an indole.



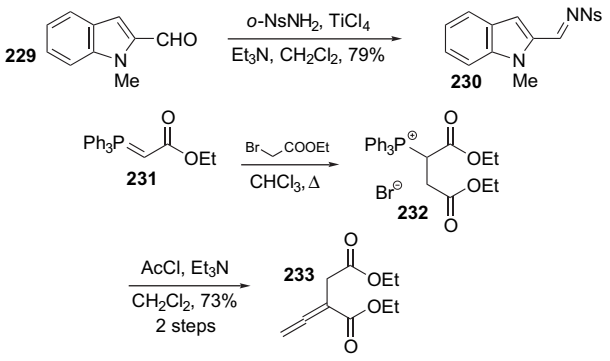
Scheme 67.

Transannular cyclisation of the bis(epoxide) starting material **222** with benzylamine led to a regioisomeric mixture of bicyclic structures. The unwanted [4.2.1]bicycle **223** may be converted into the desired [3.3.1]bicycle **224** under conditions of trifluoroacetate formation and subsequent hydrolysis. Selective monoprotection of the resultant diol to give **225** was followed by a protecting group swap, giving **226**. Oxidation to the ketone and deprotection of the other hydroxyl functionality led to the precursor **227** for Fischer indole synthesis of the tetracyclic core. This was effected in good yield with *N*-methyl-*N*-phenylhydrazine in acidic methanol at reflux overnight. Reduction to **228** regenerated the original *N*-benzyl protecting group and oxidation afforded the racemate of Cook's intermediate **10** in 25% overall yield.

6. Kwon's formal syntheses of (±)-alstonerine and (±)-macroline

Kwon and co-workers' formal syntheses[82] arose from their interest in phosphine-catalysed [4+2] annulations.[83] This key reaction occurred between an indolyl imine dienophile **230** (prepared from **229**) and a diene synthetic equivalent, allenyl diester **233** (prepared from **231** via **232**). The synthesis of these two coupling partners is shown in Scheme 68.

The cyclisation of **230** and **233** proceeded in 73% yield to give **241** as a 3:1 mixture of diastereoisomers. The proposed



Scheme 68.

mechanism (believed to proceed via intermediates **234**–**240**) is shown in Scheme 69.

Under acidic conditions, the [4+2] product **241** underwent an intramolecular Friedel–Crafts acylation (Scheme 70) to give the tetracyclic macroline skeleton **242**. Thiolate-mediated N4-deprotection and subsequent Eschweiler–Clarke N4-methylation both proceeded in essentially quantitative yield to give **243**. $NaBH_4$ and ZnI_2 effected benzylic ketone reduction (along with formation of the N4-borane adduct, **244**; the N–B bond was cleaved by heating to reflux in EtOH). DIBAL-H ester reduction gave the tetracyclic allyl alcohol *rac*-**37**.

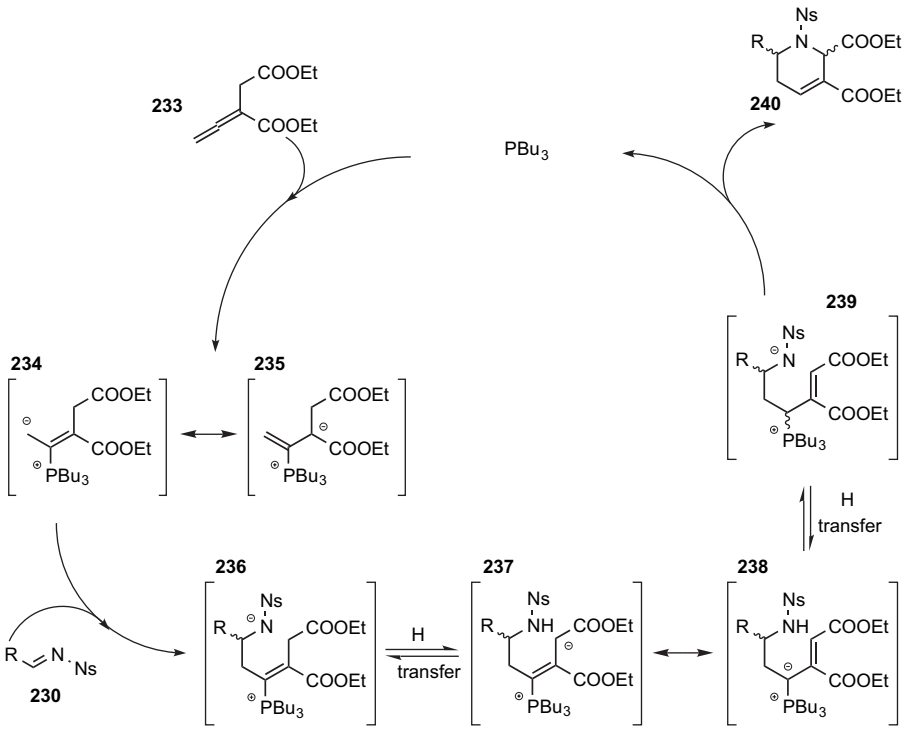
Racemic alcohol *rac*-**37** (31% yield, longest linear sequence) is an advanced intermediate in Cook's syntheses of alstonerine **44** and macroline **1** (see Sections 2.2 and 2.9).

7. Kuethe's aza-Diels–Alder/intramolecular Heck approach

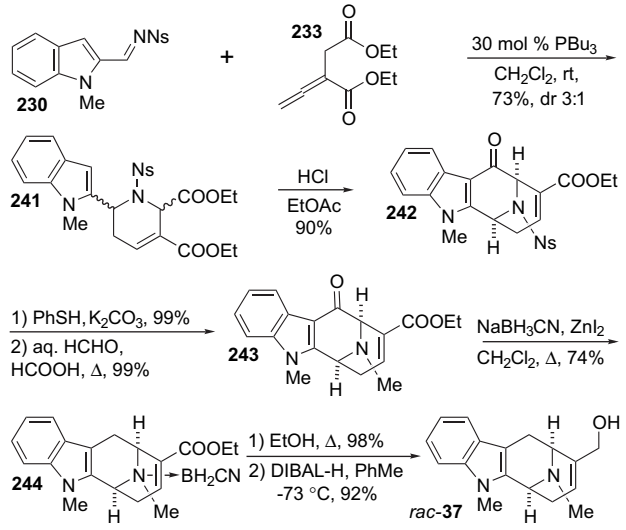
Kuethe and co-workers[84] have also adopted a [4+2] annulation strategy for construction of the tetracyclic macroline core. Adapting the work of Waldmann,[85] they employed Danishefsky's diene **248** with an imine derived from **245** (via **246** and **247**), the connectivity of which was different to that used by Martin, in that it was derived from an indole substituted at the C7-position, not the C2-position. The cyclisation is shown in Scheme 71.

Kuethe's group then attempted the synthesis of the desired tetracyclic system under conditions of both transmetallation and radical initiation. In both instances, however, the substrate **249** was simply deiodinated at the indolyl 2-position. The desired cyclisation was eventually effected by the use of palladium, giving **251** (Scheme 72).

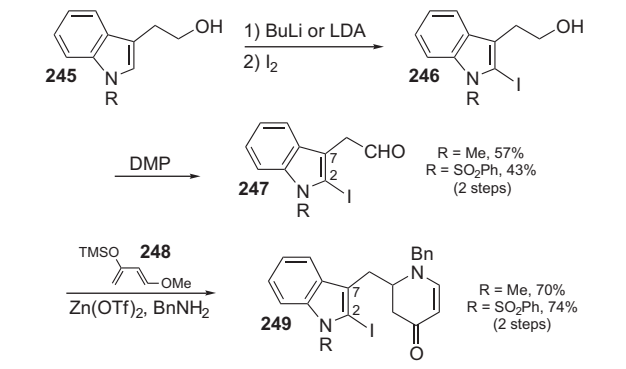
The reaction required stoichiometric amounts of Pd^{II}—rapid deposition of palladium black was observed during the course of the reaction. The inability of the reaction to go to completion under catalytic Heck conditions is presumed to arise from the lack of an appropriate β-hydrogen for elimination. The proposed intermediate *anti*-**252** (Scheme 73) has no β-hydrogen for *syn* elimination. Whilst isomerisation via a palladium enolate **253** is feasible,[86] *syn* elimination still does not occur, presumably since it would entail the formation of a high-energy *anti*-Bredt bridgehead olefin.



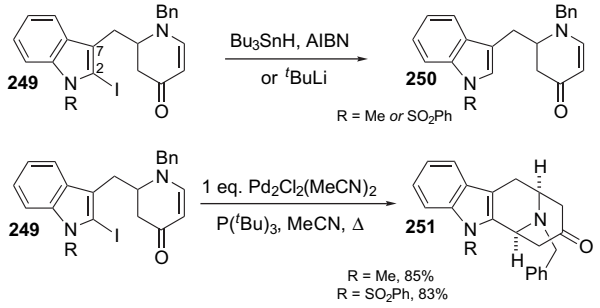
Scheme 69.



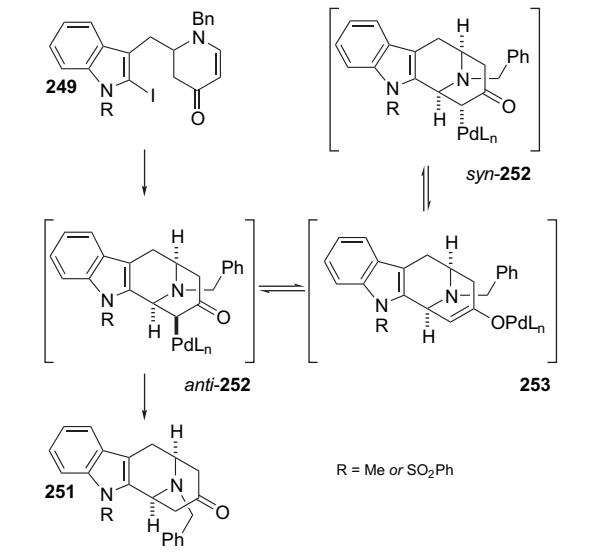
Scheme 70.



Scheme 71.

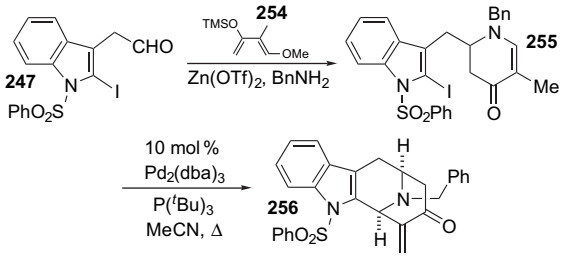


Scheme 72.



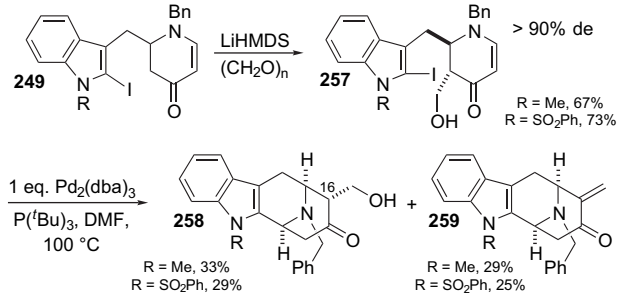
Scheme 73.

Attempts at performing the catalytic Heck reaction under reductive conditions led only to isolation of the deiodinated by-products **250**. When a modified Heck substrate **255** that contained additional β-hydrogens (the extra methyl group in **254** compared to **248**) was prepared, this smoothly underwent cyclisation with 10 mol % Pd^0 to give **256** (Scheme 74).



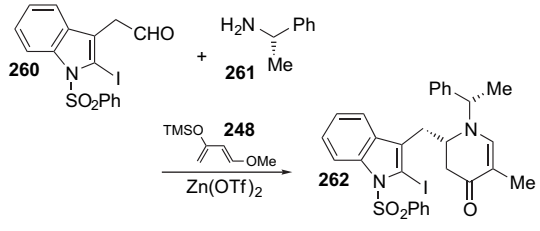
Scheme 74.

Many ajmaline/sarpagine alkaloids possess a hydroxymethyl group at the C16 position. In order to introduce such a moiety, **249** was hydroxymethylated to give **257** prior to palladium cyclisation, as before, to give **258**. Notably, appreciable amounts of α,β-unsaturated ketone **259** were isolated also. This is proposed to arise by elimination from the palladium enolate of type **253**. Whilst the use of stoichiometric amounts of palladium has obvious disadvantages, this entry to the tetracyclic macroline skeleton is novel and reasonably succinct (e.g., *N*-methyl-**258**, five steps, 9% yield, Scheme 75).



Scheme 75.

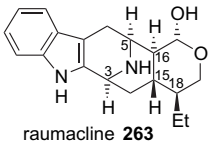
Efforts are currently under way to induce asymmetry[87] in the aza-Diels–Alder cyclisation by use of a chiral amine for imine formation. For example, the use of the imine derived from (*S*)-α-methylbenzylamine **261** and indolyl aldehyde **260** gave rise to dihydropyridone **262** in a diastereoisomeric ratio of 92:8 (Scheme 76).



Scheme 76.

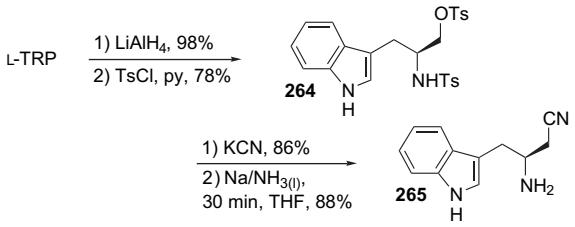
8. Bailey's synthesis of (−)-raumacline

Like Cook, Bailey and co-workers have made extensive study of the Pictet–Spengler reaction and have utilised it in previously reported formal syntheses of ajmaline, koumidine and suaveoline, amongst others.[88] Unlike Cook, Bailey's syntheses have as their core strategy the use of C3,C5-cis-specific Pictet–Spengler reactions. This permits the use of L-tryptophan to access various tetrahydro-β-carbolines having the correct configuration at C-3 and C-5 and this approach was used in Bailey's recent synthesis of raumacline[89] (**263**, Scheme 77). In contrast, Cook employs D-tryptophan in C3,C5-trans-specific Pictet–Spengler reactions, followed by selective epimerisation at C-5.



Scheme 77.

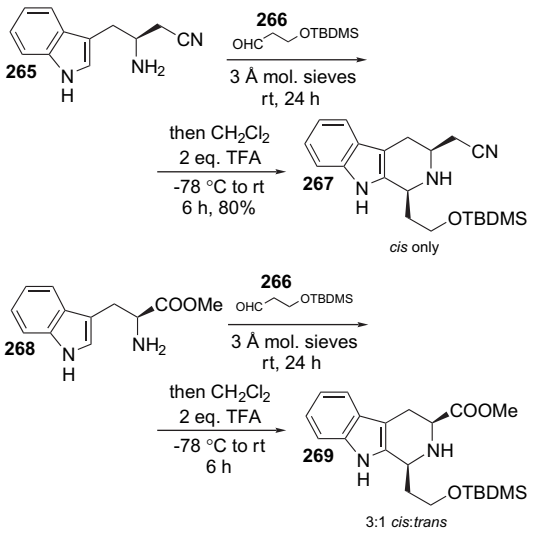
Bailey et al. employed cyanomethyltryptamine **265** as their Pictet–Spengler substrate.[90] It may be synthesised in four steps from the amino acid starting material on a large scale with no need for chromatography—the cyanosulfonamide made from **264** may be purified by crystallisation and the subsequent reductive desulfonylation has been optimised to provide pure **265** (Scheme 78).



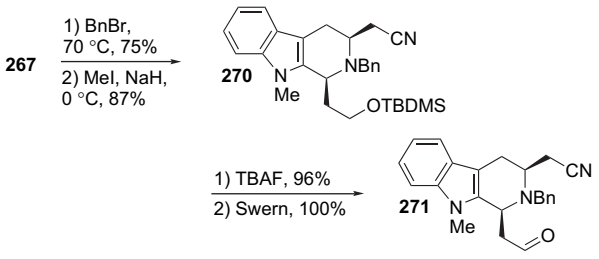
Scheme 78.

Pictet–Spengler cyclisation of **265** with a protected β-hydroxyaldehyde **266** gave C3,C5-*cis* tetrahydro-β-carboline **267** as the sole product. The factors that influence the selectivity had previously been studied[91] and it had been shown that in general, only for reactions of aryl aldehydes with tryptophan allyl ester, total C3,C5-cis selectivity was observed. A C-3 aryl substituent would not have been synthetically useful in the context of raumacline, however. A two-carbon masked aldehyde equivalent was required at the C-3 position, and the use of the silylated hydroxyaldehyde in conjunction with the cyanomethyl group is both synthetically useful and cis-specific. Such a choice of substituents likely arose from extensive optimisation; for example, cyclisation of the same aldehyde **266** with L-tryptophan methyl ester **268** gave **269** with only 3:1 cis-selectivity (Scheme 79).

Once formed, tetrahydro-β-carboline **267** was N4-benzylated and N1-methylated without complication, giving **270**. It is probably significant that the Pictet–Spengler reaction was performed on the N1,N4-unsubstituted system; Cook has observed that an N4-benzyl substituent (or any bulky substituent) enhances C3,C5-trans selectivity in the cyclisation. Hydroxyl deprotection and oxidation to **271** were routine (Scheme 80).

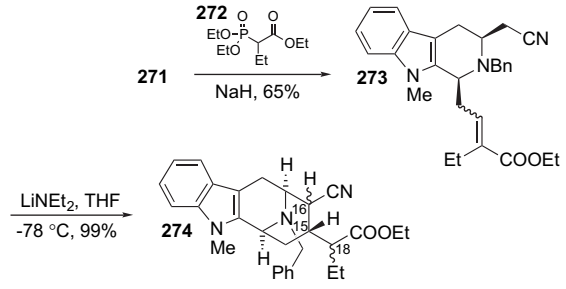


Scheme 79.



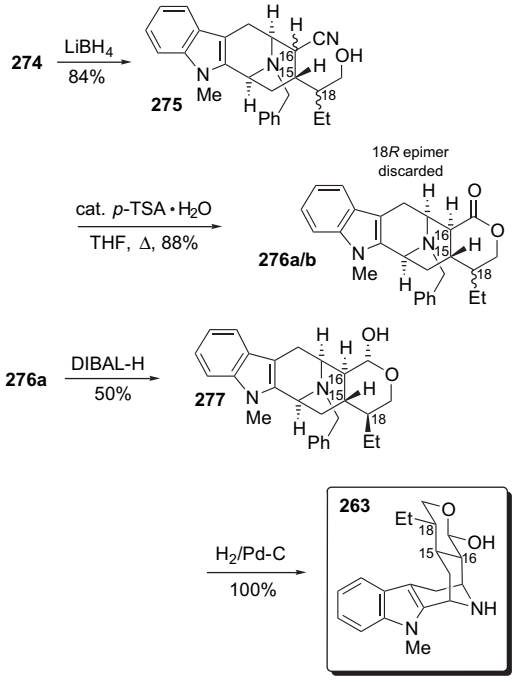
Scheme 80.

A Horner–Wadsworth–Emmons reaction with **272** furnished **273** (5:3 *E*:*Z*), the substrate for intramolecular Michael cyclisation to the tetracycle. This was induced with $LiNEt_2$, giving **274** as an inseparable mixture of diastereoisomers. C-15 was found to have entirely *R* configuration as desired and C-16 was found to be 4:1 *S*:*R*. No selectivity was observed at C-18 (1:1 *S*:*R*). Bailey makes no comment relating the C-18 stereochemistry to olefin geometry or otherwise (Scheme 81).



Scheme 81.

After reduction, heating the resultant diastereoisomeric mixture **275** to reflux with catalytic toluene-4-sulfonic acid hydrate in THF gave a mixture of two lactones **276a/b**, diastereoisomeric at C-18. Gratifyingly, both C-16 epimers had been transformed only into (16*S*) lactones **276a/b**. Presumably the (16*R*) epimer of **275** had initially cyclised to the *cis*-decalin, before base-induced epimerisation to the *trans*-decalin structure. That the *trans*-decalin would be the lower-energy configuration may be seen from the predicted 3D structure of (−)-raumacline (Scheme 82), where the all-equatorial conformation is visible. The C-18 epimeric lactones were separated by chromatography and the isomer having the correct (18*S*) configuration (**276a**) underwent DIBAL reduction to introduce lactol **277** (correctly configured) and hydrogenolytic debenzylation to afford (−)-raumacline **263** (Scheme 82).



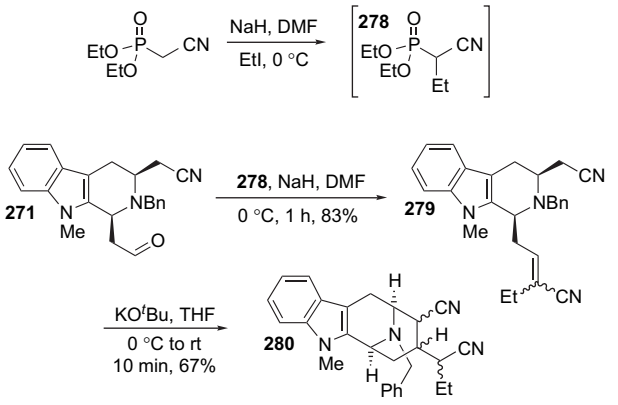
Scheme 82.

The difficulty in exerting control over the C-18 stereochemistry is regrettable, but, nevertheless, in this synthesis of (−)-raumacline (7% overall yield from L-tryptophan), five of the six stereocentres have been effectively controlled, a notable achievement and a significant improvement on previous approaches.

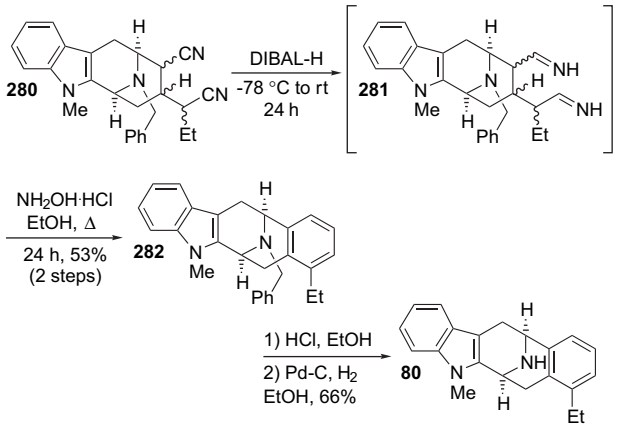
9. Bailey's synthesis of (−)-suaveoline

In addition to the earlier reported formal syntheses[88] of suaveoline and ajmaline, Bailey and co-workers have made many and varied additional contributions[92] to the field. These have culminated in a recent total synthesis of suaveoline.[93] The synthesis employs the same cis-selective Pictet–Spengler cyclisation described in Section 8, but in this instance, cyanoaldehyde **271** was homologated to an unsaturated bis(nitrile) species **279** by means of a Horner–Wadsworth–Emmons reaction. Phosphonate **278** was prepared by in situ alkylation with ethyl iodide. A vinylogous Thorpe cyclisation was then effected, giving the tetracyclic intermediate **280** (Scheme 83).

Tetracycle **280** was isolated as a mixture of diastereoisomers, all of which were suitable for further elaboration to suaveoline. Completion of the synthesis was by DIBAL-mediated reduction of **280** to an intermediate diimine **281**. This was treated with hydroxylamine hydrochloride in ethanol to effect formation of pyridine **282**. N4-Deprotection gave suaveoline **80** (6% from L-tryptophan), identical with both the natural product and a sample of semi-synthetic suaveoline prepared from ajmaline[93] (Scheme 84).



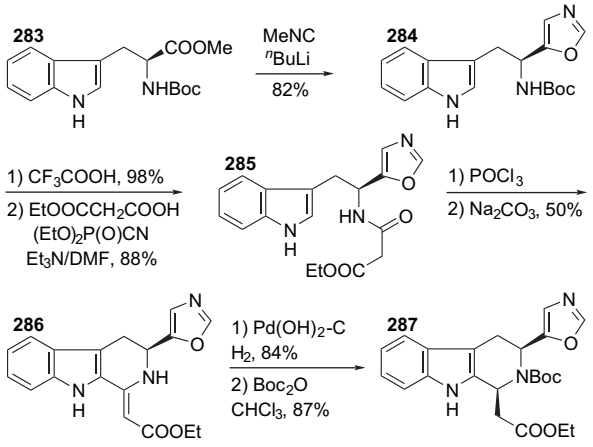
Scheme 83.



Scheme 84.

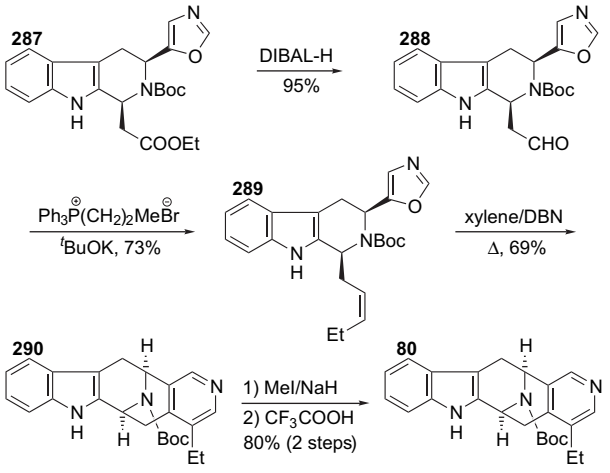
10. Ohba's synthesis of (−)-suaveoline

The total synthesis of (−)-suaveoline reported by Ohba and co-workers[94] arose from their interest in oxazole–olefin Diels–Alder reactions as a route to annulated pyridines. Formation of oxazole **284** from N4-Boc-protected L-tryptophan methyl ester **283** occurred without erosion of ee according to their previously reported methodology.[95] Temporary removal of the protecting group was necessary for N-acylation (giving **285**), Bischler–Napieralski reaction (6 days in neat $POCl_3$, giving **286**) and stereoselective hydrogenation (Scheme 85).



Scheme 85.

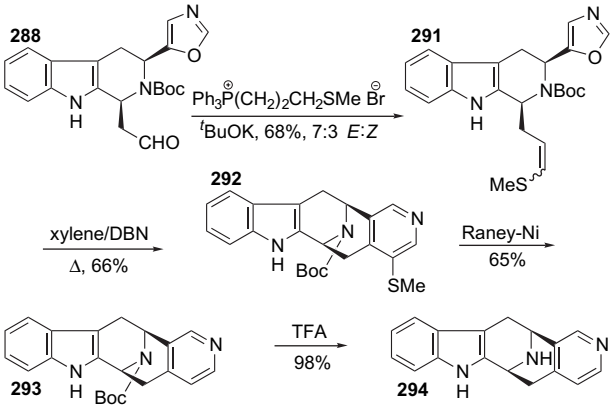
Upon re-introduction of the Boc group to give **287**, a chemoselective ester to aldehyde reduction was effected followed by Wittig reaction to introduce the ethyl side chain. The IMDA reaction of **289** was found to work best by heating in xylene at reflux, with addition of 1,5-diazabicyclo[4.3.0]-non-5-ene (suggested simply to be a scavenger for H_2O), giving pyridine **290** in 69% yield. N1-Methylation and N4-deprotection afforded (−)-suaveoline **80** in 10% yield from **283**. The route disclosed above is radically different from those of Bailey and Cook—instead of relying on a Pictet–Spengler reaction to install the crucial tetrahydro-β-carboline stereochemistry, Ohba employs a diastereoselective reduction. Whilst the synthesis was most likely conceived primarily as a showcase for the pyridine-forming IMDA reaction, the aforementioned diastereoselective reduction may be of use for the synthesis of further members of the macroline/sarpagine/ajmaline indole class. It is noteworthy that, in this succinct synthesis, N1-protection was unnecessary (Scheme 86).



Scheme 86.

11. Ohba's synthesis of 1-demethyl-20-deethylsuaveoline

In 1996, Batista et al. isolated sellowiine, a macroline-related alkaloid, from the leaves of *Rauvolfia sellowii*.[96,97] For this natural product, they proposed the structure 1-demethyl-20-deethylsuaveoline **294**. The methodology of Ohba and co-workers was ideally suited to the synthesis of this structure and they were able to achieve a total synthesis[98] (Scheme 87).

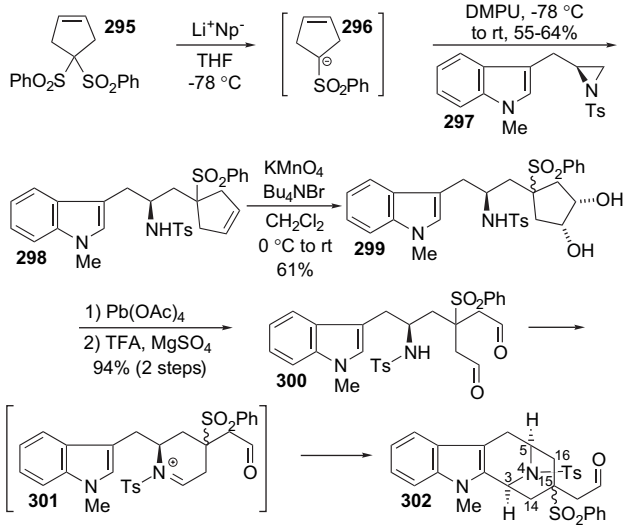


Scheme 87.

Elaboration of aldehyde **288** was by a Wittig reaction to introduce a vinyl sulfide side chain (it was found that a terminal olefin was not able to undergo the intramolecular Diels–Alder reaction). Thus the removable thiomethyl group was used instead, and the IMDA reaction of **291** gave pyridine **292** in good yield. Removal of the thiomethyl group from **292** by reduction with Raney-nickel (giving **293**) and trifluoroacetic acid-induced N4-deprotection gave 1-demethyl-20-deethylsuaveoline **294** (7% yield from N4-Boc L-tryptophan methyl ester). The spectroscopic data recorded by Ohba and co-workers for **294** did not correlate with those reported for sellowiine by Batista; the chemistry of sellowiine remains incomplete, therefore.

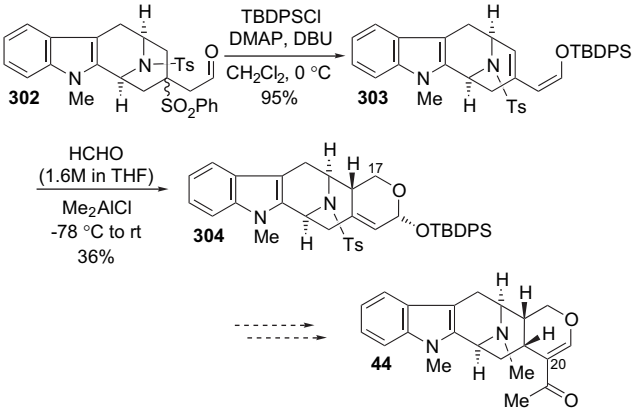
12. Craig's approach to (−)-alstonerine

Craig and co-workers have recently reported[99] the results of their studies on the syntheses of (−)-alstonerine **44** by an aziridine-based approach. Using methodology reported by Mioskowski,[100] they were able to generate anion **296** by reductive desulfonylation of bis(sulfone) **295**. This in turn was added to L-tryptophan-derived aziridine **297** to give **298**. The cyclopentene in **298** was employed as a dialdehyde surrogate; in order that it could be unmasked, a selective oxidation of the olefin in the presence of the indole was necessary. After optimisation, this was found to be viable with tetra-*n*-butylammonium permanganate in CH_2Cl_2, giving 2**99**. Subsequent diol cleavage gave dialdehyde **300**, which underwent acid-induced Pictet–Spengler cyclisation via **301** to tetracyclic monoaldehyde **302** as a mixture of diastereoisomers (Scheme 88).



Scheme 88.

Craig's use of the Pictet–Spengler reaction is strategically different from Cook's or Bailey's. In Bailey's syntheses, cis-selectivity was achieved in the Pictet–Spengler reaction by careful choice of reaction partners. In the current work, the tetrahydro-β-carboline geometry was formed exclusively cis, due to the cyclic nature of the iminium intermediate. This reversal of the order of events (formation of the C3–N4–C5–C16–C15–C14 ring prior to this *intramolecular* Pictet–Spengler cyclisation) neatly avoids stereochemical ambiguity in the cyclisation step. Monoaldehyde **302** was further elaborated by sulfone elimination and vinylogous silyl enol ether formation. The geometry shown for **303** was observed exclusively. Introduction of C17 was effected by the use of an unusual hetero-Diels–Alder reaction of formaldehyde. Monomeric formaldehyde, generated by a modified version of the Schlosser protocol,[101] was reacted with **303** under conditions of Lewis acid catalysis to give advanced pentacyclic intermediate **304** (9% from L-tryptophan). It can be seen that introduction of a pendant 2-carbon fragment at C20 would permit access to the complete alstonerine skeleton (Scheme 89).



Scheme 89.

13. Conclusions and future prospects

The chemistry detailed herein shows that considerable advances have recently been made in the field of sarpagine/macroline/ajmaline indole alkaloids since the field was last reviewed. The Pictet–Spengler reaction remains a key strategic transformation for the synthesis of molecules of this class, as evidenced by the work of Cook, Bailey and Craig. Nevertheless, a diverse array of other reaction classes have been deployed to access the targets in question. In particular, Cook's use of a common late-stage tetracyclic intermediate has allowed access to a large variety of natural products by use of varied transformations for the final elaborations. It is anticipated that further advances in the chemistry of macroline/sarpagine/ajmaline indole alkaloids will be reported in due course by many of the laboratories from which the work reviewed here originated.

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Biographical sketch

Simon E. Lewis was born in London, UK in 1978. He received his MSci degree in 2001 from Imperial College, London, where he earned the SmithKline Beecham award for excellence in organic chemistry and was jointly awarded the Neil Arnott prize. After a short period with GlaxoSmithKline, he returned to Imperial College in 2002 where he was the beneficiary of a generous Pfizer CASE scholarship. He pursued his doctoral studies under the supervision of Professor Donald Craig, on the decarboxylative Ireland–Claisen rearrangement and its application to the synthesis of suaveoline. In 2006, he joined the group of Professor Andrew G. Myers at Harvard University where he is currently working on the synthesis of tetracycline antibiotics.

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The calculated enthalpies of the nine pyrazole anions, cations, and radicals: a comparison with experiment

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Dedicated to Professor Miha Tišler on the occasion of his 80th birthday in recognition of his contribution to chemistry

Abstract—Enthalpies of 12 pyrazole species including neutral, anions, cations, and radicals have been calculated at the G3B3 level. The main conclusions are: (i) there are ten equilibria between species of which six have been measured experimentally and the agreement is excellent; (ii) two structures, cyclic and chain, have been found for the pyrazolium-radical **8** that are able to explain the electrochemistry of pyrazolium salts; (iii) the aromaticity, calculated as the NICS indexes, is related to the unexpected stability of the pyrazole anion **3**.

1. Introduction

Radicals play major role in many reactions, for instance, SET, direct electron transfer, and $S_{RN}1$,[1–3] spectroscopies such as PES,[4] ESR (EPR),[5] mass spectrometry (both positive and negative ions),[6] and electrochemistry.[7] Radicals are related, through electron and proton transfers, to neutral molecules, anions, and cations. On the other hand, we have been interested in pyrazoles for a long time.[8] It was thus natural that we wanted to explore the potential surface of the entities reported in Scheme 1 from an energy point of view.

First of all, we will summarize the information available on the nine compounds, not chronologically but in the order they appear in Scheme 1. The pyrazole cation **1** has been studied only once.[9] According to Pasto et al. the energy minimum corresponds to a closed-shell singlet with four π electrons in a $(1b_1)^2,(2b_1)^2$ configuration. This structure shows very long CN distances and a short NN distance. A second minimum with $(1b_1)^2,(1a_1)^2$ configuration with also four π electrons presents short CN distances and a long NN distance.

The 1-pyrazolyl radical **2** of Scheme 1 is, by far, the most studied radical. The question of its structure (σ-type with a $^2B_2$ state or π-type with $^2A_2$ or $^2B_1$ states) was first discussed by Janssen et al. on experimental grounds (Scheme 2),[10] and simultaneously by van der Meer and Mulder using the ab initio STO-3G basis set.[11] The result is that the most stable structure is the π-$^2B_1$, followed by the π-$^2A_2$ (34.7 kJ mol^{-1}) and the σ-$^2B_2$ (60.7 kJ mol^{-1}). The calculation level was increased to Davidson-corrected CISD/6-31G* by Bofill et al.[12] resulting in the 1-pyrazolyl radical in a different profile with the $^2B_1$ π-type being the minimum and differences of 48.1 (local minimum) and 73.6 kJ mol^{-1} (crossing) with the $^2B_2$ σ-type. It has been independently reported that the pyrazolyl radical **2** is one of the rare cases where there are three electronic states with conical intersections in the ground state.[13]

Fortunately, a very recent paper of Lineberger et al.[14] report not only calculations on **2** at the B3LYP/6-311++G(d,p) level but also important experimental data on equilibria involving **2**, **3**, and **5**, that will be very useful to test our calculations. At the same level, Flammang et al.[15] calculated the adiabatic ionization energies (IE) of the radical cation **4** that agree very well with the experimental values.

2. Computational methods

The structures have been initially optimized at the hybrid DFT/HF, B3LYP,[16] computational level and the 6-31G* basis set[17] as implemented in the Gaussian 03 package.[18] Frequency calculations have been carried out to confirm that the structures obtained correspond to energy minima. Other electronic configurations have been explored at this computational level. Further, G3//B3LYP (usually reported in the literature as G3B3) calculations[19] have been carried out in the structures selected in the first step. The reported energy values correspond to enthalpies at 298.15 K, which include zero point energy (ZPE) and thermal corrections. The most stable compound is **8** (open) (−226.570952 hartree). We will report only the structures of lower energy except in those cases where there are two structures of similar energy.

Keywords: Pyrazoles; Radicals; Aromaticity; Ab initio; NICS.
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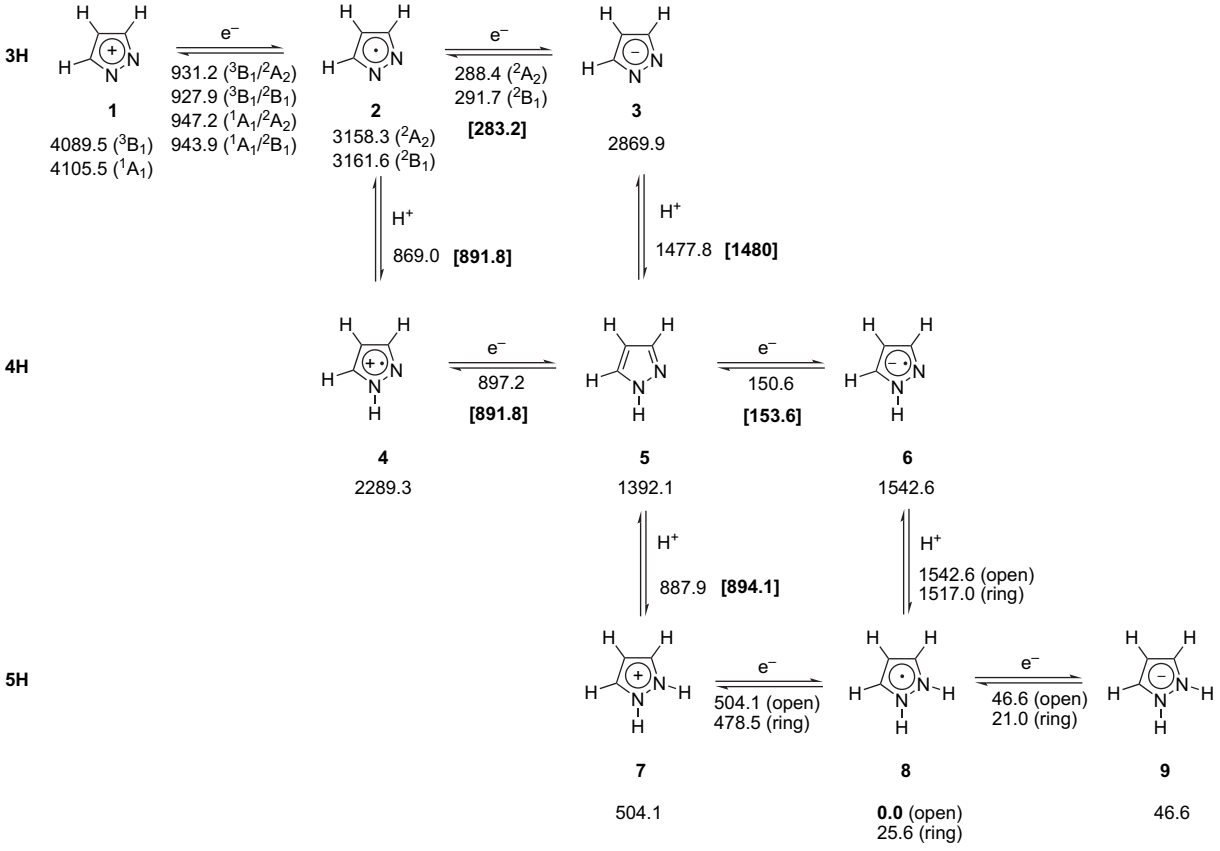
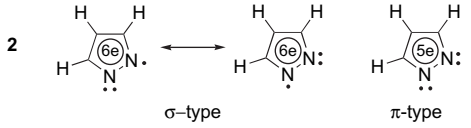


Scheme 1. The nine pyrazole derivatives under study.



Scheme 2. σ- and π-type structures of 1-pyrazolyl radical.

3. Results and discussion

3.1. Geometries

We have reported in Table 1 the main characteristics of the 12 studied compounds.

We have calculated the average bond distances (sum of the five distances/5) that reflects the size of the pyrazole ring. Leaving aside the open compound **8** (1.697 Å), these average distances belong to two groups: long distances, between 1.452 Å **9** and 1.412 Å **8** ring including **6** (1.419 Å) and **1s** (1.413 Å) and short distances, between 1.384 Å **4** and 1.365 Å **7**, the remaining compounds. We have also calculated the ratio of C–N (N2–C3/C5–N1) and C–C distances (C3–C4/C4–C5). In most cases these ratios are 1 (due to symmetry) except for four compounds: **4** (1.011 and 0.971), **5** (0.981 and 1.024), **6** (0.923 and 0.969) and **9** (0.981 and 0.958).

3.2. Energies

There are 10 equilibria in Scheme 1: the values under the formulae are the energies in kJ mol^{-1} with regard to the absolute minimum, compound **8**. The numbers close to the double arrows correspond to the equilibria and are differences between the former values. There are experimental enthalpies corresponding to six out of ten equilibria: **2/3** 283.2 kJ mol^{-1},[14] **2/4** 891.8 kJ mol^{-1},[15,16] **3/5** 1480 kJ mol^{-1},[20] **4/5** 891.8 kJ mol^{-1},[15] **5/6** 153.6 kJ mol^{-1},[14] and **5/7** 894.1 kJ mol^{-1}.[20]

Table 1. Geometries of the calculated structures at the B3LYP/6-31G* computational level. The electronic configuration and molecular symmetry are also given

	Electronic configuration	Molecular symmetry	N1–N2	N2–C3	C3–C4	C4–C5	C5–N1
1	$^3B_1$	C_{2v}	1.19	1.45	1.39	1.39	1.45
1	$^1A_1$	C_{2v}	1.23	1.54	1.38	1.38	1.54
2	$^2A_2$	C_{2v}	1.28	1.43	1.39	1.39	1.43
2	$^2B_1$	C_{2v}	1.47	1.30	1.38	1.38	1.30
3	$^1A_1$	C_{2v}	1.37	1.35	1.40	1.40	1.35
4	$^2A''$	C_s	1.42	1.32	1.42	1.46	1.30
5	$^1A'$	C_s	1.35	1.33	1.41	1.38	1.36
6	^2A	C_1	1.45	1.35	1.39	1.44	1.46
7	$^1A_1$	C_{2v}	1.35	1.35	1.39	1.39	1.34
8 ring	^2A	C_2	1.45	1.42	1.38	1.38	1.42
8 open	^2B	C_2	3.02	1.23	1.43	1.43	1.30
9	^1A	C_1	1.48	1.48	1.37	1.43	1.50

These data and the values of Scheme 1 are highly correlated (Eq. 1):

$$\Delta H^0_{298}\text{exp.} = (1.005 \pm 0.005)\Delta H^0_{298}\ \text{calcd}, \quad n = 6, \quad r^2 = 0.99989 \tag{1}$$

This gives strong confidence to the remaining four values. We have tried an empirical model to correlate the relative energies to some structural properties. Eq. 2 corresponds to the best model (the fact that the compound is a radical or not has no significant influence on the enthalpy); in the case of compounds **1**, **2**, and **8**, where there are two values (two structures) this model describes both structures as being identical.

$$\Delta\Delta H^0_{298}\ \text{calcd} = (7909 \pm 105) - (1590 \pm 25)\ \text{no. of H} + (1188 \pm 134)\ \text{charge} + (346 \pm 43)\ \text{charge}^2 - (195 \pm 34)\ \text{no. of H}^*\text{charge},\quad n = 12,\quad r^2 = 0.9986 \qquad (2)$$

No. of H is the number of hydrogen atoms (3, 4, or 5, see Scheme 1); charge is the charge −1 (anion), 0 (neutral), and +1 (cation). The largest residuals do not affect compounds **1**, **2**, and **8**, therefore treating both structures together has no significant influence on the model. To compare the coefficients, it is necessary to scale (−1, 0, +1) all the independent variables, this results in Eq. 3.

$$\Delta\Delta H^0_{298}\ \text{calcd} = (1548 \pm 33) - (1590 \pm 25)\ \text{no. of H} + (407 \pm 28)\ \text{charge} + (346 \pm 43)\ \text{charge}^2 - (195 \pm 33)\ \text{no. of H}^*\text{charge},\ n = 12,\quad r^2 = 0.9986 \qquad (3)$$

While the intercept has no physical meaning, the number of hydrogen atoms is the most important factor, to the point that it roughly explains most of the variance (Eq. 4).

$$\Delta\Delta H^0_{298}\ \text{calcd} = (1793 \pm 129) - (1668 \pm 149)\ \text{no. of H},\quad n = 12,\ r^2 = 0.926 \qquad (4)$$

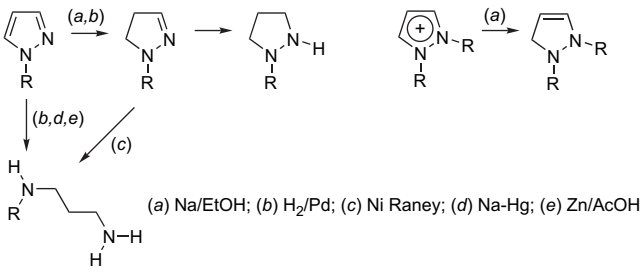
Since the number of carbon and nitrogen atoms are the same for all compounds, Eq. 4 indicates that $\Delta\Delta H^0_{298}$ calcd is an almost additive property.

In the 4H and 5H series the stability follows the neutral> anion>>cation but in the 3H series the order is different being anion>neutral>cation. May be anion **3** is overstabilized.

3.3. Structure and properties of radicals 8

Two minima have been found for **8**: a ring structure (25.6 kJ mol^{-1}) and an open structure (more stable, 0.0 kJ mol^{-1}). An examination of the literature in what concerns the reduction by Na/EtOH, Na–Hg, and H_2/Pd and electrochemistry of pyrazoles (related to **5** but *N*-substituted) and pyrazolium salts (related to **7** but *N*,*N*-disubstituted)[8,21,22] shows (Scheme 3) that these compounds are reduced to pyrazolines, pyrazolidines, and 1,3-diamines.

It is reasonable to assume that the radicals of Figure 1 are involved in some of these processes. Note from Scheme 1, that it should be easier to reduce the cation **7** than the neutral pyrazole **5**. Reciprocally, the reduction of **1** is calculated to be more difficult. This could be related to the fact that there are less H atoms to distribute the generated positive charge.



Scheme 3. The reduction of pyrazoles, pyrazolines, and pyrazolium salts.

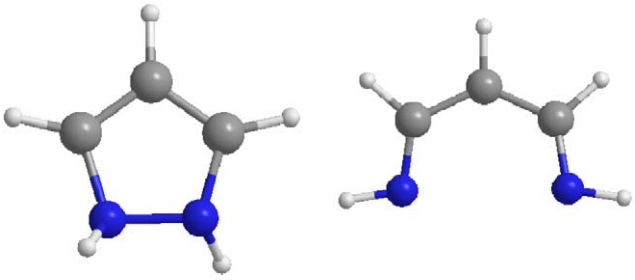


Figure 1. Structure of pyrazolium radicals **8**: both are minima and have a C_2 symmetry.

3.4. Aromaticity

In order to get some insight on the molecular properties of the structures of Scheme 1 we have calculated their NICS(0) and NICS(1) values.[23–25] The NICS(0) is calculated in the plane of the ring while the NICS(1) is calculated 1 Å above the ring plane. Note that we use[24,25] in Table 2 a sign conversion opposite to that of Schleyer et al.[23]

Although both NICS values are different they are roughly proportional [NICS(1)=(2.4±0.6)+(0.61±0.06) NICS(0), $n = 12$, $r^2 = 0.92$] so we will discuss only the NICS(1) values. Taking 11.2 ppm as the reference value, the true aromatic compounds are **3**, **5**, and **7**, the vertical column of non-radicals in Scheme 1. Slightly aromatic compounds are **6**, **8** (ring), and **9** (the bottom right side of Scheme 1) and medium (**1**($^3B_1$), **2**($^2A_2$), **2**($^2B_1$), and **4**) to strongly antiaromatic (**1s**) compounds occupy the top left side of Scheme 1. The anomalies we have reported in the energetic discussion could be related to the large aromaticity of pyrazolate

Table 2. NICS values (ppm)

Molecule	NICS(0)	NICS(1)
1 ($^3B_1$)	−5.0	−1.8
1 ($^1A_1$)	−16.4	−7.7
2 ($^2A_2$)	−8.6	−1.3
2 ($^2B_1$)	−12.4	−3.7
3	14.1	13.3
4	−10.2	−2.8
5	15.0	12.4
6	5.5	3.6
7	15.4	11.6
8 Ring	5.8	3.2
9	3.2	1.2
Benzene	9.7	11.2

anion, **3**, which is common to other five-membered anions like cyclopentadienyl.[26]

4. Conclusions

The theoretical calculation of the enthalpies of the structures involved in proton and electron transfer of pyrazole yield good results if carried out at sufficient level (G3B3). They explain the observed equilibria and allow a confident prediction of the still missing data. Aromaticity seems to play a significant role on the stability of the different structures.

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First synthesis of polyoxin M

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Abstract—Chiral enolate derived from (4*R*)-4-*tert*-butyldiphenylsilyloxymethyl-4-butanolide **10** with lithium hexamethyldisilyazide (LiHMDS) was treated with trisyl azide, followed by addition of TMSCl to give (2*S*,4*R*)-2-azido-4-[(*tert*-butyldiphenylsilyloxy)methyl]-4-butanolide **8** (53%), from which the first total synthesis of polyoxin M (**1**) was achieved in overall 3.2% yield (13 steps) from D-glutamic acid. Moreover, the synthesis of the reported synthetic intermediate (2*S*,4*R*)-4-hydroxyornithine congener **6** for biphenomycins A and B was also achieved in overall 4.1% yield (12 steps) from D-glutamic acid.

1. Introduction

Polyoxin M (**1**) is a class of peptidyl nucleoside antibiotics isolated from the culture broths of *Streptomyces cacao*i var *asoensis*.[1] All members of the polyoxin family possess 1-(5′-amino-5′-deoxy-β-D-allofuranuronosyl)pyrimidines such as thymine polyoxin C and uracil polyoxin C (**3**) as a basic component. The biological activity of the polyoxins is very characteristic because of their specific action against phytopathogenic fungi and the human fungal pathogen (e.g., *Candida albicans*), and lack of activity against other microorganisms, plants, fish, and mammals.[1] The site of action of the polyoxins was reported to be responsible for cell wall chitin biosynthesis.[2] In the preceding paper, we reported a short-path synthesis of methyl (methyl-2,3-*O*-isopropylidene-α-L-talofuranoside)uronate from methyl 2,3-*O*-isopropylidene-dialdo-D-ribofuranoside and its application to the total syntheses of thymine polyoxin C and uracil polyoxin C (**3**).[3] We also reported a convenient synthesis of the *N*-protected L-carbamoyl-polyoxamic acid derivative and its application to the total syntheses of polyoxins J,[4a] L,[4a] B,[4b] and D.[4b] Retrosynthetically, the synthesis of **1** can be achieved by amide formation between the left-half α-amino acid congener (**2**) and the right-half **3**. On the other hand, biphenomycins A (**4**) and B (**5**) were isolated from the cultured broth of *Streptomyces griseorubiginosus* No. 43708. These antibiotics are active in vitro and in vivo against bacteria and are especially potent against Gram-positive bacteria.[5] For the synthesis of **4** and **5**, (2*S*,4*R*)-4-hydroxyornithine congener (**6**), protected in three different ways, is thought to be an important intermediate.[6] We now describe the synthesis of polyoxin M (**1**) and (2*S*,4*R*)-4-hydroxyornithine congener (**6**) based on the electrophilic azide transfer to chiral enolate (Scheme 1).

2. Results and discussion

2.1. Total synthesis of polyoxin M (1)

For the synthesis of **2** or **6**, (2*S*,4*R*)-2-azido-4-protected hydroxymethyl-4-butanolide congener **7** or **8** is thought to be an important intermediate. These azide compounds, **7** or **8**, could be obtained by the diastereoselective azide transfer to chiral enolate derived from the (4*R*)-protected hydroxymethyl-4-butanolide **9** or **10**. By applying the reported method,[7] the synthesis of (4*R*)-**9** or (4*R*)-**10** was achieved by tritylation or silylation of (4*R*)-γ-hydroxymethyl-γ-butyrolactone derived from D-glutamic acid. Concerning the diastereoselective introduction of a substituent at the 2-position in (4*S*)-**9** or (4*S*)-**10**, three examples were reported as shown in Scheme 2. The first example is the efficient enantioselective construction of quarternary carbon centers by the sequential dialkylation of (4*S*)-**9**[8] and the second one is the diastereoselective introduction (2α:2β=7:1) of a hydroxyl group at the 2-position in (4*S*)-**10**.[9] The third one is the diastereoselective introduction (2β:2α=93:7) of a 1,2-bis(*N*-Boc)hydrazino group at the 2-position in (4*R*)-**10**.[10] On the other hand, treatment of chiral enolate derived from *N*-acyloxazolidone **A** with 2,4,6-triisopropylbenzenesulfonyl azide (trisyl azide), followed by addition of AcOH was reported to give (2*S*)-azido carboximides **B** with high diastereoselectivity[11] as shown in Scheme 2.

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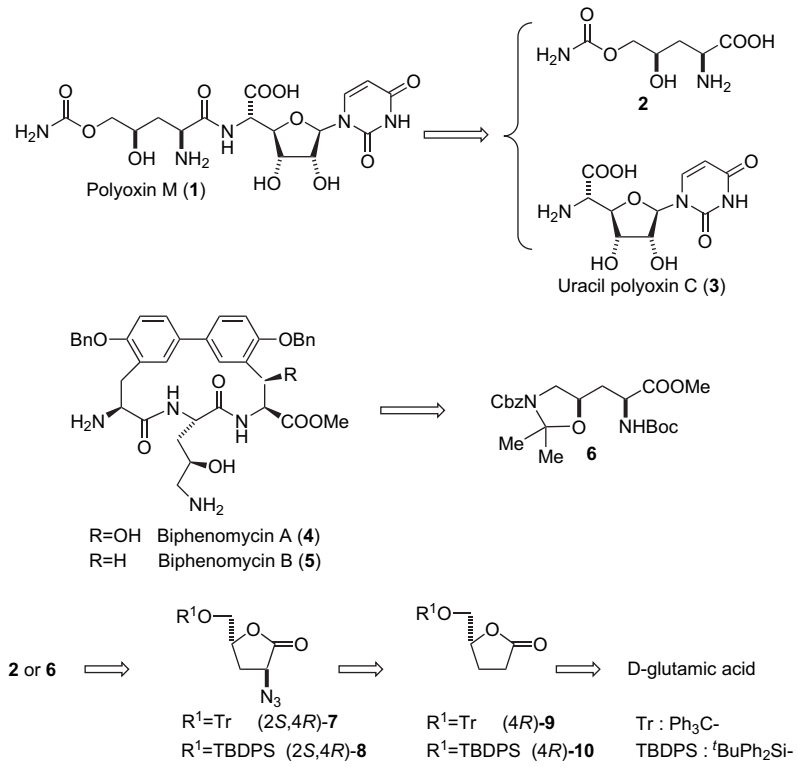
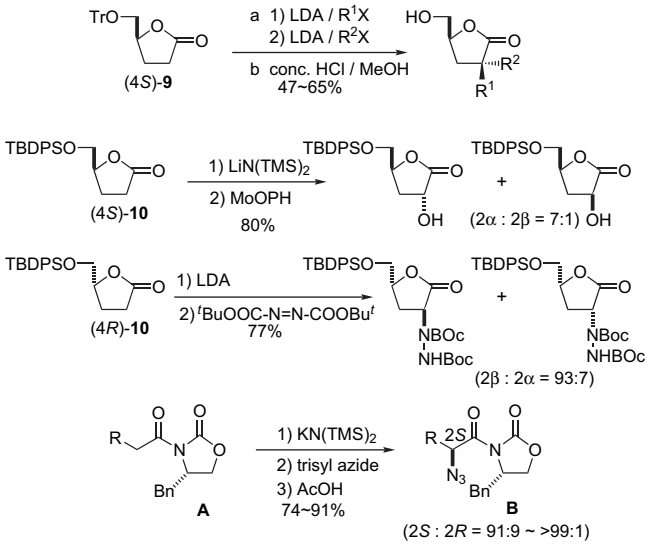


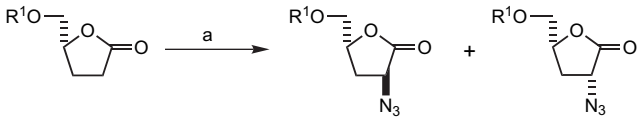
Scheme 1.



Scheme 2.

On consideration of these reports, our attention was focused only on the electrophilic azide transfer to the (4*R*)-protected hydroxymethyl-4-butanolide **9** or **10**. Chiral enolate derived from (4*R*)-**9** with lithium hexamethyldisilyazide (LiHMDS) was treated with trisyl azide, followed by addition of AcOH to give (2*S*)-**7** (37%) and (2*R*)-**11** (12%) (Table 1, entry 1). Change of the counter metal cation to sodium or potassium caused decrease of the yield of **7** (Table 1, entries 2 and 3). Treatment of chiral enolate derived from (4*R*)-**10** with trisyl azide, followed by addition of AcOH provided (2*S*)-**8** (33%) and (2*R*)-**12** (13%) (Table 1, entry 4), while change of AcOH to trimethylsilyl chloride (TMSCl) brought about a remarkable increase of the yield of (2*S*)-**8** (53%) along with (2*R*)-**12** (28%) (Table 1, entry 5). In the case of the electrophilic azide transfer to an enolate, the quench reagent was found to be an essential ingredient for successful azide transfer.[11] Surprisingly, AcOH proved to be superior to the silylating agents, TMSCl or TMSOTf, or strong acid TFA, while TMSCl was found to be a more effective quench agent in the present case. The structure of (2*S*)-**8** was determined by NMR analysis including NOE experiment as shown in Figure 1.

Table 1



a: 1) base / THF, 2) 2,4,6-triisopropylbenzenesulfonyl azide, 3) acid.

Entry	R^1	Base	Acid	Product (yield)	
1	Tr	LiHMDS	AcOH	**7** (37%)	**11** (12%)
2	Tr	NaHMDS	AcOH	**7** (25%)	**11** (trace)
3	Tr	KHMDS	AcOH	**7** (11%)	**11** (trace)
4	TBDPS	LiHMDS	AcOH	**8** (33%)	**12** (13%)
5	TBDPS	LiHMDS	TMSCl	**8** (53%)	**12** (28%)

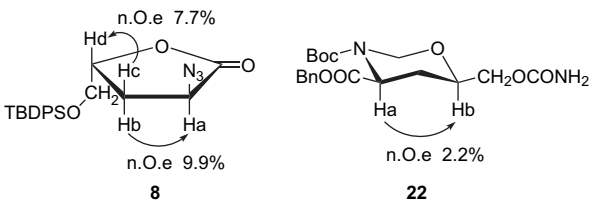
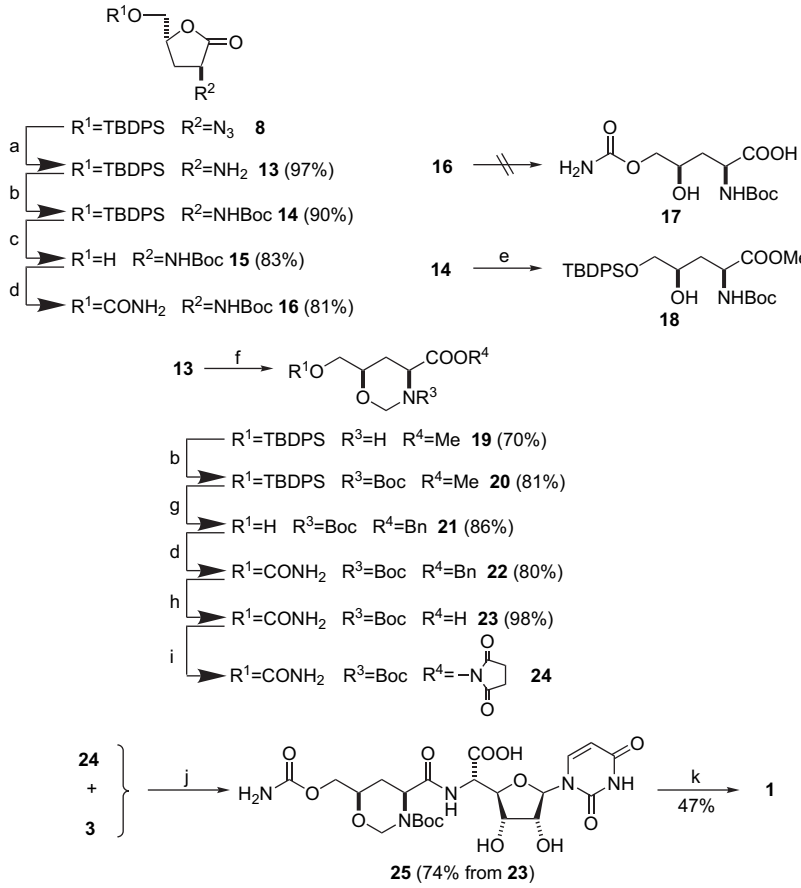


Figure 1.

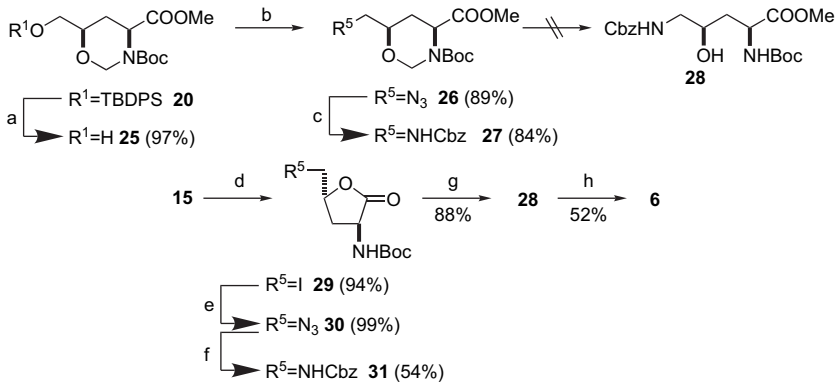
Then conversion of (2*S*)-**8** to the left-half congener **24** corresponding to **2** was carried out. Reduction of (2*S*)-**8** with Ph_3P and H_2O gave the amine **13** (97%), which was treated with $(Boc)_2O$ to afford the *N*-Boc compound **14** (90%). Deprotection of the silyl group in **14** provided an alcohol **15** (83%), which was converted to carbamoyl compound **16** in 81% yield. Alkaline hydrolysis of **16** did not give the desired γ-hydroxy acid **17**, while cleavage of the lactone ring of **14**, followed by esterification provided γ-hydroxy ester **18**. Protection of the alcohol group in **18** as a silyl group did not occur or treatment of **18** with *N*-methyl-*N*-(*tert*-butyldimethylsilyl) trifluoroacetamide gave the γ-lactone **14**. For the purpose of the double protection of the hydroxyl group and NHBoc group as a six-membered ring form, treatment of **18** with 3,3-dimethoxypropane and PPTS afforded only the starting **18**, while treatment of **18** with 3,3-dimethoxypropane and TsOH, or CSA provided γ-lactone **14**. By applying the reported procedure,[12] alkaline hydrolysis of (2*S*)-amino-γ-lactone **13**, followed by acetal formation with formaldehyde gave the six-membered ring compound **19** in 70% yield. Protection of the secondary amino group in **19** as a Boc group gave **20** (80%), which was subjected to consecutive trans-esterification and desilylation to afford an alcohol **21** in 86% yield. Conversion of **21** to the carbamoyl compound **22** (80%), followed by catalytic hydrogenation yielded the desired carboxylic acid **23** in 98% yield. The structure of **22** was reconfirmed by NMR analysis including NOE experiment as shown in Figure 1. Treatment of carboxylic acid **23** with *N*-hydroxysuccinimide in the presence of *N*,*N*-dicyclohexylcarbodiimide (DCC) in DMSO[13] provided an active ester **24**, which was coupled with uracil polyoxin C (**3**) in the presence of $(i\text{-}Pr)_2NEt$ to give the dipeptide **25** in 75% yield from **23**. Removal of the *N*-Boc and *N*,*O*-acetal protecting groups upon acid hydrolysis provided polyoxin M (**1**) ($[\alpha]_D^{25}$ +46.9 (*c* 0.29, H_2O), mp 215–220 °C (dec)) in 47% yield. The spectral data (^1H and ^{13}C NMR) of the synthetic **1** were identical with those of the natural polyoxin M (**1**) given by Dr. T. Yano. The specific rotation of synthetic **1** was in good agreement with that ($[\alpha]_D$ +49.9 (H_2O)) of the reported natural product (**1**)[1] (Scheme 3).

2.2. Synthesis of intermediate (6) for biphenomycins A and B

Deprotection of the silyl group in **20** provided an alcohol **25** (97%), which was subjected to consecutive trifluoromethanesulfonylation and azidation to give an azide **26** in overall 89% yield. A catalytic hydrogenation of **26**, followed by treatment of benzyl chloroformate (CbzCl) afforded NHCbz compound **27** (84%), deprotection of the acetal group of which did not occur. On the other hand, treatment of an alcohol **15** with iodine in the presence of Ph_3P and imidazole gave an iodide **29** (94%), which was treated with NaN_3 to provide azide **30** in 99% yield. Reduction of **30** with Ph_3P and H_2O gave the amine, which was treated with CbzCl to



Scheme 3. Reagents and conditions: (a) (1) Ph_3P, (2) H_2O; (b) $(Boc)_2O$/dioxane; (c) HF·Py/THF/pyridine; (d) (1) 4-nitrophenyl chloroformate/pyridine/Et_3N/THF, (2) NH_3/MeOH; (e) (1) NaOH aq/THF, (2) H^+, (3) CH_2N_2/Et_2O; (f) (1) NaOH aq/THF, (2) HCHO aq, (3) H^+, (4) CH_2N_2/Et_2O; (g) (1) BnOH/Ti(O-*i*-Pr)$_4$/benzene, (2) $Bu_4N^+F^-$/THF; (h) H_2/Pd–C/MeOH; (i) *N*-hydroxysuccinimide/DCC/AcOEt; (j) *i*-Pr_2NEt/DMSO; (k) $CF_3COOH/MeOH/H_2O$.



Scheme 4. Reagents and conditions: (a) HF·Py/THF/pyridine; (b) (1) Tf_2O/pyridine/CH_2Cl_2, (2) NaN_3/DMF; (c) (1) H_2/Pd–C/MeOH, (2) benzyl chloroformate/7% aq $NaHCO_3$/dioxane; (d) I_2/Ph_3P/imidazole/benzene; (e) NaN_3/DMF; (f) (1) Ph_3P/THF, (2) H_2O, (3) benzyl chloroformate/7% aq $NaHCO_3$/dioxane; (g) (1) NaOH aq, (2) H^+, (3) CH_2N_2/Et_2O; (h) 2,2-dimethoxypropane/PPTS.

afford the *N*-Cbz compound **31** (54%). Alkaline hydrolysis of **31**, followed by esterification with CH_2N_2 provided the desired methyl ester **28** (88%), which was treated with 2,2-dimethoxypropane and PPTS to afford the intermediate **6** ($[\alpha]_D^{24}$ +8.73 (*c* 1.50, $CHCl_3$)) for biphenomycins A and B in 52% yield. The spectral data (^1H and ^{13}C NMR) of the synthetic **6** were identical with those of the reported (2*S*,4*R*)-4-hydroxyornithine congener **6**.[6] The specific rotation of synthetic **6** was in good agreement with that ($[\alpha]_D^{20}$ +9.1 (*c* 1.09, $CHCl_3$)) of the reported **6**[6] (Scheme 4).

3. Conclusion

Chiral enolate derived from (4*R*)-4-*tert*-butyldiphenylsilyloxymethyl-4-butanolide **10** with lithium hexamethyldisilyazide (LiHMDS) was treated with trisyl azide, followed by addition of TMSCl to give (2*S*,4*R*)-2-azido-4-[(*tert*-butyldiphenylsilyloxy)methyl]-4-butanolide **8** (53%), from which the first total synthesis of polyoxin M (**1**) was achieved in overall 3.2% yield (13 steps) from D-glutamic acid. Moreover, the synthesis of the reported intermediate (2*S*,4*R*)-4-hydroxyornithine congener **6** for biphenomycins A and B was also achieved in overall 4.1% yield (12 steps) from D-glutamic acid.

4. Experimental

4.1. General

All melting points were measured on a Yanaco MP-3S micro melting point apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were recorded on JEOL EX 400 spectrometer in $CDCl_3$. High-resolution mass spectra (HRMS) and the fast atom bombardment mass spectra (FABMS) were obtained with JEOL JMS-DX 303 spectrometer. IR spectra were recorded with a JASCO FTIR-300 spectrometer. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. All evaporations were performed under reduced pressure. For column chromatography, silica gel (Kieselgel 60) was employed.

4.1.1. (4*R*)-4-[(Trityloxy)methyl]-4-butenolide 9 and (4*R*)-4-[(*tert*-butyldiphenylsilyloxy)methyl]-4-butanolide 10. (i) To a solution of D-glutamic acid (10.07 g, 0.068 mol) in concd HCl (20 ml) and H_2O (40 ml) was added slowly a solution of $NaNO_2$ (7.0 g, 0.102 mol) in H_2O (20 ml) at −5 °C and the whole mixture was stirred for 12 h at room temperature. The reaction mixture was evaporated in vacuo at below 50 °C to give a residue, which was shaken with AcOEt. The precipitate was filtered off and washed with AcOEt. The filtrate and washing were combined, and dried over $MgSO_4$. Evaporation of the solvent afforded (4*R*)-γ-carboxy-γ-butyrolactone (8.28 g, 93%) as a colorless syrup. $[\alpha]_D^{22}$ −5.53 (*c* 1.14, MeOH); NMR (acetone-d_6): δ 2.29–2.35 (1H, m), 2.51–2.55 (2H, m), 2.57–2.69 (1H, m), 5.00 (1H, dd, *J*=4.4, 8.4 Hz), 10.34 (1H, br s). FABMS: 131 $(M+1)^+$. (ii) To a solution of (4*R*)-γ-carboxy-γ-butyrolactone (5.48 g, 0.042 mol) in THF (100 ml) was added slowly 2 M $BH_3\cdot Me_2S$ in THF solution (25.3 ml, 0.0506 mol) at −20 °C and the whole mixture was stirred for 12 h at room temperature. The reaction mixture was diluted with aqueous NH_4Cl and AcOEt. The organic layer was washed with brine, and dried over $MgSO_4$. Evaporation of the organic solvent provided a crude oily product, which was chromatographed on silica gel (60 g, CHC_3/MeOH=100:1) to give (4*R*)-γ-hydroxymethyl-γ-butyrolactone (2.58 g, 53%) as a colorless oil. $[\alpha]_D^{24}$ −38.36 (*c* 1.35, EtOH); NMR (acetone-d_6): δ 2.05–2.15 (1H, m), 2.23–2.32 (1H, m), 2.46–2.51 (2H, m), 3.62 (1H, dd, *J*=4.4, 12.0 Hz), 3.76 (1H, dd, *J*=3.2, 12.0 Hz), 4.14 (1H, br s), 4.45–4.59 (1H, m). FABMS: 117 $(M+1)^+$. (iii) To a solution of (4*R*)-γ-hydroxymethyl-γ-butyrolactone (1.03 g, 8.9 mmol) in pyridine (5 ml) was added trityl chloride (TrCl, 3.72 g, 13.3 mmol) and the whole mixture was stirred for 12 h at room temperature. The reaction mixture was diluted with H_2O and extracted with AcOEt. The organic layer was washed with brine, and dried over $MgSO_4$. Evaporation of the organic solvent provided a residue, which was chromatographed on silica gel (40 g, *n*-hexane/AcOEt=10:1) to give **9** (2.97 g, 93%) as colorless needles. (4*R*)-**9**: mp 150–151 °C (*n*-hexane) $[\alpha]_D^{28}$ −25.3 (*c* 1.02, $CHCl_3$); IR (KBr): 1774 cm^{-1}. NMR: δ 1.99–2.07 (1H, m), 2.21–2.28 (1H, m), 2.46–2.54 (1H, m), 2.68 (1H, ddd, *J*=6.8, 10.0, 18.0 Hz), 3.15 (1H, dd, *J*=4.4, 10.6 Hz), 3.42 (1H, dd, *J*=3.6, 10.6 Hz), 4.61–4.66 (1H, m), 7.22–7.26 (3H, m), 7.28–7.33 (6H, m), 7.41–6.44 (6H, m). Anal. Calcd for $C_{24}H_{22}O_3$: C, 80.42; H, 6.19%. Found: C, 80.69; H, 6.26%. (iv) To a solution of (4*R*)-γ-hydroxymethyl-γ-butyrolactone (2.03 g, 17.5 mmol) in DMF (20 ml) were added *tert*-butyldiphenylsilyl chloride (TBDPSCl, 5.68 g,

20.7 mmol) and imidazole (2.34 g, 34.4 mmol), and the whole mixture was stirred for 1 h at room temperature. The reaction mixture was diluted with H_2O and extracted with AcOEt. The organic layer was washed with brine, and dried over $MgSO_4$. Evaporation of the organic solvent provided a residue, which was chromatographed on silica gel (60 g, *n*-hexane/AcOEt=50:1) to give **10** (5.81 g, 94%) as colorless prism. (4*R*)-**10**: mp 77–78 °C (*n*-hexane) $[\alpha]_D^{25}$ −28.55 (*c* 1.28, $CHCl_3$); IR (KBr): 1772 cm^{-1}. NMR: δ 1.06 (9H, s), 2.20–2.30 (2H, m), 2.46–2.55 (1H, m), 2.63–2.72 (1H, m), 3.69 (1H, dd, *J*=3.2, 11.2 Hz), 3.88 (1H, dd, *J*=3.2, 11.2 Hz), 4.57–4.61 (1H, m), 7.37–7.46 (6H, m), 7.65–7.68 (4H, m). Anal. Calcd for $C_{21}H_{26}O_3Si$: C, 71.15; H, 7.39%. Found: C, 71.44; H, 7.56%.

4.1.2. (2*S*,4*R*)-2-Azido-4-[(trityloxy)methyl]-4-butanolide 7 and (2*R*,4*R*)-2-azido-4-[(trityloxy)methyl]-4-butanolide 11. (i) (Entry 1, Table 1) To a well-stirred solution of (4*R*)-(trityloxy)methyl-4-butenolide **9** (0.354 g, 0.99 mmol) in THF (4 ml) at −78 °C was added 1 M solution of lithium bis(trimethylsilyl) amide (LiHMDS) in THF (1.1 ml, 1.1 mmol) and the whole mixture was stirred for 30 min. To the above reaction mixture was added a solution of 2,4,6-triisopropylbenzenesulfonyl azide (0.383 g, 1.24 mmol) in THF (4 ml) and the whole mixture was stirred for 30 min at the same temperature. To the above reaction mixture was added AcOH (0.4 ml) and the whole mixture was stirred for 12 h at room temperature. The reaction mixture was diluted with H_2O and extracted with AcOEt. The organic layer was washed with 7% aqueous $NaHCO_3$ and brine, and dried over $MgSO_4$. Evaporation of the organic solvent provided a crude oily product, which was chromatographed on silica gel (30 g) to give **7** (0.146 g, 37%) as colorless needles from *n*-hexane/AcOEt=30:1 elution and **11** (0.049 g, 12%) as colorless needles from *n*-hexane/AcOEt=20:1 elution. (2*S*,4*R*)-**7**: mp 135–137 °C (*n*-hexane); $[\alpha]_D^{25}$ −85.3 (*c* 0.83, $CHCl_3$); IR (KBr): 2114, 1779 cm^{-1}. NMR: δ 2.09–2.17 (1H, m), 2.32 (1H, ddd, *J*=2.8, 8.8, 13.2 Hz), 3.06 (1H, dd, *J*=2.8, 10.8 Hz), 3.60 (1H, dd, *J*=2.8, 10.8 Hz), 4.58 (1H, t, *J*=8.8 Hz), 4.63–4.67 (1H, m), 7.24–7.28 (3H, m), 7.30–7.34 (6H, m), 7.37–7.40 (6H, m). HRMS (FAB) Calcd for $C_{24}H_{22}O_3N_3$ (M^++H; *m*/*z*) 400.1662. Found 400.1613. (2*R*,4*R*)-**11**: mp 147–149 °C (*n*-hexane); $[\alpha]_D^{25}$ +65.3 (*c* 0.68, $CHCl_3$); IR (KBr): 2110, 1779 cm^{-1}. NMR: δ 2.04 (1H, dt, *J*=10.4, 12.8 Hz), 2.50 (1H, ddd, *J*=6.0, 8.8, 12.8 Hz), 3.26 (1H, dd, *J*=5.0, 10.6 Hz), 3.36 (1H, dd, *J*=3.8, 10.6 Hz), 4.32 (1H, dd, *J*=8.8, 10.4 Hz), 4.50–4.57 (1H, m), 7.23–7.33 (9H, m), 7.42–7.45 (6H, m). HRMS (FAB) Calcd for $C_{24}H_{22}O_3N_3$ (M^++H; *m*/*z*) 400.1662. Found 400.1602. (ii) (Entry 2, Table 1) To a well-stirred solution of (4*R*)-**9** (0.358 g, 1.0 mmol) in THF (4 ml) at −78 °C was added 0.6 M solution of sodium bis(trimethylsilyl) amide (NaHMDS) in toluene (1.9 ml, 1.1 mmol) and the whole mixture was stirred for 30 min. To the above reaction mixture was added AcOH (0.3 ml) and the whole mixture was stirred for 12 h at room temperature. The reaction mixture was worked up in the same way as (i) to give **7** (0.10 g, 25%). (iii) (Entry 3, Table 1) To a well-stirred solution of (4*R*)-**9** (0.358 g, 1.0 mmol) in THF (4 ml) at −78 °C was added 0.5 M solution of potassium bis(trimethylsilyl) amide (KHMDS) in toluene (2.2 ml, 1.1 mmol) and the whole mixture was stirred for 30 min. To the above reaction mixture was added AcOH (0.3 ml) and the whole mixture was stirred for 12 h at room temperature. The reaction mixture was worked up in the same way as (i) to give **7** (0.045 g, 11%).

4.1.3. (2*S*,4*R*)-2-Azido-4-[(*tert*-butyldiphenylsilyloxy)-methyl]-4-butanolide 8 and (2*R*,4*R*)-2-azido-4-[(*tert*-butyldiphenylsilyloxy)methyl]-4-butanolide 12. (i) (Entry 4, Table 1) To a well-stirred solution of (4*R*)-(*tert*-butyldiphenylsilyloxy)methyl-4-butenolide **10** (1.0 g, 2.8 mmol) in THF (10 ml) at −78 °C was added 1 M solution of lithium bis(trimethylsilyl) amide (LiHMDS) in THF (3.4 ml, 3.4 mmol) and the whole mixture was stirred for 30 min. To the above reaction mixture was added a solution of 2,4,6-triisopropylbenzenesulfonyl azide (1.05 g, 0.4 mmol) in THF (10 ml) and the whole mixture was stirred for 30 min at the same temperature. To the above reaction mixture was added AcOH (0.75 ml) and the whole mixture was stirred for 12 h at room temperature. The reaction mixture was diluted with H_2O and extracted with AcOEt. The organic layer was washed with 7% aqueous $NaHCO_3$ and brine, and dried over $MgSO_4$. Evaporation of the organic solvent provided a crude oily product, which was chromatographed on silica gel (50 g) to give **8** (0.368 g, 33%) as colorless needles from *n*-hexane/AcOEt=30:1 elution and **12** (0.145 g, 13%) as colorless oil from *n*-hexane/AcOEt=10:1 elution. (2*S*,4*R*)-**8**: mp 72–74 °C (*n*-hexane); $[\alpha]_D^{25}$ −108.9 (*c* 1.0, $CHCl_3$); IR (KBr): 2107, 1778 cm^{-1}. NMR: δ 1.06 (9H, s), 2.20 (1H, dt, *J*=8.8, 13.2 Hz), 2.52–2.58 (1H, m) 3.64 (1H, dd, *J*=2.4, 11.4 Hz), 3.92 (1H, dd, *J*=2.8, 11.4 Hz), 4.54 (1H, t, *J*=8.8 Hz), 4.60–4.64 (1H, m), 7.39–7.48 (6H, m), 7.61–7.65 (4H, m). Anal. Calcd for $C_{21}H_{25}N_3O_3Si$: C, 63.77; H, 6.37; N, 10.62%. Found: C, 63.91; H, 6.41; N, 10.42%. (2*R*,4*R*)-**12**: $[\alpha]_D^{24}$ +52.9 (*c* 1.13, $CHCl_3$); IR (KBr): 2110, 1784 cm^{-1}. NMR: δ 1.06 (9H, s), 2.18 (1H, ddd, *J*=9.8, 10.4, 13.0 Hz), 2.52 (1H, ddd, *J*=6.2, 9.0, 13.0 Hz), 3.72 (1H, dd, *J*=4.0, 11.6 Hz), 3.89 (1H, dd, *J*=3.6, 11.6 Hz), 4.33 (1H, dd, *J*=9.0, 10.4 Hz), 4.50 (1H, ddd, *J*=3.6, 6.2, 9.8 Hz), 7.38–7.45 (6H, m), 7.64–7.67 (4H, m). HRMS (FAB) Calcd for $C_{21}H_{26}N_3O_3Si$ (M^++H; *m*/*z*) 396.1744. Found 396.1741. (ii) (Entry 5, Table 1) To a well-stirred solution of (4*R*)-**10** (2.0 g, 5.6 mmol) in THF (20 ml) at −78 °C was added 1 M solution of lithium bis(trimethylsilyl) amide (LiHMDS) in THF (6.8 ml, 6.8 mmol) and the whole mixture was stirred for 30 min. To the above reaction mixture was added a solution of 2,4,6-triisopropylbenzenesulfonyl azide (2.1 g, 6.8 mmol) in THF (20 ml) and the whole mixture was stirred for 30 min at the same temperature. To the above reaction mixture was added trimethylsilyl chloride (TMSCl, 3.3 ml) and the whole mixture was stirred for 12 h at room temperature. The reaction mixture was worked up in the same way as (iv) to give **8** (1.182 g, 53%) and **12** (0.625 g, 28%).

4.1.4. (2*S*,4*R*)-2-Amino-4-[(*tert*-butyldiphenylsilyloxy)-methyl]-4-butanolide 13. A mixture of **7** (2.06 g, 5.2 mmol) and triphenylphosphine (Ph_3P, 1.65 g, 6.3 mmol) in THF (30 ml) was stirred for 30 min at room temperature. To the above reaction mixture was added H_2O (0.5 ml) and the whole mixture was heated with stirring for 4 h at 60 °C. The reaction mixture was evaporated to give a residue, which was chromatographed on silica gel (60 g, *n*-hexane/AcOEt=1:1) to afford (2*S*,4*R*)-**13** (1.87 g, 97%) as a colorless oil. (2*S*,4*R*)-**13**: $[\alpha]_D^{25}$ −25.94 (*c* 0.69,

$CHCl_3$); IR (KBr): 3425, 1781 cm^{-1}. NMR: δ 1.05 (9H, s), 2.07–2.17 (1H, m), 2.63 (1H, ddd, J=2.0, 9.4, 13.2 Hz), 3.65 (1H, dd, J=2.8, 11.4 Hz), 3.88 (1H, dd, J=2.8, 11.4 Hz), 3.99 (1H, t, J=9.4 Hz), 4.55–4.59 (1H, m), 7.38–7.48 (6H, m), 7.62–7.70 (4H, m). HRMS (FAB) Calcd for $C_{21}H_{28}NO_3Si$ (M^++H; m/z) 370.1838. Found 370.1852.

4.1.5. (2*S*,4*R*)-2-*tert*-Butoxycarbonylamino-4-[(*tert*-butyldiphenylsilyloxy)methyl]-4-butanolide 14. A mixture of **13** (1.33 g, 3.6 mmol), di-*tert*-butyl dicarbonate [$(Boc)_2O$, 0.9 g, 4.3 mmol] and Et_3N (0.73 g, 7.2 mmol) in dioxane (20 ml) was stirred for 12 h at room temperature. The reaction mixture was diluted with H_2O and extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. Evaporation of the organic solvent provided a crude oily product, which was chromatographed on silica gel (50 g, *n*-hexane/AcOEt=5:1) to give **14** (1.53 g, 90%) as colorless oil. (2*S*,4*R*)-**14**: $[\alpha]_D^{25}$ −22.69 (*c* 1.3, $CHCl_3$); IR (KBr): 3418, 1788, 1715 cm^{-1}. NMR: δ 1.06 (9H, s), 1.46 (9H, s), 2.35–2.43 (1H, m), 2.70–2.76 (1H, m), 3.65 (1H, dd, J=2.6, 11.6 Hz), 3.90 (1H, dd, J=2.6, 11.6 Hz), 4.53–4.59 (1H, m), 5.10 (1H, br s), 7.38–7.47 (6H, m), 7.63–7.67 (4H, m). Anal. Calcd for $C_{26}H_{35}NO_5Si\cdot H_2O$: C, 64.03; H, 7.65; N, 2.87%. Found: C, 64.07; H, 7.35; N, 2.57%.

4.1.6. (2*S*,4*R*)-2-*tert*-Butoxycarbonylamino-4-hydroxymethyl-4-butanolide 15. A mixture of **14** (2.88 g, 6.1 mmol), HF·pyridine complex (1.22 g, 12.3 mmol) in a mixed solvent [THF (20 ml)/pyridine (20 ml)] was stirred for two days at room temperature. The reaction mixture was diluted with H_2O and extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. Evaporation of the organic solvent provided a crude oily product, which was chromatographed on silica gel (50 g, $CHCl_3$/MeOH=100:1) to give **15** (1.18 g, 83%) as colorless dust. (2*S*,4*R*)-**15**: mp 202–203 °C ($CHCl_3$/MeOH); $[\alpha]_D^{25}$ −45.33 (*c* 0.3, $CHCl_3$); IR (KBr): 3348, 2963, 1735, 1696 cm^{-1}. NMR (DMSO-d_6): δ 1.37 (9H, s), 2.19–2.31 (2H, m), 3.46 (1H, ddd, J=2.4, 5.6, 12.0 Hz), 3.58 (1H, ddd, J=2.8, 5.6, 12.0 Hz), 4.34 (1H, q, J=9.4 Hz), 4.52–4.56 (1H, m), 5.15 (1H, t, J=5.6 Hz), 7.31 (1H, d, J=9.4 Hz). HRMS (FAB) Calcd for $C_{10}H_{18}NO_5$ (M^++H; m/z) 232.1185. Found 232.1204.

4.1.7. (2*S*,4*R*)-2-*tert*-Butoxycarbonylamino-4-carbamoyloxymethyl-4-butanolide 16. To a solution of **15** (0.092 g, 0.39 mmol) in THF (10 ml) was added pyridine (0.19 g, 2 mmol), Et_3N (0.075 g, 0.74 mmol), and 4-nitrophenyl chloroformate (0.23 g, 1.1 mmol) at −20 °C and the reaction mixture was stirred for 30 min at the same temperature. To the above reaction mixture was added saturated NH_3/MeOH (5 ml) and the whole mixture was stirred for 1 h at 0 °C. The reaction mixture was evaporated and the resulting residue was diluted with H_2O and extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. Evaporation of the organic solvent provided a crude oily product, which was chromatographed on silica gel (10 g, *n*-hexane/AcOEt=1:2) to give **16** (0.088 g, 81%) as colorless oil. (2*S*,4*R*)-**16**: NMR (DMSO-d_6): δ 1.38 (9H, s), 2.19–2.28 (2H, m), 4.04–4.09 (2H, m), 4.30 (1H, q, J=8.8 Hz), 4.70–4.75 (1H, m), 6.53 (1H, br s), 6.77 (1H, br s), 7.44 (1H, d, J=8.8 Hz). FABMS: 297 $(M+Na)^+$.

4.1.8. Methyl (2*S*,4*R*)-2-*tert*-butoxycarbonylamino-4-hydroxy-5-*tert*-butyldiphenylsilyloxy pentanoate 18. To a solution of **14** (0.19 g, 0.4 mmol) in THF (2 ml) was added 2 M NaOH solution (3 ml) at 0 °C and the reaction mixture was stirred for 1 h at the same temperature. The reaction mixture was acidified with 10% HCl solution and extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. Evaporation of the organic solvent provided a crude oily product, which was treated with CH_2N_2/Et_2O solution to provide a crude oily product. It was chromatographed on silica gel (10 g, *n*-hexane/AcOEt=5:1) to give **18** (0.13 g, 64%) as colorless oil. (2*S*,4*R*)-**18**: NMR: δ 1.06 (9H, s), 1.43 (9H, s), 1.85–1.93 (2H, m), 3.53 (1H, dd, J=6.8, 10.2 Hz), 3.63 (1H, dd, J=5.6, 10.2 Hz), 3.76 (3H, s), 3.84–3.90 (1H, m), 4.37–4.39 (1H, m), 5.47 (1H, br s), 7.37–7.46 (6H, m), 7.63–7.67 (4H, m). FABMS: 502 (M^++1).

4.1.9. (4*S*,6*R*)-6-*tert*-Butyldiphenylsilyloxymethyl-4-methoxycarbonyl-tetrahydro-2*H*-1,3-oxazine 19. To a solution of **13** (0.605 g, 1.6 mmol) in THF (2 ml) was added 2 M NaOH solution (3 ml) at 0 °C and the reaction mixture was stirred for 1 h at the same temperature. To the above reaction mixture was added 37% aqueous HCHO (1 ml) and the reaction mixture was stirred for 12 h at the same temperature. The reaction mixture was acidified with 10% HCl solution and extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. Evaporation of the organic solvent provided a crude oily product, which was treated with CH_2N_2/Et_2O solution to provide a crude oily product. It was chromatographed on silica gel (10 g, *n*-hexane/AcOEt=5:1) to give **19** (0.473 g, 70%) as colorless oil. (4*S*,6*R*)-**19**: $[\alpha]_D^{23}$ −4.36 (*c* 0.55, $CHCl_3$); IR (KBr): 3439, 1744 cm^{-1}. NMR: δ 1.06 (9H, s), 1.33–1.42 (1H, m), 1.93 (1H, dt, J=2.4, 12.8 Hz), 3.55–3.59 (1H, m), 3.63–3.78 (3H, m), 3.75 (3H, s), 4.23 (1H, d, J=10.8 Hz), 4.67 (1H, d, J=10.8 Hz), 7.35–7.44 (6H, m), 7.65–7.69 (4H, m). HRMS (FAB) Calcd for $C_{23}H_{32}NO_4Si$ (M^++H; m/z) 414.2132. Found 414.2163.

4.1.10. (4*S*,6*R*)-3-*tert*-Butoxycarbonylamino-6-*tert*-butyldiphenylsilyloxymethyl-4-methoxycarbonyl-tetrahydro-2*H*-1,3-oxazine 20. A mixture of **19** (0.472 g, 1.14 mmol), di-*tert*-butyl dicarbonate [$(Boc)_2O$, 0.49 g, 2.2 mmol], and Et_3N (0.34 g, 3.3 mmol) in dioxane (5 ml) was stirred for 12 h at room temperature. The reaction mixture was diluted with H_2O and extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. Evaporation of the organic solvent provided a crude oily product, which was chromatographed on silica gel (10 g, *n*-hexane/AcOEt=20:1) to give **20** (0.472 g, 81%) as colorless oil. (4*S*,6*R*)-**20**: $[\alpha]_D^{26}$ −23.29 (*c* 1.1, $CHCl_3$); IR (KBr): 1750, 1706 cm^{-1}. NMR (pyridine-d_5, 90 °C): δ 1.13 (9H, s), 1.45 (9H, s), 2.16–2.30 (2H, m), 3.67 (3H, s), 3.81 (1H, dd, J=5.0, 10.6 Hz), 3.88 (1H, dd, J=5.0, 10.6 Hz), 3.94 (1H, dq, J=5.0, 9.6 Hz), 4.51 (1H, dd, J=6.0, 10.0 Hz), 5.03 (1H, d, J=9.4 Hz), 5.23 (1H, d, J=9.4 Hz), 7.38–7.42 (6H, m), 7.79–7.83 (4H, m). Anal. Calcd for $C_{28}H_{39}NO_6Si$: C, 65.47; H, 7.65; N, 2.73%. Found: C, 65.55; H, 7.55; N, 2.72%.

4.1.11. (4*S*,6*R*)-4-Benzyloxycarbonyl-3-*tert*-butoxycarbonylamino-6-hydroxymethyl-tetrahydro-2*H*-1,3-oxazine 21. A mixture of **20** (1.2 g, 2.24 mmol), benzyl alcohol

(5.05 g, 46.7 mmol), and Ti(O-*i*-Pr)$_4$ (0.332 g, 1.17 mmol) in benzene (40 ml) was stirred for 12 h at reflux. The reaction mixture was diluted with 7% aqueous $NaHCO_3$ and extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. Evaporation of the organic solvent provided a crude oily product, which was chromatographed on silica gel (50 g, *n*-hexane/EtOAc=20:1) to give an oily product. To a solution of the above oily product in THF (10 ml) was added 1 M $Bu_4N^+F^-$/THF solution (5 ml) and the whole mixture was stirred for 12 h at room temperature. The reaction mixture was diluted with H_2O and extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. Evaporation of the organic solvent provided a crude oily product, which was chromatographed on silica gel (50 g, *n*-hexane/AcOEt=2:1) to give **21** (0.706 g, 86%) as colorless oil. (4*S*,6*R*)-**21**: $[\alpha]_D^{25}$ −29.88 (*c* 1.23, $CHCl_3$); IR (KBr): 3425, 1748, 1703 cm^{-1}. NMR (pyridine-d_5, 90 °C): δ 1.43 (9H, s), 2.22–2.37 (2H, m), 3.75 (1H, dd, *J*=4.4, 11.6 Hz), 3.81 (1H, dd, *J*=5.2, 11.6 Hz), 3.89–3.96 (1H, m), 4.58 (1H, dd, *J*=6.0, 9.6 Hz), 5.04 (1H, d, *J*=9.6 Hz), 5.10 (1H, br s), 5.24–5.31 (3H, m), 7.24–7.32 (3H, m), 7.42 (2H, d, *J*=7.2 Hz). Anal. Calcd for $C_{18}H_{25}NO_6$: C, 61.52; H, 7.17; N, 3.99%. Found: C, 61.26; H, 7.41; N, 4.06%.

4.1.12. (4*S*,6*R*)-4-Benzyloxycarbonyl-3-*tert*-butoxycarbonylamino-6-carbamoyloxymethyl-tetrahydro-2*H*-1,3-oxazine 22. To a solution of **21** (0.646 g, 1.84 mmol) in THF (15 ml) was added pyridine (0.945 g, 12 mmol), Et_3N (0.372 g, 3.7 mmol), and 4-nitrophenyl chloroformate (1.112 g, 5.52 mmol) at −20 °C and the reaction mixture was stirred for 30 min at the same temperature. To the above reaction mixture was added saturated NH_3/MeOH (5 ml) and the whole mixture was stirred for 1 h at 0 °C. The reaction mixture was evaporated and the resulting residue was diluted with H_2O and extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. Evaporation of the organic solvent provided a crude oily product, which was chromatographed on silica gel (10 g, *n*-hexane/AcOEt=2:1) to give **22** (0.58 g, 80%) as colorless oil. (4*S*,6*R*)-**22**: $[\alpha]_D^{23}$ −33.12 (*c* 0.77, $CHCl_3$); IR (KBr): 3444, 1734, 1716, 1701 cm^{-1}. NMR (pyridine-d_5, 90 °C): δ 1.42 (9H, s), 2.18–2.22 (2H, m), 4.00–4.08 (1H, m), 4.22 (1H, dd, *J*=4.8, 11.6 Hz), 4.32 (1H, dd, *J*=6.0, 11.6 Hz), 4.56 (1H, t, *J*=7.2 Hz), 5.01 (1H, d, *J*=9.6 Hz), 5.22 (1H, d, *J*=9.6 Hz), 5.26 (1H, d, *J*=12.4 Hz), 5.30 (1H, d, *J*=12.4 Hz), 6.67 (2H, br s), 7.24–7.33 (3H, m), 7.41–7.43 (2H, m). Anal. Calcd for $C_{19}H_{26}N_2O_7 \cdot 1/4H_2O$: C, 57.20; H, 6.70; N, 7.02%. Found: C, 56.91; H, 6.88; N, 6.98%.

4.1.13. (4*S*,6*R*)-3-*tert*-Butoxycarbonylamino-6-carbamoyloxymethyl-tetrahydro-2*H*-1,3-oxazin-3-carboxylic acid 23. A mixture of **22** (0.54 g, 1.37 mmol) and 10% Pd–C (0.1 g) in MeOH (10 ml) was subjected to a catalytic hydrogenation for 1 h at ordinary temperature. The reaction mixture was filtered with the aid of Celite and the filtrate was evaporated to give **23** (0.409 g, 98%) as amorphous solid. (4*S*,6*R*)-**23**: $[\alpha]_D^{20}$ −40.98 (*c* 0.82, $CHCl_3$); IR (KBr): 3443, 3367, 1733, 1715, 1703 cm^{-1}. NMR (pyridine-d_5, 90 °C): δ 1.48 (9H, s), 2.31–2.38 (2H, m), 4.08–4.14 (1H, m), 4.29 (1H, dd, *J*=4.4, 11.6 Hz), 4.44 (1H, dd, *J*=6.4, 11.6 Hz), 4.70 (1H, d, *J*=7.6 Hz), 5.20 (1H, d, *J*=9.6 Hz), 5.31 (1H, d, *J*=9.6 Hz), 6.66 (2H, br s), 7.77 (1H, br s). Anal. Calcd for $C_{12}H_{20}N_2O_7$: C, 47.36; H, 6.62; N, 9.21%. Found: C, 47.21; H, 6.84; N, 9.11%.

4.1.14. Coupling reaction of (4*S*,6*R*)-23 and uracil polyoxcin C (3). A mixture of **23** (0.077 g, 0.25 mmol), *N*-hydroxysuccinimide (0.032 g, 0.28 mmol), and *N*,*N*-dicyclohexylcarbodiimide (DCC, 0.058 g, 0.28 mmol) in AcOEt (5 ml) was stirred for 1 h at room temperature. The reaction mixture was evaporated to give a crude residue **24**. To a solution of the above residue in DMSO (3 ml) was added a mixture of uracil polyoxin C (**3**, 0.088 g, 0.28 mmol) and *i*-Pr_2NEt (0.13 ml, 0.506 mmol) in DMSO (1 ml) and the whole mixture was stirred for 12 h at room temperature. The reaction mixture was directly subjected to column chromatography (silica gel, 10 g, $CHCl_3$/MeOH=1:1) to afford **25** (0.107 g, 74%) as amorphous solid. **25**: mp 207–210 °C (dec); $[\alpha]_D^{25}$ −4.3 (*c* 0.6, MeOH); IR (KBr): 3401, 1685, 1670, 1637, 1625 cm^{-1}. ^{1}H NMR (pyridine-d_5, 90 °C): δ 1.45 (9H, s), 2.34–2.37 (2H, m), 4.08–4.12 (1H, m), 4.27 (1H, dd, *J*=4.8, 11.2 Hz), 4.40 (1H, dd, *J*=6.4, 11.2 Hz), 4.70–4.78 (2H, m), 4.91–4.94 (1H, m), 5.04–5.07 (1H, m), 5.17 (1H, d, *J*=9.8 Hz), 5.22 (1H, d, *J*=9.8 Hz), 5.29–5.32 (1H, m), 5.79 (1H, d, *J*=7.8 Hz), 6.39 (1H, d, *J*=3.6 Hz), 6.66 (2H, br s), 7.94 (1H, d, *J*=7.8 Hz). ^{13}C NMR (pyridine-d_5, 90 °C): δ 27.9, 28.4, 55.6, 56.6, 66.5, 71.3, 71.8, 71.8, 74.8, 81.3, 86.0, 90.9, 103.2, 141.5, 150.3, 152.3, 155.3, 158.0, 164.2, 172.2. HRMS (FAB) Calcd for $C_{22}H_{32}N_5O_{13}$ (M^++H; *m*/*z*) 574.1997. Found 574.1932.

4.1.15. Polyoxin M (1). To a solution of **25** (0.107 g, 0.187 mmol) in a mixed solvent [MeOH (2 ml)/H_2O (2 ml)] was added CF_3COOH (2 ml) at 0 °C and the reaction mixture was stirred for 12 h at room temperature. The reaction mixture was evaporated to give a crude residue, which was directly subjected to column chromatography (ODS, 10 g, H_2O) to afford **1** (0.040 g, 47%) as amorphous solid. **1**: mp 215–220 °C (dec); $[\alpha]_D^{25}$ +46.9 (*c* 0.29, H_2O); IR (KBr): 3423, 1677, 1655, 1648, 1637, 1631 cm^{-1}. ^{1}H NMR (D_2O): δ 1.76–1.86 (1H, m), 1.94–1.99 (1H, m), 3.79 (1H, dd, *J*=6.0, 11.2 Hz), 3.89 (1H, dd, *J*=3.6, 11.2 Hz), 3.92–3.97 (1H, m), 4.04–4.12 (2H, m), 4.21–4.28 (1H, m), 4.33 (1H, t, *J*=6.0 Hz), 4.62–4.66 (1H, m), 5.60 (1H, d, *J*=4.0 Hz), 5.70 (1H, d, *J*=8.0 Hz), 7.38 (1H, d, *J*=8.0 Hz). ^{13}C NMR (D_2O): δ 33.0, 51.3, 53.7, 66.4, 67.6, 69.4, 71.8, 81.9, 90.5, 102.1, 142.0, 151.0, 158.6, 165.4, 168.9, 170.7. HRMS (FAB) Calcd for $C_{16}H_{24}N_5O_{11}$ (M^++H; *m*/*z*) 462.1472. Found 462.1495.

4.1.16. (4*S*,6*R*)-3-*tert*-Butoxycarbonylamino-6-hydroxymethyl-4-methoxycarbonyl-tetrahydro-2*H*-1,3-oxazine 25. A mixture of **20** (0.35 g, 0.68 mmol), HF·pyridine complex (0.135 g, 1.36 mmol) in a mixed solvent [THF (5 ml)/pyridine (5 ml)] was stirred for two days at room temperature. The reaction mixture was diluted with H_2O and extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. Evaporation of the organic solvent provided a crude oily product, which was chromatographed on silica gel (10 g, *n*-hexane/AcOEt=3:2) to give **25** (0.182 g, 97%) as colorless oil. (4*S*,6*R*)-**25**: NMR (pyridine-d_5): δ 1.44 (9H, s), 2.18–2.31 (2H, m), 3.69 (3H, s), 3.75 (1H, dd, *J*=4.8, 11.2 Hz), 3.82 (1H, dd, *J*=5.4, 11.2 Hz), 3.89–3.95 (1H, m), 4.51 (1H, dd, *J*=6.4, 9.6 Hz),

4.80 (1H, br s), 5.03 (1H, d, J=9.6 Hz), 5.27 (1H, d, J=9.6 Hz). FABMS: 276 (M^++1).

4.1.17. (4*S*,6*R*)-6-Azidomethyl-3-*tert*-butoxycarbonylamino-4-methoxycarbonyl-tetrahydro-2*H*-1,3-oxazine 26. To a solution of **25** (0.615 g, 2.23 mmol) in CH_2Cl_2 (10 ml) was added pyridine (0.883 g, 11.2 mmol) and $(CF_3SO_2)_2O$ (0.892 g, 3.35 mmol) at 0 °C and reaction mixture was stirred for 15 min at the same temperature. The reaction mixture was diluted with H_2O and extracted with AcOEt. The organic layer was washed with 10% aqueous HCl, brine and dried over $MgSO_4$. Evaporation of the organic solvent provided a crude oily product. To a solution of the above crude product in DMF (10 ml) was added NaN_3 (0.219 g, 3.37 mmol) and the reaction mixture was stirred for 4 h at room temperature. The reaction mixture was diluted with H_2O and extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. Evaporation of the organic solvent provided a crude oily product, which was chromatographed on silica gel (10 g, *n*-hexane/AcOEt=10:1) to give **26** (0.596 g, 89%) as colorless needles. (4*S*,6*R*)-**26**: NMR (pyridine-d_5): δ 1.44 (9H, s), 2.04–2.18 (2H, m), 3.28 (1H, dd, J=4.4, 13.2 Hz), 3.33 (1H, dd, J=6.0, 13.2 Hz), 3.70 (3H, s), 3.87–3.93 (1H, m), 4.47 (1H, dd, J=6.0, 9.6 Hz), 4.98 (1H, d, J=9.6 Hz), 5.24 (1H, d, J=9.6 Hz). FABMS: 301 (M^++1).

4.1.18. (4*S*,6*R*)-6-Benzyloxycarbonylaminomethyl-3-*tert*-butoxycarbonylamino-4-methoxycarbonyl-tetrahydro-2*H*-1,3-oxazine 27. A mixture of **26** (0.433 g, 1.44 mmol) and 10% Pd–C (0.1 g) in MeOH (10 ml) was subjected to a catalytic hydrogenation for 1 h at ordinary temperature. The reaction mixture was filtered with the aid of Celite and the filtrate was evaporated to give a crude amine. To a solution of the crude amine in dioxane (20 ml) was added 7% aqueous $NaHCO_3$ (4 ml) and 30% benzyl chloroformate/toluene solution (1.62 g, 2.85 mmol) and the reaction mixture was stirred for 1 h at room temperature. The reaction mixture was diluted with H_2O and extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. Evaporation of the organic solvent provided a crude oily product, which was chromatographed on silica gel (10 g, *n*-hexane/AcOEt=2:1) to give **27** (0.493 g, 84%) as colorless oil. (4*S*,6*R*)-**27**: NMR (pyridine-d_5): δ 1.44 (9H, s), 2.09–2.20 (2H, m), 3.44–3.46 (1H, m), 3.67 (3H, s), 3.62–3.69 (1H, m), 3.89–3.96 (1H, m), 4.45 (1H, dd, J=6.0, 9.6 Hz), 4.92 (1H, d, J=9.6 Hz), 5.22–5.27 (3H, m), 7.21–7.34 (3H, m), 7.40–7.43 (2H, m). FABMS: 431 (M^++Na).

4.1.19. (2*S*,4*R*)-2-*tert*-Butoxycarbonylamino-4-iodomethyl-4-butanolide 29. To a solution of **15** (0.30 g, 1.3 mmol) in benzene (50 ml) were added Ph_3P (0.51 g, 1.94 mmol), imidazole (0.177 g, 2.6 mmol), and I_2 (0.495 g, 1.95 mmol) and the reaction mixture was stirred for 12 h at room temperature. The reaction mixture was diluted with saturated $NaHSO_3$ solution and extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. Evaporation of the organic solvent provided a crude oily product, which was chromatographed on silica gel (20 g, *n*-hexane/AcOEt=3:1) to give **29** (0.416 g, 94%) as colorless dust. (2*S*,4*R*)-**29**: mp 141–142 °C; $[\alpha]_D^{22}$ −44.8 (c 1.04, $CHCl_3$); IR (KBr): 3340, 1789, 1679 cm^{-1}. NMR (acetone-d_6): δ 1.42 (9H, s), 2.46 (2H, dd, J=5.0, 9.8 Hz), 3.55 (1H, dd, J=5.4, 10.6 Hz), 3.60 (1H, dd, J=5.8, 10.6 Hz), 4.47–4.54 (1H, m), 4.73–4.79 (1H, m), 6.64 (1H, br s). HRMS (FAB) Calcd for $C_{10}H_{17}INO_4$ (M^++H; m/z) 342.0203. Found 342.0312.

4.1.20. (2*S*,4*R*)-2-*tert*-Butoxycarbonylamino-4-azidomethyl-4-butanolide 30. A mixture of **29** (0.415 g, 1.2 mmol) and NaN_3 (0.119 g, 1.83 mmol) in DMF (10 ml) was stirred for 12 h at room temperature. The reaction mixture was diluted with H_2O and extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. Evaporation of the organic solvent provided a crude oily product, which was chromatographed on silica gel (20 g, *n*-hexane/AcOEt=3:1) to give **30** (0.307 g, 99%) as colorless dust. (2*S*,4*R*)-**30**: mp 103–104 °C (*n*-hexane/AcOEt); $[\alpha]_D^{22}$ −80.0 (c 0.93, $CHCl_3$); IR (KBr): 3373, 2122, 1776, 1682 cm^{-1}. NMR: δ 1.45 (9H, s), 2.38–2.55 (2H, m), 3.53 (1H, dd, J=4.0, 13.2 Hz), 3.68 (1H, dd, J=3.6, 13.2 Hz), 4.40–4.50 (1H, m), 4.73–4.80 (1H, m), 5.32 (1H, d, J=6.4 Hz). Anal. Calcd for $C_{10}H_{16}N_4O_4$: C, 46.87; H, 6.29; N, 21.86%. Found: C, 46.59; H, 6.27; N, 21.90%.

4.1.21. (2*S*,4*R*)-4-Benzyloxycarbonylaminomethyl 2-*tert*-butoxycarbonylamino-4-butanolide 31. A mixture of **30** (0.103 g, 0.4 mmol) and Ph_3P (0.125 g, 0.48 mmol) in THF (5 ml) was stirred for 5 h at room temperature. To the above reaction mixture was added H_2O (0.5 ml) and the whole mixture was heated with stirring for 5 h at 60 °C. The reaction mixture was evaporated to give a crude amine. To a solution of the crude amine in dioxane (10 ml) was added 7% aqueous $NaHCO_3$ (2 ml) and 30% benzyl chloroformate/toluene solution (0.343 g, 0.6 mmol) and the reaction mixture was stirred for 12 h at room temperature. The reaction mixture was diluted with H_2O and extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. Evaporation of the organic solvent provided a crude oily product, which was chromatographed on silica gel (10 g, *n*-hexane/AcOEt=4:1) to give **31** (0.079 g, 54%) as colorless dust. (2*S*,4*R*)-**31**: mp 73–74 °C (*n*-hexane/AcOEt); $[\alpha]_D^{25}$ −11.60 (c 0.75, $CHCl_3$); IR (KBr): 3353, 1771, 1701 cm^{-1}. NMR: δ 1.49 (9H, s), 2.27–2.34 (2H, m), 2.42–2.48 (1H, m), 3.31–3.38 (1H, m), 3.45–3.50 (1H, m), 4.21–4.30 (1H, m), 4.71 (1H, br s), 5.10 (2H, s), 5.27 (1H, d, J=6.8 Hz), 5.42–5.45 (1H, m), 7.29–7.37 (5H, m). HRMS (FAB) Calcd for $C_{18}H_{25}N_2O_6$ (M^++H; m/z) 365.1662. Found 365.1741.

4.1.22. Methyl (2*S*,4*R*)-5-benzyloxycarbonylamino-2-*tert*-butoxycarbonylamino-4-hydroxypentanoate 28. To a solution of **31** (0.078 g, 0.21 mmol) in THF (5 ml) was added 2 M NaOH solution (1 ml) at 0 °C and the reaction mixture was stirred for 30 min at the same temperature. The reaction mixture was acidified with 10% HCl solution and extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. Evaporation of the organic solvent provided a crude oily product, which was treated with CH_2N_2/Et_2O solution to provide a crude oily product. It was chromatographed on silica gel (10 g, $CHCl_3$/MeOH=100:1) to give **28** (0.073 g, 88%) as colorless oil. (2*S*,4*R*)-**28**: $[\alpha]_D^{25}$ −8.5 (c 0.6, $CHCl_3$); IR (KBr): 3370, 1771, 1701 cm^{-1}. NMR: δ 1.42 (9H, s), 1.80–1.87 (1H, m), 1.90–1.98 (1H, m), 3.08–3.15 (1H, m), 3.30–3.34 (1H, m), 3.58–3.62 (1H, m), 3.71 (3H, s), 3.83–3.89 (1H, m), 4.38 (1H, br s), 5.08 (2H, s), 5.51 (1H, t, J=5.4 Hz),

5.57–5.59 (1H, m), 7.29–7.33 (5H, m). HRMS (FAB) Calcd for $C_{19}H_{29}N_2O_7$ (M^++H; *m*/*z*) 397.1950. Found 397.1992.

4.1.23. Acetonide formation of 28 (synthesis of 6). A mixture of **28** (0.073 g, 0.18 mmol), dimethoxypropane (5 ml), and pyridinium *p*-toluenesulfonate (PPTS, 0.002 g) in DMF (1 ml) was stirred for 12 h at room temperature. The reaction mixture was diluted with H_2O and extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. Evaporation of the organic solvent provided a crude oily product, which was chromatographed on silica gel (5 g, $CHCl_3$/MeOH=200:1) to give **6** (0.042 g, 52%) as colorless oil. (2*S*,4*R*)-**6**: $[\alpha]_D^{24}$ +8.73 (*c* 1.50, $CHCl_3$); IR (KBr): 3426, 1747, 1711 cm^{-1}. ^1H NMR: δ 1.44 (9H, s), 1.50 (3H, s), 1.58 (3H, s), 2.01–2.05 (1H, m), 2.12–2.16 (1H, m), 3.11–3.18 (1H, m), 3.74 (3H, s), 3.77–3.81 (1H, m), 4.13–4.20 (1H, m), 4.37 (1H, br s), 5.10–5.15 (2H, m), 5.30–5.32 (1H, m), 7.29–7.36 (5H, m). ^{13}C NMR: δ 24.2, 26.1, 28.3, 35.8, 50.5, 51.3, 52.4, 66.5, 70.8, 80.1, 94.1, 127.9, 128.0, 128.5, 136.6, 152.2, 155.2, 172.4. HRMS (FAB) Calcd for $C_{22}H_{33}N_2O_7$ (M^++H; *m*/*z*) 437.2288. Found 437.2330.

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A D_2 symmetric tetraamide macrocycle based on 1,1′,4,4′-tetrahydro[3,3′(2*H*,2′*H*)-spirobiquinoline]-2,2′-dione: synthesis and selectivity for lithium over sodium and alkaline earth ions

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Abstract—Macrocyclic ionophores *d*,*l*-**3** and *d*,*l*-**4** with four amide carbonyl ligands were synthesized and investigated in lithium ion-selective electrodes. In solvent PVC membranes, ion selectivity of *d*,*l*-**3** and *d*,*l*-**4** for lithium relative to sodium was observed as log $K^{pot}_{Li,Na} = -1.4$ and -1.23, respectively. Spiromacrocycle *d*,*l*-**3** and analogue dibenzospiromacrocycle *d*,*l*-**4** have similar ion selectivity patterns for alkali metal ions, but *d*,*l*-**4** could discriminate against alkaline earth metal ions better than *d*,*l*-**3**. It is an example of an endopolarophilic/exolipophilic macrocyclic ionophore whose selectivity for monovalent cations over divalent cations is enhanced by thick lipophilic shells.

1. Introduction

It is well known that the amide group has high affinity for cations with high charge density. Many ionophores with amide ligands are currently employed as chemical sensors that can quantitatively and reversibly measure cationic analytes.[1] In particular, the development of ionophores with amide ligands has been frequently reported for achieving high selectivity for lithium over sodium in blood lithium measurement during treatment of manic depressive psychosis.[2] Our early reports on macrocyclic tetraamide ionophores based on the spirodiamide building block have shown reasonable lithium selectivity over sodium in solvent PVC membrane electrodes.[3] Four amide carbonyl ligands in dispiromacrocyclic ionophore **3** derived from 2,8-diazaspiro[5,5]undecan-1,7-dione (**1**) converge inward on the molecular cavity of the ionophore upon complexation with lithium ion.[3] Lithium is known to interact preferentially with four or five hard oxygen donors in complexes.[4] However, divalent cations such as calcium and magnesium frequently interfere in lithium ion-selective electrode (Li^+-ISE) measurements. Calcium and magnesium are both the important electrolytes in the human body, and are present in relatively high concentration in intra- and extracellular fluids. Reported here is an example of an endopolarophilic/exolipophilic macrocyclic ionophore whose selectivity for monovalent cations over divalent cations is enhanced by a thick lipophilic shell. Thickness of the ligand layer influences in particular the selectivity between mono and divalent cations.[5] A macrocyclic tetraamide analogue based on 1,1′,4,4′-tetrahydro[3,3′(2*H*,2′*H*)-spirobiquinoline]-2,2′-dione (**2**) was synthesized and its ion selectivity was studied in solvent PVC membrane electrodes.

2. Results and discussion

2.1. Synthesis

The key starting materials, spirodiamides **1** and **2**, have been recently reported as C_2-symmetric building blocks for ionophores capable of tetrahedral coordination of guest metal ions and as potential chiral auxiliaries. Spirodiamide **1** was synthesized in 60% overall yield by cyanoethylation of diethyl malonate by reaction with acrylonitrile, followed by reduction–cyclization.[3a] Similarly dibenzospirodiamide **2** was synthesized via reaction of malonic ester with *o*-nitrobenzyl bromide, followed by reductive cyclization in 86% yield.[6] Spiromacrocycle **3** was also reported in an earlier communication,[3a] but the detailed synthesis of **3** is given in the current paper. Spiromacrocycle **3** was synthesized by high dilution technique. Spirodiamide **1** was first converted

Keywords: Ionophore; Ion-selective electrode; Lithium ion selectivity; Dibenzospirodiamide.
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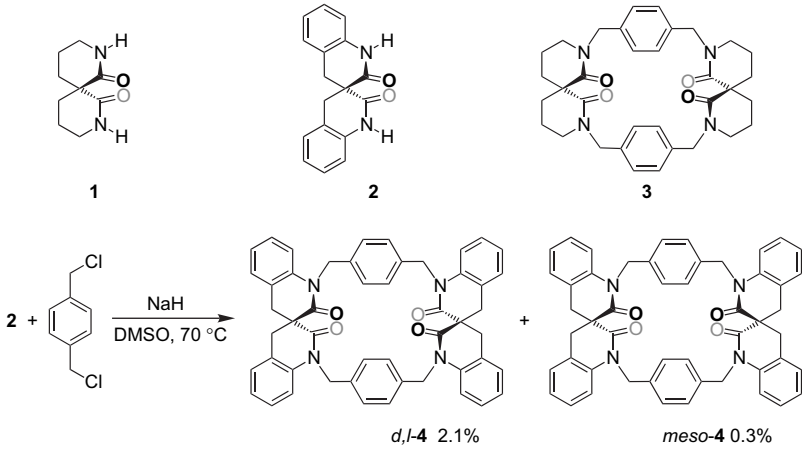
to the dianion with sodium hydride in anhydrous DMSO at 70 °C. Reaction of the sodium salt of spirodiamide **1** with 1,4-bis(chloromethyl)benzene gave the desired [2+2] product, dispiromacrocycle **3**, in 7% yield after chromatography. Similarly dibenzospiromacrocycle **4** was synthesized from dibenzospirodiamide **2**, which was converted to the dianion. Reaction of the sodium salt of dibenzospirodiamide **2** with 1,4-bis(chloromethyl)benzene gave a mixture of products containing the desired [2+2] products, higher oligomer, polymer, and aldehydes, which were apparently produced by reaction of benzylic chloride intermediates with hot DMSO. Two stereoisomeric macrocycles, apparently corresponding to the *meso* and *d*,*l* diastereomers shown in Scheme 1, were isolated from this product mixture.

The desired [2+2] products were isolated by column chromatography (silica gel, CH_2Cl_2/EtOAc 97:3) followed by preparative HPLC (silica gel, CH_2Cl_2/EtOAc 95:5). The isomers were further purified by recrystallization from CH_2Cl_2/MeOH, giving colorless needle-shaped crystals and transparent cubic crystals in 2.1% and 0.3% yield, respectively. The major needle-shaped crystals were further resolved into two components by chiral HPLC, whereas the minor transparent cubic crystals remained as a single component without further resolution. The major needle-shaped crystals were identified as a racemic mixture of chiral macrocycle *d*,*l*-**4** and the minor product as achiral macrocycle *meso*-**4**, without ambiguity. The [2+2] composition of macrocycles **4** were demonstrated by mass spectrometry. In each of these compounds the benzylic protons produce a pair of doublets in the proton NMR spectrum, demonstrating that these geminal protons are diastereotopic in both macrocycles.

2.2. Potentiometric ion selectivity of ionophores in the PVC membranes

The potentiometric response of PVC polymeric Li^+-ISEs based on amide ionophores was examined for alkali metal and alkaline earth ions and ammonium ion. The selectivity of Li^+-ISEs was found to be varied depending on the particular plasticizer used, whether the membrane contained a lipophilic anion additive, and whether the fixed interference method or the separate solution method was used for measurements.[7] The incorporation of salts of lipophilic anions such as potassium tetrakis(*p*-chlorophenyl)borate (KT*p*ClPB) into the membrane phase reduces the interference by sample anions. Selectivities for a given ionophore are very sensitive to the amount of such lipophilic anions relative to the ionophore concentration. The lithium selectivity changes drastically with respect to the stoichiomerty of anion additive KT*p*ClPB, relative to the amide ionophores. The best performance was achieved with less than 100 mol % of KT*p*ClPB for the 1:1 ionophores complexing with lithium ion. With excess additives the selectivities approached the sequence of classical ion-exchange membranes in which the selectivities are proportional to ion lipophilicity.[8] Simon's membrane composition was employed to allow comparison with previously reported results of diamide ionophore ETH 1810, *cis*-*N*,*N*-dicyclohexyl-*N*′,*N*′-diisobutylcyclohexane-1,2-dicarboxamide, which has been known as a good lithium ionophore.[7] The membranes were composed of about 1 wt % of the ionophore, 0.4 wt % of KT*p*ClPB (corresponding to about 60 mol %, relative to the ionophore), about 65.6 wt % of *o*-nitrophenyloctyl ether (*o*-NPOE) as a plasticizer, and 33 wt % of PVC.[8]

The dispiromacrocycles **3** and **4** have been tested as ionophores in PVC membranes of Li^+-ISEs. The selectivities of the resulting electrodes were determined by the separate solution method.[9] From the prior communication, the dispiromacrocyclic ionophore **3** displayed significant selectivity for lithium relative to sodium ($\log K^{pot}_{Li,Na} = -1.4$). This level of selectivity is consistent with the tentative structural assignment of **3** as the D_2-symmetric chiral isomer, *d*,*l*-**3**. In the C_{2h}-symmetric achiral *meso* isomer, *meso*-**3**, which was not isolated from the product mixture, the four-carbonyl ligands are not oriented to enable coordination of guest metal ions efficiently according to molecular models. The four amide carbonyl ligands in *d*,*l*-**3** are structurally preorganized to converge on the molecular cavity and to coordinate small metal ions such as lithium in a distorted tetrahedral geometry. For comparison of ion selectivity of these structurally similar ionophores, *d*,*l*-**3** and *d*,*l*-**4** were tested under the same conditions. The *meso* isomer of the dibenzospiromacrocycle, *meso*-**4** has not been tested as an ionophore, because a comparable PVC membrane containing *meso*-**4** could not be prepared due to its insolubility in



Scheme 1.

THF, which is a standard solvent for preparing PVC membranes for ISEs.

Selectivity coefficients for PVC membranes containing ionophores ETH 1810, *d*,*l*-**3**, and *d*,*l*-**4** are listed in Table 1. Ionophore ETH 1810 gave results that are comparable to the selectivity coefficients reported by Simon and co-workers.[8] The control membrane containing lipophilic anion additive, KT*p*ClPB shows higher selectivity for more lipophilic metal cations. This is a common phenomenon for ion-exchange membranes. Membranes containing the additive tetrakis(*p*-chlorophenyl)borate behave like ion-exchange resins in the absence of ionophores. Lipophilic solvent PVC membranes containing the spiromacrocyclic ionophores *d*,*l*-**3** and *d*,*l*-**4** gave good potentiometric responses, and also displayed significant selectivities for lithium relative to sodium ($\log K^{pot}_{Li,Na} = -1.40$ and -1.23, respectively), even though these selectivities are moderate compared to structurally optimized ionophore ETH 1810 ($\log K^{pot}_{Li,Na} = -2.22$). The selectivity patterns of the isomorphic ionophores *d*,*l*-**3** and *d*,*l*-**4** are fairly similar to each other for alkali metal ions. The dibenzo analogue *d*,*l*-**4** with thicker lipophilic benzene moiety shows slightly higher affinity toward lipophilic ions, such as rubidium and cesium. However, the dibenzo analogue *d*,*l*-**4** could better discriminate against alkaline earth metal ions such as magnesium, calcium, and barium than the analogue *d*,*l*-**3**. The selectivity coefficient differences ($\Delta\log K^{pot}_{Li,M}$) between analogous ionophores *d*,*l*-**3** and *d*,*l*-**4** are 0.68 for magnesium, 1.32 for calcium, and 1.33 for barium, which means that *d*,*l*-**4** excludes these divalent cations by factors of 5, 20, and 21, respectively. A preference for divalent relative to monovalent cations of the same size are observed with ligand *d*,*l*-**3**, which has a small thickness of the ligand layer around the central atom and high dipole moments, apparently increasing the stability of the complex.[10] In contrast, modifying the skeleton of ionophore from *d*,*l*-**3** to *d*,*l*-**4** by adding much thicker lipophilic benzene shell, gives higher lipophilicity and greater selectivity for monovalent cations relative to divalent cations.

Table 1. Selectivity coefficients, $\log K^{pot}_{Li,M}$, for solvent polymeric membranes containing ionophores, ETH 1810, *d*,*l*-**3** or *d*,*l*-**4**; plasticizer, *o*-NPOE, and lipophilic anion additive, KT*p*ClPB, and for control membrane lacking ionophore, and required selectivity coefficients, $\log K^{pot}_{Li,M_{max}}$

Interfering ion	Control	ETH 1810	*d*,*l*-**3**	*d*,*l*-**4**	$\log K^{pot}_{Li,M_{max}}$ [a]	
					Intra	Extra
Na^+	0.37	−2.22	−1.40	−1.23	−3.2	−4.3
K^+	1.97	−2.58	0.67	0.35	−4.2	−2.8
Rb^+	2.59	−2.73	1.29	1.47		
Cs^+	3.24	−2.65	1.38	2.16		
NH_4^+	1.62	−2.60	0.91	0.46		
Mg^{2+}	−0.48	−4.02	−1.61	−2.29	−3.7	−3.5
Ca^{2+}	−0.29	−2.75	−0.83	−2.15	−2.1	−3.6
Ba^{2+}	−0.29	−2.93	−0.79	−2.12		

Selectivity coefficients determined by the separate solution method with 0.1 M solutions of the chlorides.

[a] Selectivity factors required for 1% interference, based on intra- and extracellular ion activities.

The properties of novel ionophore *d*,*l*-**4** were further investigated by varying the kind of the plasticizer and amount of the lipophilic anion additive, KT*p*ClPB. The plasticizer, bis(1-butylpentyl)adipate (BBPA), is known as less lipophilic than *o*-NPOE, and has been used as a plasticizer for PVC polymeric NH_4^+-ISEs containing the ionophore nonactin.[11] Five PVC membranes containing the plasticizer *o*-NPOE and varying amount of KT*p*ClPB, 0–120 mol % with respect to the ionophore *d*,*l*-**4**, were prepared to determine the optimum amount of the lipophilic anion additive. The lithium ion selectivity coefficients of the resulting electrodes were determined and are listed in Table 2. The control membrane containing no lipophilic anion additive shows high affinity for more lipophilic alkali metal ions, in the order of cation liphophilicity, $Cs^+ > Rb^+ > K^+ > Na^+$ but discriminates against the more hydrophilic alkaline earth metal dications such as Ba^{2+}, Mg^{2+}, and Ca^{2+}. This phenomenon is commonly observed for solvent PVC membrane containing lipophilic plasticizers, such as *o*-NPOE. Such phenomena of ion-exchange membranes are enhanced when an excess of a lipophilic anion is added, with respect to the amount of ionophore. All cations examined had higher affinities to the membrane, hence poorer lithium ion selectivities when the Li^+-ISE contained 120 mol % KT*p*ClPB. The optimum amount of the lipophilic anion additive was examined for lithium selectivity. Theoretically, 100 mol % of the anion additive is required for the best sensitivity and selectivity for ionophores forming 1:1 complexes. With the addition of suitable amount of lipophilic anion additive KT*p*ClPB, lipophilic cations such as Cs^+ and Rb^+ have higher affinity to the membranes, but hydrophilic alkaline earth metal dications such as Ba^{2+}, Mg^{2+}, and Ca^{2+} have lower affinity. Response to K^+ and NH_4^+ ions was not affected strongly by the amount

Table 2. Selectivity coefficients, $\log K^{pot}_{Li,M}$, for solvent polymeric membranes based on ionophore *d*,*l*-**4** containing *o*-NPOE or BBPA as plasticizer and varying amounts of lipophilic anion additive, KT*p*ClPB

Interfering ion	*o*-NPOE					BBPA	
	KT*p*ClPB (mol %)[a]					KT*p*ClPB (mol %)[a]	
	0	30	60	90	120	0	60
Na^+	−0.07	−0.92	−1.23	−0.50	0.41	0.02	−0.75
K^+	1.22	0.85	0.35	1.21	2.06	1.03	0.17
Rb^+	1.90	1.97	1.47	2.10	2.66	1.87	1.19
Cs^+	2.45	2.61	2.16	2.74	3.32	2.64	1.97
NH_4^+	1.03	0.95	0.46	1.05	1.65	1.01	0.31
Mg^{2+}	−1.84	−2.30	−2.29	−1.47	−0.66	−1.85	−2.44
Ca^{2+}	−1.45	−1.75	−2.15	−1.42	−0.29	−1.95	−2.39
Ba^{2+}	−0.14	−1.63	−2.12	−1.23	0.02	−1.84	−1.36

[a] mol % of the anion additive KT*p*ClPB with respect to ionophore *d*,*l*-**4** in PVC membranes, which were composed of about 1 wt % of the ionophore *d*,*l*-**4**, 66 wt % of *o*-NPOE plasticizer and 33 wt % of PVC.

of the lipophilic anion additive, but the common interfering dications could be better discriminated. Specially the membrane containing 60 mol % of KT*p*ClPB with respect to the ionophore *d*,*l*-**4** shows good lithium ion selectivity over sodium, which is the most important interfering ion in blood serum during lithium measurement.

Membranes containing the plasticizer BBPA instead of *o*-NPOE could better discriminate between alkaline earth and alkali metal ions. For example, the selectivity coefficient differences ($\log K^{pot}_{Li,Cs} - \log K^{pot}_{Li,Ca}$) between relatively lipophilic Cs^+ and highly hydrophilic Ca^{2+} are 3.90 for the control membrane with *o*-NPOE and 4.59 for the control membrane with BBPA. However, with the addition of KT*p*ClPB to the membrane composed of BBPA, the lithium ion selectivities against all metal ions examined were enhanced, but practical lithium ion selectivity over sodium ion was not obtained.

2.3. Molecular modeling

Energy-minimized structures of *d*,*l*-**3**·Li^+ and *d*,*l*-**4**·Li^+ complexes are shown in Figure 1. The distances between ligand O and Li^+ are all the same in each complex: 2.196 Å for *d*,*l*-**3**·Li^+ and 2.118 Å for *d*,*l*-**4**·Li^+. Bond angles, ∠ O–Li–O, are measured as 75° and 137° in *d*,*l*-**3**·Li^+, and 76° and 136° in *d*,*l*-**4**·Li^+ for angles formed by two ligand oxygens of the same dibenzospirodiamide moiety and by oxygens of two different spirodiamide moieties, respectively. The complexing geometry of Li^+ to the ionophores *d*,*l*-**3** and *d*,*l*-**4** is very similar. Fusion of benzene rings of *d*,*l*-**4** onto the *d*,*l*-**3** skeleton does not significantly affect complex geometry. The distance from the molecular center to the *para*-xylyl moieties was unchanged, but the thickness of molecular shell is enlarged to 7.2 Å from 5.4 Å. The thicker lipophilic shell of ionophore *d*,*l*-**4** apparently enhances ion selectivities for monovalent cations over divalent cations.

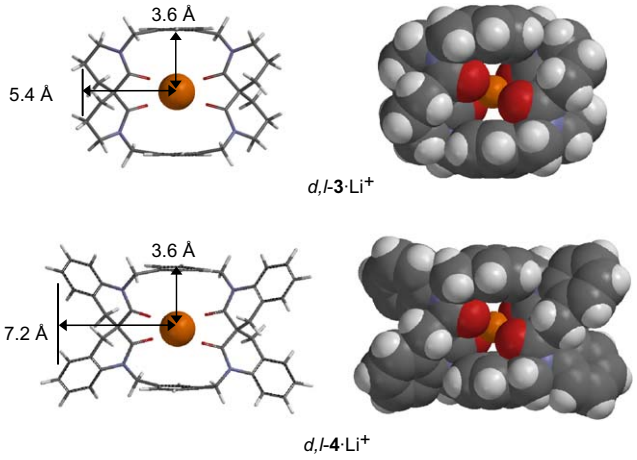


Figure 1. Energy-minimized structures of pseudo-tetrahedral complexes of *d*,*l*-**3**·Li^+ and *d*,*l*-**4**·Li^+ (Spartan'04 V1.0.1 PM3 semiempirical). In *d*,*l*-**3**·Li^+ the distances 5.4 and 3.6 Å are measured from the lithium atom to the center of the line connecting the two gamma carbons of the spirodiamide moiety, and to the center of the benzene ring in the *para*-xylyl moiety, respectively. The distance 7.2 Å in *d*,*l*-**4**·Li^+ is measured from the lithium atom to the center of the line connecting the two *para* carbons of the benzene rings in dibenzospirodiamide moiety.

3. Conclusion

In summary, macrocyclic tetraamide ionophores *d*,*l*-**3** and *d*,*l*-**4** in which four amide carbonyl ligands are structurally preorganized to converge on the molecular cavity and to coordinate tetrahedrally to small metal ions such as lithium were synthesized and tested as possible lithium ionophores in solvent PVC membranes. These PVC membranes containing the plasticizer *o*-NPOE and the lipophilic anion additive KT*p*ClPB gave good potentiometric responses to alkali metal ions and alkaline earth metal ions. Significant selectivities for lithium relative to sodium were observed as $\log K^{pot}_{Li,Na} = -1.4$ for *d*,*l*-**3** and −1.23 for *d*,*l*-**4**. Both isomorphic ionophores *d*,*l*-**3** and *d*,*l*-**4** show similar ion selectivity patterns for cations such as Na^+, K^+, and NH_4^+, but the dibenzo analogue *d*,*l*-**4** with lipophilic benzene moiety shows slightly higher affinity toward more lipophilic ions such as Rb^+ and Cs^+. However, the dibenzo ionophore *d*,*l*-**4** could discriminate against alkaline earth metal ions better than the analogue *d*,*l*-**3**. Ionophore *d*,*l*-**4** excludes these divalent cations better than *d*,*l*-**3** by factors of 5 for Mg^{2+}, 20 for Ca^{2+}, and 21 for Ba^{2+}. Modifying the skeleton of ionophore *d*,*l*-**3** to *d*,*l*-**4**, by adding a much thicker lipophilic benzene shell, gives higher lipophilicity and greater selectivity for monovalent cations relative to divalent cations. This is an example of an endopolarophilic/exolipophilic macrocyclic ionophore whose selectivity for monovalent cations over divalent cations is enhanced by thick lipophilic shells.

4. Experimental

4.1. Reagents

cis-*N*,*N*-Dicyclohexyl-*N*′,*N*′-diisobutylcyclohexane-1,2-dicarboxamide (ETH 1810) was synthesized.[12] High molecular weight poly(vinyl chloride) (PVC), 2-nitrophenyl octyl ether (*o*-NPOE), bis(1-butylpentyl)adipate (BBPA), and potassium tetrakis(*p*-chlorophenyl)borate (KT*p*ClPB) were obtained from Fluka. Analytical grade chloride salts of cesium, rubidium, potassium, sodium, lithium, barium, calcium, magnesium, and ammonium were obtained from Fluka. Doubly distilled water was used to prepare all aqueous electrolyte solutions. Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl immediately prior to use, and dimethyl sulfoxide (DMSO) was distilled from CaH_2 under reduced pressure.

4.2. Preparation of polymeric ion-selective electrodes

The typical composition of solvent polymeric membranes for Li^+-ion-selective electrodes was 33 mg PVC, 66 mg plasticizer, 1 mg ionophore, and various amounts of KT*p*ClPB (about 30 mol % in respect to the divalent carriers and 60 mol % to the tetravalent ligands). The mixture was then completely dissolved in 2 mL of THF. All membrane cocktails were cast into glass rings (1 inch i.d.) placed on glass plates for conventional ion-selective electrodes. Solvent was allowed to evaporate for 24 h at room temperature in a closed container. The thickness of the resulting membrane was about 0.3 mm.

A membrane disk (7 mm diameter) cut out with a punch was mounted in a Philips electrode body (IS-561). The electrode was filled with internal filling solution (0.1 M LiCl), and conditioned for 24 h by soaking in a 0.1 M LiCl solution. A silver/silver chloride coated wire was used as an internal reference electrode.

4.3. Potentiometric measurements

All emf measurements were performed at 24±1 °C by means of a Mettler Delta 350 meter with an Orion sleeve-type double-junction Ag/AgCl reference electrode (Model 900200). The emf readings were referenced to zero with a 0.1 M LiCl sample solution. The emf readings for interfering metal ions were used directly for selectivity coefficient calculations. The selectivity coefficients ($\log K^{pot}_{Li,M}$) were determined by the separate solution method[8] (SSM, 0.1 M chloride). Activities were calculated according to the Debye–Hückel procedure.

4.4. Synthesis

4.4.1. 3,11,18,26-Tetraazaheptacyclo[26.2.2.2^{13,16}.1^{3,7}.1^{7,11}.1^{18,22}.1^{22,26}]-octatriaconta-13,15,28,30,31,35-hexaene-33,34,37,38-tetrone, 3. To sodium hydride (60% dispersion in mineral oil, 180 mg, 4.4 mmol) washed with hexane was added dry DMSO (20 mL) and the resulting mixture was stirred at 70 °C for 30 min under nitrogen. To a clear solution was added 2,8-diazaspiro[5,5]undecane-1,7-dione, **1** (364 mg, 2 mmol) and the mixture was stirred for additional 30 min. This solution was transferred to a 50-mL syringe and the volume was adjusted to 35 mL by adding extra DMSO. A solution of 1,4-bis(chloromethyl)benzene (350 mg, 2 mmol) in DMSO (35 mL) was prepared in another 50-mL syringe. These two solutions were simultaneously added dropwise by a syringe pump to 250 mL of anhydrous DMSO containing anhydrous $MgSO_4$ (480 mg) at 70 °C for a period of 2 h, and the resulting reaction mixture was heated for additional 2 h under nitrogen. The solvent was removed in vacuo and the residue was dissolved in chloroform (100 mL). The mixture was washed with brine (100 mL). The chloroform layer was separated and the brine layer was further extracted with chloroform (2×50 mL). The combined chloroform solutions were dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The product was isolated by a column chromatography (silica gel, EtOAc/MeOH 8:2) and further purified by a preparative TLC to yield a solid product (27 mg, 7%). Mp 324–326 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.21 (s, 8H), 5.44 (d, J=14.7 Hz, 4H), 3.41 (d, J=14.7 Hz, 4H), 3.6 (m, 4H), 3.3 (m, 4H), 2.4 (m, 8H), 1.7 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.2, 135.9, 127.8, 51.9, 48.6, 48.5, 33.9, 20.5; IR (film) 2930, 1627, 1513, 1484, 1440, 1416, 1285, 1204, 728 cm^{-1}; FAB MS (NOBA) m/z 586 ([M+H_2O]$^+$, 58%), 569 ([M+1]$^+$, 100%), 568 ([M]$^+$, 11%), 284 ([M]$^+$/2, 13%).

4.4.2. 3,19,26,42-Tetraazaundecacyclo[42.2.2.2^{21,24}.1^{3,11}.1^{11,19}.1^{26,34}.1^{34,42}.0^{4,9}.0^{13,18}.0^{27,32}.0^{36,41}]-tetrapentaconta-4,6,8,13,15,17,21,23,27,29,31,36,38,40,44,46,47,51-octadecene-49,50,53,54-tetrone, 4. To sodium hydride (60% dispersion in mineral oil, 120 mg, 3 mmol) washed with hexane was added dry DMSO (10 mL) and the mixture was stirred at 70 °C for 30 min under nitrogen. To the resulting clear solution was added a solution of 1,1′,4,4′-tetrahydro[3,3′(2H,2′H)-spirobiquinoline]-2,2′-dione, **2** (278 mg, 1 mmol) in DMSO (10 mL) and the reaction mixture was stirred for additional 30 min. This solution was transferred to a 50-mL syringe. A solution of 1,4-bis(chloromethyl)benzene (175 mg, 1 mmol) in DMSO (20 mL) was prepared in another 50-mL syringe. These two solutions were simultaneously added dropwise by a syringe pump to 150 mL of anhydrous DMSO at 70 °C for a period of 2 h, and the resulting reaction mixture was heated additionally for an hour under nitrogen. The solvent was removed in vacuo and the residue was dissolved in methylene chloride (50 mL). The mixture was washed with brine (50 mL). The methylene chloride layer was separated and the brine layer was further extracted with methylene chloride (2×50 mL). The combined methylene chloride layers were dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The product was isolated by a column chromatography (silica gel, CH_2Cl_2/EtOAc 97:3) and further purified by a preparative HPLC (silica gel, CH_2Cl_2/EtOAc 95:5) to yield two diastereomeric solid products, colorless needle-shaped crystals *d*,*l*-**4** (8 mg, 2.1%, from CH_2Cl_2/MeOH) and colorless transparent cubic crystals *meso*-**4** (1 mg, 0.3%, from CH_2Cl_2/MeOH). By a chiral HPLC column (Regis (R,R)-Whelk-O2 100, 250 nm length, 4.6 mm ID, 10 μm, 100 Å, normal phase, 280 nm UV detection, 1 mL/min flow rate, hexane/CH_2Cl_2/isopropanol 49:50:1), the needle-shaped crystals could be further resolved into two components (t_R 4.9 and 7.2 min), whereas the transparent cubic crystals remained as a component without further resolution (t_R 15.6 min). *d*,*l*-**4**: R_f (silica gel, CH_2Cl_2/EtOAc 97:3) 0.47; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.19 (d, J=7.7 Hz, 4H), 7.14 (s, 8H), 7.11 (t, J=7.7 Hz, 4H), 7.00 (t, J=7.7 Hz, 4H), 6.83 (d, J=7.7 Hz, 4H), 5.60 (d, J=16 Hz, 4H), 4.37 (d, J=16 Hz, 4H), 3.41 (d, J=16 Hz, 4H), 3.08 (d, J=16 Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.5, 139.7, 135.8, 127.5, 127.4, 126.5, 124.9, 123.4, 115.2, 48.6, 47.6, 35.9; IR (KBr) 3069, 2939, 1667, 1602, 1497, 1462, 1377, 1265, 1186, 751 cm^{-1}; MS (API-ES) m/z 785.3 ([M+2+Na]$^+$, 10%), 784.3 ([M+1+Na]$^+$, 38%), 783.3 ([M+Na]$^+$, 65%), 780.3 ([M+2+H_2O]$^+$, 18%), 779.3 ([M+1+H_2O]$^+$, 74%), 778.3 ([M+H_2O]$^+$, 100%), 760.3 ([M]$^+$, 5%); Anal. Calcd for $C_{50}H_{40}N_4O_4$: C, 78.93; H, 5.30; N, 7.36. Found: C, 78.81; H, 5.47; N, 7.02. *meso*-**4**: R_f (silica gel, CH_2Cl_2/EtOAc 97:3) 0.45; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 (d, J=7.6 Hz, 4H), 7.07 (t, J=7.6 Hz, 4H), 7.02 (s, 8H), 7.02 (t, J=7.6 Hz, 4H), 6.69 (d, J=7.6 Hz, 4H), 5.72 (d, J=16.6 Hz, 4H), 4.33 (d, J=16.6 Hz, 4H), 3.35 (d, J=16 Hz, 4H), 3.26 (d, J=16 Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.4, 139.3, 135.5, 127.1, 127.0, 125.9, 125.5, 123.7, 115.5, 49.0, 47.3, 37.3; IR (KBr) 3035, 2921, 1670, 1603, 1499, 1461, 1377, 1231, 758 cm^{-1}; MS (API-ES) m/z 785.3 ([M+2+Na]$^+$, 18%), 784.3 ([M+1+Na]$^+$, 60%), 783.3 ([M+Na]$^+$, 100%), 778.3 ([M+H_2O]$^+$, 5%), 760.3 ([M]$^+$, 10%).

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Design and synthesis of novel perfluoroalkyl-containing zinc pyrithione biocide

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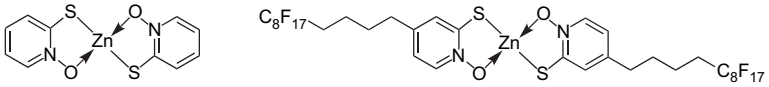
Abstract—Novel perfluoroalkyl-containing zinc pyrithione biocide **2** was designed and synthesized in six steps. Reaction of 4-methylpyridine with $C_8F_{17}(CH_2)_3I$ in the presence of LDA followed by further oxidization of the resultant pyridine derivative **6** gave the pyridine *N*-oxide **9**. Treatment of **9** with phosphorous oxychloride afforded the desirable chloride **12**. Oxidization of compound **12** with H_2O_2 gave *N*-oxide **14**, which was treated with NaSH to give the sulfide **3**. Finally, treatment of compound **3** with NaOH/$ZnSO_4$ smoothly delivered perfluoroalkyl-containing zinc pyrithione biocide **2** in good yield.

1. Introduction

Nowadays, Zinc pyrithione (also known as Zinc Omadine®, ZPT or zinc bis(2-pyridylthio)-*N*-oxide, **1**, Fig. 1) is used more and more extensively in routine life. Apart from preventing microbial degradation and deterioration of manufacturing starting materials such as plastics, polymers, and latexes, ZPT is used to prevent the growth of bacteria, fungi, mildew, and algae, which can cause various types of deterioration such as discoloration, staining, odors, etc. In addition, ZPT is the key active ingredient in various shampoos used to control dandruff and seborrheic dermatitis.[1–3] Furthermore, it is also registered for incorporation into antifoulant boat paints to control the growth of slime, algae, and marine fouling organisms below the water line on recreational and commercial boat hulls.[4,5]

Actually, biofouling is a serious problem for the shipping industry. To prevent boat hulls from fouling, lead and later copper sheathings were developed to address it in ancient years. In the 1970s, tributyltin (TBT) compounds, which were effective against both soft (e.g., algae) and hard fouling organisms, were introduced. However, although tin compounds are effective, the toxicity of released TBT is persistent in the environment and has adversely affected population of non-target organisms. Thus, TBT suffered from the international ban in antifouling paints. Fortunately, ZPT and its combination with cuprous oxide has been identified, a virtual substitute for TBT, to be effective against fouling organisms. Now, ZPT has been introduced into the market as an effective replacement for traditional TBT-based antifouling paints (for more information, see: http://www.archchemicals.com/Fed/Corporate/News).

In spite of many advantages of ZPT, it also suffers some drawbacks. For example, ZPT would stimulate eyes when it directly comes in contact with eyes when used in shampoos; it would slowly degrade when exposed to ultraviolet radiation. Thus, there keeps a strong demand for better substitution for ZPT, especially ZPT analogues.



Zinc pyrithione (ZPT) **1** Target molecule **2**

Figure 1. ZPT **1** and target compound **2**.

Keywords: Zinc pyrithione; Biocide; Perfluoroalkyl-containing compounds.
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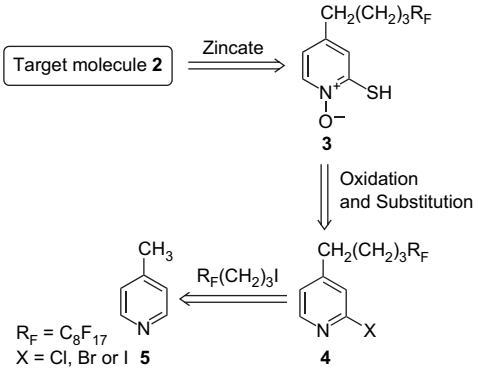
It is well known that introduction of fluorine atom(s) or fluorine-containing group into an organic compound can bring about remarkable changes in the physical, chemical, and biological properties.[6] The perfluoroalkylated tails are intended to increase the hydrophobic character[7] in order to render more waterproof or water insoluble compounds. Moreover, in some cases,[8] the introduction of perfluoroalkyl group into some compounds can not only efficiently enhance the antimicrobial activity, but also extend its application fields. Herein, we wish to report the synthesis of the novel perfluoroalkyl-containing zinc pyrithione biocides **2** (Fig. 1). Rational of target compound **2** was mainly based on the following points. Firstly, $R_F(CH_2)_4$ group was located in the C-4 position of pyrithione skeleton, which may reduce the direct influence of the blocking effect on the chelation of zinc with sulfide and oxygen atoms. In addition, R_F was connected to pyrithione through the linkage $-(CH_2)_4-$, which may eliminate the effect of strong electron-withdrawing R_F group on pyrithione ring and make oxygen atom and sulfur atom to chelate well with zinc.

2. Results and discussion

On the basis of retrosynthetic analysis (Scheme 1), 4-substituted pyrithione **3** would be reached by oxidization of pyridine derivative **4** followed by substitution of SH for halogen atom. Thus, how to prepare the intermediate **4** from 4-methyl-pyridine **5** is the key for the whole synthesis.

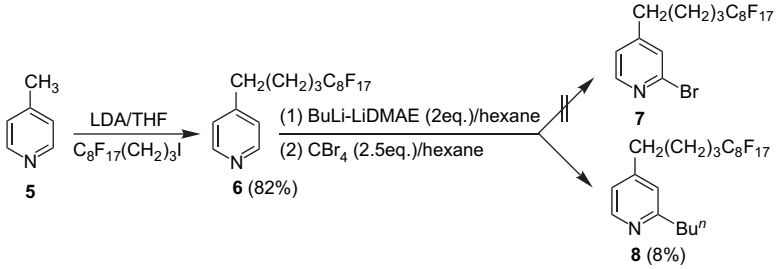


Scheme 1.

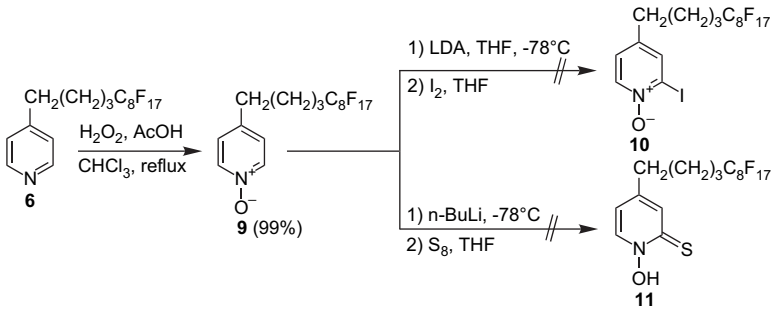
Accordingly, treatment of 4-methyl-pyridine **5** with LDA in THF followed by addition of $C_8F_{17}(CH_2)_3I$ to the resultant mixture smoothly gave the pyridine derivative **6** in 82% yield (Scheme 2).[7,9] Then, our eyesight was focused on the methodology reported by Kaminski et al.,[10] that 2,4-disubstituted pyridine derivatives were easily accessed by treatment of 4-pyridine derivatives with BuLi–$Me_2N(CH_2)_2OLi$(BuLi–LiDMAE) followed by electrophiles. However, lithiation of compound **6** with BuLi–LiDMAE/hexane followed by treatment with electrophile CBr_4 failed to afford the desired bromide **7**, only the byproduct **8** was obtained in 8% yield. Considering the low solubility of compound **6** in hexane, trifluoromethylbenzene/hexane as cosolvents and (or), addition of large excess of BuLi–LiDMAE was attempted, however, reactions still did not work. In our opinion, reaction failures were attributed to the possibility that *n*-BuLi–LiDMAE in hexane or trifluoromethylbenzene was not powerful enough to deprotonate compound **6** to form its carbanion. Kessar et al.[11] described that treatment of 4-substituted pyridine derivatives with $BF_3\cdot Et_2O$ and subsequent lithiation with an excess of lithium tetramethylpiperidide (LTMP) was also an efficient strategy for selective lithiation at C-2 position of pyridine derivatives. However, Kessar′s method still did not afford our desired product **7**.

It is well known that substitution of hydrogen in pyridine 1-oxide and its derivatives has been achieved in a number of cases by base-induced proton-abstraction followed by in situ treatment with a suitable electrophile.[12,13] Furthermore, Mongin et al.[14] successfully synthesized a series of 2-substituted pyridine *N*-oxides by the metalation of the pyridine *N*-oxide. Comparing to the corresponding pyridine derivatives, pyridine *N*-oxides indeed were more prone to metalation in C-2 position because of enhanced acidity of 2-H. Accordingly, oxidation of compound **6** with hydrogen peroxide (30% aq solution) smoothly gave the pyridine *N*-oxide **9** in almost quantitative yield (Scheme 3). However, treatment of compound **9** with LDA or LTMP at −78 °C followed by quenching the reaction with iodine failed to afford 2-iodine-4-fluoroalkyl-pyridine *N*-oxide **10**. In addition, Abramovitch et al. developed a general method for synthesis of pyridine cyclic thiohydroxamic acids, involving the addition of elemental sulfur to lithiopyridine 1-oxides.[15] Unfortunately, treatment of compound **9** with *n*-BuLi at low temperature followed by addition of elemental sulfur still did not provide our desired compound **11** (Scheme 3).

In view of the above failures, we thought that it was due to the deficiency of π-electron in the pyridine heterocycles, which resulted from the inductive effect of the perfluoroalkyl segments though the R_F group was segregated to heterocycle by four methylene units. Thus, reductive chlorination

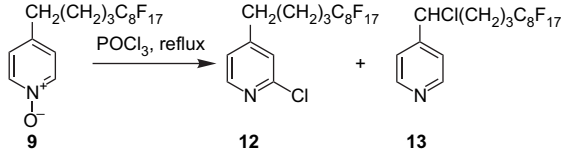


Scheme 2.



Scheme 3.

strategy developed by Queguiner et al.[16] was attempted to synthesize key intermediate **4**. To our delight, treatment of compound **9** with $POCl_3$ under reflux condition provided our desired halogenated pyridine **12** along with the byproduct **13** (Scheme 4). Interestingly, we found that the yield of byproduct **13** could be sharply decreased by lowering the amount of $POCl_3$, although the yield of compound **12** had no significant change.



$POCl_3$	reaction time	product (% yield)	
		12	**13**
10 eq.	4h	34	28
5 eq.	4h	35	7

Scheme 4.

Similar to the preparation of compound **9** from pyridine derivative **6**, pyridine *N*-oxide **14** was afforded by oxidation of chloride **12** in 79% yield (Scheme 5). Then, introduction of the requisite mercapto group was achieved via reaction of compound **14** with NaSH and sulfide **3** was furnished in 81% yield.[17,18] Finally, treatment of compound **3** with an aq NaOH followed by aq $ZnSO_4$ successfully gave our target molecule **2** in 84% yield.[19]

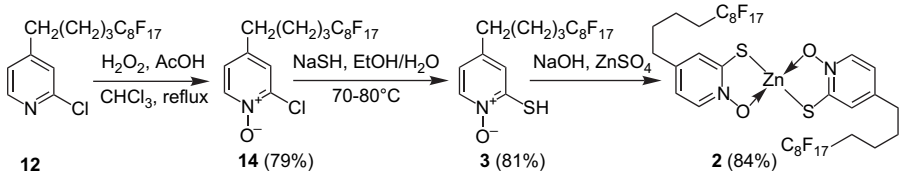
In summary, novel perfluoroalkyl-containing zinc pyrithione biocide was designed and synthesized. The key intermediate **12** was successfully afforded by reductive chlorination of compound **9** with $POCl_3$ although several lithiation strategies failed to give the desired compounds. Starting from compound **12**, target compound **2** was smoothly synthesized in a straightforward fashion. Studies on antibacterial activity and other usage of perfluoroalkyl-containing zinc pyrithione **2** are currently in progress.

3. Experimental

3.1. General methods

Melting points were determined on a Pai-ke melting point apparatus and were uncorrected. ^1H and ^{19}F NMR spectra were recorded on a Bruker AM 400 spectrometer, with TMS as an internal standard for ^1H NMR spectroscopy and $CFCl_3$ as an external standard for ^{19}F NMR spectroscopy. All chemical shifts (δ) were recorded in parts per million and coupling constants (J) were given in hertz. MS (EI, 70 eV) spectra were recorded with a Finnigan-MAT-8430 spectrometer. IR spectra were recorded on a Thermo Electron Corporation Nicolet 380 FT-IR spectrophotometer. THF and hexane were distilled from benzophenone/Na. 4-Methyl-pyridine, diisopropylamine, and 2-(dimethylamino)ethanol were distilled under nitrogen and stored over 4 Å molecular sieves. $C_8F_{17}(CH_2)_3I$ was synthesized according to literature.[20,21]

3.1.1. 4-(5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,12-Heptadecafluorododecyl)pyridine (6). *n*-Butyllithium (14.5 mL, 1.6 M in hexane, 23.2 mmol, 1.2 equiv) was added, via a syringe, to a solution of diisopropylamine (3.26 mL, 23.2 mmol, 1.2 equiv) in tetrahydrofuran (10 mL) at −78 °C. The solution was stirred at −78 °C for 0.5 h and then a solution of 4-methyl-pyridine (2.15 g, 23.09 mmol, 1.2 equiv) in THF (5 mL) was added dropwise. The resultant yellow mixture was stirred at −78 °C for 3 h. Then a solution of 1,1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8-heptadecafluoro-11-iodoundecane (11.30 g, 19.22 mmol, 1.0 equiv) in THF (15 mL) was added dropwise. The reaction mixture was slowly warmed to room temperature and stirred overnight. The reaction was quenched with methanol (5 mL) and the resultant yellow-brown solution was poured into cold water. The solution was extracted with diethyl ether (3×50 mL) and combined organic phases were dried over anhydrous Na_2SO_4. After filtration and removal of the organic solvent, the residue was purified by silica gel (petroleum ether/ethyl acetate, 5:1) to give compound **6** as white crystals (8.68 g, 82%



Scheme 5.

yield). Mp: 38.5–40.5 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.51 (d, J=8.0 Hz, 2H), 7.12 (d, J=8.0 Hz, 2H), 2.67 (t, J=8.0 Hz, 2H), 2.15–2.04 (m, 2H), 1.77–1.64 (m, 4H); ^{19}F NMR ($CDCl_3$, 376 MHz): δ −80.73 (t, J=11.3 Hz, 3F), −114.25 to −114.34 (m, 2F), −121.70 to −121.91 (m, 6F), −122.68 (s, 2F), −123.48 (s, 2F), −126.10 (s, 2F); IR (thin film) 658, 704, 955, 1073, 1116, 1147, 1171, 1216, 1251, 1332, 1372, 1469, 1603, 2919, 2949, 3440 cm^{-1}; MS (EI): m/z (%)=92 (29.5), 93 (100), 106 (12.4), 534 (13.3), 553 (M^+, 25.6), 554 (5.87, M^++1); Anal. Calcd for $C_{17}H_{12}F_{17}N$: C, 36.91; H, 2.19; N, 2.53. Found: C, 37.11; H, 2.28; N, 2.45.

3.1.2. 2-Butyl-4-(5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,12-heptadecafluorododecyl)pyridine (8). After a solution of 2-(dimethylamino)ethanol (0.4 mL, 3.9 mmol, 2.0 equiv) in hexane (1.5 mL) was cooled to 0 °C, n-butyllithium (4.9 mL, 1.6 M in hexane, 7.8 mmol, 4.0 equiv) was added dropwise. Then, the mixture was stirred at 0 °C for 0.5 h. After that, a solution of compound **6** (1.08 g, 1.95 mmol, 1.0 equiv) in hexane (2.0 mL) was added dropwise. After the reaction was further stirred for 1 h at 0 °C, the organic solution was cooled to −78 °C and treated with a solution of CBr_4 (1.62 g, 4.9 mmol, 2.5 equiv) in THF (5 mL). After 3 h at −78 °C, the mixture was warmed to room temperature and reaction was quenched with H_2O (5 mL) at 0 °C. The organic layer was then extracted with diethyl ether (3×15 mL) and the combined organic phases were dried over anhydrous $MgSO_4$. After filtration and removal of the organic solvent, the residue was purified by silica gel (petroleum ether/ethyl acetate, 5:1) to give compound **8** as a yellow oil (0.10 g, 8% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.42 (d, J=5.0 Hz, 1H), 6.96 (s, 1H), 6.91 (d, J=5.0 Hz, 1H), 2.79–2.75 (t, J=8.0 Hz, 2H), 2.64–2.60 (t, J=8.0 Hz, 2H), 2.12–2.07 (m, 2H), 1.75–1.66 (m, 6H), 1.42–1.36 (m, 2H), 0.94 (t, J=7.0 Hz, 3H); ^{19}F NMR ($CDCl_3$, 376 MHz): δ −80.99 (s, 3F), −114.32 to −114.41 (m, 2F), −121.76 to −121.99 (m, 6F), −122.79 (s, 2F), −123.58 (s, 2F), −126.21 (s, 2F); IR (thin film) 559, 656, 721, 828, 1028, 1115, 1151, 1206, 1242, 1329, 1413, 1468, 1560, 1605, 2957 cm^{-1}; MS (EI): m/z (%)=106 (23.9), 107 (32.0), 119 (124.7), 567 (100), 568 (21.2), 5580 (28.3), 94 (M^+-CH_3, 12.5), 610 (M^++1, 23.4); Anal. Calcd for $C_{21}H_{20}F_{17}N$: C, 41.39; H, 3.31; N, 2.30. Found: C, 41.50; H, 3.35; N, 2.32.

3.1.3. 4-(5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,12-Heptadecafluorododecyl)-1-hydroxypyridinium (9). Hydrogen peroxide (30% aq solution, 30 mL) was added dropwise to a solution of compound **6** (5.54 g, 10.01 mmol) in chloroform (20 mL) and then acetic acid (5 mL) was added at 0 °C. The solution was stirred at room temperature for 3 h. After that, the mixture was heated to reflux for 18 h. Then, the reaction mixture was poured into water and extracted with CH_2Cl_2 (3×30 mL). The combined organic phases were dried over anhydrous Na_2SO_4. After filtration and removal of the organic solvent, the residue was purified by silica gel (CH_2Cl_2/MeOH, 15:1) to give compound **9** as white crystals (5.66 g, 99% yield). Mp: 77.5–78.5 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.19 (d, J=8.0 Hz, 2H), 7.12 (d, J=8.0 Hz, 2H), 7.68 (t, J=8.0 Hz, 2H), 2.16–2.07 (m, 2H), 1.76–1.65 (m, 4H); ^{19}F NMR ($CDCl_3$, 376 MHz): δ −80.73 (t, J=11.3 Hz, 3F), −114.11 to −114.19 (m, 2F), −121.59 to −121.81 (m, 6F), −122.61 (s, 2F), −123.39 (s, 2F), −126.00 (t, J=11.3 Hz, 2F); IR (thin film) 566, 733, 781, 941, 1020, 1113, 1136, 1206, 1287, 1510, 1575, 1689, 1792, 2550, 2667, 2983, 3064, 3430 cm^{-1}; MS (EI): m/z (%)=91 (5.6), 93 (14.5), 108 (100), 109 (9.7), 121 (3.8), 569 (M^+, 24.4), 570 (M^++1, 5.8); Anal. Calcd for $C_{17}H_{12}F_{17}NO$: C, 35.87; H, 2.12; N, 2.46. Found: C, 35.46; H, 2.34; N, 2.32.

3.1.4. 2-Chloro-4-(5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,12-heptadecafluorododecyl)pyridine (12) and 4-(1-chloro-5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,12-heptadecafluorododecyl)pyridine (13). Phosphorus oxychloride (30 mL) was added to compound **9** (1.75 g, 3.07 mmol) and the resultant solution was heated to reflux for 4 h. Then, the solvent was removed in vacuo and H_2O (20 mL) was added to the resultant red-brown residue. After the mixture was neutralized with aq K_2CO_3, the solution was extracted with methylene dichloride (3×20 mL). The combined organic phases were dried over anhydrous Na_2SO_4. After filtration and removal of the organic solvent, the residue was purified by silica gel to give compound **12** (0.61 g, 34% yield) and compound **13** (0.50 g, 28% yield) as white solids.

Compound **12**: Mp<16.5 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.29 (d, J=4.0 Hz, 1H), 7.17 (s, 1H), 7.05 (d, J=4.0 Hz, 1H), 2.66 (t, J=8.0 Hz, 2H), 2.05–2.06 (m, 2H), 1.76–1.67 (m, 4H); ^{19}F NMR ($CDCl_3$, 376 MHz): δ −80.76 (t, J=11.3 Hz, 3F), −114.25 to −114.34 (m, 2F), −121.69 to −121.91 (m, 6F), −122.70 (s, 2F), −123.49 (s, 2F), −126.09 (s, 2F); IR (thin film) 527, 559, 653, 706, 824, 890, 948, 997, 1029, 1082, 1148, 1217, 1254, 1328, 1381, 1393, 1467, 1548, 1593, 2872, 2950, 3052, 3440 cm^{-1}; MS (EI): m/z (%)=69 (6.7), 91 (14.4), 126 (15.2), 127 (100), 129 (37.0), 587 (M^+, 11.3), 588 (M^++1, 8.5); Anal. Calcd for $C_{17}H_{11}F_{17}NCl$: C, 34.74; H, 1.89; N, 2.38. Found: C, 34.92; H, 1.67; N, 2.25.

Compound **13**: Mp: 22.5–24.5 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.65 (s, 2H), 7.32 (s, 2H), 4.92–4.80 (m, 1H), 2.36–2.31 (m, 1H), 2.19–2.06 (m, 3H), 1.74–1.72 (m, 1H), 1.30–1.27 (m, 1H); ^{19}F NMR ($CDCl_3$, 376 MHz): δ −80.72 (s, 3F), −114.21 to −114.29 (m, 2F), −121.66 to −121.88 (m, 6F), −122.68 (s, 2F), −123.25 to −123.45 (m, 2F), −126.07 (s, 2F); IR (thin film) 573, 657, 704, 722, 814, 957, 1059, 1115, 1135, 1147, 1210, 1260, 1332, 1419, 1464, 1598, 2930, 2969, 3431 cm^{-1}; MS (EI): m/z (%)=91 (23.1), 92 (100), 118 (46.1), 127 (47.4), 126 (82.7), 379 (21.4), 552 (49.9), 587 (M^+, 28.6); Anal. Calcd for $C_{17}H_{11}F_{17}NCl$: C, 34.74; H, 1.89; N, 2.38. Found: C, 34.76; H, 1.74; N, 2.32.

3.1.5. 2-Chloro-4-(5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,12-heptadecafluorododecyl)-1-hydroxypyridinium (14). To a solution of compound **12** (1.55 g, 2.64 mmol) in chloroform (10 mL), hydrogen peroxide (30% aq solution, 10 mL) and acetic acid (1.5 mL) were added dropwise at 0 °C. After dripping off, the solution was stirred at room temperature for 3 h. Then, the mixture was heated to reflux for 20 h. The reaction mixture was poured into water and then extracted with methylene dichloride (5×15 mL). The combined organic layers were dried over anhydrous Na_2SO_4. After filtration and removal of the organic solvent, the residue was purified by silica gel (methylene dichloride/methanol, 20:1) to give compound **14** as white crystals (1.26 g, 79% yield).

Mp: 58–60 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.32 (d, J=4.0 Hz, 1H), 7.34 (s, 1H), 7.05 (d, J=4.0 Hz, 1H), 2.67 (t, J=8.0 Hz, 2H), 2.19–2.10 (m, 2H), 1.75–1.68 (m, 4H); ^{19}F NMR ($CDCl_3$, 376 MHz): δ −80.72 (t, J=11.3 Hz, 3F), −114.20 to −114.33 (m, 2F), −121.67 to −121.88 (m, 6F), −122.67 (s, 2F), −123.44 (s, 2F), −126.05 (s, 2F); IR (thin film) 557, 652, 800, 837, 1025, 1115, 1150, 1205, 1250, 1373, 1331, 1419, 1465, 1605, 2852, 2921, 2950, 3064, 3097, 3432 cm^{-1}; MS (EI): *m/z* (%)=69 (5.5), 129 (4.5), 142 (100), 144 (35.4), 603 (M^+, 50.8), 604 (M^++1, 11.8), 605 (M^++2, 18.2); Anal. Calcd for $C_{17}H_{11}F_{17}NOCl$: C, 33.82; H, 1.84; N, 2.32. Found: C, 33.75; H, 1.85; N, 2.28.

3.1.6. 4-(5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,12-Heptadecafluorododecyl)-1-hydroxy-2-mercaptopyridinium (3). A solution of compound **14** (0.88 g, 1.46 mmol) in H_2O (10 mL) and EtOH (5 mL) was heated to 70 °C. Then, a solution of NaSH (0.33 g, 5.83 mmol) in H_2O (5 mL) was added dropwise. After that, the reaction mixture was further heated to 85 °C and stirred for 1 h. Then, the decolorizing carbon (0.5 g) was added and the resultant mixture was filtered. The filtrate was cooled to 15–25 °C and the pH of solution was adjusted to 1.7 with hydrochloric acid (20%). The precipitated khaki compound **3** (0.71 g, 81% yield) was recovered immediately, recrystallized from hot ethanol and dried. Mp: 107–112 °C; ^{1}H NMR (MeOD, 400 MHz): δ 8.36–8.32 (m, 1H), 7.56 (s, 1H), 7.31–7.26 (m, 1H), 2.70 (t, J=8.0 Hz, 2H), 2.19–2.03 (m, 2H), 1.70–1.61 (m, 4H); ^{19}F NMR (MeOD, 376 MHz): δ −80.39 (s, 3F), −115.37 to −115.50 (m, 2F), −122.72 to −122.93 (m, 6F), −123.76 (s, 2F), −124.47 (s, 2F), −127.30 (s, 2F); IR (thin film) 531, 560, 658, 705, 807, 872, 1027, 1115, 1150, 1204, 1332, 1465, 1538, 1608, 2949, 3435 cm^{-1}; MS (EI): *m/z* (%)=65 (34.3), 80 (89.3), 109 (100), 125 (81.5), 585 (58.5), 601 (M^+, 32.0); HRMS Calcd for $C_{17}H_{13}NOF_{17}S$ (M^++1), 602.0431. Found: 602.0441.

3.1.7. Zinc bis(4-(5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,12-heptadecafluorododecyl)-2-pyridylthio)-*N*-oxide (2). A solution of compound **3** (0.40 g, 0.67 mmol) in EtOH (10 mL) was added to a solution of NaOH (0.03 g, 0.75 mmol) in H_2O (5 mL). After stirring for half an hour, a solution of $ZnSO_4\cdot 7H_2O$ (0.098 g, 0.34 mmol) in H_2O (10 mL) was added to the mixture dropwise. A white precipitate was formed immediately and was collected by filtration. Resultant solid compound was washed with water, alcohol, and ether and air-dried. The compound **2** (0.72 g, 84% yield) was finally obtained after solid compound was kept in vacuum for about 1 h. Mp>300 °C; IR (thin film) 422, 617, 639, 657, 705, 806, 874, 1028, 1115, 1149, 1204, 1332, 1370, 1468, 1534, 1615, 2854, 2922, 3429 cm^{-1}; HRMS Calcd for $C_{34}H_{22}N_2O_2F_{17}S_2Zn$ (M^+), 1263.9819; $C_{34}H_{23}N_2O_2F_{17}S_2Zn$ (M^++1), 1264.9920. Found: 1263.9866; 1264.9944.

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Syntheses of metabolites of ethyl 4-(3,4-dimethoxyphenyl)-6,7-dimethoxy-2-(1,2,4-triazol-1-ylmethyl)quinoline-3-carboxylate (TAK-603)

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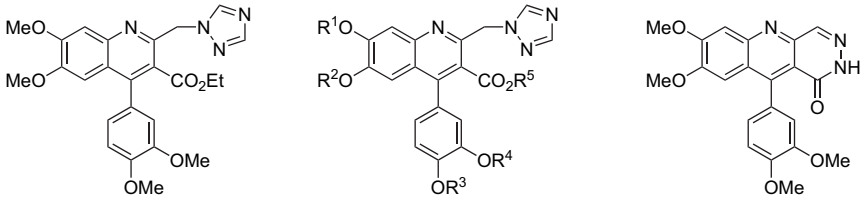
Abstract—Convenient and efficient syntheses of ethyl 4-(3-hydroxy-4-methoxyphenyl)-6,7-dimethoxy-2-(1,2,4-triazol-1-ylmethyl)quinoline-3-carboxylate (**1d**) and 10-(3,4-dimethoxyphenyl)-7,8-dimethoxy-2*H*-pyridazino[4,5-*b*]quinolin-1-one (**1e**), metabolites of TAK-603 (**1**), have been achieved. Use of the methanesulfonyl as a protective group of the phenolic hydroxy for Friedel–Crafts reaction enabled a new simpler synthetic route of **1d** in high yield. Chloromethyl derivative (**23**) was converted to formyl derivative (**32**) using the Kröhnke reaction, followed by cyclization with hydrazine, which formed a novel compound **1e**.

1. Introduction

Ethyl 4-(3,4-dimethoxyphenyl)-6,7-dimethoxy-2-(1,2,4-triazol-1-ylmethyl)quinoline-3-carboxylate (TAK-603, **1**, Fig. 1) was found to be a disease-modifying antirheumatic drug.[1] In pre-clinical and clinical studies, the metabolic mechanism and reaction of TAK-603 (**1**) have been studied in several animal species and humans.[1c,2] Analytical evaluation using liquid chromatography–mass spectrometry (LC–MS) and/or liquid chromatography–NMR (LC–NMR) suggested that **1a**–**d** existed in rat bile and **1a**,**d**,**e** (Fig. 1) existed in human serum.

Among them, the syntheses of four metabolites (**1a**–**d**, Fig. 1) have been reported.[1c] The carboxylic acid derivative (**1a**) was prepared easily by alkaline hydrolysis of **1**. The mono-demethylated compounds (**1b**–**d**) were synthesized by using the isopropyl as a protective group of the phenolic hydroxy as shown in Scheme 1. The pyridazinoquinolinone compound (**1e**) was a new metabolite. Prompt and rational synthesis of **1d** and **1e** was required for an urgent request for the toxicological studies.

Here, we wish to report the highly efficient synthesis of **1d** using methanesulfonyl as a protective group of the phenolic



1: TAK-603

1a: R^1=R^2=R^3=R^4=Me, R^5=H
1b: R^1=H, R^2=R^3=R^4=Me, R^5=Et
1c: R^3=H, R^1=R^2=R^4=Me, R^5=Et
1d: R^4=H, R^1=R^2=R^3=Me, R^5=Et

1e

Figure 1.

Keywords: TAK-603; Metabolite; Methanesulfonyl group; Friedel–Crafts reaction; Pyridazinoquinolinone; Kröhnke reaction.
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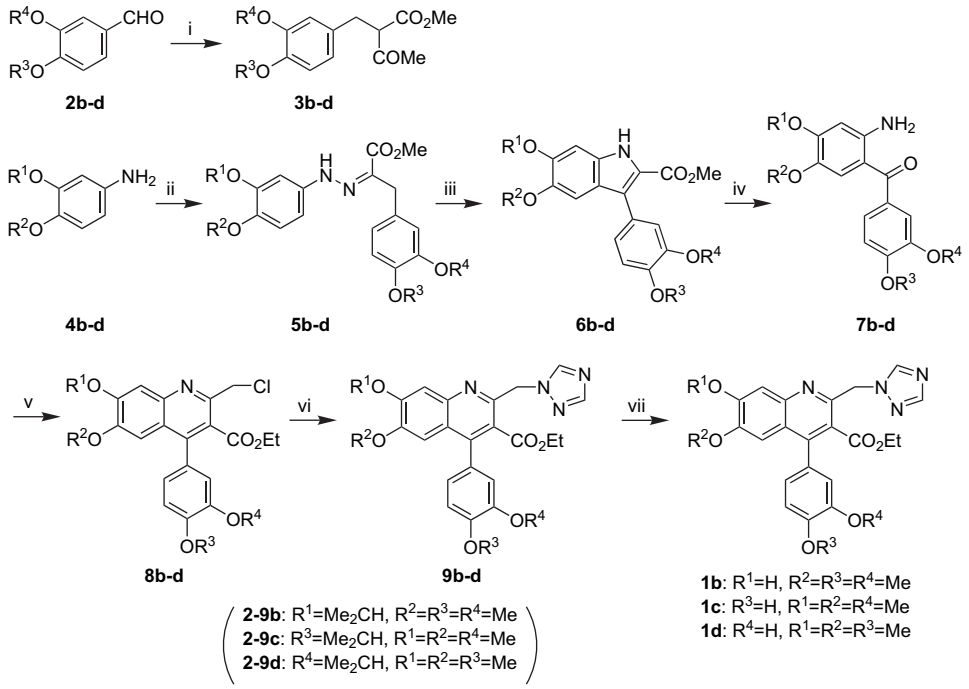


Scheme 1. Reagents and conditions: (i) (1) $MeCOCH_2CO_2Me$, piperidine, AcOH/toluene, then silica gel chromatography, (2) H_2, Pd–C/THF–EtOH; (ii) (1) $NaNO_2$, concd HCl/acetone–H_2O, (2) **3**, NaOAc, (3) KOH/MeOH; (iii) H_2SO_4/MeOH; (iv) (1) CrO_3/AcOH–H_2O, (2) KOH/H_2O; (v) $ClCH_2COCH_2CO_2Et$, H_2SO_4/AcOH, then silica gel chromatography; (vi) 1*H*-1,2,4-triazole, NaH, DMF, then silica gel chromatography; (vii) $TiCl_4/CH_2Cl_2$, then silica gel chromatography.

hydroxy by applying a Friedel–Crafts reaction. We also wish to demonstrate the synthetic route of the novel pyridazinoquinolinone metabolite (**1e**).

2. Results and discussion

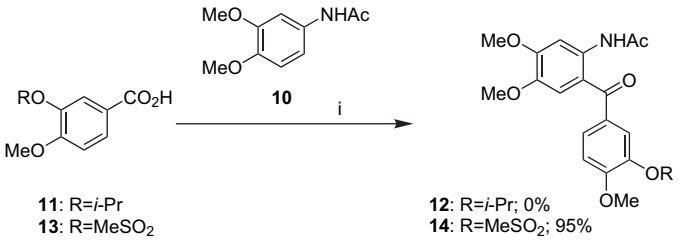
2.1. Alternative synthesis of ethyl 4-(3-hydroxy-4-methoxyphenyl)-6,7-dimethoxy-2-(1,2,4-triazol-1-ylmethyl)quinoline-3-carboxylate (1d)

In an early report,[1c] the synthetic process of **1d** involved several drawbacks from the standpoint of large scale preparation, for example, indole cyclization–cleavage–quinoline cyclization sequence, repeated tedious chromatographic methods, and low yield (overall 3%). To avoid these problems, we tried to adopt a synthetic process that uses a Friedel–Crafts reaction, which includes a basic problem where most of the protective groups of the phenolic hydroxy are vulnerable in the reaction conditions. Actually, using the isopropyl as a protective group of the phenolic hydroxy did not afford a Friedel–Crafts acylated compound (**12**) but gave a complex mixture as shown in Scheme 2.

Generally, an aryl methane- or toluenesulfonate ester is stable under the acidic conditions used for the nitration of an aromatic ring (HNO_3/AcOH),[3] and to the high temperatures (200–250 °C) of the Ullman reaction. Also, few reports[3] have shown whether an aryl methanesulfonate ester is stable under the condition of a Friedel–Crafts reaction. So we studied using methanesulfonyl as a protective group of the phenolic hydroxy of **1d**.

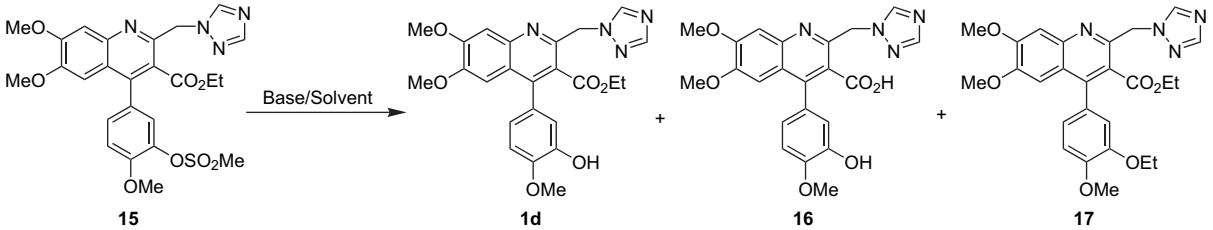
Thus, 3-mesyloxy-4-methoxybenzoic acid (**13**) reacted with *N*-(3,4-dimethoxyphenyl)acetamide (**10**) under Friedel–Crafts reaction conditions to give a benzophenone product (**14**) in 95% yield with high quality.

Initial trial using sodium hydroxide as a base reagent gave **1d** in moderate yield; however, a lot of carboxylic acid (**16**) was produced as a by-product as shown in Table 1, entries 1 and 2. Use of sodium carbonate or potassium carbonate produced a disappointing result (entries 3 and 4). Highly chemoselective deprotection of methanesulfonyl ester against ethyl ester was successfully performed using cesium carbonate in ethanol (entry 5), according to the reported method,[4] where the selective cleavage of acylphenol using



Scheme 2. Reagents and conditions: (i) $SnCl_4$, $POCl_3$, CH_2ClCH_2Cl, reflux.

Table 1. Deprotection of methanesulfonyl as a protective group of the phenolic hydroxy



15 **1d** **16** **17**

Entry	Base	Solvent	Ratio by HPLC[a]			
			15	**1d**	**16**	**17**
1	NaOH	EtOH–H_2O	0.1	67	24	9
2	NaOH	MeCN–H_2O	3	77	20	ND
3	Na_2CO_3	EtOH	91	5	ND	0.5
4	K_2CO_3	EtOH	26	47	ND	22
5	Cs_2CO_3	EtOH	ND[b]	88	ND	6
6	Cs_2CO_3	MeCN	88	6	ND	ND
7	Cs_2CO_3	THF	90	5	ND	ND
8	Cs_2CO_3	H_2O	97	0.3	ND	ND

[a] Determined at 254 nm.
[b] ND=not detected.

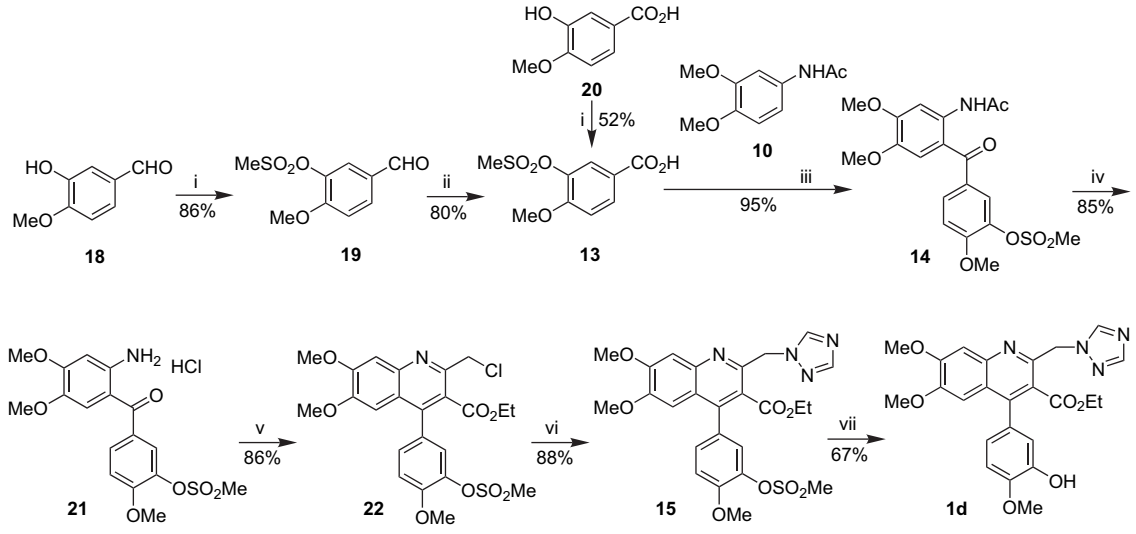
cesium carbonate without the cleavage of arylester was undertaken. Finally, under the identical reaction conditions according to entry 5 of Table 1, the ethoxybenzene by-product (**17**) was easily removed by precipitation after treatment with sodium hydroxide solution.

Compared with the early report,[1c] the present method, utilizing the synthetic method[5] of TAK-603 (**1**), for the synthesis of **1d** has a remarkable advantage in yield (3→32%) with only one chromatographic method as shown in Scheme 3.

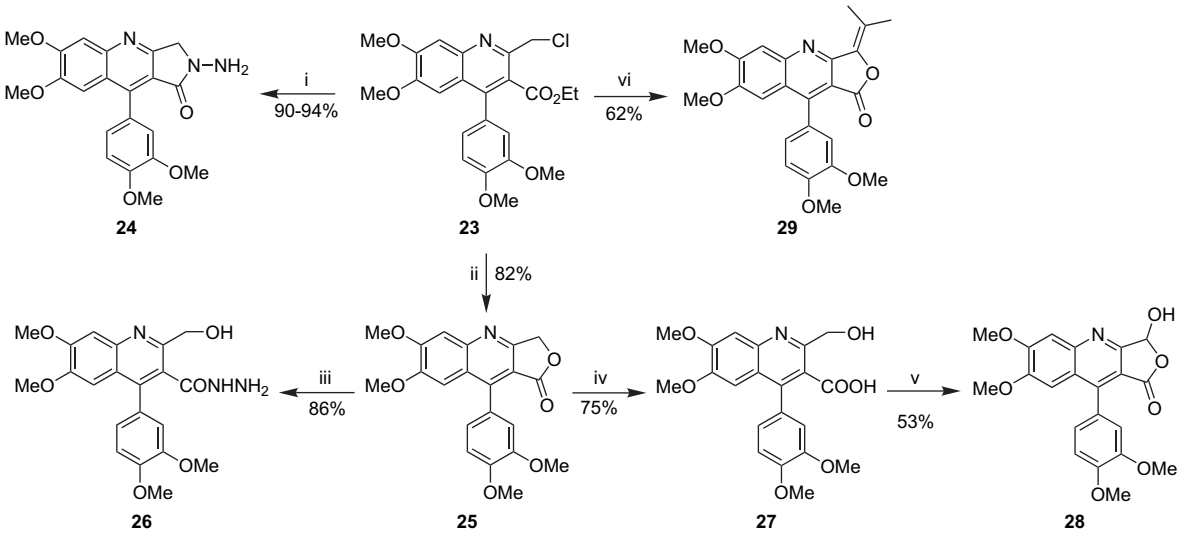
2.2. Novel synthetic route of 10-(3,4-dimethoxyphenyl)-7,8-dimethoxy-2*H*-pyridazino[4,5-*b*]quinolin-1-one (1e)

The intermediate (**23**)[5] of TAK-603 (**1**) seems to be a promising candidate for the synthesis of **1e**. At first, in order to obtain **1e**, we tried **23** to cyclize with hydrazine directly,[6] but only the lactam compound (**24**) was obtained, which cyclized as a five-member ring in 90–94% yield, as shown in Scheme 4.

This result indicated that six-member ring cyclization was more difficult than five-member ring cyclization. Using this five-member ring **24**, we studied ring expansion using silica gel,[7] but the reaction did not occur. Then, **23** was treated with dimethyl sulfoxide to obtain lactone compound (**25**), which was cyclized as a five-member ring in 82% yield. Treatment of **25** with hydrazine resulted in cleavage of the lactone ring and hydrazide compound (**26**) was obtained in 86% yield. The study of the oxidation (MnO_2, PDC, SO_3Py–DMSO) of **26** to formyl compound failed and **26** was cyclized again to obtain **25**.



Scheme 3. Reagents and conditions: (i) $MeSO_2Cl$, Et_3N, THF, 0 °C; (ii) CrO_3, AcOH; (iii) $SnCl_4$, $POCl_3$, CH_2ClCH_2Cl, reflux; (iv) concd HCl, EtOH, reflux; (v) ethyl 4-chloroacetoacetate, EtOH, reflux; (vi) 1*H*-1,2,4-triazole, K_2CO_3, DMF, then silica gel chromatography; (vii) Cs_2CO_3, EtOH, reflux.



Scheme 4. Reagents and conditions: (i) NH_2NH_2, EtOH, reflux; (ii) DMSO, reflux; (iii) NH_2NH_2, EtOH, reflux; (iv) NaOEt, EtOH, reflux; (v) MnO_2, DMF, 100 °C; (vi) 2-nitropropane, NaOEt, EtOH, DMSO, 50 °C.

Treatment of **25** with sodium ethoxide resulted in lactone ring cleavage to give carboxylic compound (**27**) in 75% yield. MnO_2-oxidation of **27** gave lactol (**28**) in 53% yield. The desired reaction of **28** with hydrazine, however, did not proceed.

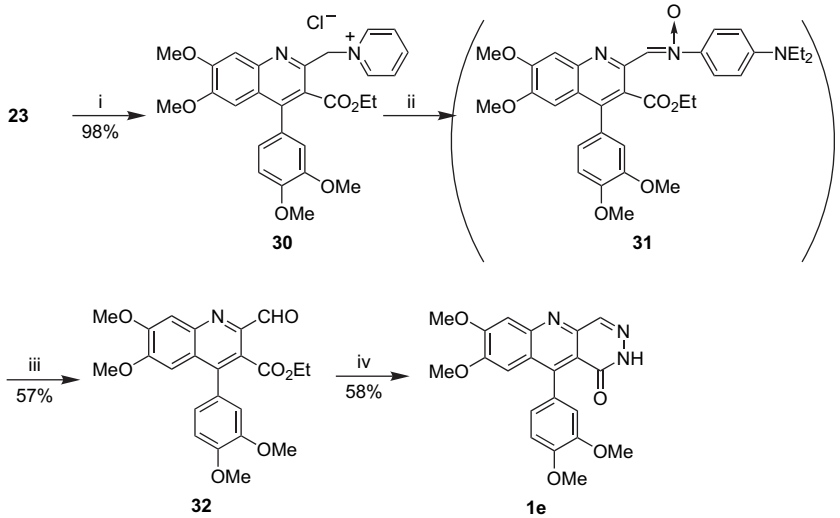
These unsuccessful results prompted us to change the synthetic strategy, that is, the stepwise route via a formyl derivative (**32**). Treatment of **23** with 2-nitropropane and sodium ethoxide [8] gave propylidene-lactone compound (**29**) in 62% yield. The application of Sommelet aldehyde synthesis (hexamethylenetetramine in acetic acid) [9] to **23** gave a complex mixture.

The failure of these formylations turned our attention on the application of a Kröhnke reaction. [10] The Kröhnke reaction was the conversion reaction from the chloromethylene moiety to the formyl moiety via a nitrone intermediate. At the first step, pyridinium salt (**30**) was obtained in 98% yield from the reaction of **23** and pyridine. Then nitrone compound (**31**) was derived from **30** by reaction with 4-nitroso-*N*,*N*-diethylaniline and sodium hydroxide in ethanol. After treatment with hydrochloric acid without isolation of **31**, formyl compound (**32**) was isolated in 57% yield as stable crystals for the first time. Finally, cyclization of **32** with hydrazine under ethanol reflux conditions gave the metabolite **1e** in 58% yield as shown in Scheme 5.

The analytical data of **1e** were in perfect agreement with the sample of human serum using liquid chromatography–mass spectrometry (LC–MS) and liquid chromatography–NMR (LC–NMR).

3. Conclusion

In summary, we have described convenient and efficient synthetic routes of ethyl 4-(3-hydroxy-4-methoxyphenyl)-6,7-dimethoxy-2-(1,2,4-triazol-1-ylmethyl)quinoline-3-carboxylate (**1d**) and 10-(3,4-dimethoxyphenyl)-7,8-dimethoxy-2*H*-pyridazino[4,5-*b*]quinolin-1-one (**1e**), metabolites of TAK-603 (**1**). In particular, using methanesulfonyl as a protective group of the phenolic hydroxy enabled Friedel–Crafts



Scheme 5. Reagents and conditions: (i) pyridine, 100 °C; (ii) 4-nitroso-*N*,*N*-diethylaniline, NaOH, EtOH–H_2O, 100 °C; (iii) HCl; (iv) NH_2NH_2, EtOH, reflux.

reaction, and a new simpler synthetic route of **1d** was established. Also, using Kröhnke reaction as a conversion reaction from chloromethyl derivative to formyl derivative enabled us to obtain a novel compound **1e**.

4. Experimental

4.1. General

Melting points were determined based on differential scanning calorimetry (DSC). Infrared spectra were recorded on a Horiba FT-210 spectrophotometer. NMR spectra were recorded on a Bruker DPX300 spectrometer. ^{1}H and ^{13}C chemical shifts were referenced to the internal deuterated solvent or tetramethylsilane. Column chromatography was performed with a Wakogel C-200 (75–150 μm) system. HPLC was performed on an YMC-Pack ODS-A302 column (150 mm×4.6 mm i.d.) with 20 mM aqueous KH_2PO_4 solution–MeCN (55:45) at 25 °C. Detection was effected with a Shimadzu SPD-6A UV spectrophotometric detector at 254 nm. Mass spectra and elemental analysis were analyzed by Takeda Analytical Research Laboratories, Ltd. All commercial chemicals and solvents used were of reagent grade and were used without further purification.

4.2. 3-Mesyloxy-4-methoxybenzaldehyde (19)

To a solution of isovanillin (**18**; 7.61 g, 50 mmol) in THF (76 mL) at 0 °C were added triethylamine (20.24 g, 200 mmol) and methanesulfonyl chloride (8.59 g, 75 mmol), and the mixture was stirred for 15 min. AcOEt (76 mL) was poured into the reaction mixture, and washed with 1 N HCl (150 mL, 75 mL), dried ($MgSO_4$), and concentrated in vacuo. To the residue was added IPE (50 mL), and the resulting precipitates were collected by filtration, washed with IPE (20 mL), and dried in vacuo to give **19** (9.92 g, 86.2%) as a white crystalline powder; t_R: 3.0 (**18**), 4.5 (**19**); mp 87.3 °C (lit.[11] 87–89 °C); IR (KBr): 1693.6, 1367.6, 1280.8, 1176.6, 1103.3, 821.7 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.24 (s, 3H), 4.00 (s, 3H), 7.15 (d, J=8.0 Hz, 1H), 7.82 (d, J=1.8 Hz, 1H), 7.85 (dd, J=8.2, 1.8 Hz, 1H), 9.89 (s, 1H); MS (FAB): m/z 231 $[M+H]^+$.

4.3. 3-Mesyloxy-4-methoxybenzoic acid (13; using 19)

To a solution of **19** (0.46 g, 2 mmol) in AcOH (2.3 mL) was added an aqueous solution (0.6 mL) of chromic acid (0.60 g, 6 mmol), and the mixture was stirred for 20 h. After the mixture was cooled, water (4.6 mL) was added and the resulting precipitates were collected by filtration, washed with water (4.6 mL×2) and EtOH (4.6 mL), and dried in vacuo to give **13** (0.44 g, 89.8%) as a white crystalline powder; t_R: 4.5 (**19**), 2.4 (**13**); mp 232.0 °C (lit.[11] 226–227 °C); IR (KBr): 1674.3, 1363.8, 1288.5, 1167.0, 825.6 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.34 (s, 3H), 3.95 (s, 3H), 7.29 (d, J=8.8 Hz, 1H), 7.80 (d, J=2.2 Hz, 1H), 7.95 (dd, J=8.8, 2.2 Hz, 1H); MS (FAB): m/z 247 $[M+H]^+$.

4.4. 3-Mesyloxy-4-methoxybenzoic acid (13; using 3-hydroxy-4-methoxybenzoic acid 20)

To a suspension of **20** (8.41 g, 50 mmol) in THF (200 mL) at 0 °C were added methanesulfonyl chloride (11.46 g, 100 mmol) and triethylamine (20.24 g, 200 mmol), and the mixture was stirred for 15 min. AcOEt (200 mL) was poured into the reaction mixture, and washed with 1 N HCl (200 mL×2), dried ($MgSO_4$), and concentrated in vacuo. To the residue was added AcOEt–IPE (1:2, 100 mL), and the resulting precipitates were collected by filtration, washed with AcOEt–IPE (1:2, 50 mL), and dried in vacuo to give **13** (6.40 g, 52.0%) as a white crystalline powder.

4.5. 2-Acetoamino-3′-mesyloxy-4,4′,5-trimethoxybenzophenone (14)

To a suspension of *N*-acetyl-3,4-dimethoxyaniline (**10**; 3.90 g, 20 mmol) and **13** in 1,2-dichloroethane (54 mL) were added phosphoryl chloride (10.25 mL) and tin chloride (4.63 mL), and the mixture was refluxed for 7 h. After cooling, dichloromethane (100 mL) was poured into the reaction mixture, and washed with water (100 mL) and 1 N NaOH (100 mL), dried ($MgSO_4$), and concentrated in vacuo. To the residue was added AcOEt–IPE (1:9, 50 mL), and the resulting precipitates were collected by filtration, washed with AcOEt–IPE (1:9, 50 mL×2), and dried in vacuo to give **14** (8.01 g, 94.5%) as a pale yellow crystalline powder; t_R: 2.4 (**13**), 5.1 (**10**), 5.5 (**14**); mp 179.2 °C; IR (KBr): 1685.9, 1604.9, 1514.2, 1367.6, 1277.0, 1128.4 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.23 (s, 3H), 3.26 (s, 3H), 3.79 (s, 3H), 4.00 (s, 6H), 7.06 (s, 1H), 7.12 (d, J=8.4 Hz, 1H), 7.68 (d, J=2.2 Hz, 1H), 7.74 (dd, J=8.4, 2.2 Hz, 1H), 8.41 (s, 1H), 11.12 (s, 1H); MS (FAB): m/z 424 $[M+H]^+$; Anal. Calcd for $C_{19}H_{21}NO_8S\cdot 0.5H_2O$: C, 52.77; H, 5.13; N, 3.24. Found: C, 52.70; H, 4.96; N, 3.44.

4.6. 2-Amino-3′-mesyloxy-4,4′,5-trimethoxybenzophenone hydrochloride (21)

To a suspension of **14** (5.08 g, 12 mmol) in EtOH (51 mL) was added concd HCl (10.2 mL), and the mixture was refluxed for 2 h. After stirring at 0 °C for 30 min, the resulting precipitates were collected by filtration, washed with EtOH (15 mL), and dried in vacuo to give **21** (4.25 g, 84.8%) as a pale yellow crystalline powder; t_R: 5.5 (**14**), 6.4 (**21**); mp 158.9 °C; IR (KBr): 2841.4, 1514.2, 1359.9, 1280.8, 1222.9, 1120.7 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.23 (s, 3H), 3.72 (s, 3H), 3.91 (s, 3H), 3.97 (s, 3H), 5.11 (br s, 2H), 6.21 (s, 1H), 6.96 (s, 1H), 7.09 (d, J=8.4 Hz, 1H), 7.62 (d, J=2.2 Hz, 1H), 7.68 (dd, J=8.4, 2.2 Hz, 1H); MS (FAB): m/z 382 $[M+H]^+$; Anal. Calcd for $C_{17}H_{20}ClNO_7S\cdot 0.6H_2O$: C, 47.63; H, 4.98; N, 3.27. Found: C, 47.59; H, 4.80; N, 3.23.

4.7. Ethyl 2-chloromethyl-4-(3-mesyloxy-4-methoxyphenyl)-6,7-dimethoxyquinoline-3-carboxylate (22)

To a suspension of **21** (4.17 g, 10 mmol) in EtOH (42 mL) was added ethyl chloroacetoacetate (2.14 g, 13 mmol), and the mixture was refluxed for 2 h. Water (14 mL) and triethylamine (1.06 g, 10.5 mmol) were added and the resulting mixture was stirred for 30 min at 0 °C. The resulting precipitates were collected by filtration, washed with water–EtOH (1:3, 25 mL×2), EtOH (10 mL), and dried in vacuo to give **22** (4.38 g, 85.9%) as a pale yellow crystalline powder; t_R: 6.4 (**21**), 24.6 (**22**); mp 168.4 °C; IR (KBr): 1716.8, 1504.6, 1367.6, 1251.9, 1230.7 cm^{-1}; ^{1}H NMR (300 MHz,

$CDCl_3$) δ: 1.05 (t, J=7.4 Hz, 3H), 3.28 (s, 3H), 3.84 (s, 3H), 4.00 (s, 3H), 4.06 (s, 3H), 4.12 (q, J=7.2 Hz, 2H), 4.89 (d, J=11.4 Hz, 1H), 5.05 (d, J=11.4 Hz, 1H), 6.91 (s, 1H), 7.14 (d, J=8.4 Hz, 1H), 7.28 (dd, J=8.4, 2.2 Hz, 1H), 7.40 (d, J=2.2 Hz, 1H), 7.46 (s, 1H); MS (FAB): m/z 510 $[M+H]^+$; Anal. Calcd for $C_{23}H_{24}ClNO_8S$: C, 54.17; H, 4.74; N, 2.75. Found: C, 54.09; H, 4.66; N, 2.70.

4.8. Ethyl 4-(3-mesyloxy-4-methoxyphenyl)-6,7-dimethoxy-2-(1,2,4-triazol-1-ylmethyl)quinoline-3-carboxylate (15)

To a solution of **22** (4.08 g, 8 mmol) in DMF (41 mL) were added 1*H*-1,2,4-triazole (0.61 g, 8.8 mmol) and potassium carbonate (3.32 g, 24 mmol), and the mixture was stirred for 4 h. After cooling, water (80 mL) and EtOH (40 mL) were added and the resulting precipitates were collected by filtration, washed with EtOH (15 mL×2), and dried in vacuo to give crude **15** (3.42 g, 78.8%) as a pale yellow crystalline powder. The mother solution was extracted with AcOEt (80 mL), and the organic extract was washed with water (80 mL) and saturated NaCl solution (80 mL), dried ($MgSO_4$), and concentrated in vacuo. To the residue was added EtOH (40 mL), and the resulting precipitates were collected by filtration, washed with EtOH (15 mL×2), and dried in vacuo to give recovered **15** (0.58 g, 13.4%) as a pale yellow crystalline powder. Both crude **15** and recovered **15** were purified together by chromatography on silica gel to give **15** (3.83 g, 88.2%) as a pale yellow crystalline powder; t_R: 24.6 (**22**), 7.1 (**15**); mp 171.9 °C; IR (KBr): 1714.8, 1504.6, 1431.3, 1367.6, 1230.7, 1209.4, 1174.7 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 0.91 (t, J=7.0 Hz, 3H), 3.27 (s, 3H), 3.83 (s, 3H), 3.99 (s, 3H), 4.00 (q, J=7.0 Hz, 2H), 4.05 (s, 3H), 5.69 (d, J=14.8 Hz, 1H), 5.80 (d, J=14.8 Hz, 1H), 6.89 (s, 1H), 7.12 (d, J=8.4 Hz, 1H), 7.25 (dd, J=8.4, 2.2 Hz, 1H), 7.37 (d, J=1.8 Hz, 1H), 7.41 (s, 1H), 7.94 (s, 1H), 8.28 (s, 1H); MS (FAB): m/z 542 $[M+H]^+$; Anal. Calcd. for $C_{25}H_{26}N_4O_8S\cdot 0.3H_2O$: C, 54.80; H, 4.89; N, 10.22. Found: C, 54.76; H, 4.90; N, 9.94.

4.9. Ethyl 4-(3-hydroxy-4-methoxyphenyl)-6,7-dimethoxy-2-(1,2,4-triazol-1-ylmethyl)quinoline-3-carboxylate (1d)

To a suspension of **15** (25.0 g, 46.1 mmol) in EtOH (2.5 L) was added cesium carbonate (60.1 g, 184 mmol), and the mixture was refluxed for 30 min. After cooling and concentration, 1 N NaOH (700 mL) was poured into the residue at 0 °C and the resulting precipitates were removed by filtration. The mother solution was extracted with AcOEt (700 mL), and the aqueous solution was adjusted to pH 7 with 1 N HCl (180 mL) and stirred at 0 °C for 30 min. The resulting precipitates were collected by filtration, washed with water (200 mL) and EtOH (50 mL), and the resulting crude powder was recrystallized from 95% EtOH (1.5 L) to give **1d** (14.3 g, 67.0%) as a white crystalline powder; t_R: 7.1 (**15**), 4.5 (**1d**); mp 233.6 °C; IR (KBr): 2978.4, 1714.8, 1504.6, 1429.3, 1211.4 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 0.89 (t, J=7.2 Hz, 3H), 3.80 (s, 3H), 3.96 (s, 3H), 3.98 (q, J=7.2 Hz, 2H), 4.04 (s, 3H), 5.73 (s, 2H), 6.37 (s, 1H), 6.80 (d, J=8.2 Hz, 1H), 6.98–6.93 (3H, m), 7.40 (s, 1H), 7.94 (s, 1H), 8.30 (s, 1H); MS (FAB): m/z 465 $[M+H]^+$; Anal. Calcd for $C_{24}H_{24}N_4O_6$: C, 62.06; H, 5.21; N, 12.06, Found: C, 62.08; H, 5.23; N, 12.05.

4.10. 2-Amino-9-(3,4-dimethoxyphenyl)-6,7-dimethoxyazolidino[3,4-*b*]quinolin-1-one (24)

To a suspension of ethyl 2-chloromethyl-4-(3,4-dimethoxyphenyl)-6,7-dimethoxyquinoline-3-carboxylate (**23**; 0.89 g, 2 mmol) in EtOH (10 mL) was added hydrazine hydrate (0.50 g, 10 mmol), and the mixture was refluxed for 2.5 h. After cooling, the resulting precipitates were collected by filtration, washed with EtOH (5 mL), and dried in vacuo to give **24** (0.74 g, 93.7%) as a pale yellow crystalline powder; t_R: 20.0 (**23**), 3.0 (**24**); mp 265.1 °C; IR (KBr): 1695.6, 1502.7, 1249.9, 1232.6 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 3.83 (s, 3H), 3.90 (s, 3H), 4.00 (s, 3H), 4.08 (s, 3H), 4.37 (s, 2H), 4.66 (s, 2H), 7.00 (s, 1H), 7.06 (s, 2H), 7.16 (s, 1H), 7.48 (s, 1H); MS (FAB): m/z 396 $[M+H]^+$; Anal. Calcd for $C_{21}H_{21}N_3O_5$: C, 63.79; H, 5.35; N, 10.63. Found: C, 63.51; H, 5.40; N, 10.40.

4.11. 9-(3,4-Dimethoxyphenyl)-6,7-dimethoxyoxolano-[3,4-*b*]quinolin-1-one (25)

A solution of **23** (0.89 g, 2 mmol) in DMSO (10 mL) was stirred for 5.5 h at 120 °C. After cooling, water (20 mL) was added and the resulting precipitates were collected by filtration, washed with water (10 mL) and EtOH (5 mL), and dried in vacuo to give **25** (0.62 g, 81.6%) as a yellow crystalline powder; t_R: 20.0 (**23**), 5.7 (**25**); mp 238.2 °C (lit.[1d] 241–242 °C); IR (KBr): 1759.2, 1518.1, 1502.7, 1483.4, 1251.9 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 3.85 (s, 3H), 3.91 (s, 3H), 4.01 (s, 3H), 4.10 (s, 3H), 5.38 (s, 2H), 7.02 (s, 1H), 7.07 (s, 2H), 7.23 (s, 1H), 7.49 (s, 1H); MS (FAB): m/z 382 $[M+H]^+$.

4.12. 2-Hydroxymethyl-4-(3,4-dimethoxyphenyl)-6,7-dimethoxyquinoline-3-carbohydrazide (26)

To a suspension of **25** (0.19 g, 0.5 mmol) in EtOH (4 mL) was added hydrazine hydrate (0.13 g, 2.5 mmol), and the mixture was refluxed for 11 h. After cooling, AcOEt (4 mL) was added and the resulting precipitates were collected by filtration, washed with AcOEt (2 mL×2), and dried in vacuo to give **26** (0.18 g, 85.7%) as an ivory crystalline powder; t_R: 5.7 (**25**), 2.2 (**26**); mp 240.9 °C; IR (KBr): 3287.0, 1657.0, 1518.1, 1504.6, 1248.0 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ: 3.70 (s, 3H), 3.77 (s, 3H), 3.85 (s, 3H), 3.97 (s, 3H), 4.21 (br s, 2H), 4.68 (d, J=5.6 Hz, 2H), 5.02 (t, J=5.2 Hz, 1H), 6.90 (s, 1H), 6.92 (d, J=8.4 Hz, 1H), 6.99 (s, 1H), 7.05 (d, J=8.4 Hz, 1H), 7.47 (s, 1H), 9.29 (s, 1H); MS (FAB): m/z 414 $[M+H]^+$; Anal. Calcd for $C_{21}H_{23}N_3O_6\cdot 0.3H_2O$: C, 60.22; H, 5.68; N, 10.03. Found: C, 60.13; H, 5.55; N, 10.00.

4.13. 2-Hydroxymethyl-4-(3,4-dimethoxyphenyl)-6,7-dimethoxyquinoline-3-carboxylic acid (27)

To a suspension of **25** (0.19 g, 0.5 mmol) in EtOH (4 mL) was added sodium ethoxide (0.14 g, 2 mmol), and the mixture was refluxed for 1 h. After cooling, 1 N HCl (3 mL) was added and the resulting precipitates were collected by filtration, washed with EtOH (4 mL), water (4 mL), and

again with EtOH (4 mL), and dried in vacuo to give **27** (0.15 g, 75.0%) as an orange crystalline powder; t_R: 5.7 (**25**), 1.7 (**27**); mp 239.3 °C; IR (KBr): 3343.0, 2548.2, 1724.5, 1500.7, 1265.4 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ: 3.76 (s, 3H), 3.79 (s, 3H), 3.89 (s, 3H), 4.02 (s, 3H), 4.92 (s, 2H), 6.96–7.03 (m, 3H), 7.14 (d, J=8.8 Hz, 1H), 7.79 (s, 1H); MS (FAB): m/z 400 $[M+H]^+$; HRMS Calcd: 400.1396. Found: 400.1425.

4.14. 3-Hydroxy-9-(3,4-dimethoxyphenyl)-6,7-dimethoxyoxolano[3,4-*b*]quinolin-1-one (28)

To a suspension of **27** (0.40 g, 1 mmol) in DMF (4 mL) was added manganese(IV) oxide (0.43 g, 5 mmol), and the mixture was stirred for 1 h at 100 °C. After cooling, the resulting precipitates were removed by filtration. To the mother solution was added water, and the resulting precipitates were collected by filtration, washed with water (8 mL) and EtOH (4 mL×2), and dried in vacuo to give **28** (0.21 g, 52.5%) as a pale brown crystalline powder; t_R: 1.7 (**27**), 3.7 (**28**); mp 249.9 °C; IR (KBr): 1761.1, 1506.5, 1259.6 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 1.25 (s, 1H), 2.92 (d, J=14.4 Hz, 1H), 3.86 (s, 3H), 3.91 (s, 3H), 4.01 (s, 3H), 4.17 (s, 3H), 6.89 (s, 1H), 6.99 (s, 1H), 7.07 (s, 1H), 7.21 (s, 1H), 7.81 (s, 1H); MS (FAB): m/z 398 $[M+H]^+$; HRMS Calcd: 398.1240. Found: 398.1264.

4.15. 3-Isopropylidene-9-(3,4-dimethoxyphenyl)-6,7-dimethoxyoxolano[3,4-*b*]quinolin-1-one (29)

To a solution of **23** (0.89 g, 2 mmol) in DMSO (13 mL) were added dropwise 2-nitropropane (0.71 g, 8 mmol) and EtOH solution (7 mL) of sodium ethoxide (0.31 g, 4.5 mmol) over 20 min, and the mixture was stirred for 5 h at 50 °C. After cooling, water (20 mL) was added and the resulting precipitates were collected by filtration, washed with EtOH (10 mL), and dried in vacuo to give **29** (0.52 g, 61.9%) as a yellow crystalline powder; t_R: 20.0 (**23**), 54.2 (**29**); mp 274.2 °C; IR (KBr): 1766.9, 1506.5, 1261.5 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 2.13 (s, 3H), 2.64 (s, 3H), 3.84 (s, 3H), 3.91 (s, 3H), 4.00 (s, 3H), 4.11 (s, 3H), 7.01 (s, 1H), 7.06 (s, 1H), 7.07 (s, 1H), 7.16 (s, 1H), 7.50 (s, 1H); MS (FAB): m/z 422 $[M+H]^+$; Anal. Calcd for $C_{24}H_{23}NO_6 \cdot 0.1H_2O$: C, 68.11; H, 5.52; N, 3.31. Found: C, 67.92; H, 5.52; N, 3.07.

4.16. 1-(3-Ethoxycarbonyl-4-(3,4-dimethoxyphenyl)-6,7-dimethoxyquinoline-2-yl)methylpyridinium chloride (30)

A suspension of **23** (8.92 g, 20 mmol) and pyridine (27 mL) was stirred for 1 h at 100 °C. After cooling, AcOEt (27 mL) was added and the resulting precipitates were collected by filtration, washed with AcOEt (27 mL×2), and dried in vacuo to give **30** (10.31 g, 98.2%) as a white crystalline powder; t_R: 20.0 (**23**), 5.1 (**30**); mp 230.4 °C; IR (KBr): 3406.6, 1712.9, 1502.7, 1255.7, 1232.6 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 0.95 (t, J=7.4 Hz, 3H), 3.79 (s, 3H), 3.87 (s, 3H), 3.98 (s, 3H), 4.02 (s, 3H), 4.15 (q, J=7.4 Hz, 2H), 6.49 (d, J=3.0 Hz, 1H), 6.86 (s, 1H), 6.88 (d, J=8.4 Hz, 1H), 7.00 (d, J=8.4 Hz, 1H), 7.21 (s, 1H), 8.21 (t, J=7.6 Hz, 2H), 8.60 (t, J=7.6 Hz, 1H), 9.55 (d, J=5.6 Hz, 2H); MS (EI): m/z 489 $[M-Cl]^+$; Anal. Calcd for $C_{28}H_{29}ClN_2O_6 \cdot 0.7H_2O$: C, 62.56; H, 5.70; N, 5.21. Found: C, 62.50; H, 5.65; N, 5.13.

4.17. Ethyl 2-formyl-4-(3,4-dimethoxyphenyl)-6,7-dimethoxyquinoline-3-carboxylate (32)

To a suspension of **30** (8.92 g, 17 mmol) in EtOH (45 mL) were added 4-nitroso-*N*,*N*-diethylaniline (3.49 g, 19.6 mol) and 1 N NaOH (21.3 mL), and the mixture was stirred for 17 h. Water (45 mL) was added and the resulting mixture was stirred for 2 h at 100 °C. After cooling, AcOEt (180 mL) was poured into the reaction mixture, and the organic layer was washed with water (45 mL), 1 N HCl (45 mL), and again with water (45 mL), and concentrated in vacuo. To the residue was added EtOH (30 mL), and the resulting precipitates were collected by filtration, washed with EtOH (30 mL×2) and dried in vacuo to give **32** (4.14 g, 57.3%) as a orange crystalline powder; t_R: 5.1 (**30**), 14.3 (**32**); mp 176.4 °C; IR (KBr): 1732.2, 1709.1, 1504.6, 1471.8, 1429.3, 1259.6 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 1.15 (t, J=7.2 Hz, 3H), 3.84 (s, 3H), 3.88 (s, 3H), 3.98 (s, 3H), 4.10 (s, 3H), 4.22 (dq, J=7.2 Hz, 1.8H, 2H), 6.9–7.0 (m, 4H), 7.58 (s, 1H), 10.17 (s, 1H); MS (FAB): m/z 426 $[M+H]^+$; Anal. Calcd for $C_{23}H_{23}NO_7$: C, 64.93; H, 5.45; N, 2.75. Found: C, 64.70; H, 5.45; N, 2.70.

4.18. 10-(3,4-Dimethoxyphenyl)-7,8-dimethoxy-2*H*-pyridazino[4,5-*b*]quinolin-1-one (1e)

To a suspension of **32** (2.98 g, 7 mmol) in EtOH (30 mL) was added hydrazine hydrate (1.75 g, 35 mmol), and the mixture was refluxed for 24 h. After cooling, the resulting precipitates were collected by filtration and washed with EtOH (20 mL×2). The resulting crystalline powder was dissolved in hot $CHCl_3$ (150 mL). After cooling, silica gel (12 g) was added to the resulting solution, and stirred for 30 min. The silica gel was filtered off and washed with $CHCl_3$–AcOEt (1:1; 400 mL) and the mother solution was concentrated in vacuo. To the residue was added AcOEt (30 mL), and the resulting precipitates were collected by filtration and washed with AcOEt (15 mL). To the resulting crystalline powder in $CHCl_3$ (150 mL) was added activated charcoal (0.15 g), and the resulting suspension was stirred for 30 min. The charcoal was filtered off and the mother solution was concentrated in vacuo. To the residue was added EtOH (50 mL), and the resulting precipitates were collected by filtration, washed with AcOEt (25 mL×2) and dried in vacuo to give **1e** (1.59 g, 57.8%) as a pale yellow crystalline powder; t_R: 14.3 (**32**), 3.8 (**1e**); mp 289.3 °C; IR (KBr): 3071.0, 2939.8, 1674.3, 1496.9, 1255.7 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 3.80 (s, 3H), 3.87 (s, 3H), 4.00 (s, 3H), 4.12 (s, 3H), 6.83 (d, J=1.8 Hz, 1H), 6.87 (dd, J=8.2, 1.8 Hz, 1H), 6.90 (s, 1H), 7.06 (d, J=8.2 Hz, 1H), 7.55 (s, 1H), 8.41 (s, 1H), 9.64 (s, 1H); MS (FAB): m/z 394 $[M+H]^+$; Anal. Calcd for $C_{21}H_{19}N_3O_5$: C, 64.12; H, 4.87; N, 10.68. Found: C, 64.11; H, 4.96; N, 10.56.

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Alkyl phosphines promoted reductive coupling of acyl cyanides: formation of *O*-acyl cyanohydrins

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Abstract—The reductive coupling of acyl cyanides promoted by alkyl phosphines has been discovered. Under mild reaction conditions, the substituted cyanohydrins were obtained in moderate to high yields by using trimethylphosphine or tributylphosphine as a promoter. The possible mechanism involved in the reaction was discussed on the basis of deuterium labeling and control experiments, indicating that one hydride transfer took place from alkyl phosphine to *O*-acyl cyanohydrin.

1. Introduction

Cyanohydrins are of synthetic interest as they can be transformed into a number of relevant functional groups such as β-amino alcohols, α-hydroxyacids and α-hydroxyesters, α-sulfonyloxynitriles, α-aminonitriles, α-fluoronitriles, 3-amino-2-alkenoates, and substituted azacycloalkenes.[1] The synthesis of cyanohydrins and their *O*-protected derivatives can be accomplished by enzymes,[2] organocatalysts,[3] and metal complexes.[4–7] Among various cyanide ion sources, trimethylsilyl cyanide,[8] methyl cyanoformate,[9] and acyl cyanides[10] are safer and commercially available reagents. With these cyanide ion sources, reactions between acyl cyanides and aldehydes are very efficient synthetic methods to give cyanohydrin derivatives in good yields.[11] While reductive coupling of acyl cyanides themselves was attained through Zn/HCl or hydrogenation in the presence of a Ni catalyst,[12] titanous chloride,[13] photoreduction,[14] and electrochemical reduction.[15] To the best of our knowledge, reductive coupling of acyl cyanides using organocatalysts has not been studied thoroughly.

More recently, organocatalysts, metal-free organic compounds, which exhibit catalytic abilities in organic reactions, have received much attention because of their advantages from an environmental as well as a resource standpoint.[16] Phosphines are widely used as Lewis bases or ligands in organic chemistry.[17] Some selected examples are their applications in the Baylis–Hillman reaction.[18] Another significant application of phosphines in organic reactions is the reduction of azides to amines in the Staudinger reaction.[19] Recently, we reported the reduction of activated carbonyl groups in α-keto esters, benzils, 1,2-cyclohexanedione, and α-ketophosphonates to the corresponding hydroxyl compounds by alkyl phosphines.[20] Herein, we report another application of alkyl phosphines in the reductive coupling of various acyl cyanides to produce *O*-acyl cyanohydrins in good yields under mild conditions, which is an effective novel reducing agent for converting carbonyl group into hydroxyl group containing compounds.

2. Results and discussion

Initially, we examine the phosphine Lewis base effects for the reduction of benzoyl cyanide. We found that the reaction of benzoyl cyanide **1a** with 1.0 equiv of trimethylphosphine (PMe_3) in THF (a THF solution) at room temperature for 6 h afforded the corresponding cyanohydrin **2a**, a reductive coupling product, in 80% yield (Table 1, entry 1). The results are summarized in Table 1. Additionally, when tributylphosphine (PBu_3) was used as a promoter, the isolated yield of **2a** was 51% under identical conditions (Table 1, entry 2). In the case of using diphenylmethylphosphine (PPh_2Me) or dimethylphenylphosphine ($PPhMe_2$) as a promoter, complicated products were obtained on the basis of TLC analysis (Table 1, entries 3 and 4). Triphenylphosphine (PPh_3), tricyclohexylphosphine (PCy_3), ethyl phosphite [$P(OEt)_3$], and various amines such as DBU, DMAP, DABCO, and pyridine have no activities in the reductive coupling of benzoyl cyanide (Table 1, entries 5–11).

Keywords: Acyl cyanide; Alkyl phosphine; Cyanohydrins; Hydride transfer; Deuterium labeling experiment.
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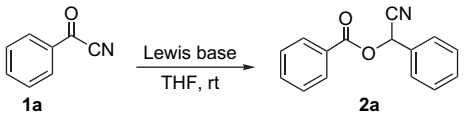
Table 1. Reductive coupling of benzoyl cyanide using various Lewis base promoters[a]



1a **2a**

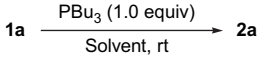
Entry	Lewis base	Time/h	**2a**[b]/%
1	PMe_3	6	80
2	P^nBu_3	6	51
3	PPh_2Me	12	—[c]
4	$PPhMe_2$	12	—[c]
5	PPh_3	24	NR[d]
6	PCy_3	24	NR[d]
7	$P(OEt)_3$	24	NR[d]
8	DBU	24	NR[d]
9	DMAP	24	NR[d]
10	DABCO	24	NR[d]
11	Pyridine	24	NR[d]

[a] All reactions were carried out using benzoyl cyanide **1a** (0.5 mmol) and Lewis base (0.5 mmol) in THF (0.5 mL, 1.0 M) at room temperature under argon atmosphere.
[b] Isolated yields.
[c] Complicated reactions took place.
[d] No reaction took place. DBU: 1,8-diazabicyclo[5.4.0]undec-7-ene, DMAP: 4-dimethylaminopyridine, DABCO: 1,4-diazabicyclo[2.2.2]-octane, THF: tetrahydrofuran.

Next, to examine the solvent effect of this reductive coupling reaction, we carried out the reaction of **1a** with PBu_3 in various solvents (Table 2). Using toluene or dichloromethane as solvent, **2a** was obtained in 78 and 72% yields, respectively (Table 2, entries 2 and 4). On the other hand, using THF or MeCN as solvent, the achieved yields were slightly lower (Table 2, entries 1 and 3). In MeOH, no reaction occurred (Table 2, entry 5). Therefore, the optimized condition is using PMe_3 as a reductive coupling reagent in THF at room temperature for 6 h.

A variety of acyl cyanides were tested under these optimized conditions (Table 3). For a variety of aromatic acyl cyanides, the corresponding reductive coupling products **2** were obtained in good yields. Using sterically encumbered acyl cyanide **1d** as a substrate, the corresponding reductive coupling product **2d** was obtained in slightly lower yield (52%) compared with the *para*- or *meta*-chloro substituted substrates **1b** and **1c** (Table 3, entries 2–4). With respect to the electron-neutral and electron-rich acyl cyanides, the reactions proceeded smoothly to provide the corresponding cyanohydrin products **2** in good to high yields (Table 3, entries 5–7). The reductive coupling reaction of furan-2-carbonyl cyanide **1h** also proceeded smoothly under the standard conditions to give the corresponding product **2h** in 74% yield (Table 3, entry 8).

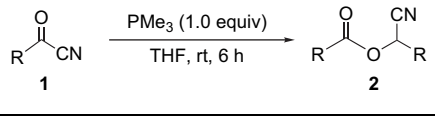
Table 2. Reductive coupling of benzoyl cyanide using tributylphosphine in various solvents[a]



Entry	Solvent	Time/h	**2a**[b]/%
1	THF	6	51
2	PhMe	6	78
3	MeCN	6	50
4	CH_2Cl_2	6	72
5	MeOH	24	NR[c]

[a] All reactions were carried out using benzoyl cyanide **1a** (0.5 mmol) and PBu_3 (0.5 mmol) in solvent (0.5 mL, 1.0 M) at room temperature under argon.
[b] Isolated yields.
[c] No reaction took place.

Table 3. Reductive coupling of acyl cyanides in the presence of trimethylphosphine[a]

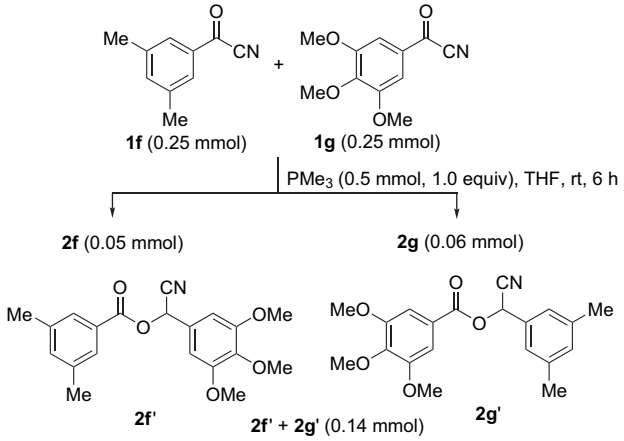


1 **2**

Entry	R	**2**, %[b]
1	C_6H_5 (**1a**)	**2a**, 80
2	4-ClC_6H_4 (**1b**)	**2b**, 89
3	3-ClC_6H_4 (**1c**)	**2c**, 78
4	2-ClC_6H_4 (**1d**)	**2d**, 52
5	4-MeC_6H_4 (**1e**)	**2e**, 78
6	3,5-$Me_2C_6H_3$ (**1f**)	**2f**, 91
7	3,4,5-$(MeO)_3C_6H_2$ (**1g**)	**2g**, 74
8	2-Furan (**1h**)	**2h**, 74

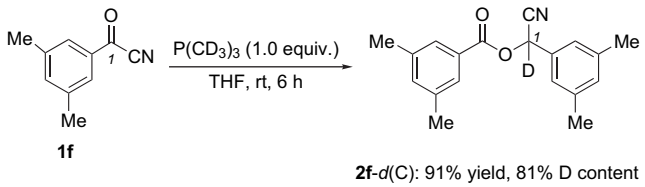
[a] All reactions were carried out using acyl cyanides (0.5 mmol) and PMe_3 (0.5 mL, 0.5 mmol, 1.0 M in THF) at room temperature under argon atmosphere for 6 h.
[b] Isolated yields.

The reaction mechanism is the most interesting issue in this context. In order to clarify the reaction mechanism, an intercrossing experiment between substrates **1f** and **1g** was carried out in the presence of PMe_3 under identical conditions (Scheme 1). As can be seen from Scheme 1, four reductive coupling products were formed in this reaction as isolated products **2f** (0.05 mmol) and **2g** (0.06 mmol) as well as a mixture of **2f′/2g′** (0.14 mmol). This result indicated that the coupling reaction proceeded through an intermolecular reductive condensation of **1**.



Scheme 1. Intercrossing experiment between **1f** and **1g** in the presence of trimethylphosphine.

In order to identify the hydride source in these reductive coupling reactions, the reductive coupling of **1f** with trimethylphosphine-d_9, prepared from the reaction of CD_3MgI with tri-*o*-tolyl phosphite,[21] was carried out under the standard conditions and the product of **2f**-*d*(C) was produced in 91% isolated yield with 81% D incorporation at the C_1 position (Scheme 2).[22]

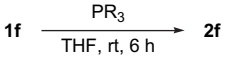


Scheme 2. Isotopic labeling experiment.

The deuterium labeling experiment indicated that only one D was transferred from phosphine to the product. This result suggests that only 0.5 equiv of PMe_3 is necessary in this reaction. Thus, we carried out this reaction using 0.5 equiv of PMe_3 under the standard conditions. As a result, the yield of **2f** decreased to 47%, indicating that 1.0 equiv of phosphine is essential in the reaction (Table 4, entry 1). Next, this reaction was carried out using 0.5 equiv of PMe_3 and 0.5 equiv of PPh_3 under otherwise identical conditions. We found that the yield of **2f** decreased to 41% (Table 4, entry 2). While using 0.5 equiv of PMe_3 and 0.5 equiv of $P(OBu)_3$ or PBu_3 or non-nucleophilic $^iPr_2NEt$, **2f** was obtained in 75, 85, and 65% yields, respectively (Table 4, entries 3–5). These results suggested that 50 mol % of phosphine such as PMe_3 or PBu_3 acted as a reductant and other 50 mol % of phosphine such as PMe_3, PBu_3 and $P(OBu)_3$ (phosphite), or $^iPr_2NEt$ acted as a promoter to quench the generated CN^- in the reaction process since $P(OBu)_3$ itself and amine cannot promote this reaction. Triphenylphosphine did not indicate the promoter effect in combination with trimethylphosphine presumably due to its steric bulkiness (Table 4, entry 2). In addition, by adding 30 mol % of 2,6-di(*tert*-butyl)-4-methylphenol (BHT) as a radical inhibitor in this reaction, the yield of **2f** was unaffected (87%), rendering unlikely the intervention of a radical pathway (Table 4, entry 6 and Table 3, entry 6).

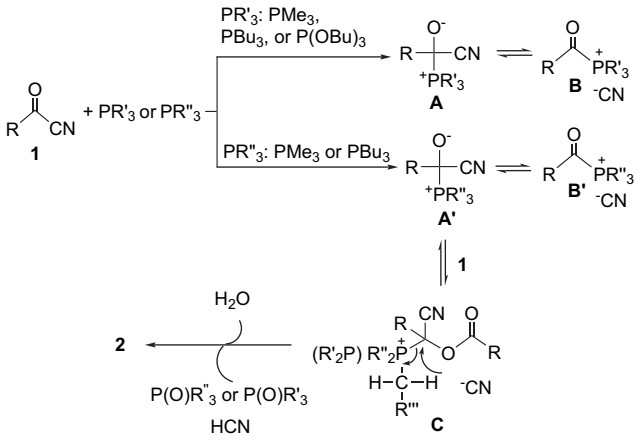
According to the relative control experiments, a plausible mechanism for the reductive coupling of acyl cyanides is proposed in Scheme 3. Initially, acyl cyanide **1** is probably activated by phosphine or phosphite to form intermediates **A** or **A′**, which is in equilibrium with the corresponding intermediate **B** or **B′**.[23] Then the nucleophilic attack of **1** by intermediate **A** or **A′** in the following step to give intermediate **C**. The subsequent one hydride transfer from trimethylphosphine or tributylphosphine to the carbon connected to

Table 4. Reduction of benzyl cyanide **1f** in the presence of various phosphines, phosphite, and amine[a]



Entry	PR_3	**2f**[b]/%
1	PMe_3 (0.5 equiv)	47
2	PMe_3 (0.5 equiv), PPh_3 (0.5 equiv)	41
3	PMe_3 (0.5 equiv), $P(OBu)_3$ (0.5 equiv)	75
4	PMe_3 (0.5 equiv), PBu_3 (0.5 equiv)	85
5	PMe_3 (0.5 equiv), $^iPr_2NEt$ (0.5 equiv)	65
6[c]	PMe_3 (1.0 equiv)	87

[a] All reactions were carried out using benzoyl cyanide **1f** (0.5 mmol) and PR_3 (0.5 mmol) in THF (0.5 mL, 1.0 M) at room temperature under argon atmosphere.
[b] Isolated yields.
[c] 2,6-Di(*tert*-butyl)-4-methylphenol (0.15 mmol) was added in the reaction.



Scheme 3. A proposed reaction mechanism.

CN group takes place to give product **2** and the corresponding phosphine oxide[24] and HCN by ambient moisture or during work-up (Supplementary data).[20] This H transfer only takes place when R′ or R″ is an alkyl group that is the driving force to move these equilibriums forward to form the final product. When PR'_3 is $P(OBu)_3$ in intermediate **C**, the reaction will not take place to produce the final product and will go back to the reversed way to intermediates **A** and **A′** or **B** and **B′** to generate the reactive intermediate. In addition, the generated HCN is quenched by other 0.5 equiv of phosphine or phosphite during the reaction. This is why using 50 mol % of PMe_3 and 50 mol % of $P(OBu)_3$, **2f** can be still obtained in good yield. The more detailed mechanistic investigation is undergoing to figure out the key factor of this interesting reductive coupling reaction promoted by alkyl phosphine.

3. Conclusion

In summary, we disclosed an efficient reductive coupling reaction of acyl cyanides to form *O*-acyl cyanohydrin products. These reactions could take place at room temperature in the presence of alkyl phosphines such as trimethylphosphine in THF or tributylphosphine in various solvents within 6 h to give the corresponding products in good yields. We confirmed that this was an intermolecular reductive coupling reaction of acyl cyanide by a hydride transfer from alkyl phosphine to the carbonyl group. Efforts are underway to elucidate the mechanistic details of this reductive system and to extend the scope of substrates in this reductive coupling reaction.

4. Experimental

4.1. General

Melting points were obtained with a Yanagimoto micro melting point apparatus and were uncorrected. ^1H NMR and ^{13}C NMR spectra were recorded for a solution in $CDCl_3$ with tetramethylsilane (TMS) as internal standard. *J*-values are in hertz. Mass spectra were recorded with an HP-5989 instrument and HRMS was measured by a Finnigan MA^+ mass spectrometer. The solid compounds reported in this paper gave satisfactory CHN microanalyses with

a Carlo-Erba 1106 analyzer. Commercially obtained reagents were used without further purification. All reactions were monitored by TLC with Huanghai GF_{254} silica gel coated plates. Flash column chromatography was carried out using 300–400 mesh silica gel at increased pressure. Reaction experiments were performed under argon condition. The starting materials **1b**–**1h**[25] were synthesized according to the previous literature.

4.2. Typical reaction procedure for the preparation of 2

A mixture of acyl cyanide (0.5 mmol) and PMe_3 (0.5 mL, 0.5 mmol, 1.0 M in THF) was stirred under argon at room temperature for the required time indicated in tables. After the reaction solution was concentrated under reduced pressure, the residue was purified by flash chromatography on silica gel (eluent: EtOAc/petroleum=1/20) to afford pure product **2**.

4.2.1. Benzoic acid cyano-phenyl-methyl ester (2a) (a known compound).[26] An off-white solid: 47 mg, 80% yield. ^{1}H NMR ($CDCl_3$, 300 MHz, TMS): δ 6.68 (s, 1H, CH), 7.45–7.50 (m, 6H, Ar), 7.60–7.65 (m, 2H, Ar), 8.06–8.09 (m, 2H, Ar). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 63.3, 116.2, 127.8, 128.0, 128.6, 129.2, 130.0, 130.4, 131.8, 134.1, 164.6. MS (EI) *m/z*: 237 (M^+, 3.73), 116 (28.30), 105 (100), 77 (25.62).

4.2.2. 4-Chlorobenzoic acid (4-chlorophenyl)-cyano-methyl ester (2b) (a known compound).[15] A light yellow solid: 68 mg, 89% yield. ^{1}H NMR ($CDCl_3$, 300 MHz, TMS): δ 6.63 (s, 1H, CH), 7.44–7.48 (m, 4H, Ar), 7.55 (d, J=8.1 Hz, 2H, Ar), 7.98 (d, J=8.4 Hz, 2H, Ar). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 62.8, 115.6, 126.2, 129.0, 129.3, 129.5, 130.1, 131.3, 136.7, 140.8, 163.6.

4.2.3. 3-Chlorobenzoic acid (3-chlorophenyl)-cyano-methyl ester (2c). A light yellow oil: 60 mg, 78% yield. IR (CH_2Cl_2) ν 1738, 1428, 1243, 1196, 1120, 1069 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz, TMS): δ 6.63 (s, 1H, CH), 7.41–7.51 (m, 4H, Ar), 7.59–7.63 (m, 2H, Ar), 7.94–7.98 (m, 1H, Ar), 8.02–8.04 (m, 1H, Ar). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 62.9, 115.4, 126.0, 128.0, 128.2, 129.4, 130.0, 130.1, 130.7, 130.8, 133.2, 134.3, 134.9, 135.3, 163.3. MS (EI) *m/z*: 306 (M^+, 2.26), 150 (22.58), 139 (100), 111 (22.35), 75 (23.83). HRMS (EI) for $C_{15}H_9NO_2Cl_2$: 305.0010; found: 305.0016.

4.2.4. 2-Chlorobenzoic acid (2-chlorophenyl)-cyano-methyl ester (2d) (a known compound).[15] A white solid: 40 mg, 52% yield. ^{1}H NMR ($CDCl_3$, 300 MHz, TMS): δ 6.96 (s, 1H, CH), 7.33–7.51 (m, 6H, Ar), 7.81–7.84 (m, 1H, Ar), 7.91 (d, J=7.5 Hz, 1H, Ar). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 61.1, 115.1, 126.8, 127.4, 127.7, 129.2, 129.7, 130.3, 131.5, 131.8, 132.0, 133.6, 133.8, 134.7, 163.1.

4.2.5. 4-Methylbenzoic acid cyano(4-methylphenyl)-methyl ester (2e) (a known compound).[15] A white solid: 52 mg, 78% yield. ^{1}H NMR ($CDCl_3$, 300 MHz, TMS): δ 2.40 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 6.62 (s, 1H, CH), 7.26 (t, J=6.6 Hz, 4H, Ar), 7.50 (d, J=8.1 Hz, 2H, Ar), 7.94 (d, J=8.1 Hz, 2H, Ar). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 21.2, 21.7, 63.0, 116.4, 125.3, 127.8, 129.0, 129.3, 129.8, 130.0, 140.5, 144.9, 164.6. MS (EI) *m/z*: 265 (M^+, 8.14), 130 (42.40), 119 (100), 91 (29.56), 65 (32.29).

4.2.6. 3,5-Dimethylbenzoic acid cyano(3,5-dimethyl-phenyl)methyl ester (2f). A light yellow oil: 67 mg, 91% yield. IR (CH_2Cl_2) ν 3010, 2922, 2859, 1727, 1609, 1462, 1301 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz, TMS): δ 2.35 (s, 6H, $2CH_3$), 2.36 (s, 6H, $2CH_3$), 6.60 (s, 1H, CH), 7.10 (s, 1H, Ar), 7.21 (s, 2H, Ar), 7.24 (s, 1H, Ar), 7.67 (s, 2H, Ar). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 21.0, 21.1, 63.2, 116.4, 125.6, 127.6, 127.9, 131.7, 131.9, 135.6, 138.2, 138.9, 164.8. MS (EI) *m/z*: 293 (M^+, 4.18), 144 (16.48), 133 (100), 105 (11.43). HRMS (MALDI) for $C_{19}H_{19}NO_2Na^+$: 316.1315; found: 316.1308.

4.2.7. 3,4,5-Trimethoxybenzoic acid cyano(3,4,5-trimethoxyphenyl)methyl ester (2g). A yellow solid: 77 mg, 74% yield. Mp: 137–139 °C. IR (CH_2Cl_2) ν 2941, 1727, 1593, 1464, 1338, 1211 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz, TMS): δ 3.88 (s, 3H, OCH_3), 3.91 (s, 6H, $2OCH_3$), 3.92 (s, 6H, $2OCH_3$), 3.93 (s, 3H, OCH_3), 6.63 (s, 1H, CH), 6.82 (s, 2H, Ar), 7.31 (s, 2H, Ar). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 56.1, 56.2, 60.7, 60.8, 63.5, 105.0, 107.2, 116.1, 122.7, 127.0, 139.4, 143.1, 152.9, 153.6, 164.2. MS (EI) *m/z*: 418 (M^++1, 18.88), 205 (100), 194 (88.24), 93 (1.41), 81 (4.68). Anal. Calcd for $C_{21}H_{23}NO_8$: C, 60.43%; H, 5.55%; N, 3.36%; found: C, 60.40%; H, 5.64%; N, 3.13%.

4.2.8. Cyano(furan-2-yl)methyl furan-2-carboxylate (2h). A yellow solid: 40 mg, 74% yield. Mp: 122–124 °C. IR (CH_2Cl_2) ν 2923, 1732, 1470, 1394, 1280, 1099 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz, TMS): δ 6.46–6.48 (m, 1H, Fu), 6.55–6.57 (m, 1H, Fu), 6.72 (s, 1H, CH), 6.76–6.78 (m, 1H, Fu), 7.32–7.33 (m, 1H, Fu), 7.53–7.55 (m, 1H, Fu), 7.64–7.66 (m, 1H, Fu). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 55.8, 111.1, 112.3, 113.0, 116.9, 120.5, 142.3, 145.2, 146.3, 147.8, 156.1. MS (EI) *m/z*: 217 (M^+, 1.74), 106 (89.00), 95 (100), 77 (15.07), 51 (19.96). HRMS (EI) for $C_{11}H_7NO_4$: 217.0375; found: 217.0380.

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3-Nitrochromene derivatives as 2π components in 1,3-dipolar cycloadditions of azomethine ylides

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Abstract—The 1,3-dipolar cycloaddition of 2-aryl-3-nitrochromenes with various azomethine ylides has been investigated. The structure and stereochemistry of cycloadducts were studied in detail by NMR spectroscopic methods.

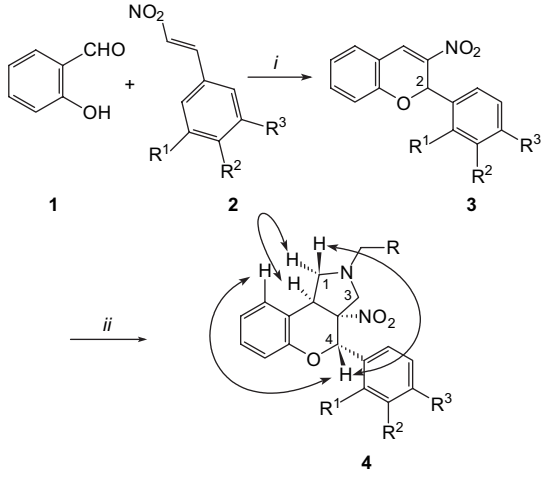
1. Introduction

1,3-Dipolar cycloadditions delineate one of the simplest approaches for the construction of five-membered heterocyclic rings.[1] The ease of generation of 1,3-dipoles, coupled with the often observed highly regio- and stereoselective nature of their cycloaddition reactions has led to a number of syntheses, which utilize such a reaction as the key step.[2] Recently, we have demonstrated the usefulness of the intermolecular 1,3-dipolar cycloaddition of azomethine ylides in the synthesis of aza-cephalotaxine analogues[3] or alkaloid derivatives with a spiro-indolenine framework.[4] This method gives rapid access to the pyrrolo[3,2-*c*]quinoline ring system of martinellines[5] and to pyrrolo[3,4-*c*]quinolines.[6] The abundance of naturally occurring chromene and chromane derivatives, and their interesting physiological properties along with the known selective dopamine D_3 receptor antagonist action of some benzopyrano[3,4-*c*]pyrrolidine derivatives,[7] suggested the study of easily available 2-aryl-3-nitrochromene derivatives as 2π components in 1,3-dipolar cycloadditions of azomethine ylides.

To our knowledge, 3-nitrochromenes[8] have never been used as 2π components in cycloaddition reactions, although they are promising starting materials for the synthesis of functionalized heterocyclic frameworks. Some examples involving coumarins such as 3-nitro-,[9] 3-cyano-[10] and 4-phenylsulfonyl-[11] derivatives have been reported. With the intention to develop a new procedure for the synthesis of benzopyrano[3,4-*c*]pyrrolidine derivatives in this paper, we report the 1,3-dipolar cycloadditions of azomethine ylides with 2-aryl-3-nitrochromenes.[12]

2. Results and discussion

The 3-nitrochromene derivatives (**3a–j**) were prepared by modification of the method described by Yao[8b] from the corresponding 2-aryl-nitroethylenes (**2a–j**)[13] by the treatment with salicylaldehyde, in the presence of DABCO, without any solvent, in a single step (Scheme 1).



Scheme 1. Reagents and conditions: (i) DABCO, 40 °C; (ii) $CH_3NHCH_2CO_2H$ (R=H) or $BnNHCH_2CO_2H$ (R=Ph), $(CH_2O)_n$, toluene, reflux.

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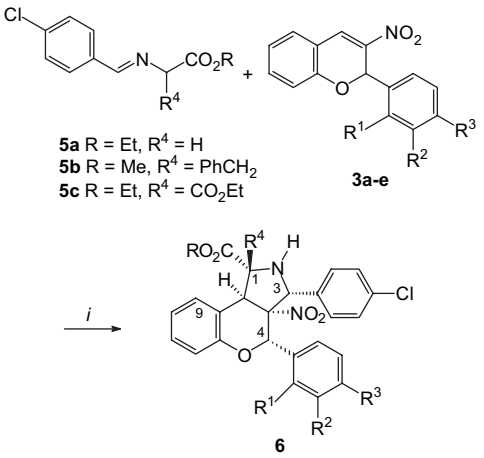
In the first set of experiments, we used the most simple non-stabilized azomethine ylides, which were generated from paraformaldehyde and sarcosine or *N*-benzyl-glycine using the decarboxylation method.[10] The reaction of 3-nitrochromenes (**3a–j**) with these unstable intermediates in refluxing toluene proceeds smoothly, to give the expected 3a-nitro-4-aryl-benzopyrano[3,4-*c*]-pyrrolidines (**4a–q**) (Scheme 1). The results, summarized in Table 1, showed that dipolarophiles (**2**) with more electron-donating substituents on the 2-aryl group are less reactive than those either without electron-donating groups or with electron-withdrawing substituents. This result is similar to our earlier experiments with β-nitro-styrenes.[14]

The structures of compounds **4** were elucidated by NMR spectroscopy using ^{1}H, ^{13}C, ^{1}H–^{1}H COSY, ^{1}H–^{13}C HSQC and ^{1}H–^{13}C HMQC techniques. The relative stereochemistry of these cycloadducts (**4**) was established on **4a** and **4n** mostly by $^{1}H\{^{1}H\}$ NOE studies. The most important proof of their stereochemistry was the NOE enhancements indicated with arrows in Scheme 1.

Thermally generated dipoles from the imines of glycine or other α-amino acid esters undergo stereoselective cycloadditions with highly activated cyclic dipolarophiles such as maleimides leading to the exclusive formation of *endo*-adducts of *E*,*E*-ylides.[15] However, their cycloadditions with less reactive olefin dipolarophiles such as maleates and fumarates were found to be no longer stereoselective.[16] Activation with a wide range of metal salt/tertiary amine combinations proved to be effective for increasing the rate of cycloaddition of aryl imines to less reactive dipolarophiles, allowing the reaction to run at room temperature with excellent regio- and stereocontrol.[17] The cycloaddition of **3** with the azomethine ylides derived from the imines of ethyl glycinate, phenylalanine ethylester or diethyl aminomalonate in the presence of AgOAc and Et_3N occurred smoothly at room temperature giving pure benzopyrano[3,4-*c*]-pyrrolidine derivatives **6a–k** in 60–77% yield (Scheme 2, Table 2). Representative ^{1}H and ^{13}C NMR data for compounds **6c** and **6f**, which verify the structure are collected in Tables 3 and 4. Assignments and stereochemistry were confirmed as noted above in the case of compounds **4**.



Scheme 2. Reagents and conditions: AgOAc, Et_3N, toluene, rt.

The condensation of various aldehydes with *N*-alkyl or *N*-aryl α-amino esters leads to *N*-substituted azomethine ylides. These ylides can be trapped smoothly by the added dipolarophiles, since there are no other reactive reagents (e.g., base, Lewis acids) in the reaction mixture.[18] The cycloaddition of **3** with the azomethine ylides derived from methyl sarcosinate and benzaldehyde in refluxing toluene under Dean–Stark conditions resulted in the formation of cycloadducts **7a–f** in moderate yield (Scheme 3, Table 5). Selected NMR data for cycloadduct **7a** are collected in Table 6.

Table 2. Products of the cycloaddition of **3** with the azomethine ylides derived from the imines **5a–c**

Entry	R^1	R^2	R^3	Starting material	R	R^4	Product	Yield (%)
1	H	H	H	**3a**	Et	H	**6a**	72
2	H	H	H	**3a**	Me	$PhCH_2$	**6b**	75
3	H	H	MeO	**3c**	Et	H	**6c**	60
4	H	H	MeO	**3c**	Me	$PhCH_2$	**6d**	65
5	H	H	Cl	**3f**	Et	H	**6e**	70
6	H	H	Cl	**3f**	Me	$PhCH_2$	**6f**	72
7	Cl	H	H	**3e**	Me	$PhCH_2$	**6g**	60
8	H	MeO	MeO	**3h**	Et	H	**6h**	61
9	H	MeO	MeO	**3h**	Me	$PhCH_2$	**6i**	62
10	H	NO_2	H	**3j**	Me	$PhCH_2$	**6j**	75
11	H	H	H	**3a**	Et	CO_2Et	**6k**	48

Table 1. Reaction times and yields of compounds **4a–q**

Entry	R^1	R^2	R^3	Nitro-chromene	R	Product	Time (h)	Yield (%)
1	H	H	H	**3a**	H	**4a**	3	93
2	H	H	H	**3a**	Ph	**4b**	5	72
3	MeO	H	H	**3b**	H	**4c**	7	79
4	H	H	MeO	**3c**	H	**4d**	5	94
5	H	H	MeO	**3c**	Ph	**4e**	5	85
6	H	MeO	H	**3d**	H	**4f**	7	76
7	H	MeO	H	**3d**	Ph	**4g**	11	68
8	Cl	H	H	**3e**	H	**4h**	7	71
9	H	Cl	H	**3f**	H	**4i**	5	89
10	H	Cl	H	**3f**	Ph	**4j**	5	83
11	H	OBn	H	**3g**	H	**4k**	5	96
12	H	OBn	H	**3g**	Ph	**4l**	5	83
13	H	MeO	MeO	**3h**	H	**4m**	12	75
14	H	MeO	MeO	**3h**	Ph	**4n**	16	68
15	H	OCH_2O		**3i**	H	**4o**	12	66
16	H	H	NO_2	**3j**	H	**4p**	1	84
17	H	H	NO_2	**3j**	Ph	**4q**	1	79

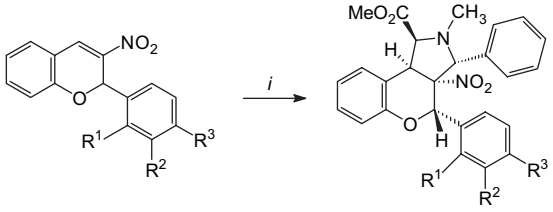
Table 3. ^{1}H and ^{13}C NMR chemical shifts, selected H–H couplings and measured NOE and HMQC connectivities for compound **6c**

	δ_H	$J_{H,H}$ (Hz)	$^1H\{^1H\}$ NOE connections	δ_C	HMQC correlations
1	4.12, br s		H-9, H-4, H-3, H-9b, OCH_2, H-3	68.3	H-9b
3	4.94, d	7.4	H-4, H-1, Ar^3-2′ and 6′H	69.4	Ar^3-2′ and 6′H, H-4
3a	—			96.6	H-3, H-4, H-9b
4	5.49, s		Ar^3-2′ and 6′H, Ar^4-2′ and 6′H, H-9b	75.2	Ar^4-2′ and 6′H, H-9b
9b	4.79	3.8	H-9, Ar^3-2′ and 6′H, Ar^4-2′ and 6′H, H-1	45.6	H-9, H-4, H-1

Table 4. ^{1}H and ^{13}C NMR chemical shifts, selected H–H couplings and measured NOE and HMBC connectivities for compound **6f**

	δ_H	$J_{H,H}$ (Hz)	$^1H\{^1H\}$ NOE[a]	δ_C	HMQC
1	—			72.2	CH_2, H-9b, H-2
2 (NH)	2.94, d	7.8	Ar^3-2′,6′H, Bn-2′,6′H, H-3, H-9b	—	—
3[a]	5.09, d	7.8	H-9, Ar^3-2′,6′H, Ar^4-2′,6′H, H-4, *O*Me, H-2	67.4	Ar^3-2′,6′H, H-4, H-9b
3a	—			98.5	H-4, H-3, H-2, H-9b.
4	5.55, s		Ar^3-2′,6′H, Ar^4-2′,6′H, H-3, β-CH_2; H-6	77.0	Ar^4-2′ and 6′H, H-9b
CH_2-α	2.81, d	13.7	H-9, Bn-2′,6′H, *O*Me, β-CH_2		
CH_2-β	2.37, d	13.7	Bn-2′,6′H, H-4, H-3, H-9b, *O*Me, α-CH_2	42.2	H-9b
9b[a]	5.10, s		H-9, Ar^3-2′,6′H, Ar^4-2′,6′H, H-4, OMe, H-2, β-CH_2	49.8	H-9, H-4

[a] H-3 and H-9b were irradiated together.



3 7

Scheme 3. Reagents and conditions: $CH_3NHCH_2CO_2Me$, PhCHO, toluene, reflux.

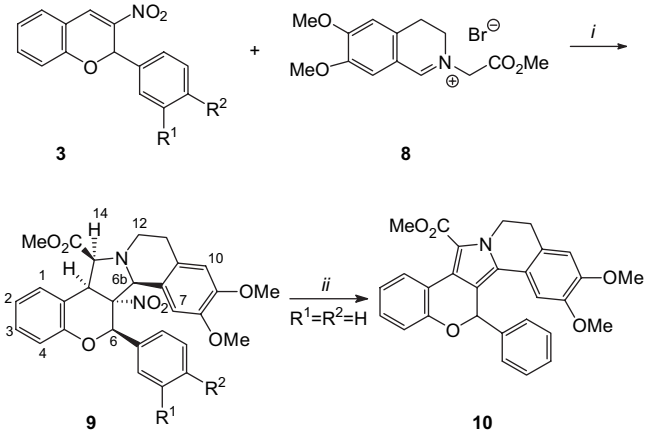
Table 5. Products of the cycloaddition of **3** with the azomethine ylides derived from methyl sarcosinate and benzaldehyde

Entry	R^1	R^2	R^3	Starting material	Product	Yield (%)
1	H	H	H	**3a**	**7a**	57
2	OMe	H	H	**3b**	**7b**	44
3	Cl	H	H	**3e**	**7c**	50
4	H	H	Cl	**3f**	**7d**	63
5	H	MeO	MeO	**3h**	**7e**	47
6	H	OCH_2O		**3i**	**7f**	38

Table 6. ^{1}H and ^{13}C NMR chemical shifts, selected H–H couplings and measured NOE and HMBC connectivities for compound **7a**

	δ_H	$^1H\{^1H\}$ NOE	δ_C	HMQC
1	3.88, d[a]	H-3, H-9b, NMe	73.3	H-9b, H-9a, NMe
3	3.85, s	H-1, H-4, NMe, Ph^3-2′ and 6′H	81.8	H-3a, H-4, NMe
3a	—	—	95.6	H-3, H-4, H-9b
4	5.16, s	H-3, Ph^4-2′ and 6′H, Ph^3-2′ and 6′H	76.8	H-3, Ph^4-2′ and 6′H
9b[a]	5.02, s	H-1, Ph^4-2′ and 6′H, H-9	43.1	H-9, H-4, H-2

1,3-Dipolar cycloadditions of azomethine ylides derived from isoquinolinium salt **8** by deprotonation have previously been studied in detail by us.[19] Reaction with suitably active dipolarophiles afford pyrrolo[2,1-*a*]isoquinoline cycloadducts in practically quantitative yield as single diastereoisomers. The cycloaddition of **3** with the azomethine ylide derived from isoquinolinium salt **8** at ambient temperature with the exclusion of air gave rise to the formation of cycloadducts **9a–c** in virtually quantitative yield as a single diastereoisomer (Scheme 4, Table 7). However, as observed during the earlier experiments, the solution of **9a** in the presence of oxygen transforms into pyrrole derivative **10** at room temperature in a short period of time.



Scheme 4. Reagents and conditions: (i) Et_3N, EtOH, rt; (ii) O_2, $CDCl_3$, rt.

Table 7. Cycloaddition of **3** with azomethine ylide derived from isoquinolinium salt **8**

Entry	R^1	R^2	Nitro-chromene	Product	Yield (%)
1	H	H	**3a**	**9a**	92
2	H	MeO	**3c**	**9b**	95
3	MeO	MeO	**3h**	**9c**	93

The structures of compounds **9** were elucidated again by NMR spectroscopy: the ^{1}H–^{1}H NOE experiments proved the all-cis relationship of the 6, 6b, 14, 14a protons. The strongly shielded aromatic H-7 proton, probably result as a consequence of the anisotropy of the aromatic ring connected at C-6 exhibiting a chemical shift of 6.10 ppm, further corroborated the proposed structure. Selected NMR data are collected in Table 8.

Table 8. ^{1}H and ^{13}C NMR chemical shifts, selected H–H couplings and measured NOE and HMQC connectivities for compound **9c**

	δ_H	$J_{H,H}$ (Hz)	$^{1}H\{^{1}H\}$ NOE connections	δ_C	HMQC correlations
6	5.77, s	—	Ar^6-2′ and 6′H, H-7, H-6b, H-14, H-14a	75.8	Ar^6-2′ and 6′H, H-6b
6a	—	—	—	90.4	H-6b
6b	4.86, s	—	H-7, H-6, H-14, H-14a, H-12α	65.7	H-7, H-12, H-14
7	6.10, s	—	H-6, H-6b, 8-OMe	109.8	H-6b
14	4.11, d	11.3	—	67.7	H-12, H-14a
14a	4.12, d	11.3	—	47.2	H-1, H-14

In summary, the use of 3-nitrochromene derivatives as 2π components in 1,3-dipolar cycloadditions of azomethine ylides allows the assembly of polysubstituted benzopyrano[3,4-*c*]-pyrrolidines from simple precursors in one-pot reaction.

3. Experimental

3.1. General

Melting points were determined on a Gallenkamp apparatus and are uncorrected. Column chromatography was performed using Merck Kieselgel 60 (70–230 mesh), TLC on aluminium sheets coated with Kieselgel 60 F_{254}. Plates were stained with anisaldehyde solution (100 ml glacial acetic acid, 2 ml concd sulfuric acid and 1 ml anisaldehyde) and heated at ca. 150 °C. IR spectra were obtained on a Bruker VECTOR 22 FT-IR instrument. NMR spectra were obtained on Varian INOVA 500, Bruker DRX-500 and Bruker 250 instruments. Chemical shifts are given relative to δ_{TMS}. All solvents were purified according to standard procedures.

3.1.1. Synthesis of 2-methyl- and 2-benzyl-3a-nitro-4-aryl-benzopyrano[3,4-*c*]-pyrrolidines (4). General procedure. A mixture of sarcosine (2.5 equiv) or *N*-benzylglycine (2.5 equiv), paraformaldehyde (6 equiv) and the corresponding 3-nitrochromene (1 equiv) was heated under reflux in toluene (10 ml for 1 mmol of dipolarophile). The water formed was removed by the aid of a Dean–Stark trap. After completion of the reaction, the reaction mixture was filtered through a pad of Celite and the solvent was evaporated in vacuo. The residue was crystallized from ether to give the title products. The reaction times and yields (based on the dipolarophiles) are summarized in Table 1.

3.1.1.1. 2-Methyl-3a-nitro-4-phenyl-benzopyrano[3,4-*c*]-pyrrolidine (4a). White powder, mp 138–139 °C; [Found: C, 69.9; H, 5.8; N, 9.0. $C_{18}H_{18}N_2O_3$ requires C, 69.7; H, 5.9; N, 9.0%]; ^{1}H NMR (250 MHz, $CDCl_3$): 7.44 (5H, m, Ph-H), 7.23 (2H, m, H-7 and H-9), 7.04 (2H, m, H-6 and H-8), 5.01 (1H, s, H-4), 4.03 (1H, t, *J* 8.5 Hz, H-9b), 3.62 (1H, d, *J* 11.4 Hz, H-3), 3.50 (1H, t, *J* 8.5 Hz, H-1), 2.85 (1H, d, *J* 11.4 Hz, H-3), 2.71 (1H, t, *J* 8.5 Hz, H-1), 2.41 (3H, s, NMe); ^{13}C NMR (62.5 MHz, $CDCl_3$): 154.0 (q), 134.0 (q), 129.4 (CH), 128.5 (2×CH), 128.3 (CH), 127.8 (CH), 126.8 (2×CH), 122.6 (q), 122.5 (CH), 117.6 (CH), 95.9 (q), 80.1 (CH), 62.8 (CH_2), 61.8 (CH_2), 43.3 (CH), 41.3 (CH_3); IR (KBr, cm^{-1}): 2976, 2947, 2823, 1535, 1489, 1479, 1452, 1371, 1254, 1238, 1149, 1045, 1024.

3.1.1.2. 2-Benzyl-3a-nitro-4-phenyl-benzopyrano[3,4-*c*]-pyrrolidine (4b). White powder, mp 144 °C; [Found: C, 74.5; H, 5.7; N, 7.0. $C_{24}H_{22}N_2O_3$ requires C, 74.6; H, 5.7; N, 7.2%]; ^{1}H NMR (500 MHz, $CDCl_3$): 7.43 (10H, m, Ar-H), 7.28 (1H, t, *J* 7.5 Hz, H-7), 7.23 (1H, d, *J* 7.5 Hz, H-9), 7.12 (2H, m, H-6 and H-8), 5.13 (1H, s, H-4), 4.08 (1H, t, *J* 7.5 Hz, H-9b), 3.80 (1H, d, *J* 12.5 Hz, C*H*$_2$Ph), 3.69 (1H, d, *J* 12.5 Hz, C*H*$_2$Ph), 3.56 (2H, m, H-3 and H-1), 3.03 (1H, d, *J* 11.5 Hz, H-3), 2.9.0 (1H, t, *J* 7.5 Hz, H-1); ^{13}C NMR (62.5 MHz, $CDCl_3$): 154.3 (q), 137.7 (q), 134.2 (q), 129.5 (CH), 128.8 (2×CH), 128.7 (2×CH), 128.65 (2×CH), 128.6 (CH), 128.0 (CH), 127.7 (CH), 127.1 (2×CH), 123.1 (q), 122.7 (CH), 117.8 (CH), 95.1 (q), 80.3 (CH), 60.7 (CH_2), 59.3 (CH_2), 59.2 (CH_2), 42.8 (CH); IR (KBr, cm^{-1}): 3029, 2926, 2839, 2808, 1534, 1488, 1452, 1370, 1306, 1231, 1048, 1028.

3.1.1.3. 2-Methyl-4-(2-methoxyphenyl)-3a-nitro-benzopyrano[3,4-*c*]-pyrrolidine (4c). White powder, mp 152–153 °C; [Found: C, 67.1; H, 5.7; N, 8.2. $C_{19}H_{20}N_2O_4$ requires C, 67.0; H, 5.9; N, 8.2%]; ^{1}H NMR (500 MHz, DMSO-d_6): 7.41 (1H, t, *J* 7.5 Hz, Ar^4-5′H), 7.32 (1H, d, *J* 7.5 Hz, Ar^4-6′H), 7.21 (1H, t, *J* 7.5 Hz, H-7), 7.14 (1H, d, *J* 7.5 Hz, H-9), 7.12 (1H, d, *J* 7.5 Hz, H-8), 7.07 (1H, t, *J* 7.5 Hz, H-6), 6.99 (1H, d, *J* 7.5 Hz, Ar^4-3′H), 6.95 (1H, d, *J* 7.5 Hz, Ar^4-4′H), 5.49 (1H, s, H-4), 3.99 (1H, t, *J* 8.3 Hz, H-9b), 3.83 (3H, s, OMe), 3.51 (1H, t, *J* 8.3 Hz, H-3), 3.39 (1H, d, *J* 11.1 Hz, H-1), 2.86 (1H, d, *J* 11.1 Hz, H-3), 2.59 (1H, t, *J* 8.3 Hz, H-1), 2.29 (3H, s, NMe); ^{13}C NMR (125 MHz, DMSO-d_6): 156.1 (q), 154.2 (q), 130.6 (q), 129.9 (CH), 127.7 (CH), 127.1 (CH), 123.5 (CH), 122.65 (q), 122.6 (CH), 120.8 (CH), 117.1 (CH), 111.5 (CH), 96.9 (q), 73.1 (CH), 62.4 (CH_2), 61.4 (CH_2), 55.8 (CH_3), 43.4 (CH), 41.1 (CH_3); IR (KBr, cm^{-1}): 2944, 2841, 2824, 2761, 1585, 1532, 1494, 1456, 1365, 1289, 1256, 1232, 1115, 1045.

3.1.1.4. 2-Methyl-4-(3-methoxyphenyl)-3a-nitro-benzopyrano[3,4-*c*]-pyrrolidine (4d). White powder, mp 141–142 °C; [Found: C, 67.2; H, 6.0; N, 8.1. $C_{19}H_{20}N_2O_4$ requires C, 67.0; H, 5.9; N, 8.2%]; ^{1}H NMR (500 MHz, $CDCl_3$): 7.30 (1H, t, *J* 7.5 Hz, H-7), 7.23 (4H, m, H-6, H-8, H-9 and Ar^4-6′H), 7.03 (1H, d, *J* 7.8 Hz, Ar^4-4′H), 6.90 (2H, m, Ar^4-2′ and 5′H), 4.96 (1H, s, H-4), 4.01 (1H, t, *J* 8.1 Hz, H-9b), 3.77 (3H, s, OMe), 3.62 (1H, d, *J* 11.5 Hz, H-1), 3.50 (1H, t, *J* 8.5 Hz, H-3), 2.85 (1H, d, *J* 11.5 Hz, H-3), 2.70 (1H, t, *J* 8.6 Hz, H-1), 2.40 (3H, s, NMe); ^{13}C NMR (125 MHz, $CDCl_3$): 159.7 (q, Ar^4-1′C), 152.3 (q, C-5a), 135.0 (q), 129.6 (CH), 128.4 (CH, C-7), 127.9 (CH, C-9), 123.1 (CH, C-8), 122.6 (q, C-9a), 119.2 (CH), 117.7 (CH), 116.9 (CH, C-6), 112.5 (CH), 94.8 (q, C-3a), 79.1 (CH, C-4), 63.0 (CH_2), 62.1 (CH_2), 55.3 (OMe), 43.5 (CH, H-9b), 41.5 (NCH_3); IR (KBr, cm^{-1}): 2990, 2920, 2830, 1601, 1543, 1486, 1458, 1358, 1261, 1206, 1173, 1036.

3.1.1.5. 2-Benzyl-4-(3-methoxyphenyl)-3a-nitro-benzopyrano[3,4-*c*]-pyrrolidine (4e). White powder, mp 149 °C; [Found: C, 71.9; H, 6.0; N, 6.7. $C_{25}H_{24}N_2O_4$ requires C,

72.1; H, 5.8; N, 6.7%]; ^{1}H NMR (500 MHz, $CDCl_3$): 7.25 (8H, m, Ph-H and H-6, H-7, H-8), 7.03 (2H, m, H-9 and Ar^4-6′H), 6.88 (3H, m, Ar^4-2′, 4′ and 5′H), 5.02 (1H, s, H-4), 4.00 (1H, t, *J* 7.6 Hz, H-9b), 3.79 (1H, d, *J* 12.3 Hz, C*H*$_2$Ph), 3.78 (3H, s, OMe), 3.74 (1H, d, *J* 12.3 Hz, C*H*$_2$Ph), 3.62 (1H, t, *J* 8.1 Hz, H-3), 3.48 (1H, d, *J* 11.3 Hz, H-1), 2.98 (1H, d, *J* 11.3 Hz, H-3), 2.86 (1H, t, *J* 8.1 Hz, H-1); ^{13}C NMR (125 MHz, $CDCl_3$): 159.7 (q), 153.3 (q), 135.0 (q), 130.8 (q), 129.7 (2×CH), 129.6 (CH), 128.6 (2×CH), 127.5 (CH), 128.5 (CH), 127.9 (CH), 123.1 (CH), 122.6 (q), 119.2 (CH), 118.9 (CH), 116.9 (CH), 112.3 (CH), 88.7 (q), 80.0 (CH), 60.6 (CH_2), 59.3 (CH_2), 59.1 (CH_2), 55.3 (CH_3), 42.7 (CH); IR (KBr, cm^{-1}): 3020, 2961, 2810, 1600, 1543, 1486, 1457, 1358, 1261, 1206, 1173, 1137, 1035, 1016.

3.1.1.6. 2-Methyl-4-(4-methoxyphenyl)-3a-nitro-benzopyrano[3,4-*c*]-pyrrolidine (4f). White powder, mp 135–136 °C; [Found: C, 67.1; H, 5.9; N, 8.2. $C_{19}H_{20}N_2O_4$ requires C, 67.0; H, 5.9; N, 8.2%]; ^{1}H NMR (250 MHz, $CDCl_3$): 7.22 (2H, s, *J* 8.6 Hz, Ar^4-3′ and 5′H), 7.20 (1H, t, *J* 7.5 Hz, H-7), 7.14 (1H, d, *J* 7.5 Hz, H-9), 7.00 (1H, t, *J* 7.5 Hz, H-8), 6.97 (1H, d, *J* 7.5 Hz, H-6), 6.86 (2H, s, *J* 8.6 Hz, Ar^4-2′ and 6′H), 4.90 (1H, s, H-4), 3.95 (1H, t, *J* 8.3 Hz, H-9b), 3.75 (3H, s, *O*Me), 3.54 (1H, d, *J* 11.3 Hz, H-3), 3.43 (1H, t, *J* 8.3 Hz, H-1), 2.74 (1H, d, *J* 11.3 Hz, H-3), 2.63 (1H, t, *J* 8.5 Hz, H-1), 2.33 (3H, s, *N*Me); ^{13}C NMR (62.5 MHz, $CDCl_3$): 160.3 (q), 154.1 (q), 128.3 (CH), 128.1 (2×CH), 127.8 (CH), 125.9 (q), 122.6 (q), 122.5 (CH), 117.6 (CH), 114.0 (2×CH), 95.9 (q), 79.9 (CH), 62.9 (CH_2), 61.9 (CH_2), 55.2 (CH_3), 43.3 (CH), 41.4 (CH_3); IR (KBr, cm^{-1}): 2949, 2822, 1537, 1515, 1475, 1454, 1307, 1253, 1231, 1176, 1045, 1034.

3.1.1.7. 2-Benzyl-4-(4-methoxyphenyl)-3a-nitro-benzopyrano[3,4-*c*]-pyrrolidine (4g). White powder, mp 158 °C; [Found: C, 72.2; H, 5.7; N, 6.8. $C_{25}H_{24}N_2O_4$ requires C, 72.1; H, 5.8; N, 6.7%]; ^{1}H NMR (500 MHz, DMSO-d_6+MeOD): 7.31 (5H, m, Ar-H), 7.27 (2H, m, H-9 and H-7), 7.18 (2H, d, *J* 8.0 Hz, Ar^4-3′ and 5′H), 7.02 (1H, t, *J* 8.0 Hz, H-8), 6.96 (1H, d, *J* 8.0 Hz, H-6), 6.89 (2H, s, *J* 8.0 Hz, Ar^4-2′ and 6′H), 5.18 (1H, s, H-4), 3.85 (1H, t, *J* 8.5 Hz, H-9b), 3.79 (1H, d, *J* 13.4 Hz, C*H*$_2$Ph), 3.76 (1H, d, *J* 13.4 Hz, C*H*$_2$Ph), 3.74 (3H, s, *O*Me), 3.57 (1H, t, *J* 8.5 Hz, H-1), 3.43 (1H, d, *J* 11.0 Hz, H-3), 2.84 (1H, d, *J* 11.0 Hz, H-3), 2.70 (1H, t, *J* 8.5 Hz, H-1); ^{13}C NMR (125 MHz, DMSO-d_6+MeOD): 160.3 (q), 154.3 (q), 138.6 (q), 129.2 (CH), 128.8 (2×CH), 128.7 (2×CH), 128.5 (CH), 128.4 (2×CH), 128.0 (CH), 126.5 (q), 123.4 (q), 122.7 (CH), 117.4 (CH), 114.1 (2×CH), 95.9 (q), 79.2 (CH), 60.3 (CH_2), 58.9 (CH_2), 58.5 (CH_2), 55.3 (CH_3), 43.1 (CH); IR (KBr, cm^{-1}): 3030, 2964, 2940, 2802, 1613, 1584, 1534, 1515, 1488, 1452, 1370, 1255, 1238, 1179, 1054, 1046, 1026.

3.1.1.8. 4-(2-Chlorophenyl)-2-methyl-3a-nitro-benzopyrano[3,4-*c*]-pyrrolidine (4h). White powder, mp 143–144 °C; [Found: C, 62.7; H, 4.9; N, 8.1. $C_{18}H_{17}ClN_2O_3$ requires C, 62.7; H, 5.0; N, 8.1%]; ^{1}H NMR (500 MHz, DMSO-d_6): 7.59 (1H, d, *J* 7.8 Hz, Ar^4-3′H), 7.48 (1H, t, *J* 7.8 Hz, Ar^4-4′H), 7.42 (1H, t, *J* 7.8 Hz, Ar^4-5′H), 7.34 (1H, d, *J* 7.6 Hz, H-9), 7.26 (1H, d, *J* 7.8 Hz, Ar^4-6′H), 7.22 (1H, t, *J* 7.6 Hz, H-7), 7.10 (1H, t, *J* 7.6 Hz, H-8), 6.98 (1H, d, *J* 7.6 Hz, H-6), 5.61 (1H, s, H-4), 4.08 (1H, t, *J* 7.5 Hz, H-9b), 3.45 (1H, t, *J* 8.9 Hz, H-1), 3.31 (1H, d, *J* 10.9 Hz, H-3), 2.97 (1H, d, *J* 10.9 Hz, H-3), 2.80 (1H, t, *J* 8.9 Hz, H-1), 2.30 (3H, s, NMe); ^{13}C NMR (125 MHz, DMSO-d_6): 153.6 (q), 132.4 (q), 132.3 (q), 131.3 (CH), 129.9 (CH), 129.2 (CH), 128.7 (q), 128.4 (CH), 127.9 (CH), 127.85 (CH), 122.9 (CH), 117.1 (CH), 96.1 (q), 75.3 (CH), 62.3 (CH_2), 60.1 (CH_2), 42.8 (CH), 40.8 (CH_3); IR (KBr, cm^{-1}): 2943, 2843, 2828, 2793, 2765, 1585, 1532, 1489, 1479, 1455, 1363, 1301, 1280, 1268, 1256, 1237, 1161, 1134, 1114, 1093, 1061, 1048, 1038, 1025.

3.1.1.9. 4-(4-Chlorophenyl)-2-methyl-3a-nitro-benzopyrano[3,4-*c*]-pyrrolidine (4i). White powder, mp 150 °C; [Found: C, 62.4; H, 4.8; N, 8.3. $C_{18}H_{17}ClN_2O_3$ requires C, 62.7; H, 5.0; N, 8.1%]; ^{1}H NMR (250 MHz, $CDCl_3$): 7.35 (2H, d, *J* 8.5 Hz, Ar^4-2′ and 6′H), 7.27 (2H, d, *J* 8.5 Hz, Ar^4-3′ and 5′H), 7.17 (2H, m, H-7 and H-9), 7.05 (1H, t, *J* 7.5 Hz, H-8), 6.99 (1H, d, *J* 7.5 Hz, H-6), 4.97 (1H, s, H-4), 4.00 (1H, t, *J* 8.4 Hz, H-9b), 3.54 (1H, d, *J* 11.1 Hz, H-3), 3.43 (1H, t, *J* 8.4 Hz, H-1), 2.80 (1H, d, *J* 11.1 Hz, H-3), 2.70 (1H, t, *J* 8.4 Hz, H-1), 2.37 (3H, s, *N*Me); ^{13}C NMR (62.5 MHz, $CDCl_3$): 154.0 (q), 135.6 (q), 132.9 (q), 129.0 (2×CH), 128.6 (CH), 128.4 (2×CH), 128.1 (CH), 123.0 (CH), 122.9 (q), 117.8 (CH), 96.0 (q), 79.7 (CH), 63.1 (CH_2), 62.1 (CH_2), 43.4 (CH), 41.6 (CH_3); IR (KBr, cm^{-1}): 2948, 2843, 2793, 1534, 1490, 1456, 1366, 1254, 1227, 1152, 1090, 1060, 1014.

3.1.1.10. 2-Benzyl-4-(4-chlorophenyl)-3a-nitro-benzopyrano[3,4-*c*]-pyrrolidine (4j). White powder, mp 155–157 °C; [Found: C, 68.8; H, 4.9; N, 6.6. $C_{24}H_{21}ClN_2O_3$ requires C, 68.5; H, 5.0; N, 6.7%]; ^{1}H NMR (250 MHz, $CDCl_3$): 7.37–7.23 (8H, m, Ph-H, Ar^4-2′ and 6′H and H-9), 7.21 (1H, t, *J* 7.5 Hz, H-7), 7.16 (2H, d, *J* 8.5 Hz, Ar^4-3′ and 5′H), 7.02 (1H, t, *J* 7.5 Hz, H-8), 7.00 (1H, d, *J* 7.5 Hz, H-6), 5.03 (1H, s, H-4), 3.97 (1H, t, *J* 8.4 Hz, H-9b), 3.71 (1H, d, *J* 12.9 Hz, CH_2Ph), 3.57 (1H, d, *J* 12.9 Hz, CH_2Ph), 3.46 (1H, t, *J* 8.4 Hz, H-1), 3.41 (1H, d, *J* 11.4 Hz, H-3), 2.87 (1H, d, *J* 11.4 Hz, H-3), 2.86 (1H, t, *J* 8.4 Hz, H-1); ^{13}C NMR (62.5 MHz, $CDCl_3$): 154.0 (q), 137.6 (q), 135.5 (q), 132.8 (q), 129.1 (CH), 128.9 (2×CH), 128.8 (2×CH), 128.7 (2×CH), 128.4 (2×CH), 128.1 (CH), 127.8 (CH), 123.1 (CH), 122.9 (q), 117.8 (CH), 94.9 (q), 79.6 (CH), 60.7 (CH_2), 59.2 (CH_2), 59.1 (CH_2), 42.6 (CH); IR (KBr, cm^{-1}): 3061, 3025, 2968, 2920, 2824, 1537, 1490, 1455, 1380, 1260, 1233, 1210, 1153, 1092, 1057, 1014.

3.1.1.11. 4-(4-Benzyloxyphenyl)-2-methyl-3a-nitro-benzopyrano[3,4-*c*]-pyrrolidine (4k). White powder, mp 139–140 °C; [Found: C, 72.1; H, 5.9; N, 6.6. $C_{25}H_{24}N_2O_4$ requires C, 72.1; H, 5.8; N, 6.7%]; ^{1}H NMR (500 MHz, $CDCl_3$): 7.42 (2H, d, *J* 7.2 Hz, Bn-2′ and 6′H), 7.38 (2H, t, *J* 7.2 Hz, Bn-3′ and 5′H), 7.32 (1H, t, *J* 7.2 Hz, Bn-4′H), 7.26 (2H, d, *J* 8.5 Hz, Ar^4-2′ and 6′H), 7.17 (2H, m, H-9 and H-7), 7.04 (1H, t, *J* 7.4 Hz, H-8), 7.01 (1H, d, *J* 7.4 Hz, H-6), 6.98 (2H, d, *J* 8.5 Hz, Ar^4-2′ and 6′H), 5.05 (2H, s, OCH_2), 4.94 (1H, s, H-4), 4.00 (1H, t, *J* 8.3 Hz, H-9b), 3.58 (1H, d, *J* 11.2 Hz, H-3), 3.47 (1H, t, *J* 8.7 Hz, H-1), 2.80 (1H, d, *J* 11.2 Hz, H-3), 2.69 (1H, t, *J* 8.7 Hz, H-1), 2.38 (3H, s, *N*Me); ^{13}C NMR (125 MHz, $CDCl_3$): 159.6 (q), 154.1 (q), 136.6 (q), 128.6 (2×CH), 128.4 (CH), 128.2 (2×CH), 128.0 (q), 127.8 (CH), 127.5 (2×CH), 126.3 (CH), 122.7 (q), 122.5 (CH), 117.6 (CH), 114.9 (2×CH),

95.9 (q), 79.9 (CH), 70.0 (CH_2), 63.0 (CH_2), 62.0 (CH_2), 43.4 (CH), 41.4 (CH_3); IR (KBr, cm^{-1}): 3026, 2937, 2910, 2796, 1614, 1588, 1533, 1514, 1457, 1423, 1395, 1372, 1331, 1233, 1178, 1117, 1095, 1056, 1040, 1027.

3.1.1.12. 2-Benzyl-4-(4-benzyloxyphenyl)-3a-nitro-benzopyrano[3,4-*c*]-pyrrolidine (4l). White powder, mp 139–140 °C; [Found: C, 75.6; H, 5.9; N, 5.6. $C_{31}H_{28}N_2O_4$ requires C, 75.6; H, 5.7; N, 5.7%]; ^{1}H NMR (250 MHz, $CDCl_3$): 7.43–7.27 (10H, m, Ar-H), 7.17 (4H, m, Ar-H), 7.03 (1H, t, *J* 7.6 Hz, H-8), 7.00 (1H, d, *J* 7.4 Hz, H-6), 6.93 (2H, d, *J* 8.2 Hz, Ar^4-2′ and 6′H), 5.04 (2H, s, OCH_2), 5.00 (1H, s, H-4), 3.99 (1H, t, *J* 7.9 Hz, H-9b), 3.71 (1H, d, *J* 12.9 Hz, NCH_2), 3.62 (1H, d, *J* 12.9 Hz, NCH_2), 3.46 (2H, m, H-3 and H-1), 2.90 (1H, d, *J* 11.1 Hz, H-3), 2.84 (1H, t, *J* 7.7 Hz, H-1); ^{13}C NMR (125 MHz, $CDCl_3$): 159.6 (q), 154.2 (q), 137.5 (q), 137.1 (q), 136.7 (q), 128.65 (2×CH), 128.6 (2×CH), 128.5 (2×CH), 128.2 (2×CH), 128.0 (CH), 127.9 (CH), 127.51 (CH), 127.5 (2×CH), 126.2 (CH), 122.9 (q), 122.5 (CH), 117.6 (CH), 114.9 (2×CH), 94.9 (q), 79.8 (CH), 70.0 (CH_2), 60.6 (CH_2), 59.2 (CH_2), 59.1 (CH_2), 42.6 (CH); IR (KBr, cm^{-1}): 3050, 3020, 2930, 2800, 1612, 1539, 1513, 1490, 1455, 1235, 1177, 1027.

3.1.1.13. 4-(3,4-Dimethoxyphenyl)-2-methyl-3a-nitro-benzopyrano[3,4-*c*]-pyrrolidine (4m). White powder, mp 129–130 °C; [Found: C, 64.5; H, 5.9; N, 7.5. $C_{20}H_{22}N_2O_5$ requires C, 64.8; H, 6.0; N, 7.6%]; ^{1}H NMR (250 MHz, $CDCl_3$): 7.04 (1H, t, *J* 7.8 Hz, H-7), 7.02 (1H, d, *J* 7.8 Hz, H-9), 6.89 (1H, t, *J* 7.8 Hz, H-8), 6.87 (1H, d, *J* 7.8 Hz, H-6), 6.70 (3H, m, Ar^4-H), 4.77 (1H, s, H-4), 3.83 (1H, t, *J* 8.2 Hz, H-9b), 3.72 (3H, s, OMe), 3.71 (3H, s, OMe), 3.45 (1H, d, *J* 11.2 Hz, H-3), 3.32 (1H, t, *J* 8.2 Hz, H-1), 2.64 (1H, d, *J* 11.2 Hz, H-3), 2.52 (1H, t, *J* 8.2 Hz, H-1), 2.23 (3H, s, *N*Me); ^{13}C NMR (62.5 MHz, $CDCl_3$): 154.0 (q), 149.8 (q), 149.1 (q), 128.9 (CH), 128.3 (CH), 126.2 (q), 122.6 (q), 122.5 (CH), 119.7 (CH), 117.6 (CH), 110.6 (CH), 109.4 (CH), 95.8 (q), 80.1 (CH), 62.9 (CH_2), 61.9 (CH_2), 55.9 (CH_3), 55.7 (CH_3), 43.4 (CH), 41.4 (CH_3); IR (KBr, cm^{-1}): 2959, 2844, 2785, 1536, 1518, 1492, 1456, 1365, 1266, 1230, 1147, 1052, 1020.

3.1.1.14. 2-Benzyl-4-(3,4-dimethoxyphenyl)-3a-nitro-benzopyrano[3,4-*c*]-pyrrolidine (4n). White powder, mp 134 °C; [Found: C, 69.7; H, 5.9; N, 6.5. $C_{26}H_{26}N_2O_5$ requires C, 69.9; H, 5.9; N, 6.3%]; ^{1}H NMR (500 MHz, $CDCl_3$): 7.34 (5H, m, Bn-H), 7.23 (1H, t, *J* 7.6 Hz, H-7), 7.18 (1H, d, *J* 7.6 Hz, H-9), 7.06 (1H, t, *J* 7.6 Hz, H-8), 7.05 (1H, d, *J* 7.6 Hz, H-6), 6.82 (2H, m, Ar^4-5′H and 6′H), 6.76 (1H, s, Ar^4-2′H), 5.03 (1H, s, H-4), 4.00 (1H, t, *J* 8.0 Hz, H-9b), 3.88 (3H, s, *O*Me), 3.78 (3H, s, *O*Me), 3.78 (1H, d, *J* 12.8 Hz, CH_2Ph), 3.59 (1H, d, *J* 12.8 Hz, CH_2Ph), 3.49 (1H, t, *J* 8.0 Hz, H-1), 3.44 (1H, d, *J* 11.1 Hz, H-3), 2.98 (1H, d, *J* 11.1 Hz, H-3), 2.94 (1H, t, *J* 8.0 Hz, H-1); ^{13}C NMR (125 MHz, $CDCl_3$): 154.0 (q), 149.8 (q), 149.0 (q), 137.6 (q), 128.6 (2×CH), 128.5 (2×CH), 128.2 (CH), 127.8 (CH), 127.4 (CH), 126.3 (q), 122.9 (q), 122.5 (CH), 119.6 (CH), 117.6 (CH), 110.7 (CH), 109.7 (CH), 94.7 (q), 79.9 (CH), 60.8 (CH_2), 59.03 (CH_2), 59.01 (CH_2), 55.9 (CH_3), 55.8 (CH_3), 42.4 (CH); IR (KBr, cm^{-1}): 2956, 2933, 2825, 1537, 1515, 1489, 1455, 1378, 1266, 1232, 1160, 1143, 1057, 1024.

3.1.1.15. 2-Methyl-4-(3,4-methylenedioxyphenyl)-3a-nitro-benzopyrano[3,4-*c*]-pyrrolidine (4o). White powder, mp 122–124 °C; [Found: C, 64.7; H, 4.9; N, 7.9. $C_{19}H_{18}N_2O_5$ requires C, 64.4; H, 5.1; N, 7.9%]; ^{1}H NMR (500 MHz, $CDCl_3$): 7.20 (1H, t, *J* 7.5 Hz, H-7), 7.17 (1H, d, *J* 7.5 Hz, H-9), 7.05 (1H, t, *J* 7.5 Hz, H-8), 7.01 (1H, d, *J* 7.5 Hz, H-6), 6.82 (3H, m, Ar^4-H), 6.98 (2H, s, OCH_2O), 4.92 (1H, s, H-4), 4.00 (1H, t, *J* 8.2 Hz, H-9b), 3.60 (1H, d, *J* 11.4 Hz, H-3), 3.47 (1H, t, *J* 8.2 Hz, H-1), 2.82 (1H, d, *J* 11.4 Hz, H-3), 2.70 (1H, t, *J* 8.2 Hz, H-1), 2.39 (3H, s, *N*Me); ^{13}C NMR (125 MHz, $CDCl_3$): 154.0 (q), 148.5 (q), 148.0 (q), 128.4 (CH), 127.9 (CH), 127.7 (q), 122.7 (q), 122.6 (CH), 120.8 (CH), 117.6 (CH), 108.3 (CH), 107.1 (CH), 101.3 (CH_2), 95.9 (q), 80.1 (CH), 63.0 (CH_2), 62.1 (CH_2), 43.4 (CH), 41.4 (CH_3); IR (KBr, cm^{-1}): 2984, 2943, 2898, 2843, 2776, 1588, 1542, 1505, 1491, 1445, 1336, 1295, 1263, 1251, 1231, 1180, 1166, 1103, 1086, 1060, 1041.

3.1.1.16. 2-Methyl-4-(3-nitrophenyl)-3a-nitro-benzopyrano[3,4-*c*]-pyrrolidine (4p). White powder, mp 152–153 °C; [Found: C, 60.7; H, 4.8; N, 11.9. $C_{18}H_{17}N_3O_5$ requires C, 60.8; H, 4.8; N, 11.8%]; ^{1}H NMR (250 MHz, $CDCl_3$+DMSO-d_6): 8.03 (1H, d, *J* 7.6 Hz, Ar^4-4′H), 8.01 (1H, s, Ar^4-2′H), 7.52 (1H, d, *J* 7.6 Hz, Ar^4-6′H), 7.40 (1H, t, *J* 7.6 Hz, Ar^4-5′H), 7.01 (1H, d, *J* 8.0 Hz, H-9), 6.99 (1H, t, *J* 8.0 Hz, H-7), 6.86 (1H, t, *J* 8.0 Hz, H-8), 6.78 (1H, d, *J* 8.0 Hz, H-6), 5.01 (1H, s, H-4), 3.82 (1H, t, *J* 8.3 Hz, H-9b), 3.34 (1H, d, *J* 11.2 Hz, H-3), 3.30 (1H, t, *J* 8.3 Hz, H-1), 2.68 (1H, d, *J* 11.2 Hz, H-3), 2.52 (1H, t, *J* 8.2 Hz, H-1), 2.20 (3H, s, *N*Me); ^{13}C NMR (62.5 MHz, $CDCl_3$+DMSO-d_6): 152.8 (q), 147.4 (q), 135.9 (q), 132.3 (CH), 129.0 (CH), 127.8 (CH), 127.3 (CH), 123.4 (CH), 122.3 (CH), 121.9 (q), 121.1 (CH), 116.7 (CH), 95.5 (q), 80.0 (CH), 61.8 (CH_2), 60.9 (CH_2), 42.5 (CH), 40.5 (CH_3); IR (KBr, cm^{-1}): 3104, 3042, 2949, 2840, 2793, 1527, 1485, 1455, 1353, 1224, 1168, 1092, 1053.

3.1.1.17. 2-Benzyl-4-(3-nitrophenyl)-3a-nitro-benzopyrano[3,4-*c*]-pyrrolidine (4q). White powder, mp 155 °C; [Found: C, 66.7; H, 4.8; N, 9.9. $C_{24}H_{21}N_3O_5$ requires C, 66.8; H, 4.9; N, 9.7%]; ^{1}H NMR (250 MHz, $CDCl_3$): 8.19 (1H, d, *J* 8.0 Hz, Ar^4-4′H), 8.11 (1H, s, Ar^4-2′H), 7.57 (1H, d, *J* 8.0 Hz, Ar^4-6′H), 7.48 (1H, t, *J* 8.0 Hz, Ar^4-5′H), 7.34 (5H, m, Bn-H), 7.20 (1H, t, *J* 8.1 Hz, H-7), 7.17 (1H, d, *J* 8.1 Hz, H-9), 7.06 (1H, t, *J* 8.1 Hz, H-8), 7.02 (1H, d, *J* 8.1 Hz, H-6), 5.18 (1H, s, H-4), 4.03 (1H, t, *J* 8.5 Hz, H-9b), 3.78 (1H, d, *J* 12.9 Hz, CH_2Ph), 3.58 (1H, d, *J* 12.9 Hz, CH_2Ph), 3.49 (1H, t, *J* 8.5 Hz, H-1), 3.38 (1H, d, *J* 11.1 Hz, H-3), 2.95 (1H, d, *J* 11.1 Hz, H-3), 2.93 (1H, t, *J* 8.5 Hz, H-1); ^{13}C NMR (62.5 MHz, $CDCl_3$): 153.6 (q), 148.2 (q), 137.4 (q), 136.6 (q), 133.0 (CH), 129.8 (CH), 128.9 (CH), 128.8 (CH), 128.7 (2×CH), 128.2 (CH), 127.9 (CH), 125.8 (CH), 124.4 (CH), 123.2 (CH), 122.9 (q), 122.3 (CH), 117.7 (CH), 94.9 (q), 79.1 (CH), 60.6 (CH_2), 59.05 (CH_2), 59.0 (CH_2), 42.4 (CH); IR (KBr, cm^{-1}): 2802, 1536, 1489, 1455, 1356, 1265, 1234, 1212, 1144, 1091, 1054.

3.1.2. Synthesis of 3-(4-chlorophenyl)-3a-nitro-4-aryl-benzopyrano[3,4-*c*]-pyrrolidine-1-carboxylates (6). General procedure. The appropriate 3-nitro-2*H*-chromene (**3**) (1 mmol) was dissolved in dry toluene (15 ml), and ethyl (4-chlorobenzylideneamino)acetate (**5a**) (0.25 g,

1.1 mmol) or ethyl 2-[1-(4-chlorophenyl)methylideneamino]-3-phenylpropanoate (**5b**) (0.35 g, 1.1 mmol) or diethyl 2-[1-(4-chlorophenyl)methylideneamino]malonate (**5c**) (0.33 g, 1.1 mmol), silver acetate (0.25 g, 1.5 mmol) and triethylamine (0.11 g, 0.16 ml, 1.1 mmol) were added. The reaction mixture was stirred at room temperature for 12 h. After the completion of the reaction (judged by TLC) saturated NH_4Cl solution (15 ml) was added to the reaction mixture and this was washed with water (2×10 ml) and brine (10 ml). The combined organic fractions were dried over magnesium sulfate, evaporated and the residue was trituated with ether. The crystallized product was collected to yield white powder, which could be recrystallized from ethanol. The yields (based on the dipolarophiles) are summarized in Table 2.

3.1.2.1. Ethyl 3-(4-chlorophenyl)-3a-nitro-4-phenyl-benzopyrano[3,4-*c*]-pyrrolidine-1-carboxylate (6a). White powder, mp 137–138 °C; [Found: C, 65.4; H, 4.8; N, 5.9. $C_{26}H_{23}N_2O_5Cl$ requires C, 65.2; H, 4.8; N, 5.8%]; ^{1}H NMR (250 MHz, $CDCl_3$): 7.51 (1H, d, *J* 7.6 Hz, H-9), 7.35 (2H, d, *J* 8.7 Hz, Ar^3-3′ and 5′H), 7.27 (2H, d, *J* 8.7 Hz, Ar^3-2′ and 6′H), 7.12 (7H, m, Ar-H), 6.77 (1H, d, *J* 7.5 Hz, H-6), 5.48 (1H, s, H-4), 4.88 (1H, br m, H-3), 4.74 (1H, d, *J* 3.6 Hz, H-9b), 4.43 (2H, q, *J* 7.1 Hz, OCH_2), 4.05 (1H, br s, H-1), 2.99 (1H, br s, H-2), 1.41 (3H, t, *J* 7.1 Hz, CH_2CH_3); ^{13}C NMR (62.5 MHz, $CDCl_3$): 171.8 (q), 149.7 (q), 135.4 (q), 134.7 (q), 129.1 (2×CH), 129.0 (CH), 128.9 (CH), 128.8 (CH), 128.5 (2×CH), 128.4 (q), 128.3 (2×CH), 128.2 (2×CH), 124.8 (CH), 123.2 (CH), 118.2 (CH), 96.4 (q), 75.5 (CH), 69.4 (CH), 68.3 (CH), 62.2 (CH_2), 45.6 (CH), 14.3 (CH_3); IR (KBr, cm^{-1}): 3334, 2979, 1733, 1586, 1540, 1487, 1453, 1368, 1298, 1228, 1212, 1114, 1094, 1015.

3.1.2.2. Methyl 1-benzyl-3-(4-chlorophenyl)-3a-nitro-4-phenyl-benzopyrano[3,4-*c*]-pyrrolidine-1-carboxylate (6b). White powder, mp 162–163 °C; [Found: C, 69.4; H, 4.8; N, 4.9. $C_{32}H_{27}N_2O_5Cl$ requires C, 69.2; H, 4.9; N, 5.0%]; ^{1}H NMR (250 MHz, $CDCl_3$): 7.84 (1H, d, *J* 7.5 Hz, H-9), 7.40 (2H, d, *J* 8.5 Hz, Ar^3-3′ and 5′H), 7.35 (2H, d, *J* 8.5 Hz, Ar^3-2′ and 6′H), 7.23 (9H, m, Ar-H), 7.13 (1H, t, *J* 7.5 Hz, H-8), 7.05 (2H, m, Bn-H), 6.83 (1H, d, *J* 7.5 Hz, H-6), 5.67 (1H, s, H-4), 5.21 (1H, s, H-9b), 5.20 (1H, d, *J* 7.2 Hz, H-3), 3.84 (3H, s, *O*Me), 3.01 (1H, br d, *J* 7.2 Hz, H-2), 2.89 (1H, d, *J* 14.0 Hz, α-CH_2), 2.45 (1H, d, *J* 14.0 Hz, β-CH_2); ^{13}C NMR (125 MHz, $CDCl_3$): 174.6 (q), 152.5 (q), 136.3 (q), 135.5 (q), 134.5 (q), 133.8 (q), 130.7 (CH), 130.7 (CH), 130.2 (2×CH), 129.3 (2×CH), 129.2 (q), 129.1 (CH), 128.6 (2×CH), 128.5 (4×CH), 128.3 (2×CH), 127.2 (CH), 123.1 (CH), 122.7 (q), 118.9 (CH), 98.8 (q), 78.0 (CH), 72.4 (q), 67.6 (CH), 52.8 (CH_3), 50.2 (CH), 42.5 (CH_2); IR (KBr, cm^{-1}): 3373, 3059, 3032, 2954, 1725, 1541, 1533, 1488, 1456, 1361, 1337, 1260, 1245, 1187, 1110, 1096, 1033, 1015.

3.1.2.3. Ethyl 3-(4-chlorophenyl)-4-(4-methoxyphenyl)-3a-nitro-benzopyrano[3,4-*c*]-pyrrolidine-1-carboxylate (6c). White powder, mp 145–146 °C; [Found: C, 63.4; H, 4.8; N, 5.5. $C_{27}H_{25}N_2O_6Cl$ requires C, 63.7; H, 4.9; N, 5.5%]; ^{1}H NMR (500 MHz, $CDCl_3$): 7.57 (d, 1H, *J* 7.5 Hz, H-9), 7.40 (2H, d, *J* 8.8 Hz, Ar^3-3′ and 5′H), 7.33 (2H, d, *J* 8.8 Hz, Ar^3-2′ and 6′H), 7.17 (1H, t, *J* 7.5 Hz, H-7), 7.09 (1H, t, *J* 7.5 Hz, H-8), 7.08 (2H, d, *J* 8.7 Hz, Ar^4-2′ and 6′H), 6.84 (d, 1H, *J* 7.5 Hz, H-6), 6.70 (2H, d, *J* 8.7 Hz, Ar^4-3′ and 5′H), 5.49 (1H, s, H-4), 4.94 (1H, d, *J* 7.4 Hz, H-3), 4.79 (1H, d, *J* 3.8 Hz, H-9b), 4.48 (2H, q, *J* 7.1 Hz, OCH_2), 4.12 (1H, br s, H-1), 3.69 (3H, s, *O*Me), 3.02 (1H, br s, H-2), 1.48 (3H, t, *J* 7.1 Hz, CH_2CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): 171.8 (q), 159.8 (q), 149.9 (q), 135.4 (q), 132.7 (q), 129.6 (2×CH), 129.1 (2×CH), 128.9 (CH), 128.7 (CH), 128.3 (2×CH), 126.9 (q), 124.9 (q), 123.2 (CH), 118.3 (CH), 113.8 (2×CH), 96.6 (q), 75.2 (CH), 69.4 (CH), 68.3 (CH), 62.1 (CH_2), 55.1 (CH_3), 45.6 (CH), 14.3 (CH_3); IR (KBr, cm^{-1}): 3331, 2936, 2837, 1733, 1612, 1586, 1539, 1515, 1489, 1457, 1300, 1253, 1212, 1185, 1111, 1036, 1015.

3.1.2.4. Methyl 1-benzyl-3-(4-chlorophenyl)-4-(4-methoxyphenyl)-3a-nitro-benzopyrano[3,4-*c*]-pyrrolidine-1-carboxylate (6d). White powder, mp 159–160 °C; [Found: C, 67.7; H, 4.8; N, 4.9. $C_{33}H_{29}N_2O_6Cl$ requires C, 67.7; H, 5.00; N, 4.8%]; ^{1}H NMR (250 MHz, $CDCl_3$): 7.90 (1H, d, *J* 8.0 Hz, H-9), 7.70 (2H, d, *J* 8.3 Hz, Ar^3-3′ and 5′H), 7.45 (2H, d, *J* 8.3 Hz, Ar^3-2′ and 6′H), 7.35 (8H, m, Ar-H), 7.18 (1H, t, *J* 8.0 Hz, H-8), 7.07 (2H, m, Bn-H), 6.77 (1H, d, *J* 7.5 Hz, H-6), 5.65 (1H, s, H-4), 5.22 (1H, s, H-9b), 5.21 (1H, d, *J* 7.0 Hz, H-3), 3.84 (3H, s, *O*Me), 3.70 (3H, s, *O*Me), 3.00 (1H, br d, *J* 7.0 Hz, H-2), 2.89 (1H, d, *J* 14.0 Hz, CH_2), 2.46 (1H, d, *J* 14.0 Hz, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$): 174.4 (q), 159.6 (q), 152.7 (q), 137.3 (q), 136.1 (q), 133.6 (q), 130.5 (CH), 129.9 (2×CH), 129.7 (2×CH), 129.6 (2×CH), 129.1 (CH), 128.8 (2×CH), 128.4 (CH), 128.3 (2×CH), 126.6 (q), 122.9 (CH), 122.5 (q), 118.8 (CH), 113.6 (2×CH), 98.8 (q), 77.5 (CH), 72.1 (q), 67.4 (CH), 55.1 (CH_3), 52.3 (CH_3), 49.9 (CH), 42.3 (CH_2); IR (KBr, cm^{-1}): 3436, 2954, 1746, 1544, 1513, 1490, 1455, 1244, 1184, 1112, 1028, 1014.

3.1.2.5. Ethyl 3,4-bis-(4-chlorophenyl)-3a-nitro-benzopyrano[3,4-*c*]-pyrrolidine-1-carboxylate (6e). White powder, mp 144 °C; [Found: C, 60.7; H, 4.6; N, 5.4. $C_{26}H_{22}N_2O_5Cl_2$ requires C, 60.8; H, 4.3; N, 5.5%]; ^{1}H NMR (250 MHz, $CDCl_3$): 7.54 (d, 1H, *J* 8.1 Hz, H-9), 7.38–7.22 (6H, m, Ar-H), 7.18–7.04 (4H, m, Ar-H), 6.82 (1H, d, *J* 8.1 Hz, H-6), 5.49 (1H, s, H-4), 4.91 (1H, s, H-3), 4.71 (1H, d, *J* 3.7 Hz, H-9b), 4.43 (2H, q, *J* 7.1 Hz, OCH_2), 4.12 (1H, d, *J* 3.7 Hz, H-1), 3.11 (1H, br s, H-2), 1.44 (3H, t, *J* 7.1 Hz, CH_2CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): 171.6 (q), 149.5 (q), 137.8 (q), 135.4 (q), 133.3 (q), 132.5 (q), 129.5 (2×CH), 129.1 (2×CH), 129.0 (CH), 128.7 (CH), 128.7 (2×CH), 128.2 (2×CH), 124.5 (q), 123.4 (CH), 118.2 (CH), 96.3 (q), 74.7 (CH), 69.3 (CH), 68.1 (CH), 62.1 (CH_2), 45.4 (CH), 14.2 (CH_3); IR (KBr, cm^{-1}): 2981, 2903, 1734, 1587, 1543, 1490, 1456, 1371, 1208, 1092, 1013.

3.1.2.6. Methyl 1-benzyl-3,4-bis-(4-chlorophenyl)-3a-nitro-benzopyrano[3,4-*c*]-pyrrolidine-1-carboxylate (6f). White powder, mp 168–169 °C; [Found: C, 65.0; H, 4.6; N, 4.4. $C_{32}H_{26}N_2O_5Cl_2$ requires C, 65.2; H, 4.4; N, 4.7%]; ^{1}H NMR (250 MHz, $CDCl_3$): 7.76 (1H, dd, *J* 1.7 and 7.8 Hz, H-9), 7.35 (2H, d, *J* 8.6 Hz, Ar^3-3′ and 5′H), 7.27 (2H, d, *J* 8.6 Hz, Ar^3-2′ and 6′H), 7.15 (4H, m, Bn-H and H-7), 7.14 (2H, d, *J* 8.5 Hz, Ar^4-3′ and 5′H), 7.10 (1H, dt, *J* 1.7 and 7.8 Hz, H-8), 7.05 (2H, d, *J* 8.5 Hz, Ar^4-2′ and 6′H), 6.96 (2H, m, Bn-H), 6.76 (1H, dd, *J* 1.7 and 7.8 Hz, H-6), 5.55 (1H, s, H-4), 5.10 (1H, s, H-9b), 5.09

(1H, d, *J* 7.8 Hz, H-3), 3.78 (3H, s, *O*Me), 2.94 (1H, br d, *J* 7.8 Hz, H-2), 2.81 (1H, d, *J* 13.7 Hz, CH_2), 2.37 (1H, d, *J* 13.7 Hz, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$): 174.4 (q), 152.1 (q), 136.0 (q), 135.4 (q), 134.9 (q), 133.3 (q), 132.9 (q), 130.6 (CH), 129.9 (2×CH), 129.6 (2×CH), 129.3 (CH), 129.2 (2×CH), 128.5 (2×CH), 128.3 (2×CH), 128.1 (2×CH), 127.0 (CH), 123.1 (CH), 122.2 (q), 118.7 (CH), 98.5 (q), 77.0 (CH), 72.2 (q), 67.4 (CH), 52.7 (CH_3), 49.8 (CH), 42.2 (CH_2); IR (KBr, cm^{-1}): 3341, 3031, 1751, 1601, 1542, 1491, 1456, 1436, 1239, 1208, 1130, 1111, 1096, 1079, 1042, 1014, 1006.

3.1.2.7. Methyl 1-benzyl-3-(4-chlorophenyl)-4-(2-chlorophenyl)-3a-nitro-benzopyrano[3,4-*c*]-pyrrolidine-1-carboxylate (6g). White powder, mp 151–152 °C; [Found: C, 65.3; H, 4.2; N, 4.6. $C_{32}H_{26}N_2O_5Cl_2$ requires C, 65.2; H, 4.4; N, 4.7%]; ^{1}H NMR (500 MHz, $CDCl_3$): 7.75 (1H, d, *J* 7.6 Hz, H-9), 7.36 (5H, m, Bn-H), 7.17 (3H, m, Ar^3-3′ and 5′H, Ar^4-3′H), 7.11 (2H, m, H-7 and Ar^4-4′H), 7.08 (1H, t, *J* 7.6 Hz, H-8), 6.98 (2H, d, *J* 7.7 Hz, Ar^3-2′ and 6′H), 6.89 (1H, t, *J* 7.6 Hz, Ar^4-5′H), 6.78 (1H, d, *J* 7.6 Hz, H-6), 6.75 (1H, d, *J* 7.6 Hz, Ar^4-6′H), 6.18 (1H, s, H-4), 5.21 (1H, d, *J* 8.5 Hz, H-3), 5.13 (1H, s, H-9b), 3.82 (3H, s, *O*Me), 3.01 (1H, d, *J* 8.5 Hz, H-2), 2.75 (1H, d, *J* 13.6 Hz, CH_2), 2.40 (1H, d, *J* 13.6 Hz, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$): 174.2 (q), 152.0 (q), 136.1 (q), 135.5 (q), 135.3 (q), 131.8 (q), 130.3 (CH), 130.2 (CH), 130.0 (CH), 127.0 (q), 129.9 (2×CH), 129.3 (CH), 129.2 (2×CH), 128.25 (2×CH), 128.2 (CH), 128.1 (2×CH), 128.0 (CH), 126.1 (CH), 123.3 (CH), 123.0 (q), 118.7 (CH), 99.1 (q), 73.8 (CH), 72.6 (q), 68.0 (CH), 52.7 (CH_3), 50.5 (CH), 41.6 (CH_2); IR (KBr, cm^{-1}): 3364, 3021, 1723, 1542, 1535, 1489, 1472, 1439, 1261, 1209, 1186, 1112, 1094, 1052, 1034, 1014.

3.1.2.8. Ethyl 3-(4-chlorophenyl)-4-(3,4-dimethoxyphenyl)-3a-nitro-benzopyrano[3,4-*c*]-pyrrolidine-1-carboxylate (6h). White powder, mp 137–139 °C; [Found: C, 62.5; H, 4.9; N, 5.4. $C_{28}H_{27}N_2O_7Cl$ requires C, 62.4; H, 5.0; N, 5.2%]; ^{1}H NMR (250 MHz, $CDCl_3$): 7.54 (1H, d, *J* 7.5 Hz, H-9), 7.38 (2H, d, *J* 8.4 Hz, Ar^3-3′ and 5′H), 7.31 (2H, d, *J* 8.4 Hz, Ar^3-2′ and 6′H), 7.16 (1H, t, *J* 7.5 Hz, H-7), 7.07 (1H, t, *J* 7.5 Hz, H-8), 6.83 (1H, d, *J* 8.1 Hz, H-6), 6.70 (1H, s, Ar^4-2′H), 6.61 (2H, m, Ar^4-5′ and 6′H), 5.46 (1H, s, H-4), 4.92 (1H, s, H-3), 4.76 (1H, d, *J* 3.6 Hz, H-9b), 4.45 (2H, q, *J* 7.1 Hz, OCH_2), 4.09 (1H, d, *J* 3.7 Hz, H-1), 3.73 (3H, s, OMe), 3.70 (3H, s, OMe), 3.00 (1H, br s, H-2), 1.45 (3H, t, *J* 7.1 Hz, CH_2CH_3); ^{13}C NMR (62.5 MHz, $CDCl_3$): 172.0 (q), 150.1 (q), 149.6 (q), 148.9 (q), 135.5 (q), 132.9 (q), 129.3 (2×CH), 129.1 (CH), 128.8 (CH), 128.5 (2×CH), 127.3 (q), 125.2 (q), 123.4 (CH), 120.3 (CH), 118.5 (CH), 112.3 (CH), 110.7 (CH), 96.8 (q), 75.5 (CH), 69.5 (CH), 68.3 (CH), 62.3 (CH_2), 55.9 (CH_3), 55.8 (CH_3), 45.7 (CH), 14.5 (CH_3); IR (KBr, cm^{-1}): 3336, 2981, 2935, 2836, 1737, 1589, 1543, 1519, 1490, 1453, 1366, 1261, 1245, 1210, 1146, 1092, 1020.

3.1.2.9. Methyl 1-benzyl-3-(4-chlorophenyl)-4-(3,4-dimethoxyphenyl)-3a-nitro-benzopyrano[3,4-*c*]-pyrrolidine-1-carboxylate (6i). White powder, mp 143–144 °C; [Found: C, 66.5; H, 4.9; N, 4.3. $C_{34}H_{31}N_2O_7Cl$ requires C, 66.4; H, 5.1; N, 4.5%]; ^{1}H NMR (250 MHz, $CDCl_3$): 7.77 (1H, d, *J* 8.0 Hz, H-9), 7.35 (2H, d, *J* 8.5 Hz, Ar^3-3′ and 5′H), 7.30 (2H, d, *J* 8.5 Hz, Ar^3-2′ and 6′H), 7.26 (4H, m, H-7 and Bn-H), 7.19 (1H, t, *J* 7.5 Hz, H-8), 7.06 (2H, m, Bn-H), 6.87 (1H, d, *J* 7.5 Hz, H-6), 6.75 (1H, s, Ar^4-2′H), 6.67 (2H, m, Ar^4-5′ and 6′H), 5.62 (1H, s, H-4), 5.23 (1H, s, H-9b), 5.23 (1H, d, *J* 7.0 Hz, H-3), 3.88 (3H, s, *O*Me), 3.83 (3H, s, *O*Me), 3.82 (3H, s, *O*Me), 3.00 (1H, br d, *J* 7.0 Hz, H-2), 2.92 (1H, d, *J* 13.5 Hz, CH_2), 2.47 (1H, d, *J* 13.5 Hz, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$): 174.5 (q), 152.3 (q), 149.3 (q), 148.6 (q), 136.1 (q), 135.3 (q), 133.5 (q), 130.4 (CH), 129.9 (2×CH), 129.2 (CH), 129.1 (2×CH), 128.4 (2×CH), 128.1 (2×CH), 127.0 (CH), 126.6 (q), 122.9 (CH), 122.7 (q), 120.7 (CH), 118.9 (CH), 111.8 (CH), 110.3 (CH), 98.9 (q), 77.7 (CH), 72.3 (q), 67.4 (CH), 55.8 (CH_3), 55.6 (CH_3), 52.7 (CH_3), 49.9 (CH), 42.1 (CH_2); IR (KBr, cm^{-1}): 3337, 2986, 2950, 1747, 1545, 1515, 1490, 1454, 1261, 1240, 1209, 1113, 1030, 1010.

3.1.2.10. Methyl 1-benzyl-3-(4-chlorophenyl)-4-(3-nitrophenyl)-3a-nitro-benzopyrano[3,4-*c*]-pyrrolidine-1-carboxylate (6j). White powder, mp 165 °C; [Found: C, 64.1; H, 4.2; N, 6.9. $C_{32}H_{26}N_3O_7Cl$ requires C, 64.1; H, 4.4; N, 7.0%]; ^{1}H NMR (250 MHz, DMSO-d_6): 8.29 (1H, s, Ar^3-2′H), 8.11 (1H, d, *J* 8.1 Hz, Ar^3-4′H), 7.81 (1H, d, *J* 7.0 Hz, H-9), 7.59 (1H, d, *J* 8.1 Hz, Ar^4-6′H), 7.51–7.38 (5H, m, Ar-H), 7.28–7.11 (5H, m, Ar-H), 7.03 (2H, m, Bn-H), 6.82 (1H, d, *J* 7.5 Hz, H-6), 5.97 (1H, s, H-4), 5.27 (1H, d, *J* 9.0 Hz, H-3), 5.28 (1H, s, H-9b), 3.82 (3H, s, *O*Me), 3.26 (1H, br d, *J* 9.0 Hz, H-2), 2.65 (1H, d, *J* 13.5 Hz, CH_2), 2.52 (1H, d, *J* 13.5 Hz, CH_2); ^{13}C NMR (62.5 MHz, DMSO-d_6): 173.6 (q), 151.8 (q), 148.1 (q), 136.8 (q), 136.1 (q), 134.1 (q), 134.0 (CH), 133.7 (q), 130.5 (CH), 130.1 (2×CH), 129.6 (CH), 129.3 (CH), 129.1 (CH), 129.0 (2×CH), 128.7 (2×CH), 127.7 (2×CH), 126.6 (CH), 123.7 (CH), 123.0 (CH), 122.4 (q), 118.0 (CH), 98.9 (q), 76.0 (CH), 71.9 (q), 66.7 (CH), 52.7 (CH_3), 50.4 (CH), 41.0 (CH_2); IR (KBr, cm^{-1}): 3370, 3097, 2952, 1730, 1585, 1534, 1488, 1456, 1356, 1288, 1241, 1208, 1187, 1112, 1094, 1040, 1013.

3.1.2.11. Diethyl 3-(4-chlorophenyl)-4-phenyl-3a-nitro-benzopyrano[3,4-*c*]-pyrrolidine-1,1-dicarboxylate (6k). White powder, mp 126–127 °C; [Found: C, 63.1; H, 5.0; N, 5.9. $C_{29}H_{27}N_2O_7Cl$ requires C, 63.2; H, 4.9; N, 5.1%]; ^{1}H NMR (500 MHz, $CDCl_3$): 7.54 (1H, d, *J* 7.5 Hz, H-9), 7.27 (4H, s, Ar^3-H), 7.18 (5H, m, Ar^4-H), 7.05 (1H, t, *J* 7.5 Hz, H-7), 6.93 (1H, t, *J* 7.5 Hz, H-8), 6.72 (1H, d, *J* 7.5 Hz, H-6), 5.71 (1H, s, H-9b), 5.57 (1H, s, H-4), 5.54–5.57 (1H, s, H-3), 4.45 (1H, m, OCH_2), 4.37 (1H, m, OCH_2), 3.74 (1H, m, OCH_2), 3.60 (1H, m, OCH_2), 3.30 (1H, br s, H-2), 1.35 (3H, t, *J* 7.1 Hz, CH_2CH_3), 0.89 (3H, t, *J* 7.1 Hz, CH_2CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): 170.5 (q), 169.4 (q), 151.9 (q), 134.9 (q), 134.8 (q), 134.1 (q), 130.0 (CH), 129.0 (CH), 128.7 (2×CH), 128.6 (2×CH), 128.2 (2×CH), 128.1 (2×CH), 128.05 (CH), 122.1 (CH), 121.1 (q), 118.0 (CH), 96.7 (q), 77.3 (CH), 76.3 (q), 67.8 (CH), 62.5 (CH_2), 62.0 (CH_2), 47.1 (CH), 13.9 (CH_3), 13.3 (CH_2); IR (KBr, cm^{-1}): 3340, 3067, 3037, 2985, 2899, 1739, 1586, 1546, 1490, 1456, 1367, 1271, 1255, 1215, 1174, 1143, 1120, 1092, 1038, 1016.

3.1.3. Synthesis of methyl 4-aryl-3-phenyl-3a-nitro-2-methyl-benzopyrano[3,4-*c*]-pyrrolidine-1-carboxylates (7). General procedure. Methyl sarcosinate hydrochloride

(0.23 g, 2 mmol), the corresponding 3-nitrochromene (**3**) (1 mmol), benzaldehyde (0.15 g, 1.4 mmol) and triethylamine (0.20 g, 0.29 ml, 2 mmol) were heated under reflux in toluene (20 ml). The water formed was continuously removed by the aid of a Dean–Stark trap. After the completion of reaction, the mixture was poured into saturated NH_4Cl solution (20 ml) and was extracted with ethyl acetate (2×15 ml). The combined organic extracts were dried over $MgSO_4$, filtered and evaporated in vacuo. The residue was recrystallized from ethanol to yield the title products. The reaction yields (based on the dipolarophiles) are summarized in Table 5.

3.1.3.1. Methyl 3,4-diphenyl-3a-nitro-2-methyl-benzopyrano[3,4-*c*]-pyrrolidine-1-carboxylate (7a). White powder, mp 112–113 °C; [Found: C, 70.2; H, 5.4; N, 6.1. $C_{26}H_{24}N_2O_5$ requires C, 70.3; H, 5.4; N, 6.3%]; ^{1}H NMR (500 MHz, $CDCl_3$): 7.45 (5H, m, Ph^3-H), 7.09 (1H, m, Ph^4-4′H), 7.08 (1H, dd, *J* 1.5 and 7.5 Hz, H-9), 7.05 (1H, dt, *J* 1.5 and 7.5 Hz, H-7), 7.02 (2H, t, *J* 8.7 Hz, Ph^4-3′ and 5′H), 6.90 (1H, dt, *J* 1.5 and 7.5 Hz, H-8), 6.85 (2H, d, *J* 8.7 Hz, Ph^4-2′ and 6′H), 6.66 (1H, dd, *J* 1.5 and 7.5 Hz, H-6), 5.16 (1H, s, H-4), 5.02 (1H, d, *J* 6.5 Hz, H-9b), 3.88 (1H, d, *J* 6.5 Hz, H-1), 3.85 (1H, s, H-3), 3.69 (3H, s, *O*Me), 2.18 (3H, s, *N*Me); ^{13}C NMR (125 MHz, $CDCl_3$): 169.4 (q), 151.9 (q), 134.9 (q), 134.4 (q), 129.2 (CH), 129.0 (CH), 128.8 (CH), 128.2 (4×CH), 128.15 (4×CH), 127.7 (CH), 122.5 (q), 121.9 (CH), 118.7 (CH), 95.6 (q), 81.8 (CH), 76.8 (CH), 73.3 (CH), 51.8 (CH_3), 43.1 (CH), 39.9 (CH_3); IR (KBr, cm^{-1}): 2848, 2807, 1761, 1588, 1543, 1490, 1454, 1386, 1280, 1240, 1211, 1197, 1132, 1116, 1084, 1072.

3.1.3.2. Methyl 4-(2-methoxyphenyl)-3a-nitro-2-methyl-3-phenyl-benzopyrano[3,4-*c*]-pyrrolidine-1-carboxylate (7b). White powder, mp 118–119 °C; [Found: C, 68.2; H, 5.4; N, 6.0. $C_{27}H_{26}N_2O_6$ requires C, 68.3; H, 5.5; N, 5.9%]; ^{1}H NMR (500 MHz, $CDCl_3$): 7.33 (2H, br s, Ph^3-3′ and 5′H), 7.14 (1H, t, *J* 7.5 Hz, H-7), 7.00–7.10 (4H, m, H-8, H-9, Ar^4-H, Ph^3-4′H), 6.88 (1H, d, *J* 7.5 Hz, H-6), 6.84 (1H, d, *J* 7.9 Hz, Ar^4-H), 6.78 (1H, t, *J* 7.9 Hz, Ar^4-H), 6.67 (2H, d, *J* 7.9 Hz, Ph^3-2′ and 6′H), 6.53 (1H, t, *J* 7.9 Hz, Ar^4-H), 5.67 (1H, s, H-4), 5.06 (1H, d, *J* 6.7 Hz, H-9b), 3.91 (1H, d, *J* 6.5 Hz, H-1), 3.84 (1H, s, H-3), 3.69 (3H, s, *O*Me), 3.61 (3H, s, *O*Me), 2.19 (3H, s, *N*Me); ^{13}C NMR (125 MHz, $CDCl_3$): 170.2 (q, C═O), 158.4 (q), 153.1 (q), 135.3 (q), 131.0 (q), 130.8 (CH), 129.9 (CH), 129.6 (2×CH), 129.2 (CH), 128.4 (2×CH), 128.3 (CH), 124.3 (CH), 122.6 (CH), 121.2 (q), 120.6 (CH), 119.6 (CH), 112.0 (CH), 96.2 (q), 83.2 (CH), 75.5 (CH), 74.5 (CH), 56.5 (CH_3), 52.5 (CH_3), 44.5 (CH), 40.7 (CH_3); IR (KBr, cm^{-1}): 2964, 2840, 1763, 1723, 1588, 1545, 1492, 1457, 1295, 1252, 1225, 1213, 1173, 1107, 1073, 1030.

3.1.3.3. Methyl 4-(2-chlorophenyl)-3a-nitro-2-methyl-3-phenyl-benzopyrano[3,4-*c*]-pyrrolidine-1-carboxylate (7c). White powder, mp 111–112 °C; [Found: C, 65.2; H, 5.0; N, 6.0. $C_{26}H_{23}ClN_2O_5$ requires C, 65.2; H, 4.8; N, 5.8%]; ^{1}H NMR (500 MHz, $CDCl_3$): 7.49 (4H, m, Ph), 7.25 (2H, m, Ph and Ar^4-6′H), 7.11 (1H, d, *J* 7.8 Hz, H-9), 7.04 (1H, t, *J* 7.8 Hz, H-7), 7.03 (1H, d, *J* 7.7 Hz, Ar^4-4′H), 6.92 (1H, t, *J* 7.8 Hz, H-8), 6.83 (1H, t, *J* 7.7 Hz, Ar^4-5′H), 6.71 (2H, m, H-6 and Ar^4-3′H), 5.83 (1H, s, H-4), 5.06 (1H, d, *J* 6.9 Hz, H-9b), 3.96 (1H, d, *J* 6.9 Hz, H-1), 3.87 (1H, s, H-3), 3.69 (3H, s, *O*Me), 2.23 (3H, s, *N*Me); ^{13}C NMR (125 MHz, $CDCl_3$): 169.2 (q), 151.9 (q), 135.6 (q), 134.0 (q), 132.5 (q), 130.2 (CH), 129.8 (CH), 129.4 (CH), 129.2 (CH), 128.3 (CH), 127.7 (CH), 127.2 (2×CH), 126.1 (2×CH), 122.8 (q), 122.1 (CH), 118.9 (CH), 94.9 (q), 82.4 (CH), 73.8 (CH), 72.2 (CH), 51.8 (CH_3), 43.7 (CH), 39.9 (CH_3); IR (KBr, cm^{-1}): 3335, 2977, 2905, 1727, 1542, 1507, 1489, 1456, 1359, 1300, 1275, 1232, 1215, 1130, 1114, 1097, 1052, 1016.

3.1.3.4. Methyl 4-(4-chlorophenyl)-3a-nitro-2-methyl-3-phenyl-benzopyrano[3,4-*c*]-pyrrolidine-1-carboxylate (7d). White powder, mp 131–132 °C; [Found: C, 65.1; H, 5.1; N, 5.9. $C_{26}H_{23}ClN_2O_5$ requires C, 65.2; H, 4.8; N, 5.8%]; ^{1}H NMR (500 MHz, $CDCl_3$): 7.48 (2H, d, *J* 8.1 Hz, Ar^4-H), 7.41 (2H, d, *J* 8.1 Hz, Ar^4-H), 7.44 (1H, d, *J* 7.6 Hz, H-9), 7.25 (3H, m, Ph^3-C), 7.17 (1H, t, *J* 7.6 Hz, H-7), 7.05 (2H, m, Ph^3-C), 7.03 (1H, t, *J* 7.6 Hz, H-8), 6.91 (1H, d, *J* 7.6 Hz, H-6), 5.85 (1H, s, H-4), 5.14 (1H, d, *J* 9.8 Hz, H-9b), 4.87 (1H, s, H-3), 4.76 (1H, d, *J* 6.5 Hz, H-1), 3.44 (3H, s, *O*Me), 2.31 (3H, s, *N*Me); ^{13}C NMR (125 MHz, $CDCl_3$): 171.9 (q), 154.5 (q), 135.7 (q), 134.9 (q), 133.9 (q), 129.9 (2×CH), 129.5 (CH), 128.7 (CH), 128.6 (CH), 128.5 (3×CH), 128.3 (q), 128.0 (2×CH), 122.0 (CH), 119.1 (CH), 117.4 (CH), 97.8 (q), 78.6 (CH), 71.9 (CH), 69.7 (CH), 51.5 (CH_3), 44.3 (CH), 35.6 (CH_3); IR (KBr, cm^{-1}): 2950, 2811, 1739, 1588, 1541, 1491, 1457, 1438, 1372, 1261, 1233, 1219, 1179, 1089, 1014.

3.1.3.5. Methyl 4-(3,4-dimethoxyphenyl)-3a-nitro-2-methyl-3-phenyl-benzopyrano[3,4-*c*]-pyrrolidine-1-carboxylate (7e). White powder, mp 125–126 °C; [Found: C, 66.6; H, 5.6; N, 5.5. $C_{28}H_{28}N_2O_7$ requires C, 66.7; H, 5.6; N, 5.5%]; ^{1}H NMR (300 MHz, $CDCl_3$): 7.29 (1H, d, *J* 7.5 Hz, H-9), 7.24 (3H, m, Ph-H and H-9), 7.15 (1H, t, *J* 7.5 Hz, H-7), 7.04 (3H, m, Ph-H), 7.00 (1H, d, *J* 7.5 Hz, H-6), 6.95 (1H, s, Ar^4-2′H), 6.89 (1H, d, *J* 8.1 Hz, Ar^4-5′H), 6.87 (1H, d, *J* 8.1 Hz, Ar^4-6′H), 5.77 (1H, s, H-4), 5.10 (1H, d, *J* 9.9 Hz, H-9b), 4.90 (1H, s, H-3), 4.72 (1H, d, *J* 9.6 Hz, H-1), 3.91 (3H, s, *O*Me), 3.86 (3H, s, *O*Me), 3.40 (3H, s, *O*Me), 2.27 (3H, s, *N*Me); ^{13}C NMR (125 MHz, $CDCl_3$): 171.9 (q), 154.6 (q), 149.6 (q), 148.5 (q), 135.9 (q), 129.5 (CH), 128.6 (CH), 128.5 (4×CH), 128.4 (q), 127.5 (CH), 121.8 (q), 121.3 (CH), 119.6 (CH), 117.6 (CH), 111.7 (CH), 110.0 (CH), 97.8 (q), 79.2 (CH), 76.6 (CH), 72.1 (CH), 56.0 (CH_3), 55.9 (CH_3), 51.5 (CH_3), 44.5 (CH), 35.6 (CH_3); IR (KBr, cm^{-1}): 2952, 2838, 1735, 1588, 1545, 1519, 1491, 1465, 1261, 1236, 1209, 1145, 1027.

3.1.3.6. Methyl 4-(3,4-methylenedioxyphenyl)-3a-nitro-2-methyl-3-phenyl-benzopyrano[3,4-*c*]-pyrrolidine-1-carboxylate (7f). White powder, mp 119–120 °C; [Found: C, 66.5; H, 5.1; N, 5.6. $C_{27}H_{24}N_2O_7$ requires C, 66.4; H, 4.9; N, 5.7%]; ^{1}H NMR (500 MHz, DMSO-d_6): 7.42 (1H, d, *J* 7.5 Hz, H-9), 7.31 (3H, m, Ph-H and H-9), 7.18 (3H, m, Ph-H), 7.05 (1H, t, *J* 7.5 Hz, H-7), 7.00 (1H, d, *J* 8.2 Hz, Ar^4-5′H), 6.93 (1H, d, *J* 7.5 Hz, H-6), 6.89 (1H, d, *J* 8.2 Hz, Ar^4-6′H), 6.79 (1H, s, Ar^4-2′H), 6.09 (1H, s, OCH_2O), 6.07 (1H, s, OCH_2O), 5.86 (1H, s, H-4), 5.21 (1H, d, *J* 10.2 Hz, H-9b), 4.75 (1H, s, H-3), 4.73 (1H, d, *J* 10.2 Hz, H-1), 3.31 (3H, s, *O*Me), 2.14 (3H, s, *N*Me); ^{13}C NMR (125 MHz, DMSO-d_6): 171.8 (q), 153.7 (q), 148.0 (q), 147.2 (q), 135.5 (q), 130.1 (CH), 128.65

(5×CH), 128.6 (CH), 128.5 (q), 122.1 (q), 121.8 (CH), 119.9 (CH), 116.8 (CH), 107.95 (CH), 107.9 (CH), 101.5 (CH_2), 96.7 (q), 78.2 (CH), 71.6 (CH), 69.4 (CH), 51.6 (CH_3), 43.2 (CH), 35.6 (CH_3); IR (KBr, cm^{-1}): 2949, 2867, 2808, 1733, 1544, 1506, 1491, 1457, 1443, 1391, 1368, 1334, 1305, 1250, 1240, 1211, 1173, 1112, 1096, 1040.

3.1.4. Synthesis of methyl 8,9-dimethoxy-6a-nitro-6-aryl-6a,6b,11,12,14,14a-hexahydro-6*H*-chromeno[3′,4′:3,4]-pyrrolidino[2,1-*a*]isoquinoline-14-carboxylates (9). General procedure. The corresponding 3-nitrochromene (**3**) (0.8 mmol) and 6,7-dimethoxy-(2-methoxycarbonylmethyl)-3,4-dihydroisoquinolinium bromide (**8**) (0.29 g, 0.85 mmol) were dissolved in dry methanol (10 ml) and triethylamine (0.14 ml, 0.10 g, 1 mmol) was added under argon atmosphere. The reaction mixture was stirred at room temperature for 4 h. The solvent was removed in vacuo and the residue was suspended in ether (20 ml). The ethereal solution was washed with water (10 ml) and brine (5 ml), dried over $MgSO_4$ and evaporated in vacuo to yield a white solid, which was recrystallized from ethanol. The reaction yields (based on the dipolarophiles) are summarized in Table 7.

3.1.4.1. Methyl 8,9-dimethoxy-6a-nitro-6-phenyl-6a,6b,11,12,14,14a-hexahydro-6*H*-chromeno[3′,4′:3,4]-pyrrolidino[2,1-*a*]isoquinoline-14-carboxylate (9a). White powder, mp 152–153 °C; [Found: C, 67.5; H, 5.3; N, 5.3. $C_{29}H_{28}N_2O_7$ requires C, 67.4; H, 5.5; N, 5.4%]; ^{1}H NMR (250 MHz, $CDCl_3$): 7.22 (1H, t, *J* 7.5 Hz, H-3), 7.12 (1H, d, *J* 7.5 Hz, H-1), 7.06 (1H, d, *J* 7.5 Hz, H-4), 7.02 (1H, t, *J* 7.5 Hz, H-2), 6.95 (5H, m, Ph-H), 6.51 (1H, s, H-10), 6.05 (1H, s, H-7), 5.81 (1H, s, H-6), 4.88 (1H, s, H-6b), 4.18 (1H, d, *J* 11.2 Hz, H-14a), 4.12 (1H, d, *J* 11.2 Hz, H-14), 3.83 (3H, s, *O*Me), 3.37 (3H, s, *O*Me), 3.25 (3H, s, *O*Me), 3.20 (1H, m, H-11), 3.03 (1H, dd, *J* 6.6 and 10.8 Hz, H-12), 2.74 (1H, dd, *J* 3.2 and 11.1 Hz, H-12), 2.64 (1H, m, H-11); ^{13}C NMR (125 MHz, $CDCl_3$): 170.5 (q), 153.5 (q), 147.9 (q), 146.5 (q), 135.9 (q), 129.9 (CH), 129.5 (CH), 128.9 (2×CH), 128.4 (CH), 128.2 (q), 127.4 (q), 127.4 (2×CH), 123.2 (q), 120.7 (CH), 117.1 (CH), 110.8 (CH), 109.8 (CH), 90.4 (q), 76.4 (CH), 67.8 (CH), 65.9 (CH), 55.8 (CH_3), 54.8 (CH_3), 51.9 (CH_3), 47.4 (CH), 46.9 (CH_2), 29.7 (CH_2); IR (KBr, cm^{-1}): 3019, 2949, 2886, 2843, 1754, 1615, 1586, 1537, 1518, 1493, 1454, 1390, 1357, 1290, 1265, 1249, 1234, 1213, 1178, 1152, 1119, 1041, 1024.

3.1.4.2. Methyl 8,9-dimethoxy-6a-nitro-6-(4-methoxyphenyl)-6a,6b,11,12,14,14a-hexahydro-6*H*-chromeno[3′,4′:3,4]pyrrolidino[2,1-*a*]isoquinoline-14-carboxylate (9b). White powder, mp 159–160 °C; [Found: C, 65.9; H, 5.5; N, 5.1. $C_{30}H_{30}N_2O_8$ requires C, 65.9; H, 5.5; N, 5.1%]; ^{1}H NMR (500 MHz, $CDCl_3$): 7.18 (1H, t, *J* 7.5 Hz, H-3), 7.06 (1H, d, *J* 7.5 Hz, H-1), 6.93 (1H, d, *J* 7.5 Hz, H-4), 6.90 (1H, t, *J* 7.5 Hz, H-2), 6.85 (2H, d, *J* 8.2 Hz, Ar^6-2′ and 6′H), 6.51 (1H, s, H-10), 6.46 (2H, d, *J* 8.2 Hz, Ar^6-3′ and 5′H), 6.10 (1H, s, H-7), 5.77 (1H, s, H-6), 4.86 (1H, s, H-6b), 4.12 (1H, d, *J* 11.3 Hz, H-14a), 4.11 (1H, d, *J* 11.3 Hz, H-14), 3.83 (3H, s, *O*Me), 3.70 (3H, s, *O*Me), 3.36 (3H, s, *O*Me), 3.32 (3H, s, *O*Me), 3.18 (1H, m, H-11), 3.01 (1H, m, H-12), 2.70 (1H, m, H-12), 2.62 (1H, m, H-11); ^{13}C NMR (125 MHz, $CDCl_3$): 170.4 (q), 159.6 (q), 153.6 (q), 147.8 (q), 146.7 (q), 129.9 (2×CH), 129.4 (CH), 128.8 (CH), 128.0 (q), 127.5 (q), 127.4 (q), 123.4 (q), 120.5 (CH), 116.2 (CH), 113.2 (CH), 112.9 (2×CH), 109.8 (CH), 90.4 (q), 75.8 (CH), 67.7 (CH), 65.7 (CH), 55.8 (CH_3), 55.2 (CH_3), 54.6 (CH_3), 51.7 (CH_3), 47.2 (CH), 46.8 (CH_2), 29.7 (CH_2); IR (KBr, cm^{-1}): 2990, 2945, 2913, 2835, 1749, 1612, 1585, 1552, 1519, 1490, 1459, 1437, 1353, 1249, 1212, 1193, 1150, 1117, 1076, 1042, 1021.

3.1.4.3. Methyl 8,9-dimethoxy-6a-nitro-6-(3,4-dimethoxyphenyl)-6a,6b,11,12,14,14a-hexahydro-6*H*-chromeno[3′,4′:3,4]pyrrolidino[2,1-*a*]isoquinoline-14-carboxylate (9c). White powder, mp 146–147 °C; [Found: C, 64.6; H, 5.4; N, 5.0. $C_{31}H_{32}N_2O_9$ requires C, 64.6; H, 5.6; N, 4.9%]; ^{1}H NMR (500 MHz, $CDCl_3$): 7.19 (1H, t, *J* 7.5 Hz, H-3), 7.07 (1H, d, *J* 7.5 Hz, H-1), 6.96 (1H, d, *J* 7.5 Hz, H-4), 6.92 (1H, t, *J* 7.5 Hz, H-2), 6.58 (1H, s, Ar^6-2′H), 6.51 (1H, s, H-10), 6.43 (1H, d, *J* 8.3 Hz, Ar^6-5′H), 6.37 (1H, d, *J* 8.3 Hz, Ar^6-6′H), 6.12 (1H, s, H-7), 5.75 (1H, s, H-6), 4.86 (1H, s, H-6b), 4.13 (1H, d, *J* 11.2 Hz, H-14a), 4.10 (1H, d, *J* 11.2 Hz, H-14), 3.82 (3H, s, *O*Me), 3.78 (3H, s, *O*Me), 3.69 (3H, s, *O*Me), 3.38 (3H, s, *O*Me), 3.34 (3H, s, *O*Me), 3.18 (1H, m, H-11), 3.03 (1H, dd, *J* 6.0 and 10.5 Hz, H-12), 2.71 (1H, m, H-12), 2.64 (1H, m, H-11); ^{13}C NMR (125 MHz, $CDCl_3$): 170.4 (q), 153.5 (q), 149.1 (q), 148.2 (q), 147.8 (q), 146.5 (q), 129.4 (CH), 128.8 (CH), 128.2 (q), 127.6 (q), 127.2 (q), 123.5 (q), 121.6, 120.6 (CH), 117.0 (CH), 116.2 (CH), 111.4 (CH), 110.8 (CH), 110.3 (CH), 109.8 (CH), 90.4 (q), 76.1 (CH), 67.6 (CH), 65.7 (CH), 55.9 (CH_3), 55.7 (CH_3), 55.3 (CH_3), 54.5 (CH_3), 51.7 (CH_3), 47.3 (CH), 46.9 (CH_2), 29.7 (CH_2); IR (KBr, cm^{-1}): 2995, 2953, 2908, 2834, 2797, 1733, 1610, 1589, 1540, 1520, 1458, 1324, 1266, 1234, 1143, 1027.

3.1.4.4. Methyl 8,9-dimethoxy-6a-nitro-6-phenyl-11,12-dihydro-6*H*-chromeno[3′,4′:3,4]pyrrolidino[2,1-*a*]isoquinoline-14-carboxylate (10). Compound **10** was prepared analogously to **9a** from **3a** but *not under argon atmosphere*. The product was isolated after column chromatography (eluent: hexanes–acetone 3:1 v/v) as a white powder (0.13 g, 32%); mp 133–134 °C; [Found: C, 74.7; H, 5.6; N, 3.0. $C_{29}H_{25}NO_5$ requires C, 74.5; H, 5.4; N, 3.0%]; ^{1}H NMR (250 MHz, $CDCl_3$): 7.95 (1H, d, *J* 7.5 Hz, H-1), 7.40 (2H, m, Ph-H), 7.29 (3H, m, Ph-H), 6.95 (1H, t, *J* 7.5 Hz, H-3), 6.84 (1H, t, *J* 7.5 Hz, H-2), 6.74 (1H, d, *J* 7.5 Hz, H-4), 6.66 (1H, s, H-10), 6.43 (1H, s, H-7), 6.31 (1H, s, H-6), 5.06 (1H, ddd, *J* 13.4, 5.4 and 2.7 Hz, H-12), 3.83 (3H, s, OMe), 3.81 (3H, s, OMe), 3.79 (1H, m, H-12), 3.75 (3H, s, OMe), 3.00 (1H, m, H-11), 2.80 (1H, m, H-11); ^{13}C NMR (125 MHz, $CDCl_3$): 162.1 (q), 151.7 (q), 148.2 (q), 147.8 (q), 138.5 (q), 129.6 (q), 128.4 (2×CH), 128.3 (2×CH), 128.1 (CH), 126.5 (q), 125.6 (q), 122.2 (q), 121.4 (CH), 120.7 (q), 119.9 (q), 118.3 (CH), 116.1 (q), 114.7 (q), 110.8 (CH), 74.2 (CH), 55.8 (CH_3), 55.3 (CH_3), 51.2 (CH_3), 43.0 (CH_2), 28.9 (CH_2); IR (KBr, cm^{-1}): 2949, 2886, 2843, 1754, 1615, 1586, 1537, 1518, 1493, 1390, 1265, 1235, 1178, 1055.

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Studies toward the construction of substituted piperidine-2-ones and pyridine-2-ones from Baylis–Hillman adducts: discovery of a facile synthesis of 5-methyl-4-oxo-6-aryl-3-aza-bicyclo[3.1.0]hexane-1-carboxylates☆

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Abstract—Studies toward the construction of functionalized piperidone derivatives from derivatives of Baylis–Hillman adducts are described. Interestingly the 6-oxo-4-aryl-piperidine-3-carboxylates generated during the study serve as precursor for the facile synthesis of 4-oxo-6-aryl-3-aza-bicyclo[3.1.0]hexane-1-carboxylates.

1. Introduction

The multifunctional nature of the backbone of Baylis–Hillman adducts provides an excellent opportunity to generate a variety of heterocycles employing simple synthetic manipulations. Notably, the last decade has witnessed an extraordinary growth in the number of reports describing different approaches to achieve the syntheses of an array of cyclic compounds using Baylis–Hillman chemistry.[1] Recently, we too have reported the synthesis of a variety of heterocycles in solution and on solid phase utilizing Baylis–Hillman chemistry.[2] In our continuing efforts aimed at this objective, we describe herein the results of our studies toward the synthesis of substituted piperidine-2-ones and pyridine-2-ones from the nucleophilic substitution reaction products afforded by the reaction between the acetyl derivatives of Baylis–Hillman adducts and ethyl cyanoacetate. We have discovered that the substituted piperidine-2-one generated during the endeavor may serve as precursor for the efficient synthesis of 5-methyl-4-oxo-6-aryl-3-aza-bicyclo[3.1.0]-hexane-1-carboxylates.

The piperidine ring system is a structural component of numerous naturally occurring alkaloids, biologically active synthetic molecules, and organic chemicals. The syntheses of piperidones and piperidines have been exhaustively reviewed recently by Sabol and co-workers.[3] We envisaged the synthesis of piperidine-2-ones from the nucleophilic addition products afforded by reactions between acetates of the Baylis–Hillman adducts and ethyl cyanoacetate by reduction of the cyano group and subsequent intramolecular cyclization between the amino group and the ester moiety. On the other hand, the conversion of the cyano group to an amide in the same substrate should lead to 3-methylene-piperidine-2,6-diones (Fig. 1). The substituted piperidine-2,6-diones are structural component of several natural products and biologically active molecules.[4]

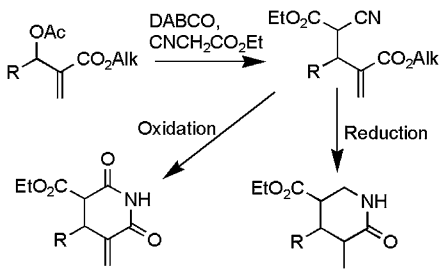

Figure 1. Strategy for the synthesis of substituted piperidine-2-ones and piperidine-2,6-diones.

2. Results and discussion

The preparation of the starting materials in our synthetic sequence (Scheme 1, acetates **3a**–**g**) was accomplished by

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Keywords: Baylis–Hillman; Piperidine-2-one; DBU; 4-Oxo-3-aza-bicyclo[3.1.0]hexane; 3-Methylene-piperidine-2,6-dione.
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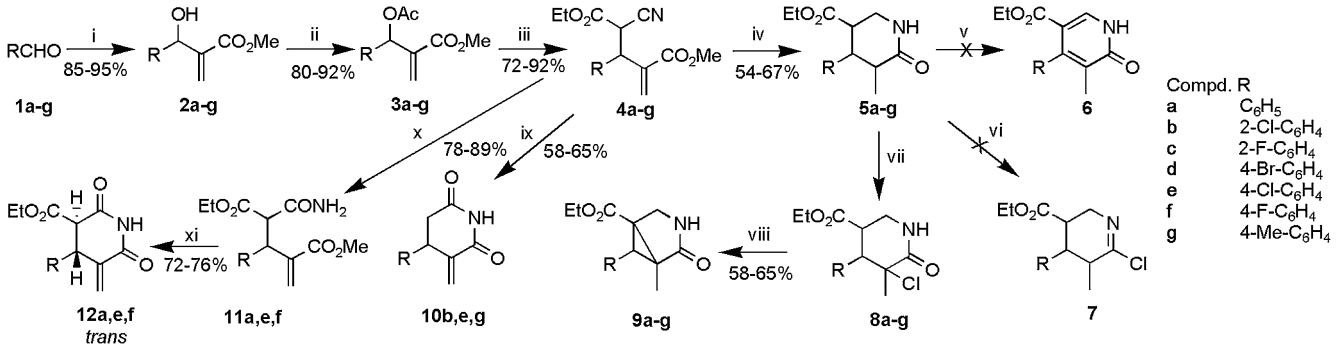

Scheme 1. Reagents and conditions: (i) $CH_2{=}CHCO_2Me$, DABCO, rt, 2–5 days; (ii) AcCl, pyridine, CH_2Cl_2, rt, 4–6 h; (iii) $CNCH_2CO_2Et$, DABCO, THF:H_2O, rt, 2 h; (iv) Raney-Ni, H_2, 40 psi, rt, 3 h; (v) DDQ, dioxane, reflux, 24 h; (vi) $POCl_3$, neat, reflux, 24 h; (vii) PCl_5, $POCl_3$, reflux, 2 h; (viii) DBU, CH_3CN, reflux, 14 h; (ix) $FeCl_3{\cdot}6H_2O$, propionic acid, reflux, 2 h; (x) TFA:H_2SO_4, neat, rt, 3 h; (xi) NaH, toluene, rt, 30 min.

acetylating the Baylis–Hillman adducts **2a**–**g**, which in turn were obtained from substituted benzaldehydes (**1a**–**g**) following the literature procedure.[5] The nucleophilic substitution of the acetates **3a**–**g** with ethyl cyanoacetate in the presence of DABCO in a THF:water system following a standard procedure yielded the substituted 1,5-dipentanoate derivatives **4a**–**g** as diastereoisomeric mixture in excellent yields.[2a] As would be expected, the reduction of these compounds in the presence of Raney-nickel under hydrogenation conditions yielded 5-methyl-6-oxo-4-aryl-piperidine-3-carboxylic acid ethyl esters **5a**–**g** in 54–67% yields. These compounds were obtained as mixtures of diastereoisomers. In principle, oxidation of these piperidinones with a suitable reagent should furnish pyridine-2-one derivatives **6**. Accordingly, the aromatization of these compounds was investigated in the presence of DDQ.[6] However, in our hands, the desired oxidation did not take place and only the starting material was recovered.

At this point, we envisaged that if compound **5** was converted to its chloro-derivative **7** with $POCl_3$ it would easily afford the desired pyridinone through oxidation.[7] Unfortunately, chlorination in the presence of $POCl_3$ under several conditions failed to afford the chloro-derivative **7**. Subsequently, the chlorination of compound **5** was attempted with a mixture of PCl_5 and $POCl_3$. Interestingly, this reaction yielded a less polar product **8**, the structure of which was established on the basis of spectroscopic analysis. The general nature of halogenation was confirmed by the synthesis of compounds **8a**–**g**. In principle, the tertiary nature of the chloro-group in compound **8** should make it an appropriate substrate for dehydrohalogenation followed by oxidation. In order to achieve the envisaged product, several reactions were attempted. It was gratifying to note that compounds **8a**–**g** undergo reaction in the presence of DBU to furnish products in good yields. On the basis of spectroscopic evidence the structure of these compounds was identified as 5-methyl-4-oxo-6-aryl-3-aza-bicyclo[3.1.0]hexane-1-carboxylates **9a**–**g**. The structure of these products was ascertained unambiguously via X-ray analysis of a representative compound **9e** (Fig. 2).[8] The formation of these products could be explained on the basis of the fact that the hydrogen atom on the carbon bearing the alkoxycarbonyl group being more acidic participates in the elimination of the chloride ion.

In a different strategy it was envisaged that the conversion of the cyano group to an amide would lead to an intermediate, which should undergo intramolecular cyclization resulting in 3-methylene-piperidine-2,6-dione. Recently Wang et al. have described an interesting $FeCl_3$-mediated highly efficient synthesis of 1,2-dihydro-2-oxo-3-pyridine-carboxylate

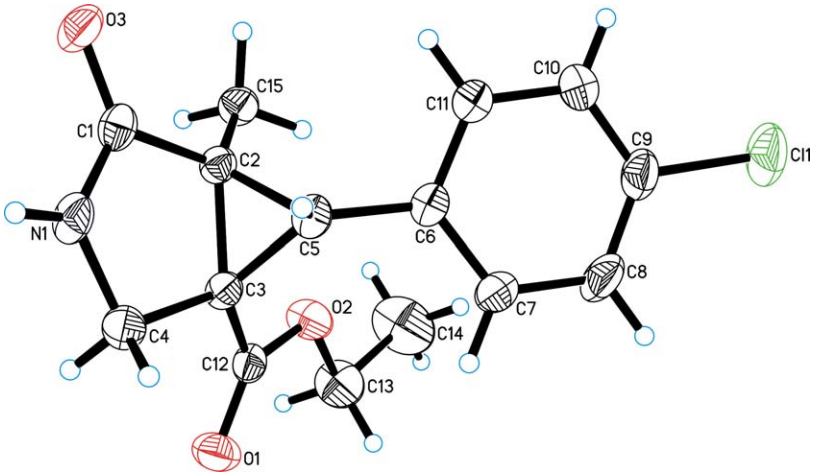

Figure 2. ORTEP diagram showing the crystal structure of **9e** with atomic numbering scheme for non-H atoms only at 30% probability level.

starting from enones and ethyl cyanoacetate.[9] Following their strategy, treatment of compounds **4b**,**e**,**g** with 3 equiv of $FeCl_3 \cdot 6H_2O$ in propionic acid at reflux for 2 h afforded 3-methylene-4-substituted phenyl-piperidine-2,6-diones **10b**,**e**,**g** in 60–65% yields. However, in contrast to their results, we observed that the carboxylate group was lost during the reaction and dehydrogenation did not occurred. In order to investigate the scope of our substrates for the generation of the carboxylate group containing piperidine-2,6-dione derivative, **4a**,**e**,**f** were hydrolyzed in the presence of TFA:H_2SO_4 mixture to afford the products **11a**,**e**,**f** in good yields. Subsequent treatment of the amides **11a**,**e**,**f** with NaH at room temperature furnished the desired 5-methylene-2,6-dioxo-4-phenyl-piperidine-3-carboxylic acid ethyl esters **12a**,**e**,**f** in good yields. Interestingly, the formation of compounds **12a**,**e**,**f** was highly stereoselective in favor of the *trans*-isomer. The stereochemistry was assigned on the basis of selective 1D NOESY experiment with compound **12a**. During the progress of this work, Kim et al. reported the synthesis of 3,5-dimethylene-4-phenyl-piperidine-2,6-dione and mono-alkylidene glutarimide by hydrolysis of the cyano group with sulfuric acid in methanol followed by cyclization in the presence of sodium bicarbonate.[10] However, in our hand, the sodium bicarbonate-mediated cyclization of substrates **11a**,**e**,**f** led to a complex mixture of product from which the corresponding 3-methylene-piperidine-2,6-diones **12a**,**e**,**f** were isolated in only low yields.

3. Conclusions

In summary, we have demonstrated a convenient and general process for the synthesis of 5-methyl-4-oxo-6-aryl-3-aza-bicyclo[3.1.0]hexane-1-carboxylates via products afforded by the nucleophilic addition reaction of ethyl cyanoacetate with acetyl derivatives of the Baylis–Hillman adducts. Additionally these products have been readily hydrolyzed and subsequently cyclized to yield new substituted piperidine-2,6-diones in good yields.

4. Experimental

4.1. General

Melting points were uncorrected and determined in capillary tubes on a hot stage apparatus containing silicon oil. IR spectra were recorded using a Perkin–Elmer RX I FTIR spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on either 300 or 200 MHz FT spectrometer, using TMS as an internal standard (chemical shifts in δ values, J in hertz). The FABMS were recorded on a JEOL/SX-102 spectrometer and ESMS were recorded in a Micromass LCMS system. Elemental analyses as performed on a Carlo Erba 1108 microanalyzer or Elementar's Vario EL *III* microanalyzer. All compounds were obtained in the powder form unless otherwise stated.

4.2. General procedure for the synthesis of compounds 4a–c, as exemplified for compound 4a

To a stirred solution of compound **3a** (2.0 g, 8.5 mmol) in THF:H_2O (20 mL, 50:50, v/v) was added DABCO (1.5 g, 12.8 mmol) at room temperature and the reaction was allowed to continue for 20 min. Thereafter, ethyl cyanoacetate (1.1 mL, 10.3 mmol) was added to the reaction mixture and the reaction was allowed to proceed at room temperature for 2 h. Then THF was removed from the reaction mixture via rotary evaporation and the residue was diluted with water (100 mL) and extracted with EtOAc (3×40 mL). The organic layers were pooled, washed with brine (50 mL), dried (Na_2SO_4), and evaporated in vacuo to yield a residue, which was purified by silica gel chromatography employing hexane:EtOAc (80:20, v/v) to afford 2.0 g (82%) of product **4a** as colorless oil.

4.2.1. 2-Cyano-4-methylene-3-phenyl-pentanedioic acid-1-ethyl ester-5-methyl ester (4a). ν_{max} (neat) 1747 (CO_2Me and CO_2Et), 2255 (CN) cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ=1.15 (t, 3H, J=7.1 Hz, CH_3CH_2), 1.33 (t, 3H, J=7.1 Hz, CH_3CH_2), 3.71 (s, 3H, CO_2CH_3), 3.76 (s, 3H, CO_2CH_3), 4.10–4.30 (m, 6H, 2×CH_2CH_3 and 2×CHAr), 4.45 (d, 1H, J=8.2 Hz, CHCN), 4.64 (d, 1H, J=8.2 Hz, CHCN), 5.75 (d, 1H, J=1.2 Hz, =CH), 5.99 (d, 1H, J=0.9 Hz, =CH), 6.49 (s, 1H, =CH), 6.51 (s, 1H, =CH), 7.29–7.37 (m, 10H, 2×5ArH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ=14.2, 14.3, 42.3, 42.6, 47.0, 47.6, 52.6, 52.7, 61.8, 63.3, 113.5, 116.0, 127.7, 128.5, 128.6, 128.9, 129.2, 136.9, 137.4, 139.0, 139.3, 163.4, 165.2, 166.4, 166.5; mass (FAB+) m/z 288 (M^++1); Anal. Calcd for $C_{16}H_{17}NO_4$: C, 66.89; H, 5.96; N, 4.88. Found: C, 67.08; H, 5.76; N, 4.98.

4.2.2. 3-(2-Chloro-phenyl)-2-cyano-4-methylene-pentanedioic acid-1-ethyl ester-5-methyl ester (4b). Yield 84% (2.3 g from 2.3 g) as a colorless oil; ν_{max} (neat) 1746 (CO_2Me and CO_2Et), 2253 (CN) cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ=1.13 (t, 3H, J=7.1 Hz, CH_3CH_2), 1.24 (t, 3H, J=7.1 Hz, CH_3CH_2), 3.69 (s, 3H, CO_2CH_3), 3.80 (s, 3H, CO_2CH_3), 4.13–4.25 (m, 4H, 2×CH_2CH_3), 4.33 (d, 1H, J=7.2 Hz, CHAr), 4.61 (d, 1H, J=7.2 Hz, CHAr), 5.08–5.02 (m, 2H, 2×CHCN), 5.61 (d, 1H, J=0.9 Hz, =CH), 6.15 (s, 1H, =CH), 5.50 (s, 1H, =CH), 6.60 (s, 1H, =CH), 7.28–7.63 (m, 8H, 2×4ArH); mass (ES+) m/z 322.0 (M^++1); Anal. Calcd for $C_{16}H_{16}ClNO_4$: C, 59.73; H, 5.01; N, 4.35. Found: C, 59.79; H, 4.87; N, 4.51.

4.2.3. 2-Cyano-3-(2-fluoro-phenyl)-4-methylene-pentanedioic acid-1-ethyl ester-5-methyl ester (4c). Yield 92% (1.1 g from 1.0 g) as a pale yellow oil; ν_{max} (neat) 1744 (CO_2Me and CO_2Et), 2259 (CN) cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ=1.13–1.33 (m, 6H, 2×CH_3CH_2), 3.71 (s, 3H, CO_2CH_3), 3.78 (s, 3H, CO_2CH_3), 4.13–4.23 (m, 5H, 2×CH_2CH_3 and CHAr), 4.39 (d, 1H, J=7.3 Hz, CHAr), 4.93 (d, 2H, J=7.1 Hz, 2×CHCN), 5.75 (s, 1H, =CH), 6.00 (s, 1H, =CH), 6.50 (s, 1H, =CH), 6.58 (s, 1H, =CH), 7.11–7.30 (m, 6H, 2×3ArH), 7.53–7.56 (m, 2H, 2×1ArH); mass (ES+) m/z 306.1 (M^++1); Anal. Calcd for $C_{16}H_{16}FNO_4$: C, 62.94; H, 5.28; N, 4.59. Found: C, 63.01; H, 5.22; N, 4.58.

4.2.4. 3-(4-Bromo-phenyl)-2-cyano-4-methylene-pentanedioic acid-1-ethyl ester-5-methyl ester (4d). Yield 82% (2.2 g from 2.3 g) as a colorless oil; ν_{max} (neat) 1748 (CO_2Me and CO_2Et), 2254 (CN) cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ=1.18 (t, 3H, J=7.1 Hz, CH_3CH_2), 1.32 (t, 3H,

J=7.1 Hz, CH_3CH$_2$), 3.71 (s, 3H, CO$_2$CH$_3$), 3.75 (s, 3H, CO$_2$CH$_3$), 4.13–4.41 (m, 6H, 2×CH_2CH$_3$ and 2×CHAr), 4.55–4.58 (m, 2H, 2×CHCN), 5.75 (s, 1H, =CH), 6.00 (s, 1H, =CH), 6.50 (s, 1H, =CH), 6.52 (s, 1H, =CH), 7.15–7.27 (m, 4H, 2×2ArH), 7.43–7.51 (m, 4H, 2×2ArH); mass (FAB+) m/z 366 (M$^+$+1); Anal. Calcd for $C_{16}H_{16}BrNO_4$: C, 52.48; H, 4.40; N, 3.82. Found: C, 52.63; H, 4.54; N, 3.77.

4.2.5. 3-(4-Chloro-phenyl)-2-cyano-4-methylene-pentanedioic acid-1-ethyl ester-5-methyl ester (4e). Yield 76% (1.8 g from 2.0 g) as a colorless oil; ν_{max} (neat) 1747 (CO$_2$Me and CO$_2$Et), 2255 (CN) cm^{-1}; ^{1}H NMR (CDCl$_3$, 200 MHz) δ=1.18 (t, 3H, J=7.1 Hz, CH_3CH$_2$), 1.33 (t, 3H, J=7.1 Hz, CH_3CH$_2$), 3.71 (s, 3H, CO$_2$CH$_3$), 3.75 (s, 3H, CO$_2$CH$_3$), 4.09–4.30 (m, 5H, 2×CH_2CH$_3$ and CHAr), 4.39 (d, 1H, J=7.3 Hz, CHAr), 4.56–4.63 (m, 2H, 2×CHCN), 5.76 (d, 1H, J=1.1 Hz, =CH), 6.00 (s, 1H, =CH), 6.50 (s, 1H, =CH), 6.52 (s, 1H, =CH), 7.25–7.32 (m, 8H, 2×4ArH); mass (ES+) m/z 344 (M$^+$+Na); Anal. Calcd for $C_{16}H_{16}ClNO_4$: C, 59.73; H, 5.01; N, 4.35. Found: C, 59.89; H, 4.78; N, 4.31.

4.2.6. 2-Cyano-3-(4-fluoro-phenyl)-4-methylene-pentanedioic acid-1-ethyl ester-5-methyl ester (4f). Yield 72% (1.3 g from 1.5 g) as a colorless oil; ν_{max} (neat) 1746 (CO$_2$Me and CO$_2$Et), 2254 (CN) cm^{-1}; ^{1}H NMR (CDCl$_3$, 200 MHz) δ=1.16 (t, 3H, J=7.1 Hz, CH_3CH$_2$), 1.33 (t, 3H, J=7.1 Hz, CH_3CH$_2$), 3.71 (s, 3H, CO$_2$CH$_3$), 3.76 (s, 3H, CO$_2$CH$_3$), 4.12–4.22 (m, 4H, 2×CH_2CH$_3$), 4.28 (d, 1H, J=7.1 Hz, CHAr), 4.39 (d, 1H, J=7.3 Hz, CHAr), 4.58–4.60 (m, 2H, 2×CHCN), 5.75 (s, 1H, =CH), 6.00 (s, 1H, =CH), 6.49 (s, 1H, =CH), 6.51 (s, 1H, =CH), 7.00–7.09 (m, 4H, 2×2ArH), 7.23–7.39 (m, 4H, 2×2ArH); ^{13}C NMR (CDCl$_3$, 50 MHz) δ=14.2, 14.3, 42.3, 42.6, 46.3, 47.1, 52.7, 52.8, 63.4, 116.0, 116.4, 127.7, 128.7, 130.2, 130.4, 130.6, 130.8, 132.6, 133.1, 138.8, 139.2, 165.0, 165.3, 166.3, 166.4; mass (ES+) m/z 306.2 (M$^+$+1); Anal. Calcd for $C_{16}H_{16}FNO_4$: C, 62.94; H, 5.28; N, 4.59. Found: C, 63.11; H, 5.41; N, 4.67.

4.2.7. 2-Cyano-4-methylene-3-*p*-tolyl-pentanedioic acid-1-ethyl ester-5-methyl ester (4g). Yield 78% (1.89 g from 2.0 g) as a colorless oil; ν_{max} (neat) 1742 (CO$_2$Me and CO$_2$Et), 2256 (CN) cm^{-1}; ^{1}H NMR (CDCl$_3$, 200 MHz) δ=1.16 (t, 3H, J=7.2 Hz, CH_3CH$_2$), 1.29 (t, 3H, J=7.2 Hz, CH_3CH$_2$), 2.31 (s, 3H, ArCH$_3$), 2.32 (s, 3H, ArCH$_3$), 3.71 (s, 3H, CO$_2$CH$_3$), 3.75 (s, 3H, CO$_2$CH$_3$), 4.11–4.22 (m, 5H, 2×CH_2CH$_3$ and CHAr), 4.40 (d, 1H, J=7.6 Hz, CHAr), 4.57–4.61 (m, 2H, 2×CHCN), 5.75 (d, 1H, J=1.0 Hz, =CH), 5.97 (s, 1H, =CH), 6.47 (s, 1H, =CH), 6.49 (s, 1H, =CH), 7.09–7.23 (m, 8H, 2×4ArH); ^{13}C NMR (CDCl$_3$, 50 MHz) δ=14.2, 14.5, 21.5, 42.4, 42.7, 46.7, 47.4, 52.6, 52.7, 61.9, 63.3, 116.1, 127.4, 128.3, 128.8, 129.9, 133.7, 134.3, 138.3, 139.1, 139.4, 165.3, 166.5, 166.6; mass (ES+) m/z 302.1 (M$^+$+1); Anal. Calcd for $C_{17}H_{19}NO_4$: C, 67.76; H, 6.36; N, 4.65. Found: C, 67.49; H, 6.51; N, 4.67.

4.3. General procedure for the synthesis of compounds 5a–g, as exemplified for compound 5a

A mixture of compound **4a** (1.2 g, 4.18 mmol) and Raney-Ni (0.3 g, wet) in MeOH (20 mL) was hydrogenated at 40 psi in the hydrogenation assembly (Parr) at room temperature. After completion, the catalyst was filtered over a bed of Celite and the filtrate was evaporated in vacuo to yield the crude product. Purification via silica gel column chromatography (hexane:EtOAc, 40:60, v/v) gave 0.65 g (60%) of product **5a** as a white solid.

4.3.1. 5-Methyl-6-oxo-4-phenyl-piperidine-3-carboxylic acid ethyl ester (5a). Mp 102–104 °C; ν_{max} (KBr) 1661 (CONH), 1730 (CO$_2$Et), 3402 (NH) cm^{-1}; ^{1}H NMR (CDCl$_3$, 200 MHz) δ=0.86 (t, 3H, J=7.1 Hz, CH_3CH$_2$), 1.00–1.18 (m, 9H, CH_3CH$_2$ and 2×CH_3CH), 2.43–2.61 (m, 1H, CHCH$_3$), 2.71–2.78 (m, 1H, CHCH$_3$), 2.81–3.00 (m, 1H, CHCH$_2$), 3.07–3.22 (m, 1H, CHCH$_2$), 3.31–3.64 (m, 6H, 2×CH_2NH and 2×CHAr), 3.83 (q, 2H, J=7.1 Hz, CH_2CH$_3$), 4.08 (q, 2H, J=7.1 Hz, CH_2CH$_3$), 6.27 (s, 2H, 2×NH), 7.17–7.36 (m, 10H, 2×5ArH); ^{13}C NMR (CDCl$_3$, 50 MHz) δ=13.6, 14.0, 14.4, 15.2, 38.9, 42.2, 42.5, 43.9, 44.0, 45.7, 47.4, 50.2, 61.0, 61.5, 127.04, 127.7, 128.3, 128.6, 129.0, 140.4, 140.9, 172.2, 172.7, 175.2, 176.4; mass (ES+) m/z 262.1 (M$^+$+1); Anal. Calcd for $C_{15}H_{19}NO_3$: C, 68.94; H, 7.33; N, 5.36. Found: C, 69.08; H, 7.55; N, 5.57.

4.3.2. 4-(2-Chloro-phenyl)-5-methyl-6-oxo-piperidine-3-carboxylic acid ethyl ester (5b). Yield 67% (0.98 g from 1.6 g) as a pale yellow oil; ν_{max} (neat) 1667 (CONH), 1731 (CO$_2$Et), 3222 (NH) cm^{-1}; ^{1}H NMR (CDCl$_3$, 200 MHz) δ=0.93 (t, 3H, J=7.1 Hz, CH_3CH$_2$), 1.01–1.18 (m, 9H, CH_3CH$_2$ and 2×CH_3CH), 2.80–2.98 (m, 1H, CHCH$_3$), 3.17–3.20 (m, 2H, CHCH$_3$ and CHCH$_2$), 3.59–3.60 (m, 1H, CHCH$_2$), 3.63–3.66 (m, 4H, 2×CH_2NH), 3.87–3.92 (m, 2H, 2×CHAr), 4.09–4.24 (m, 4H, 2×CH_2CH$_3$), 6.19 (s, 2H, 2×NH), 7.14–7.21 (m, 6H, 2×3ArH), 7.32–7.42 (m, 2H, 2×1ArH); ^{13}C NMR (CDCl$_3$, 50 MHz) δ=13.7, 14.0, 14.3, 14.6, 37.2, 37.6, 41.5, 41.8, 42.6, 42.8, 43.4, 43.5, 61.2, 61.6, 127.1, 127.7, 128.6, 129.0, 130.2, 130.4, 134.7, 135.0, 137.4, 138.8, 171.7, 172.3, 174.9, 176.0; mass (FAB+) m/z 296 (M$^+$+1); Anal. Calcd for $C_{15}H_{18}ClNO_3$: C, 60.91; H, 6.13; N, 4.74. Found: 60.82; H, 5.94; N, 4.89.

4.3.3. 4-(2-Fluoro-phenyl)-5-methyl-6-oxo-piperidine-3-carboxylic acid ethyl ester (5c). Yield 57% (0.47 g from 0.9 g) as a colorless oil; ν_{max} (neat) 1652 (CONH), 1723 (CO$_2$Et), 3436 (NH) cm^{-1}; ^{1}H NMR (CDCl$_3$, 200 MHz) δ=0.90 (t, 3H, J=7.1 Hz, CH_3CH$_2$), 1.02–1.29 (m, 9H, CH_3CH$_2$ and 2×CH_3CH), 2.62–2.93 (m, 2H, 2×CHCH$_3$), 3.24–3.26 (m, 2H, 2×CHCH$_2$), 3.58–3.61 (m, 4H, 2×CH_2NH), 3.87–3.91 (m, 2H, 2×CHAr), 4.06–4.13 (m, 4H, 2×CH_2CH$_3$), 6.37 (s, 2H, 2×NH), 7.00–7.22 (m, 8H, 2×4ArH); ^{13}C NMR (CDCl$_3$, 50 MHz) δ=13.6, 14.0, 14.3, 15.2, 37.9, 38.5, 41.2, 42.8, 43.1, 43.9, 44.7, 45.8, 61.3, 61.5, 115.9, 116.4, 124.5, 124.7, 127.2, 127.5, 127.8, 128.0, 128.9, 129.2, 129.5, 130.1, 172.0, 172.4, 174.8, 176.0; mass (FAB+) m/z 280 (M$^+$+1); Anal. Calcd for $C_{15}H_{18}FNO_3$: C, 64.50; H, 6.50; N, 5.01. Found: C, 64.55; H, 6.41; N, 5.09.

4.3.4. 4-(4-Bromo-phenyl)-5-methyl-6-oxo-piperidine-3-carboxylic acid ethyl ester (5d). Yield 58% (0.7 g from 1.3 g) as a white solid, mp 126–128 °C; ν_{max} (KBr) 1667 (CONH), 1724 (CO$_2$Et), 3313 (NH) cm^{-1}; ^{1}H NMR

($CDCl_3$, 200 MHz) δ=0.93 (t, 3H, J=7.1 Hz, CH_3CH$_2$), 1.00 (d, 3H, J=7.3 Hz, CH_3CH), 1.08 (d, 3H, J=6.9 Hz, CH_3CH), 1.17 (t, 3H, J=7.1 Hz, CH_3CH$_2$), 2.38–2.60 (m, 1H, CHCH$_3$), 2.68–2.82 (m, 1H, CHCH$_3$), 2.75–3.10 (m, 2H, 2×CHCH$_2$), 3.45–3.68 (m, 6H, 2×CH_2NH and 2×CHAr), 3.85 (q, 2H, J=7.1 Hz, CH_2CH$_3$), 4.10 (q, 2H, J=7.2 Hz, CH_2CH$_3$), 6.21 (s, 2H, 2×NH), 7.07–7.10 (m, 4H, 2×2ArH), 7.44–7.54 (m, 4H, 2×2ArH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ=13.6, 14.2, 14.4, 15.2, 38.8, 42.2, 42.4, 44.0, 44.1, 45.3, 47.2, 49.7, 61.2, 61.7, 121.4, 121.5, 130.0, 130.3, 132.2, 139.6, 140.0, 171.9, 172.4, 174.7, 176.0; mass (FAB+) m/z 340 (M^++1); Anal. Calcd for $C_{15}H_{18}BrNO_3$: C, 52.96; H, 5.33; N, 4.12. Found: C, 52.58; H, 5.49; N, 3.97.

4.3.5. 4-(4-Chloro-phenyl)-5-methyl-6-oxo-piperidine-3-carboxylic acid ethyl ester (5e). Yield 62% (0.45 g from 0.79 g) as a white solid, mp 118–120 °C; ν_{max} (KBr) 1665 (CONH), 1733 (CO_2Et), 3303 (NH) cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ=0.92 (t, 3H, J=7.1 Hz, CH_3CH$_2$), 1.00 (d, 3H, J=7.2 Hz, CH_3CH), 1.08 (d, 3H, J=7.0 Hz, CH_3CH), 1.17 (t, 3H, J=7.1 Hz, CH_3CH$_2$), 2.40–2.58 (m, 1H, CHCH$_3$), 2.66–2.78 (m, 1H, CHCH$_3$), 2.93–3.10 (m, 2H, 2×CHCH$_2$), 3.57–3.69 (m, 6H, 2×CH_2NH and 2×CHAr), 3.85 (q, 2H, J=7.1 Hz, CH_2CH$_3$), 4.09 (q, 2H, J=7.1 Hz, CH_2CH$_3$), 6.23 (s, 2H, 2×NH), 7.09–7.15 (m, 4H, 2×2ArH), 7.29–7.33 (m, 4H, 2×2ArH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ=13.5, 14.1, 14.4, 15.2, 38.9, 42.3, 42.5, 44.0, 44.2, 45.2, 47.3, 49.7, 61.2, 61.6, 129.1, 129.2, 129.6, 130.0, 133.3, 133.5, 139.1, 139.5, 171.9, 172.5, 174.7, 176.2; mass (FAB+) m/z 296 (M^++1); Anal. Calcd for $C_{15}H_{18}ClNO_3$: C, 60.91; H, 6.13; N, 4.74. Found: C, 61.15; H, 5.85; N, 4.88.

4.3.6. 4-(4-Fluoro-phenyl)-5-methyl-6-oxo-piperidine-3-carboxylic acid ethyl ester (5f). Yield 54% (0.26 g from 0.53 g) as a yellow solid, mp 125–127 °C; ν_{max} (KBr) 1663 (CONH), 1729 (CO_2Et), 3407 (NH) cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ=0.90 (t, 3H, J=7.1 Hz, CH_3CH$_2$), 1.02–1.29 (m, 9H, CH_3CH$_2$ and 2×CH_3CH), 2.38–2.76 (m, 2H, 2×CHCH$_3$), 2.80–3.18 (m, 2H, CHCH$_2$), 3.53–3.63 (m, 6H, 2×CH$_2$NH and 2×CHAr), 3.85 (q, 2H, J=7.0 Hz, CH_2CH$_3$), 4.11 (q, 2H, J=7.1 Hz, CH_2CH$_3$), 6.15 (s, 2H, 2×NH), 6.96–7.06 (m, 4H, 2×2ArH), 7.11–7.20 (m, 4H, 2×2ArH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ=13.6, 14.1, 14.4, 15.1, 39.0, 42.5, 44.0, 44.2, 45.0, 47.5, 49.6, 61.1, 61.6, 115.6, 116.0, 129.7, 129.8. 130.0, 130.2, 136.2, 136.7, 159.8, 164.6, 172.0, 172.6, 174.8, 176.2; mass (FAB+) m/z 280 (M^++1); Anal. Calcd for $C_{15}H_{18}FNO_3$: C, 64.50; H, 6.50; N, 5.01. Found: C, 64.80; H, 6.36; N, 4.88.

4.3.7. 5-Methyl-6-oxo-4-*p*-tolyl-piperidine-3-carboxylic acid ethyl ester (5g). Yield 56% (0.56 g from 1.1 g) as a white solid, mp 105–107 °C; ν_{max} (KBr) 1663 (CONH), 1726 (CO_2Et), 3233 (NH) cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ=0.89 (t, 3H, J=7.2 Hz, CH_3CH$_2$), 1.01 (d, 3H, J=7.2 Hz, CH_3CH), 1.08 (d, 3H, J=7.2 Hz, CH_3CH), 1.17 (t, 3H, J=7.1 Hz, CH_3CH$_2$), 2.32 (s, 6H, 2×ArCH$_3$), 2.46–2.58 (m, 1H, CHCH$_3$), 2.68–2.76 (m, 1H, CHCH$_3$), 2.89–3.11 (m, 2H, 2×CHCH$_2$), 3.55–3.63 (m, 6H, 2×CH_2NH and 2×CHAr), 3.84 (q, 2H, J=7.1 Hz, CH_2CH$_3$), 4.09 (q, 2H, J=7.1 Hz, CH_2CH$_3$), 6.10 (s, 2H, 2×NH), 7.04–7.14 (m, 8H, 2×4ArH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ=13.6, 14.1, 14.4, 15.2, 21.4, 38.9, 41.6, 42.4, 44.0, 44.1, 45.4, 47.5, 49.9, 61.0, 61.5, 128.1, 128.5, 129.6, 137.0, 137.2, 137.3, 137.9, 171.8, 172.2, 172.8, 175.3, 176.5; mass (FAB+) m/z 276 (M^++1); Anal. Calcd for $C_{16}H_{21}NO_3$: C, 69.79; H, 7.69; N, 5.09. Found: C, 69.98; H, 7.54; N, 4.89.

4.4. General procedure for the synthesis of compounds 8a–g, as exemplified for compound 8a

A mixture of compound **5a** (0.51 g, 1.95 mmol) and PCl_5 (1.62 g, 7.8 mmol) in $POCl_3$ (4 mL) was refluxed for 3 h. Thereafter, the reaction mixture was poured into ice water, neutralized with $NaHCO_3$, and extracted with EtOAc (2×25 mL). The combined organic layer was washed with brine (50 mL), dried (Na_2SO_4), and the solvent was in vacuo. The crude product obtained was purified by silica gel column chromatography (hexane:EtOAc, 50:50, v/v) to give 0.39 g (68%) of chloro-derivative **8a** as a white solid.

4.4.1. 5-Chloro-5-methyl-6-oxo-4-phenyl-piperidine-3-carboxylic acid ethyl ester (8a). Mp 142–144 °C; ν_{max} (KBr) 1678 (CONH), 1736 (CO_2Et), 3200 (NH) cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ=0.90 (t, 3H, J=7.1 Hz, CH_3CH$_2$), 1.03 (t, 3H, J=7.1 Hz, CH_3CH$_2$), 1.56 (s, 3H, CH$_3$), 1.61 (s, 3H, CH$_3$), 3.32–3.38 (m, 2H, 2×CHAr), 3.62–3.72 (m, 4H, 2×CH_2NH), 3.78–4.05 (m, 6H, 2×CHCH$_2$ and 2×CH_2CH$_3$), 6.36 (br s, 1H, NH), 6.74 (br s, 1H, NH), 7.24–7.40 (m, 10H, 2×5ArH);^{13}C NMR ($CDCl_3$, 50 MHz) δ=14.1, 14.2, 26.0, 26.2, 42.4, 43.1, 43.9, 44.5, 52.9, 54.8, 61.4, 61.7, 67.8, 68.1, 128.2, 128.3, 128.4, 128.7, 130.0, 136.1, 138.0, 170.7, 171.3, 171.6, 172.0; mass (FAB+) m/z 296 (M^++1); Anal. Calcd for $C_{15}H_{18}ClNO_3$: C, 60.91; H, 6.13; N, 4.74. Found: C, 61.19; H, 5.87; N, 4.78.

4.4.2. 5-Chloro-4-(2-chloro-phenyl)-5-methyl-6-oxo-piperidine-3-carboxylic acid ethyl ester (8b). Yield 65% (0.59 g from 0.81 g) as a white crystalline solid, mp 142–144 °C; ν_{max} (KBr) 1685 (CONH), 1735 (CO_2Et), 3250 (NH) cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ=0.93 (t, 3H, J=6.0 Hz, CH_3CH$_2$), 1.17 (t, 3H, J=6.0 Hz, CH_3CH$_2$), 1.58 (s, 3H, CH$_3$), 1.60 (s, 3H, CH$_3$), 3.26–3.40 (m, 2H, 2×CHAr), 3.50–3.61 (m, 4H, 2×CH$_2$N), 3.86–4.03 (m, 6H, 2×CH_2CH$_3$ and 2×CHCO$_2$Et), 6.18 (s, 1H, NH), 6.47 (s, 1H, NH), 7.20–7.30 (m, 6H, 2×3ArH), 7.42–7.46 (m, 2H, 2×1ArH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ=14.2, 14.3, 25.9, 26.0, 41.5, 42.0, 45.6, 45.9, 49.2, 50.1, 61.5, 61.9, 67.0, 68.0, 126.8, 127.4, 127.6, 129.3, 129.4, 129.9, 130.6, 131.5, 135.7, 137.8, 171.2, 171.4, 171.7, 171.9; mass (ES+) m/z 330.0 (M^++1), 332.1 (M^++3); Anal. Calcd for $C_{15}H_{17}Cl_2NO_3$: C, 54.56; H, 5.19; N, 4.24. Found: C, 54.33; H, 5.11; N, 4.52.

4.4.3. 5-Chloro-4-(2-fluoro-phenyl)-5-methyl-6-oxo-piperidine-3-carboxylic acid ethyl ester (8c). Yield 63% (1.39 g from 2.0 g) as a white solid, mp 144–146 °C; ν_{max} (KBr) 1679 (CONH), 1735 (CO_2Et), 3401 (NH) cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ=0.93 (t, 3H, J=7.1 Hz, CH_3CH$_2$), 1.06 (t, 3H, J=7.1 Hz, CH_3CH$_2$), 1.62 (s, 3H, CH$_3$), 1.65 (s, 3H, CH$_3$), 3.28–3.41 (m, 2H, 2×CHAr), 3.55–3.63 (m, 4H, 2×CH$_2$N), 3.88–4.07 (m, 6H,

2×C*H*$_2$CH$_3$ and 2×CHCO$_2$Et), 6.52 (s, 1H, NH), 6.83 (s, 1H, NH), 7.03–7.18 (m, 6H, 2×3ArH), 7.27–7.31 (m, 2H, 2×1ArH); mass (ES+) *m*/*z* 314.0 (M$^+$+1); Anal. Calcd for $C_{15}H_{17}ClFNO_3$: C, 57.42; H, 5.46; N, 4.46. Found: C, 57.60; H, 5.64; N, 4.31.

4.4.4. 4-(4-Bromo-phenyl)-5-chloro-5-methyl-6-oxo-piperidine-3-carboxylic acid ethyl ester (8d). Yield 60% (0.13 g from 0.2 g) as a white solid, mp 148–150 °C; ν_{max} (KBr) 1697 (CONH), 1726 (CO$_2$Et), 3439 (NH) cm^{-1}; ^{1}H NMR (CDCl$_3$, 200 MHz) δ=0.93–1.09 (m, 6H, 2×C*H*$_3$CH$_2$), 1.55 (s, 3H, CH$_3$), 1.59 (s, 3H, CH$_3$), 3.28–3.47 (m, 2H, 2×CHAr), 3.58–3.83 (m, 4H, 2×CHCO$_2$Et and 2×C*H*HN), 3.89–4.06 (m, 6H, 2×C*H*$_2$CH$_3$ and 2×CH*H*N), 6.32 (s, 1H, NH), 6.67 (s, 1H, NH), 7.13–7.23 (m, 4H, 2×2ArH), 7.45–7.49 (m, 4H, 2×2ArH); mass (ES+) *m*/*z* 374 (M$^+$+1); Anal. Calcd for $C_{15}H_{17}BrClNO_3$: C, 48.09; H, 4.57; N, 3.74. Found: C, 47.85; H, 4.83; N, 3.78.

4.4.5. 5-Chloro-4-(4-chloro-phenyl)-5-methyl-6-oxo-piperidine-3-carboxylic acid ethyl ester (8e). Yield 54% (1.2 g from 2.0 g) as a white solid, mp 158–160 °C; ν_{max} (KBr) 1690 (CONH), 1728 (CO$_2$Et), 3312 (NH) cm^{-1}; ^{1}H NMR (CDCl$_3$, 200 MHz) δ=0.96 (t, 3H, *J*=7.2 Hz, C*H*$_3$CH$_2$), 1.05 (t, 3H, *J*=7.2 Hz, C*H*$_3$CH$_2$), 1.55 (s, 3H, CH$_3$), 1.59 (s, 3H, CH$_3$), 3.28–3.37 (m, 2H, 2×CHAr), 3.58–3.88 (m, 4H, 2×CHCO$_2$Et and 2×C*H*HN), 3.90–4.06 (m, 6H, 2×C*H*$_2$CH$_3$ and 2×CH*H*N), 6.45 (s, 1H, NH), 6.81 (s, 1H, NH), 7.19–7.34 (m, 8H, 2×4ArH); ^{13}C NMR (CDCl$_3$, 50 MHz) δ=14.2, 14.3, 25.9, 26.1, 42.4, 43.2, 43.8, 44.3, 52.3, 54.2, 61.6, 61.8, 67.5, 67.8, 128.6, 128.9, 131.3, 131.8, 134.3, 134.5, 134.6, 136.2, 170.3, 170.9, 171.5, 171.8; mass (FAB+) *m*/*z* 330 (M$^+$+1); Anal. Calcd for $C_{15}H_{17}Cl_2NO_3$: C, 54.56; H, 5.19; N, 4.24. Found: C, 54.77; H, 5.02; N, 4.31.

4.4.6. 5-Chloro-4-(4-fluoro-phenyl)-5-methyl-6-oxo-piperidine-3-carboxylic acid ethyl ester (8f). Yield 72% (0.97 g from 1.2 g) as a white solid, mp 110–112 °C; ν_{max} (KBr) 1696 (CONH), 1728 (CO$_2$Et), 3412 (NH) cm^{-1}; ^{1}H NMR (CDCl$_3$, 200 MHz) δ=0.94 (t, 3H, *J*=7.2 Hz, CH$_3$CH$_2$), 1.04 (t, 3H, *J*=7.2 Hz, CH$_3$CH$_2$), 1.56 (s, 3H, CH$_3$), 1.59 (s, 3H, CH$_3$), 3.29–3.44 (m, 2H, 2×CHAr), 3.57–3.67 (m, 4H, 2×CH$_2$N), 3.83–4.01 (m, 6H, 2×CH$_2$CH$_3$ and 2×CHCO$_2$Et), 6.36 (s, 1H, NH), 6.72 (s, 1H, NH), 6.99–7.07 (m, 4H, 2×2ArH), 7.31–7.34 (m, 4H, 2×2ArH), ^{13}C NMR (CDCl$_3$, 50 MHz) δ=14.1, 14.2, 25.9, 26.0, 42.5, 43.2, 43.8, 44.4, 52.2, 54.1, 61.5, 61.8, 67.7, 68.1, 115.1, 115.4, 115.5, 115.8, 131.6, 132.0, 133.4, 170.5, 171.0, 171.7, 171.9; mass (ES+) *m*/*z* 314.0 (M$^+$+1); Anal. Calcd for $C_{15}H_{17}ClFNO_3$: C, 57.42; H, 5.46; N, 4.46. Found: C, 57.71; H, 5.40; N, 4.44.

4.4.7. 5-Chloro-5-methyl-6-oxo-4-*p*-tolyl-piperidine-3-carboxylic acid ethyl ester (8g). Yield 67% (0.45 g from 0.6 g) as a white solid, mp 134–136 °C; ν_{max} (KBr) 1679 (CONH), 1724 (CO$_2$Et), 3365 (NH) cm^{-1}; ^{1}H NMR (CDCl$_3$, 300 MHz) δ=0.94 (t, 3H, *J*=7.5 Hz, C*H*$_3$CH$_2$), 1.07 (t, 3H, *J*=7.5 Hz, C*H*$_3$CH$_2$), 1.56 (s, 3H, CH$_3$), 1.61 (s, 3H, CH$_3$), 2.34 (s, 6H, 2×ArCH$_3$), 3.23–3.40 (m, 2H, 2×CHAr), 3.78–3.80 (m, 3H, 2×CHCO$_2$Et and C*H*HN), 3.84–4.06 (m, 7H, 2×C*H*$_2$CH$_3$, C*H*HN and 2×CH*H*N), 6.18 (s, 1H, NH), 6.56 (s, 1H, NH), 7.12–7.14 (m, 4H, 2×2ArH), 7.24–7.27 (m, 4H, 2×2ArH); ^{13}C NMR (CDCl$_3$, 50 MHz) δ=14.1, 14.2, 21.6, 26.0, 26.2, 42.4, 43.0, 43.9, 44.5, 52.5, 54.4, 61.4, 61.6, 68.0, 68.2, 129.0, 129.4, 129.8, 130.4, 133.0, 135.0, 137.9, 138.1, 170.8, 171.3, 171.9, 172.0; mass (ES+) *m*/*z* 310 (M$^+$+1), 332.0 (M$^+$+Na); Anal. Calcd for $C_{16}H_{20}ClNO_3$: C, 62.03; H, 6.51; N, 4.52. Found: C, 61.86; H, 6.42; N, 4.60.

4.5. General procedure for the synthesis of compounds 9a–g, as exemplified for compound 9a

To the solution of compound **8a** (0.5 g, 1.69 mmol) in anhydrous acetonitrile was added DBU (0.52 mL, 3.39 mmol) and the reaction mixture was heated at reflux for 14 h. Thereafter, the solvent was evaporated in vacuo to yield a residue, which via silica gel column chromatography (hexane: EtOAc, 40:60 v/v) afforded 0.28 g (64%) of compound **9a** as a white solid.

4.5.1. 5-Methyl-4-oxo-6-phenyl-3-aza-bicyclo[3.1.0]hexane-1-carboxylic acid ethyl ester (9a). Mp 115–117 °C; ν_{max} (KBr) 1678 (CONH), 1718 (CO$_2$Et), 3413 (NH) cm^{-1}; ^{1}H NMR (CDCl$_3$, 200 MHz) δ=1.02 (t, 3H, *J*=7.2 Hz, C*H*$_3$CH$_2$), 1.52 (s, 3H, CH$_3$), 2.66 (s, 1H, CHAr), 3.66 (d, 1H, *J*=10.4 Hz, C*H*HN), 3.90 (d, 1H, *J*=10.6 Hz, CH*H*NH), 4.05 (q, 2H, *J*=7.2 Hz, C*H*$_2$CH$_3$), 5.98 (s, 1H, NH), 7.15–7.28 (m, 3H, ArH), 7.32–7.40 (m, 2H, ArH); ^{13}C NMR (CDCl$_3$, 75 MHz) δ=9.1, 14.1, 35.0, 35.4, 37.9, 45.1, 61.3, 128.2, 128.4, 129.6, 136.0, 170.1, 178; mass (ES+) *m*/*z* 260.2 (M$^+$+1); Anal. Calcd for $C_{15}H_{17}NO_3$: C, 69.48; H, 6.61; N, 5.40. Found: C, 69.42; H, 6.55; N, 5.31.

4.5.2. 6-(2-Chloro-phenyl)-5-methyl-4-oxo-3-aza-bicyclo[3.1.0]hexane-1-carboxylic acid ethyl ester (9b). Yield 61% (0.27 g from 0.5 g) as a yellow oil; ν_{max} (neat) 1679 (CONH), 1732 (CO$_2$Et), 3406 (NH) cm^{-1}; ^{1}H NMR (CDCl$_3$, 200 MHz) δ=0.99 (t, 3H, *J*=7.2 Hz, C*H*$_3$CH$_2$), 1.60 (s, 3H, CH$_3$), 2.49 (s, 1H, CHAr), 3.75 (d, 1H, *J*=10.5 Hz, C*H*HNH), 3.93–4.07 (m, 3H, CH*H*NH and C*H*$_2$CH$_3$), 6.11 (s, 1H, NH), 7.21–7.32 (m, 3H, ArH), 7.34–7.40 (m, 1H, ArH); ^{13}C NMR (CDCl$_3$, 75 MHz) δ=9.3, 14.2, 34.5, 35.6, 37.9, 45.2, 52.2, 61.3, 127.2, 128.6, 130.2, 134.6, 137.4, 171.2, 175.4; mass (ES+) *m*/*z* 294.1 (M$^+$+1); Anal. Calcd for $C_{15}H_{16}ClNO_3$: C, 61.31; H, 5.49; N, 4.77. Found: C, 61.59; H, 5.70; N, 4.68.

4.5.3. 6-(2-Fluoro-phenyl)-5-methyl-4-oxo-3-aza-bicyclo[3.1.0]hexane-1-carboxylic acid ethyl ester (9c). Yield 65% (0.16 g from 0.28 g) as a white solid, mp 142–144 °C; ν_{max} (KBr) 1657 (CONH), 1729 (CO$_2$Et), 3280 (NH) cm^{-1}; ^{1}H NMR (CDCl$_3$, 200 MHz) δ=1.04 (t, 3H, *J*=7.2 Hz, C*H*$_3$CH$_2$), 1.52 (s, 3H, CH$_3$), 2.48 (s, 1H, CHAr), 3.65 (d, 1H, *J*=10.8 Hz, C*H*HN), 3.97–4.09 (m, 3H, C*H*$_2$CH$_3$ and CH*H*N), 5.95 (s, 1H, NH), 6.98–7.15 (m, 2H, ArH), 7.23–7.27 (m, 2H, ArH); ^{13}C NMR (CDCl$_3$, 50 MHz) δ=9.2, 14.2, 34.4, 35.4, 38.0, 45.3, 61.3, 115.4, 115.8, 121.0, 121.3, 124.2, 129.4, 129.6, 131.8, 169.1, 178.5; mass (FAB+) *m*/*z* 278 (M$^+$+1); Anal. Calcd for $C_{15}H_{16}FNO_3$: C, 64.57; H, 5.82; N, 5.05. Found: C, 64.77; H, 5.70; N, 4.86.

4.5.4. 6-(4-Bromo-phenyl)-5-methyl-4-oxo-3-aza-bicyclo[3.1.0]hexane-1-carboxylic acid ethyl ester (9d). Yield

58% (0.05 g from 0.1 g) as a white solid, mp 136–138 °C; ν_{max} (KBr) 1704 (CONH), 1728 (CO_2Et), 3268 (NH) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ=1.08 (t, 3H, J=7.5 Hz, CH_3CH_2), 1.51 (s, 3H, CH_3), 2.59 (s, 1H, CHAr), 3.66 (d, 1H, J=12.0 Hz, C*H*HN), 3.93 (d, 1H, J=12.0 Hz, CH*H*N), 4.08 (q, 2H, J=7.5 Hz, CH_2CH_3), 5.72 (s, 1H, NH), 7.07 (d, 2H, J=8.5 Hz, ArH), 7.45 (d, 2H, J=8.5 Hz, ArH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ=8.8, 13.9, 32.2, 37.4, 38.5, 44.8, 61.1, 121.4, 131.4, 131.5, 132.0, 168.8, 177.7; mass (ES+) m/z 338.1 (M^++1), 340.1 (M^++3); Anal. Calcd for $C_{15}H_{16}BrNO_3$: C, 53.27; H, 4.77; N, 4.14. Found: C, 53.55; H, 4.91; N, 3.86.

4.5.5. 6-(4-Chloro-phenyl)-5-methyl-4-oxo-3-aza-bicyclo[3.1.0]hexane-1-carboxylic acid ethyl ester (9e). Yield 60% (0.16 g from 0.3 g) as a white crystalline solid, mp 125–127 °C; ν_{max} (KBr) 1705 (CONH), 1732 (CO_2Et), 3312 (NH) cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ=1.07 (t, 3H, J=7.1 Hz, CH_3CH_2), 1.50 (s, 3H, CH_3), 2.60 (s, 1H, CHAr), 3.65 (d, 1H, J=10.0 Hz, C*H*HN), 3.98 (d, 1H, J=10.5 Hz, CH*H*N), 4.00 (q, 2H, J=7.1 Hz, CH_2CH_3), 5.69 (s, 1H, NH), 7.10 (d, 2H, J=8.3 Hz, ArH), 7.29 (d, 2H, J=8.2 Hz, ArH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ=9.2, 14.3, 35.7, 37.9, 38.8, 45.4, 61.4, 128.9, 131.6, 131.8, 133.6, 169.3, 178.3; mass (FAB+) m/z 294 (M^++1); Anal. Calcd for $C_{15}H_{16}ClNO_3$: C, 61.33; H, 5.49; N, 4.77. Found: C, 61.68; H, 5.41; N, 4.49.

4.5.6. 6-(4-Fluoro-phenyl)-5-methyl-4-oxo-3-aza-bicyclo[3.1.0]hexane-1-carboxylic acid ethyl ester (9f). Yield 63% (0.14 g from 0.25 g) as a yellow oil; ν_{max} (neat) 1701 (CONH), 1738 (CO_2Et), 3400 (NH) cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ=1.06 (t, 3H, J=7.2 Hz, CH_3CH_2), 1.50 (s, 3H, CH_3), 2.61 (s, 1H, CHAr), 3.65 (d, 1H, J=11.2 Hz, C*H*HNH), 3.92 (d, 1H, J=11.0 Hz, CH*H*NH), 3.98–4.13 (m, 2H, CH_2CH_3), 5.89 (s, 1H, NH), 6.98–7.22 (m, 4H, ArH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ=9.2, 14.3, 35.7, 38.2, 38.8, 45.6, 61.4, 115.4, 115.8, 128.2, 129.2, 131.7, 131.9, 169.4, 178.9; mass (ES+) m/z 278.1 (M^++1); Anal. Calcd for $C_{15}H_{16}FNO_3$: C, 64.97; H, 5.82; N, 5.05. Found: C, 64.90; H, 5.69; N, 4.98.

4.5.7. 5-Methyl-4-oxo-6-*p*-tolyl-3-aza-bicyclo[3.1.0] hexane-1-carboxylic acid ethyl ester (9g). Yield 65% (0.23 g from 0.4 g) as a white solid, mp 156–158 °C; ν_{max} (KBr) 1696 (CONH), 1735 (CO_2Et), 3426 (NH) cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ=1.05 (t, 3H, J=7.1 Hz, CH_3CH_2), 1.50 (s, 3H, CH_3), 2.33 (s, 3H, $ArCH_3$), 2.62 (s, 1H, CHAr), 3.65 (d, 1H, J=10.5 Hz, C*H*HN), 3.89 (d, 1H, J=10.4 Hz, CH*H*N), 4.08 (q, 2H, J=7.1 Hz, CH_2CH_3), 5.80 (s, 1H, NH), 7.04 (d, 2H, J=8.1 Hz, ArH), 7.12 (d, 2H, J=8.2 Hz, ArH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ=9.3, 14.3, 21.6, 35.6, 38.0, 39.5, 42.6, 61.8, 128.5, 129.4, 136.7, 139.6, 168.0, 171.2; mass (ES+) m/z 274.1 (M^++1); Anal. Calcd for $C_{16}H_{19}NO_3$: C, 70.31; H, 7.01; N, 5.12. Found: C, 70.10; H, 6.75; N, 4.88.

4.6. General procedure for the synthesis of compounds 10b,e,g, as exemplified for compound 10g

Compound **4g** (0.3 g, 1.0 mmol) and $FeCl_3 \cdot 6H_2O$ (0.80 g, 3.0 mmol) were dissolved in propionic acid (6 mL) and the mixture was heated at reflux for 2 h. After cooling the mixture to room temperature, it was poured into 1 N HCl (20 mL) and extracted with EtOAc (3×30 mL). The organic layers were pooled and washed with $NaHCO_3$ (50 mL), dried over Na_2SO_4, and evaporated to yield a residue, which was purified via silica gel column chromatography. Elution with hexane:EtOAc (60:40, v/v) yielded 0.13 g (60%) of product **10g** as a brown solid.

4.6.1. 4-(2-Chloro-phenyl)-3-methylene-piperidine-2,6-dione (10b). Yield 58% (0.26 g from 0.62 g) as a white solid, 108–110 °C; ν_{max} (KBr) 1701 (CONH), 3408 (NH) cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ=2.94–3.05 (m, 2H, CH_2CH), 4.54–4.59 (m, 1H, CHAr), 5.33 (s, 1H, =CH), 6.52 (d, 1H, J=0.9 Hz, =CH), 7.15–7.32 (m, 3H, ArH), 7.43–7.48 (m, 1H, ArH), 8.24 (s, 1H, NH);^{13}C NMR ($CDCl_3$, 50 MHz) δ=37.7, 39.1, 127.2, 127.9, 128.5, 129.4, 130.6, 137.1, 137.7, 166.2, 171.8; mass (FAB+) m/z 236 (M^++1); Anal. Calcd for $C_{12}H_{10}ClNO_2$: C, 61.16; H, 4.28; N, 5.94. Found: C, 60.80; H, 4.49; N, 6.01.

4.6.2. 4-(4-Chloro-phenyl)-3-methylene-piperidine-2,6-dione (10e). Yield 65% (0.23 g from 0.49 g) as a white solid, 182–184 °C; ν_{max} (KBr) 1699 (CONH), 3404 (NH) cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ=2.96–2.99 (m, 2H, CH_2CH), 4.02–4.12 (m, 1H, CHAr), 5.39 (s, 1H, =CH), 6.50 (s, 1H, =CH), 7.15 (d, 2H, J=8.5 Hz, ArH), 7.36 (d, 2H, J=8.5 Hz, ArH), 8.19 (br s, 1H, NH);^{13}C NMR ($CDCl_3$, 50 MHz) δ=43.3, 46.5, 131.2, 134.2, 134.4, 138.1, 143.3, 144.5, 171.1, 177.0; mass (FAB+) m/z 236 (M^++1); Anal. Calcd for $C_{12}H_{10}ClNO_2$: C, 61.16; H, 4.28; N, 5.94. Found: C, 61.22; H, 4.03; N, 5.78.

4.6.3. 3-Methylene-4-*p*-tolyl-piperidine-2,6-dione (10g). Mp 152–154 °C; ν_{max} (KBr) 1697 (CONH), 3427 (NH) cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ=2.35 (s, 3H, $ArCH_3$), 2.89–3.02 (m, 2H, CH_2CH), 3.99–4.15 (m, 1H, CHAr), 5.39 (s, 1H, =CH), 6.47 (s, 1H, =CH), 7.09 (d, 2H, J=8.0 Hz, ArH), 7.19 (d, 2H, J=8.0 Hz, ArH), 8.18 (s, 1H, NH); mass (FAB+) m/z 216 (M^++1); Anal. Calcd for $C_{13}H_{13}NO_2$: C, 72.54; H, 6.09; N, 6.51. Found: C, 72.67; H, 5.85; N, 6.82.

4.7. General procedure for the synthesis of compounds 11a,e,f, as exemplified for compound 11a

To a flask charged with compound **4a** (0.9 g, 3.14 mmol) was added 6 mL mixture of TFA and H_2SO_4 (1: 1) at room temperature and the reaction was continued for 4 h. Thereafter, the reaction mixture was poured into ice cold water (50 mL) and neutralized with $NaHCO_3$ and extracted with EtOAc (2×30 mL). Combined organic layer was dried over Na_2SO_4 and evaporated in vacuo to afford a residue, which was purified via column chromatography over silica gel using hexane:EtOAc (30:70, v/v) to furnish 0.76 g (80%) of amide **11a** as a white solid.

4.7.1. 2-Carbamoyl-4-methylene-3-phenyl-pentanedioic acid-1-ethyl ester-5-methyl ester (11a). Mp 120–122 °C; ν_{max} (KBr) 1666 ($CONH_2$), 1724 (CO_2Me and CO_2Et), 3396 (NH_2) cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ=1.01 (t, 3H, J=7.1 Hz, CH_3CH_2), 1.26 (t, 3H, J=7.1 Hz, CH_3CH_2), 3.69 (s, 6H, 2×CO_2CH_3), 3.97 (q, 2H, J=7.1 Hz, CH_2CH_3), 4.10–4.21 (m, 4H, CH_2CH_3 and

2×CHAr), 4.53 (d, 1H, *J*=12.4 Hz, CHCON), 4.62 (d, 1H, *J*=12.4 Hz, CHCON), 5.36 (s, 1H, 1H of NH_2), 5.53 (s, 1H, 1H of NH_2), 5.80 (s, 1H, =CH), 5.94 (s, 1H, =CH), 5.98 (s, 1H, 1H of NH_2), 6.28 (s, 1H, 1H of NH_2), 6.32 (s, 1H, =CH), 6.33 (s, 1H, =CH), 7.14–7.20 (m, 6H, 2×3ArH), 7.38–7.42 (m, 4H, 2×2ArH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ=19.0, 19.3, 50.7, 50.8, 57.1, 61.5, 61.7, 66.4, 66.5, 125.9, 129.6, 130.5, 135.6, 135.9, 136.3, 143.6, 144.0, 145.3, 146.5, 171.4, 173.5, 173.8; mass (FAB+) *m/z* 306 (M^++1); Anal. Calcd for $C_{16}H_{19}NO_5$: C, 62.94; H, 6.27; N, 4.59. Found: C, 63.05; H, 6.13; N, 4.83.

4.7.2. 2-Carbamoyl-3-(4-chloro-phenyl)-4-methylene-pentanedioic acid-1-ethyl ester-5-methyl ester (11e). Yield 89% (0.63 g from 0.67 g) as a white solid, mp 102–104 °C; ν_{max} (KBr) 1667 ($CONH_2$), 1725 (CO_2Me and CO_2Et), 3389 (NH_2) cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ=1.02 (t, 3H, *J*=7.2 Hz, CH_3CH_2), 1.25 (t, 3H, *J*=7.2 Hz, CH_3CH_2), 3.69 (s, 6H, 2×CO_2CH_3), 3.96 (q, 2H, *J*=7.2 Hz, CH_2CH_3), 4.11–4.25 (m, 4H, CH_2CH_3 and 2×CHAr), 4.54 (d, 1H, *J*=12.4 Hz, CHCON), 4.63 (d, 1H, *J*=12.4 Hz, CHCON), 5.62 (s, 1H, 1H of NH_2), 5.73 (s, 1H, 1H of NH_2), 5.81 (s, 1H, =CH), 5.85 (s, 1H, 1H of NH_2), 5.94 (s, 1H, =CH), 5.98 (s, 1H, 1H of NH_2), 6.32 (s, 1H, =CH), 6.33 (s, 1H, =CH), 7.23–7.31 (m, 8H, 2×4ArH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ=19.0, 19.2, 50.8, 51.1, 57.3, 61.7, 61.8, 66.3, 66.5, 129.5, 130.5, 133.4, 135.2, 135.4, 137.7, 142.9, 143.4, 145.4, 146.5, 171.4, 173.6, 173.8; mass (ES+) *m/z* 340.0 (M^++1); 342.0 (M^++3); Anal. Calcd for $C_{16}H_{18}ClNO_5$: C, 56.56; H, 5.34; N, 4.12. Found: C, 56.79; H, 5.22; N, 3.95.

4.7.3. 2-Carbamoyl-3-(4-fluoro-phenyl)-4-methylene-pentanedioic acid-1-ethyl ester-5-methyl ester (11f). Yield 78% (0.68 g from 0.83 g) as a white solid, mp 110–112 °C; ν_{max} (KBr) 1664 ($CONH_2$), 1726 (CO_2Me and CO_2Et), 3403 (NH_2) cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ=1.02 (t, 3H, *J*=7.5 Hz, CH_3CH_2), 1.27 (t, 3H, *J*=7.5 Hz, CH_3CH_2), 3.70 (s, 3H, CO_2CH_3), 3.71 (s, 3H, CO_2CH_3), 3.94–4.01 (m, 3H, CH_2CH_3 and CHAr), 4.12–4.25 (m, 3H, CH_2CH_3 and CHAr), 4.57 (d, 1H, *J*=12.0 Hz, CHCON), 4.66 (d, 1H, *J*=12.0 Hz, CHCON), 5.28 (s, 1H, 1H of NH_2), 5.43 (s, 1H, 1H of NH_2), 5.81 (s, 1H, =CH), 5.95 (s, 2H, =CH and 1H of NH_2), 6.28 (s, 1H, 1H of NH_2), 6.34 (s, 2H, 2×=CH), 6.94–7.00 (m, 4H, 2×2ArH), 7.26–7.32 (m, 4H, 2×2ArH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ=14.1, 14.3, 46.1, 46.6, 52.3, 57.5, 61.7, 61.8, 115.1, 115.6, 124.5, 125.7, 130.3, 130.5, 130.6, 134.9, 135.2, 140.5, 141.7, 159.6, 164.4, 166.6, 166.7, 168.6, 169.2; mass (ES+) *m/z* 324.1 (M^++1); Anal. Calcd for $C_{16}H_{18}FNO_5$: C, 59.44; H, 5.61; N, 4.33. Found: C, 59.63; H, 5.40; N, 4.47.

4.8. General procedure for the synthesis of compounds 12a,e,f, as exemplified for compound 12a

To the stirred solution of compound **11a** (0.5 g, 1.64 mmol) in anhydrous toluene was added NaH (0.098 g in 60% oil, 2.46 mmol) at ambient temperature. After 30 min reaction mixture was quenched carefully with water and extracted with ethyl acetate (2×25 mL). The organic layer was dried (Na_2SO_4) and evaporated in vacuo to obtain a residue that was subjected to column chromatography using hexane:EtOAc (40:60 v/v) over silica gel to yield 0.32 g (72%) of compound **12a** as a white solid.

4.8.1. 5-Methylene-2,6-dioxo-4-phenyl-piperidine-3-carboxylic acid ethyl ester (12a). Mp 114–116 °C; ν_{max} (KBr) 1705 (CONH), 1748 (CO_2Et), 3418 (NH) cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ=1.13 (t, 3H, *J*=7.5 Hz, CH_3CH_2), 3.40 (d, 1H, *J*=9.0 Hz, CHAr), 4.14 (q, 2H, *J*=7.5 Hz, CH_2CH), 4.43 (d, 1H, *J*=9.0 Hz, $CHCO_2Et$), 5.45 (s, 1H, =CH), 6.57 (s, 1H, =CH), 7.21–7.33 (m, 3H, ArH), 7.35–7.41 (m, 2H, ArH), 8.15 (s, 1H, NH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ=14.3, 46.2, 55.3, 62.5, 128.3, 128.6, 129.6, 137.2, 137.4, 165.1, 167.5, 168.3; mass (ES+) *m/z* 274.1 (M^++1); Anal. Calcd for $C_{15}H_{15}NO_4$: C, 65.52; H, 5.53; N, 5.13. Found: C, 65.59; H, 5.71; N, 4.93.

4.8.2. 4-(4-Chloro-phenyl)-5-methylene-2,6-dioxo-piperidine-3-carboxylic acid ethyl ester (12e). Yield 74% (0.15 g from 0.22 g) as a white solid, mp 108–110 °C; ν_{max} (KBr) 1695 (CONH), 1748 (CO_2Et), 3418 (NH) cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ=1.14 (t, 3H, *J*=7.1 Hz, CH_3CH_2), 3.40 (d, 1H, *J*=9.5 Hz, CHAr), 4.14 (q, 2H, *J*=7.1 Hz, CH_2CH), 4.38 (d, 1H, *J*=9.2 Hz, $CHCO_2Et$), 5.40 (s, 1H, =CH), 6.58 (s, 1H, =CH), 7.16 (d, 2H, *J*=7.8 Hz, ArH), 7.36 (d, 2H, *J*=8.0 Hz, ArH), 8.21 (s, 1H, NH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ=14.3, 45.5, 51.1, 62.6, 129.3, 130.5, 134.5, 135.7, 137.1, 164.8, 167.2, 167.9; mass (FAB+) *m/z* 308 (M^++1); Anal. Calcd for $C_{15}H_{14}ClNO_4$: C, 58.54; H, 4.59; N, 4.55. Found: C, 58.50; H, 4.69; N, 4.58.

4.8.3. 4-(4-Fluoro-phenyl)-5-methylene-2,6-dioxo-piperidine-3-carboxylic acid ethyl ester (12f). Yield 76% (0.25 g from 0.36 g) as a white solid, mp 135–137 °C; ν_{max} (KBr) 1703 (CONH), 1740 (CO_2Et), 3372 (NH) cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ=1.15 (t, 3H, *J*=7.5 Hz, CH_3CH_2), 3.95 (d, 1H, *J*=9.0 Hz, CHAr), 4.15 (q, 2H, *J*=7.5 Hz, CH_2CH), 4.40 (d, 1H, *J*=9.0 Hz, $CHCO_2Et$), 5.41 (s, 1H, =CH), 6.58 (s, 1H, =CH), 7.06–7.12 (m, 2H, ArH), 7.19–7.24 (m, 2H, ArH), 8.12 (s, 1H, NH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ=14.3, 45.4, 55.4, 62.6, 116.3, 116.8, 129.6, 130.0, 130.2, 132.9, 137.4, 165.0, 167.3, 168.1; mass (FAB+) *m/z* 292 (M^++1); Anal. Calcd for $C_{15}H_{14}FNO_4$: C, 61.58; H, 4.85; N, 4.81. Found: C, 61.55; H, 4.56; N, 4.77.

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8. Crystal data of compound **9e**: $C_{15}H_{16}NO_3Cl$, M=293.75, orthorhombic, $P2_12_12_1$, a=6.323(1), b=9.076(2), c=26.253(2) Å, V=1506.5(4) Å^3, Z=4, D_c=1.295 g cm^{-3}, μ(Mo K_α)=0.026 mm^{-1}, F(000)=616.0, colorless block, dimension 0.3×0.25×0.2 mm, 2196 reflections measured (R_{int}=0.0234), 1978 unique, wR_2=0.098, conventional R=0.0401 on F values of 1485 reflections with $I>2\sigma(I)$, $(\Delta/\sigma)_{max}$=000), S=1.02 for all data and 184 parameters. Unit cell determination and intensity data collection (2θ=50°) was performed on a Bruker P4 diffractometer at 293(2) K. Structure solutions by direct methods and refinements by full-matrix least-squares methods on F^2. Programs: XSCANS (Siemens Analytical X-ray Instrument Inc.: Madison, WI, USA, 1996) for data collection and data processing; SHELXTL-NT (Bruker AXS Inc.: Madison, Wisconsin, USA, 1997) for structure determination, refinements, and molecular graphics. Further details of the crystal structure investigation can be obtained from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK (CCDC deposition no. of **9e**: 609070).
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An alternate approach to quinoline architecture via Baylis–Hillman chemistry: $SnCl_2$-mediated tandem reaction toward synthesis of 4-(substituted vinyl)-quinolines

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Abstract—An alternate approach to densely substituted quinolines from the products of S_N2 nucleophilic substitution reaction between the acetyl derivatives of the Baylis–Hillman adducts obtained from 2-nitrobenzaldehydes and the carbonyl group containing carbon nucleophiles is described. Treatment of these compounds with $SnCl_2$, triggers a tandem reaction wherein reduction of the nitro group is followed by a remarkably regioselective intramolecular cyclization and subsequent dehydrogenation to afford 4-(substituted vinyl)-quinolines.

1. Introduction

Substituted quinolines are one of the oldest known classes of pharmaceutical agents and their relevance in chemotherapy especially against malaria is widely known.[1] Beside antimalarials, a spectrum of other pharmacological activities[2] has been the major reason for the development of novel and efficient syntheses of this heterocycle. As a result, the recent past has witnessed the publication of several simple and elegant syntheses of substituted quinolines.[3] Nevertheless, a new mild one-pot method, from readily accessible starting materials, which would permit delivery of this motif decorated with functional groups amenable to further diversification, should be of great synthetic relevance.

In the recent times, Baylis–Hillman adducts have been illustrated as suitable starting materials for the synthesis of variety of heterocyclic systems.[4] The generation of substituted quinolines either directly from the Baylis–Hillman adducts or their derivatives has therefore received considerable attention. Historically, since the first such synthesis reported by Familoni et al. in 1998, several variants of this approach have been demonstrated.[5–16] A summary of these methods is provided in Figure 1.

With our ongoing interest in the synthesis of heterocycles employing derivatives of Baylis–Hillman adducts,[17] it

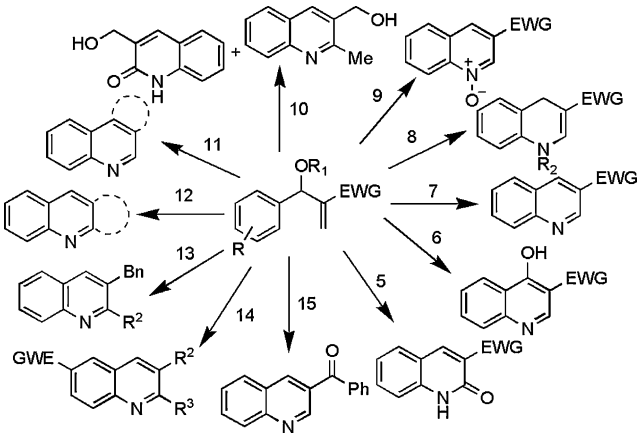

Figure 1. Summary of synthesis of quinoline motif from Baylis–Hillman adducts or their derivatives. Numbers on the arrows indicate the corresponding reference no. (also see Ref. 16).

occurred to us that substrates resulting from the S_N2 nucleophilic substitution reaction between the acetyl derivatives of Baylis–Hillman adducts of 2-nitrobenzaldehydes and a nucleophile containing a keto group or an ester group would represent an interesting carbon framework for the construction of a quinoline architecture. In these compounds beside the three carbon chain originating from the Baylis–Hillman reaction, which generally participates in the intramolecular cyclization toward construction of the quinoline, there would also be another three carbon chain containing a terminal keto or an ester group, which could also undergo cyclization. In the latter case cyclization would result in a dihydroquinoline derivative with a vinyl chain at the 4-position, which could be dehydrogenated to yield the desired quinoline. We have

Keywords: Quinolines; Baylis–Hillman; 2-Nitrobenzaldehyde; Reduction; $SnCl_2$; Regioselective.
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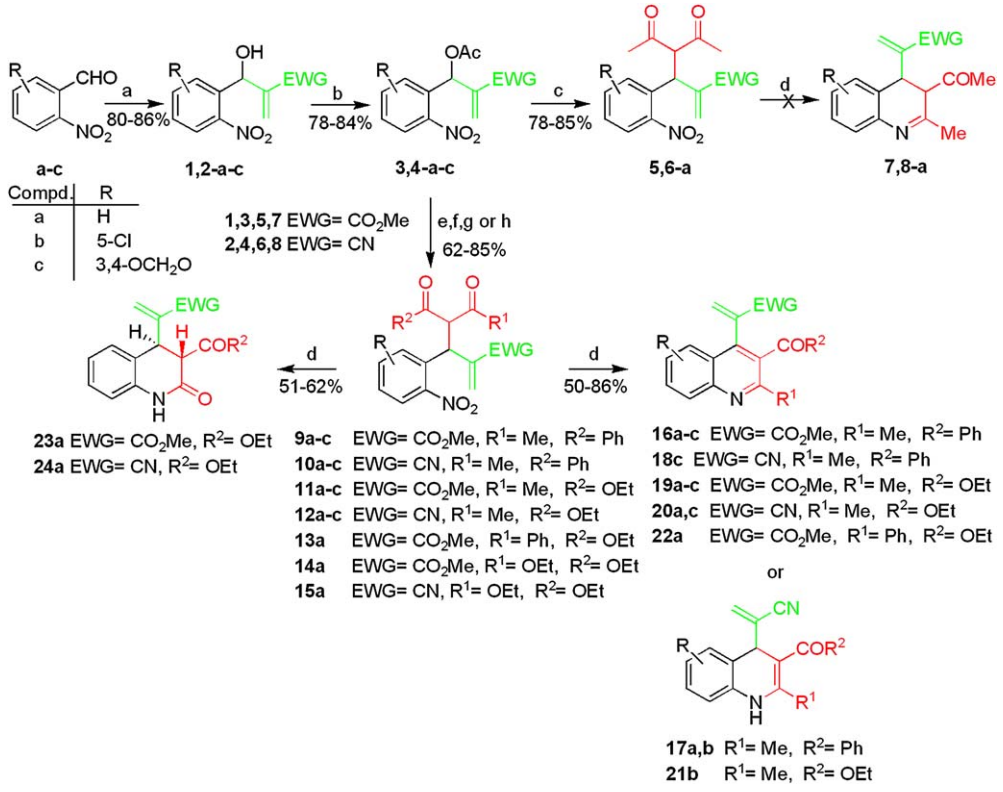
therefore carried out S_N2 reactions of the acetates of the Baylis–Hillman adducts of 2-nitrobenzaldehydes and substituted 2-nitrobenzaldehydes with nucleophiles such as acetyl acetone, benzoyl acetone, methylacetoacetate, and benzoylacetoacetate and subjected the resulting products to chemoselective reduction in the presence of $SnCl_2$. Interestingly, the chemoselective reduction led to a tandem reaction wherein the reduction, a regioselective cyclization involving the carbonyl group of the added nucleophile dehydrogenation occurred in a single step to furnish the 4-(substituted vinyl)-quinolines. This observation prompted us to disclose the details of results of our study in this paper.

2. Results and discussion

Preparation of the starting materials, the acetates **3a–c**,**4a–c**, was accomplished by acetylating the corresponding Baylis–Hillman adducts **1a–c**,**2a–c** in the presence of acetyl chloride and pyridine in dichloromethane at room temperature (Scheme 1). Initially, the S_N2 reaction of acetyl acetone was carried out with the acetate **3a** following the reported procedure to obtain product **5a**.[17d] The chemoselective reduction of the nitro group employing anhydrous $SnCl_2$ in compound **5a**, instead of yielding the desired dihydroquinoline **7a**, resulted in an inseparable mixture of products. It is likely that both the carbonyl group of the added nucleophile and the ester group originally present in the substrate could have participated in the cyclization reaction leading to a complex mixture. In principle, if the acetate **4a** is used as the starting substrate for a similar reaction then the possibility of simultaneous cyclizations could be eliminated. Accordingly, compound **6a** was prepared and subjected to similar reduction. However, instead of the desired product **8a**, a complex mixture of products was formed, which could not be characterized.

In the next step we decided to investigate the same reaction sequence by replacing the nucleophile with benzoyl acetone. Thus, the S_N2 nucleophilic substitution reaction of benzoyl acetone with the acetate **3a** in the presence of DABCO in a THF/H_2O system led to the synthesis of compound **9a** as a diastereoisomeric mixture in good yield. The nitro group in **9a** was then chemoselectively reduced with anhydrous $SnCl_2$. Gratifyingly this reaction proceeded smoothly to yield a product, which was established as the quinoline **16a** via spectroscopic analysis. The change in the chemical shift of the protons of methyl group indicated that the acetyl group of the nucleophile was involved in the intramolecular cyclization with the amino group. The isolation of quinoline **16a** implied that the $SnCl_2$ had triggered a tandem reaction wherein the reduction of the nitro group was followed by cyclization and subsequent dehydrogenation. This reaction was found to be general in nature since substrates **9b**,**c** also furnished the quinolines **16b**,**c**. During optimization it was observed that replacing anhydrous $SnCl_2$ with $SnCl_2\cdot 2H_2O$ did not have any effect on the outcome of the reaction. Encouraged with these results we decided to evaluate the reaction with compounds **10a–c**, which were synthesized from **4a–c**, respectively, following a similar synthetic route to that for the preparation of **9a–c**. Interestingly when **10a** was subjected to $SnCl_2$-promoted reaction, unlike compound **9a–c**, the dihydroquinoline derivative **17a** was isolated. ^{1}H NMR analysis of product **17a** showed



Scheme 1. Reagents and conditions: (a) CH_2=CHEWG, DABCO, rt, 15 min to 1 h; (b) AcCl, pyridine, CH_2Cl_2, rt, 2–3 h; (c) DABCO, $MeCOCH_2COMe$, THF/H_2O (1:1), rt, 30 min; (d) $SnCl_2$, MeOH, reflux, 1 h; (e) DABCO, $PhCOCH_2COMe$, THF/H_2O (1:1), rt, 30 min; (f) DABCO, $MeCOCH_2CO_2Et$, THF/H_2O (1:1), rt, 30 min; (g) DABCO, $PhCOCH_2CO_2Et$, THF/H_2O (1:1), rt, 30 min; (h) DABCO, $CO_2EtCH_2CO_2Et$, THF/H_2O (1:1), rt, 30 min.

the presence of peaks for the NH and the CH protons and ^{13}C NMR exhibited the CH carbon instead of the signal for tertiary carbon. The mass spectrum supported the assigned structure. Treatment of compound **10b** with $SnCl_2$ also yielded the dihydroquinoline **17b** but substrate **10c** gave the usual quinoline **18c**.

The results generated interest in studying the outcome of reactions with other carbonyl group containing carbon nucleophiles in order to further explore the scope of this strategy. Therefore, compounds **11a–c**,**12a–c** were synthesized using ethylacetoacetate as the nucleophile in the S_N2 reaction of the acetates **3a–c**,**4a–c**, respectively. We were pleased to observe that the $SnCl_2$ reduction of the nitro group proceeded smoothly in these compounds to furnish the quinolines **19a–c**, **20a**,**c** in good yields. Like compounds **10a**,**b**, compound **12b** also yielded dihydroquinoline **21b** exclusively. These results indicated that the acetyl carbonyl introduced through the S_N2 reaction reacts in preference to other activated carbonyl moieties present in the molecule with the amine generated during the reduction reaction.

We next examined the reduction in compound **13a**. Gratifyingly treatment with $SnCl_2$ yielded the quinoline **22a** in good yield. This result again indicated that the carbonyl group are more reactive in the cyclization reaction and precedes the ester moiety. In our quest to find out whether the ester group can at all participate in the intramolecular cyclization, diethylmalonate was selected as nucleophile for the S_N2 reaction. Hence, compounds **14a** and **15a** generated by the reaction of compounds **3a** and **4a**, respectively, with diethylmalonate in the presence of DABCO, were treated with $SnCl_2$. The reaction led to isolation of products, which were established to be tetrahydro quinolin-2-ones **23a**, **24a**. Interestingly, this cyclization was found to be diastereoselective in favor of *trans* isomer, as evident from the NOE correlations for H-3 and H-4 protons. Thus in the absence of a ketone moiety, the ester group can also participate in the intramolecular cyclization. However, the subsequent dehydrogenation does not occur.

3. Conclusions

In summary, we have demonstrated a new alternate strategy for the synthesis of highly functionalized quinolines from easily accessible derivatives of Baylis–Hillman adducts, which has general applicability. The $SnCl_2$-mediated reduction of the nitro group initiates a highly regioselective intramolecular cyclization between the amino group and the carbonyl moiety from the nucleophile introduced through the S_N2 substitution reaction. This study indicates that the preference of the activated carbonyl group COR for cyclization has the following order: R=Me>Ph>*O*-alkyl. We believe that the quinoline derivatives generated during the present study would serve as good building blocks for the synthesis of quinoline-annulated ring systems.

4. Experimental

4.1. General

Melting points are uncorrected and were determined using a hot stage apparatus containing silicon oil. IR spectra were recorded using a Perkin–Elmer RX I FTIR spectrophotometer. ^1H NMR and ^{13}C NMR spectra were recorded on either a 300 or a 200 MHz FT spectrometer, using TMS as an internal standard (chemical shifts are expressed as δ values, *J* in hertz). FABMS were recorded on a JEOL/SX-102 spectrometer and ESMS were recorded using a Micromass LC–MS system. Elemental analyses were performed on a Carlo Erba 1108 microanalyzer or Elementar's Vario EL *III* microanalyzer. All yields are the isolated yields from column chromatography. Spectroscopic data of compounds **9–13** are reported as diastereoisomeric mixtures unless otherwise stated.

4.2. General procedure for the S_N2 nucleophilic substitution reaction with the acetyl derivatives

To a stirred solution of appropriate acetate (1.0 equiv) in THF/H_2O (50:50, v/v) was added DABCO (1.5 equiv) at room temperature and the reaction was allowed to continue for 20 min. Thereafter, the appropriate nucleophile (1.2 equiv) was added to the reaction mixture, and was further stirred for 30 min at room temperature. The organic solvent was removed and the residue diluted with water and extracted with EtOAc (3×50 mL). Combined organic layer was washed with brine solution (70 mL), dried (Na_2SO_4), and evaporated to yield a crude product, which was purified via silica gel column chromatography using hexanes/EtOAc (85:15, v/v) as eluent to furnish pure products in 62–85% yield.

4.2.1. 4-Acetyl-2-methylene-3-(2-nitro-phenyl)-5-oxo-hexanoic acid methyl ester (5a). Ref. 17d.

4.2.2. 4-Acetyl-2-methylene-3-(2-nitro-phenyl)-5-oxo-hexanenitrile (6a). Yield 78% (0.9 g from 1.0 g) as a brown solid; mp 135–137 °C; ν_{max} (KBr) 1707 (2×CO), 2227 (CN) cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 1.98 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 4.69 (d, 1H, *J*=11.8 Hz, CH), 5.21 (d, 1H, *J*=11.8 Hz, CH), 6.02 (s, 1H, =CH), 6.19 (s, 1H, =CH), 7.45–7.65 (m, 3H, ArH), 7.87 (d, 1H, *J*=7.4 Hz, CH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 29.3, 30.0, 42.6, 72.0, 117.0, 121.4, 125.8, 129.0, 129.6, 131.3, 135.7, 150.3, 200.0, 200.3; mass (ES+) *m*/*z* 309 (M^++Na); Anal. Calcd for $C_{15}H_{14}N_2O_4$: C, 62.93; H, 4.93; N, 9.79. Found: C, 63.04; H, 4.96; N, 9.65.

4.2.3. 4-Benzoyl-2-methylene-3-(2-nitro-phenyl)-5-oxo-hexanoic acid methyl ester (9a). Yield 81% (1.1 g from 1.0 g) as a brown solid; mp 102–104 °C; ν_{max} (KBr) 1679 (CO), 1724 (CO and CO_2Me) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 2.09 (s, 3H, CH_3), 2.19 (s, 3H, CH_3), 3.57 (s, 3H, CO_2CH_3), 3.71 (s, 3H, CO_2CH_3), 5.55–5.82 (m, 5H, 4×CH and =CH), 5.99 (s, 1H, =CH), 6.18 (s, 1H, =CH), 6.39 (s, 1H, =CH), 7.21–7.72 (m, 16H, ArH), 7.92 (d, 1H, *J*=11.8 Hz, ArH), 8.08 (d, 1H, *J*=11.8 Hz, ArH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 26.8, 27.0, 40.1, 40.8, 50.7, 50.8, 64.8, 65.3, 123.4, 123.6, 126.6, 126.9, 127.4, 127.5, 127.7, 128.3, 128.6, 129.5, 131.0, 131.1, 131.8, 132.6, 132.8, 135.1, 135.3, 136.8, 137.5, 149.0, 149.1, 152.4, 164.7, 165.0, 189.7, 192.5, 200.7, 200.8; mass (ES+) *m*/*z* 382.1 (M^++1), 404.1 (M^++Na); Anal. Calcd for $C_{21}H_{19}NO_6$: C, 66.13; H, 5.02; N, 3.67. Found: C, 66.10; H, 4.97; N, 3.66.

4.2.4. 4-Benzoyl-3-(5-chloro-2-nitro-phenyl)-2-methylene-5-oxo-hexanoic acid methyl ester (9b). Yield 63% (0.5 g from 0.6 g) as a brown semisolid; ν_{max} (Neat) 1680 (CO), 1721 (CO and CO_2Me) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 2.11 (s, 3H, CH_3), 2.17 (s, 3H, CH_3), 3.59 (s, 6H, 2×CO_2CH_3), 5.45–5.60 (m, 4H, 4×CH), 5.65 (s, 2H, 2×=CH), 5.75 (s, 1H, =CH), 6.22 (s, 1H, =CH), 7.34 (d, 2H, J=6.8 Hz, ArH), 7.49–7.76 (m, 10H, ArH), 8.07 (d, 4H, J=7.2 Hz, ArH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 29.6, 30.0, 42.6, 43.2, 53.6, 53.7, 67.2, 67.7, 127.6, 127.7, 129.5, 127.5, 129.3, 129.9, 130.1, 130.3, 130.5, 131.7, 132.2, 135.5, 135.7, 136.3, 138.8, 139.6, 140.1, 149.9, 167.2, 167.5, 195.0, 202.9; mass (ES+) m/z 415.9 (M^++1); Anal. Calcd for $C_{21}H_{18}ClNO_6$: C, 60.66; H, 4.36; N, 3.37. Found: C, 60.69; H, 4.44; N, 3.67.

4.2.5. 4-Benzoyl-2-methylene-3-(6-nitro-benzo[1,3]-dioxol-5-yl)-5-oxo-hexanoic acid methyl ester (9c). Yield 69% (0.9 g from 1.0 g) as a brown solid; mp 127–129 °C; ν_{max} (KBr) 1680 (CO), 1721 (CO and CO_2Me) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 2.12 (s, 3H, CH_3), 2.19 (s, 3H, CH_3), 3.62 (s, 3H, CO_2CH_3), 3.75 (s, 3H, CO_2CH_3), 5.58–5.79 (m, 4H, 3×CH and =CH), 5.96–6.03 (m, 2H, CH and =CH), 6.10 (s, 4H, 2×OCH_2O), 6.20 (s, 1H, =CH), 6.40 (s, 1H, =CH), 7.01 (s, 1H, ArH), 7.06 (s, 1H, ArH), 7.28–7.30 (m, 4H, ArH), 7.43–7.62 (m, 6H, ArH), 7.97 (d, 1H, J=12.0 Hz, ArH), 8.08 (d, 1H, J=12.0 Hz, ArH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 14.6, 21.4, 28.5, 28.7, 41.5, 42.3, 52.5, 60.8, 66.7, 67.1, 103.2, 103.4, 106.1, 106.3, 109.8, 127.9, 129.2, 129.5, 130.1, 134.6, 136.9, 139.4, 144.8, 151.6, 166.5, 194.2, 202.7; mass (ES+) m/z 425.9 (M^++1), 448.1 (M^++Na); Anal. Calcd for $C_{22}H_{19}NO_8$: C, 62.12; H, 4.50; N, 3.29. Found: C, 62.00; H, 4.41; N, 3.11.

4.2.6. 4-Benzoyl-2-methylene-3-(2-nitro-phenyl)-5-oxo-hexanenitrile (10a). Yield 78% (1.1 g from 1.0 g) as a brown solid; mp 102–104 °C; ν_{max} (KBr) 1674 (CO), 1724 (CO), 2221 (CN) cm^{-1}; ^1H NMR ($CDCl_3$, 200 Hz) δ 1.95 (s, 3H, CH_3), 2.25 (s, 3H, CH_3), 5.41–5.53 (m, 4H, 4×CH), 5.91 (s, 1H, =CH), 6.09 (s, 1H, =CH), 6.17 (s, 1H, =CH), 6.30 (s, 1H, =CH), 7.43–8.11 (m, 16H, ArH), 8.12–8.15 (d, 2H, J=7.2 Hz, ArH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 28.9, 29.6, 43.1, 43.5, 65.8, 66.7, 117.4, 117.5, 121.3, 121.7, 125.7, 128.6, 129.1, 129.3, 129.5, 129.6, 131.3, 133.6, 133.7, 134.8, 135.1, 135.7, 135.9, 136.5, 150.4, 192.5, 192.8, 199.9, 200.2; mass (ES+) m/z 349.0 (M^++1); Anal. Calcd for $C_{20}H_{16}N_2O_4$: C, 68.96; H, 4.63; N, 8.04. Found: C, 69.15; H, 4.55; N, 7.90.

4.2.7. 4-Benzoyl-3-(5-chloro-2-nitro-phenyl)-2-methylene-5-oxo-hexanenitrile (10b). Yield 71% (1.1 g from 1.15 g) as a white solid; mp 183–185 °C; ν_{max} (KBr) 1672 (CO), 1723 (CO), 2221 (CN) cm^{-1}; ^1H NMR ($CDCl_3$, 200 Hz) δ 1.99 (s, 3H, CH_3), 2.25 (s, 3H, CH_3), 5.36–5.50 (m, 4H, 4×CH), 5.94 (s, 1H, =CH), 6.13 (s, 1H, =CH), 6.18 (s, 1H, =CH), 6.32 (s, 1H, =CH), 7.48–7.91 (m, 14H, ArH), 8.15 (d, 2H, J=7.6 Hz, ArH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 29.4, 29.9, 42.9, 43.3, 65.5, 66.4, 117.3, 120.6, 121.1, 127.2, 129.1, 129.3, 129.6, 133.9, 134.5, 135.0, 135.2, 136.2, 136.5, 140.0, 140.2, 148.6, 192.3, 192.5, 199.3, 199.7; mass (ES+) m/z 405.0 (M^++Na); Anal. Calcd for $C_{20}H_{15}ClN_2O_4$: C, 62.75; H, 3.95; N, 7.32. Found: C, 62.95; H, 4.02; N, 7.33.

4.2.8. 4-Benzoyl-2-methylene-3-(6-nitro-benzo[1,3]-dioxol-5-yl)-5-oxo-hexanenitrile (10c). Yield 81% (0.75 g from 0.8 g) as a yellow solid; mp 189–191 °C; ν_{max} (KBr) 1676 (CO), 1722 (CO and CO_2Me) cm^{-1}; ^1H NMR ($CDCl_3$, 300 Hz) δ 1.99 (s, 3H, CH_3), 5.42 (d, 1H, J=12.0 Hz, CH), 5.61 (s, 1H, J=12.0 Hz, CH), 5.92 (s, 1H, =CH), 6.17 (s, 3H, =CH, CH_2), 7.14 (s, 1H, ArH), 7.44 (s, 1H, ArH), 7.54–7.71 (m, 3H, ArH), 8.13 (d, 2H, J=9.0 Hz, ArH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 28.5, 29.4, 43.0, 43.4, 66.1, 66.9, 103.6, 103.8, 106.8, 108.2, 117.3, 121.3, 121.7, 127.6, 128.6, 129.1, 129.5, 129.6, 134.8, 135.0, 135.7, 136.6, 144.4, 148.1, 152.3, 192.7, 200.1; mass (ES+) m/z 393.0 (M^++1), 415.0 (M^++Na); Anal. Calcd for $C_{21}H_{16}N_2O_6$: C, 64.28; H, 4.11; N, 7.14. Found: C, 64.21; H, 4.19; N, 7.31.

4.2.9. 2-Acetyl-4-methylene-3-(2-nitro-phenyl)-pentanedioic acid 1-ethyl ester 5-methyl ester (11a). Yield 85% (0.80 g from 0.75 g) as a brown oil; ν_{max} (Neat) 1722 (CO, CO_2Me, CO_2Et) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.03 (t, 3H, J=7.5 Hz, CH_3), 1.26 (t, 3H, J=7.5 Hz, CH_3), 2.18 (s, 3H, CH_3), 2.31 (s, 3H, CH_3), 3.68 (s, 3H, CO_2CH_3), 3.69 (s, 3H, CO_2CH_3), 3.98 (q, 2H, J=7.0 Hz, CH_2), 4.20 (q, 2H, J=7.0 Hz, CH_2), 4.57 (d, 1H, J=12.0 Hz, CH), 4.63 (d, 1H, J=9.0 Hz, CH), 5.32 (d, 2H, J=12.0 Hz, CH), 5.88 (s, 1H, =CH), 5.92 (s, 1H, =CH), 6.35 (s, 1H, =CH), 6.36 (s, 1H, =CH), 7.35–7.41 (m, 2H, ArH), 7.51–7.62 (m, 4H, ArH), 7.74–7.79 (m, 2H, ArH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 14.0, 14.4, 29.6, 29.8, 41.0, 41.1, 50.5, 52.5, 62.2, 62.3, 63.4, 63.5, 125.0, 125.1, 128.2, 128.6, 129.1, 130.4, 130.6, 132.8, 133.7, 138.8, 139.2, 150.7, 166.3, 166.7, 167.6, 168.1, 210.2, 201.3; mass (ES+) m/z 349.9 (M^++1); Anal. Calcd for $C_{17}H_{19}NO_7$: C, 58.45; H, 5.48; N, 4.01. Found: C, 58.36; H, 5.44; N, 3.98.

4.2.10. 2-Acetyl-3-(5-chloro-2-nitro-phenyl)-4-methylene-pentanedioic acid 1-ethyl ester 5-methyl ester (11b). Yield 75% (1.0 g from 0.9 g) as a brown oil; ν_{max} (Neat) 1720 (CO, CO_2Me, CO_2Et) cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 1.06 (t, 3H, J=7.0 Hz, CH_2CH_3), 1.24 (t, 3H, J=7.0 Hz, CH_2CH_3), 2.20 (s, 3H, CH_3), 2.26 (s, 3H, CH_3), 3.68 (s, 3H, CO_2CH_3), 3.69 (s, 3H, CO_2CH_3), 3.99–4.21 (2q merged, 4H, 2×CH_2CH_3), 4.15 (d, 1H, J=12.0 Hz, CH), 4.19 (d, 1H, J=12.0 Hz, CH), 5.34 (dd, 2H, J_1=12.0 Hz, J_1=3.4 Hz, 2×CH), 5.90 (s, 1H, =CH), 5.96 (s, 1H, =CH), 6.38 (s, 2H, 2×=CH), 7.32 (d, 2H, J=8.4 Hz, ArH), 7.53 (d, 2H, J=9.6 Hz, ArH), 7.75 (dd, 2H, J_1=8.4 Hz, J_2=2.2 Hz, ArH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 14.1, 14.4, 26.1, 29.7, 30.0, 40.9, 42.6, 58.1, 61.9, 62.4, 63.0, 63.3, 126.6, 128.6, 128.9, 129.6, 130.4, 131.0, 135.7, 136.1, 138.2, 138.6, 139.1, 148.4, 166.2, 166.5, 167.4, 167.8, 200.7, 200.8; mass (ES+) m/z 383.9 (M^++1); HR-EIMS calculated for $C_{17}H_{18}ClNO_7$: 383.0772, found: 383.0770.

4.2.11. 2-Acetyl-4-methylene-3-(6-nitro-benzo[1,3]-dioxol-5-yl)-pentanedioic acid 1-ethyl ester 5-methyl ester (11c) (single diastereoisomer). Yield 77% (0.75 g from 0.8 g) as a white solid; mp 125–127 °C; ν_{max} (KBr) 1710 (CO, CO_2Me, CO_2Et) cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 1.10 (t, 3H, J=7.0 Hz, CH_2CH_3), 2.28 (s, 3H, CH_3), 3.68 (s, 3H, CO_2CH_3), 4.01 (q, 2H, J=7.0 Hz,

CH_2CH_3), 4.49 (d, 1H, J=11.8 Hz, CH), 5.38 (d, 1H, J=11.8 Hz, CH), 5.86 (s, 1H, =CH), 6.07 (s, 2H, OCH_2O), 6.34 (s, 1H, =CH), 6.97 (s, 1H, ArH), 7.31 (s, 1H, ArH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 14.1, 29.7, 40.9, 52.8, 62.2, 63.4, 103.4, 106.0, 109.0, 128.9, 130.3, 138.9, 144.6, 147.2, 151.6, 166.7, 167.6, 201.3; mass (ES+) m/z 393.9 (M^++1); Anal. Calcd for $C_{18}H_{19}NO_9$: C, 54.96; H, 4.87; N, 3.56. Found: C, 55.12; H, 4.99; N, 3.67.

4.2.12. 2-Acetyl-4-cyano-3-(2-nitro-phenyl)-pent-4-enoic acid ethyl ester (12a). Yield 78% (1.0 g from 1.0 g) as a brown solid; mp 85–87 °C; ν_{max} (KBr) 1723 (CO and CO_2Et), 2226 (CN) cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.96 (t, 3H, J=7.0 Hz, CH_2CH_3), 1.31 (t, 3H, J=7.0 Hz, CH_2CH_3), 2.12 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 3.92 (q, 2H, J=7.0 Hz, CH_2CH_3), 4.25 (q, 2H, J=7.0 Hz, CH_2CH_3), 4.41 (d, 1H, J=6.8 Hz, CH), 4.47 (d, 1H, J=6.8 Hz, CH), 5.06 (d, 2H, J=11.8 Hz, 2×CH), 6.05 (s, 1H, =CH), 6.06 (s, 1H, =CH), 6.24 (s, 1H, =CH), 6.28 (s, 2H, 2×=CH), 7.42–7.48 (m, 2H, ArH), 7.56–7.65 (m, 4H, ArH), 7.83–7.88 (m, 2H, ArH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 14.5, 30.1, 42.5, 63.0, 63.1, 117.6, 121.6, 125.7, 128.3, 129.3, 132.1, 133.7, 135.5, 166.6, 199.2; mass (ES+) m/z 317.1 (M^++1), 339.1 (M^++Na); Anal. Calcd for $C_{16}H_{16}N_2O_5$: C, 60.75; H, 5.10; N, 8.86. Found: C, 60.88; H, 5.18; N, 8.99.

4.2.13. 2-Acetyl-3-(5-chloro-2-nitro-phenyl)-4-cyano-pent-4-enoic acid ethyl ester (12b). Yield 64% (0.8 g from 1.0 g) as a white solid; mp 119–121 °C; ν_{max} (KBr) 1708 (CO), 1738 (CO_2Et), 2228 (CN) cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.03 (t, 3H, J=7.0 Hz, CH_2CH_3), 1.32 (t, 3H, J=7.0 Hz, CH_2CH_3), 2.17 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 3.97 (q, 2H, J=7.0 Hz, CH_2CH_3), 4.29 (q, 2H, J=7.0 Hz, CH_2CH_3), 4.37 (d, 1H, J=8.2 Hz, CH), 4.43 (d, 1H, J=8.0 Hz, CH), 5.10 (dd, 2H, J_1=11.6 Hz, J_2=3.6 Hz, 2×CH), 6.08 (s, 2H, 2×=CH), 6.25 (s, 1H, =CH), 6.28 (s, 1H, =CH), 7.39–7.44 (m, 2H, ArH), 7.52 (s, 1H, ArH), 7.59 (s, 1H, ArH), 7.85 (dd, 2H, J_1=8.8 Hz, J_2=2.8 Hz, 2×CH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 14.0, 14.4, 30.1, 30.9, 41.8, 42.2, 62.7, 62.8, 63.0, 63.1, 117.1, 120.7, 121.1, 127.0, 127.2, 128.5, 129.1, 129.4, 134.5, 135.8, 136.6, 140.0, 148.6, 148.7, 165.8, 166.3, 198.8, 199.0; mass (ES+) m/z 351.1 (M^++1), 373.0 (M^++Na); HR-EIMS calculated for $C_{16}H_{15}ClN_2O_5$: 350.0670, found: 350.0671.

4.2.14. 2-Acetyl-4-cyano-3-(6-nitro-benzo[1,3]dioxol-5-yl)-pent-4-enoic acid ethyl ester (12c). Yield 79% (0.68 g from 0.7 g) as a yellow solid; mp 130–132 °C; ν_{max} (KBr) 1710 (CO), 1743 (CO_2Et), 2225 (CN) cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.07 (t, 3H, J=7.0 Hz, CH_2CH_3), 1.32 (t, 3H, J=7.0 Hz, CH_2CH_3), 2.16 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 4.00 (q, 2H, J=7.0 Hz, CH_2CH_3), 4.26 (q, 2H, J=7.0 Hz, CH_2CH_3), 4.32 (d, 1H, J=9.0 Hz, CH), 4.45 (d, 1H, J=9.0 Hz, CH), 5.19 (d, 2H, J=12.0 Hz, 2×CH), 6.04 (s, 1H, =CH), 6.08 (s, 1H, =CH), 6.14 (s, 4H, 2×OCH_2O), 6.22 (s, 1H, =CH), 6.28 (s, 1H, =CH), 6.95 (s, 1H, ArH), 7.02 (s, 1H, ArH), 7.39 (s, 1H, ArH), 7.41 (s, 1H, ArH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 14.0, 14.4, 29.9, 30.8, 41.9, 42.6, 62.9, 63.2, 103.7, 106.4, 106.7, 107.1, 117.3, 121.2, 121.6, 128.3, 135.2, 136.1, 147.8, 152.2, 165.9, 166.6, 199.1; mass (ES+) m/z 383.1 (M^++Na); HR-EIMS calculated for $C_{17}H_{16}N_2O_7$: 360.0958, found: 360.0961.

4.2.15. 2-Benzoyl-4-methylene-3-(2-nitro-phenyl)-pentanedioic acid 1-ethyl ester 5-methyl ester (13a). Yield 82% (1.2 g from 1.0 g) as a brown oil; ν_{max} (Neat) 1691 (CO), 1729 (CO_2Me and CO_2Et) cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.94 (t, 3H, J=6.0 Hz, CH_2CH_3), 1.15 (t, 3H, J=6.0 Hz, CH_2CH_3), 3.60 (s, 3H, CH_3), 3.72 (s, 3H, CH_3), 3.82 (q, 2H, J=6.0 Hz, CH_2CH_3), 4.11 (q, 2H, J=6.0 Hz, CH_2CH_3), 5.43–5.63 (m, 3H, 3×CH), 5.72 (d, 1H, J=12.0 Hz, CH), 5.81 (s, 1H, =CH), 6.06 (s, 1H, =CH), 6.26 (s, 1H, =CH), 6.39 (s, 1H, =CH), 7.37–7.62 (m, 11H, ArH), 7.69–7.72 (m, 1H, ArH), 7.76–7.78 (m, 2H, ArH), 7.97–8.00 (m, 2H, ArH), 8.08–8.11 (m, 2H, ArH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 13.9; 14.3, 42.1, 43.1, 52.4, 57.6, 60.8, 62.3, 125.0, 125.1, 128.2, 128.5, 128.8, 129.1, 129.2, 130.3, 131.2, 132.7, 133.3, 133.4, 134.3, 136.4, 136.6, 138.1, 138.8, 150.9, 166.6, 167.6, 168.0, 192.7; mass (ES+) m/z 411.9 (M^++1), 434.1 (M^++Na); Anal. Calcd for $C_{22}H_{21}NO_7$: C, 64.23; H, 5.14; N, 3.40. Found: C, 64.22; H, 5.01; N, 3.32.

4.2.16. 2-Ethoxycarbonyl-4-methoxycarbonyl-3-(2-nitro-phenyl)-pent-4-enoic acid ethyl ester (14a). Yield 74% (1.0 g from 1.0 g) as brown oil; ν_{max} (Neat) 1727 (CO_2Me and 2×CO_2Et) cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.85 (t, 3H, J=7.0 Hz, CH_2CH_3), 1.05 (t, 3H, J=7.0 Hz, CH_2CH_3), 3.68 (s, 6H, CO_2CH_3), 4.01 (q, 2H, J=7.0 Hz, CH_2CH_3), 4.20 (q, 2H, J=7.0 Hz, CH_2CH_3), 4.41 (d, 1H, J=12.0 Hz, CH), 5.27 (d, 1H, J=12.0 Hz, CH), 5.89 (s, 1H, =CH), 6.38 (s, 1H, =CH), 7.35–7.41 (m, 1H, ArH), 7.51–7.62 (m, 2H, ArH), 7.76 (d, 1H, J=7.5.0 Hz, ArH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 14.0, 14.3, 41.4, 52.5, 55.9, 62.1, 124.9, 126.7, 128.0, 128.5, 130.4, 132.8, 133.4, 139.4, 150.7, 166.2, 167.4, 167.7; mass (ES+) m/z 379.9 (M^++1), 402.0 (M^++Na); Anal. Calcd for $C_{18}H_{21}NO_8$: C, 56.99; H, 5.58; N, 3.69. Found: C, 57.17; H, 5.66; N, 3.85.

4.2.17. 2-[2-Cyano-1-(2-nitro-phenyl)-allyl]-malonic acid diethyl ester (15a). Yield 85% (1.25 g from 1.0 g) as a white solid; mp 78–80 °C; ν_{max} (KBr) 1739 (2×CO_2Et), 2226 (CN) cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.99 (t, 3H, J=7.0 Hz, CH_2CH_3), 1.30 (t, 3H, J=7.0 Hz, CH_2CH_3), 3.93 (q, 2H, J=7.0 Hz, CH_2CH_3), 4.10–4.30 (m, 3H, CH_2CH_3 and 1×CH), 5.01 (d, 1H, J=12.0 Hz, CH), 6.07 (s, 1H, =CH), 6.26 (s, 1H, =CH), 7.43–7.51 (m, 1H, ArH), 7.65 (d, 2H, J=4.0 Hz, ArH), 7.87 (m, 1H, J=8.0 Hz, ArH); mass (ES+) m/z 347.2 (M^++1), 369.1 (M^++Na); Anal. Calcd for $C_{17}H_{18}N_2O_6$: C, 58.96; H, 5.24; N, 8.09. Found: C, 59.08; H, 5.49; N, 7.87.

4.3. General procedure for $SnCl_2$-mediated reactions

To a solution of an appropriate nitro derivative (1.0 equiv) in methanol (10 mL) was added anhydrous $SnCl_2$ (5.0 equiv) and the reaction mixture was heated at reflux in a nitrogen atmosphere for 1.0 h. The excess solvent was removed and the residue was made basic with $NaHCO_3$ solution and taken in EtOAc (50 mL). The resultant suspension was passed through a bed of Celite and the organic layer separated, dried (Na_2SO_4), and evaporated to yield the crude product, which

was purified by silica gel column chromatography using hexanes/EtOAc (80–70:20–30, v/v) to yield corresponding products in 50–86% yield.

4.3.1. 2-(3-Benzoyl-2-methyl-quinolin-4-yl)-acrylic acid methyl ester (16a). Yield 67% (0.35 g from 0.6 g) as a white solid; mp 164–166 °C; ν_{max} (KBr) 1661 (CO), 1727 (CO_2Me) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 2.61 (s, 3H, CH_3), 3.54 (s, 3H, CO_2CH_3), 5.81 (d, 1H, J=0.6 Hz, =CH), 6.66 (d, 1H, J=0.6 Hz, =CH), 7.43 (t, 2H, J=8.6 Hz, ArH), 7.54–7.59 (m, 2H, ArH), 7.66–7.68 (m, 1H, ArH), 7.74–7.79 (m, 3H, ArH), 8.13 (d, 1H, J=6.0 Hz, ArH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 22.7, 50.9, 123.7, 124.0, 125.5, 127.4, 127.6, 128.4, 129.1, 131.3, 132.1, 132.8, 134.1, 135.3, 139.8, 145.9, 153.4, 164.0, 195.9; mass (FAB+) m/z 332 (M^++1); HR-EIMS calculated for $C_{21}H_{17}NO_3$: 331.1208, found: 331.1210.

4.3.2. 2-(3-Benzoyl-6-chloro-2-methyl-quinolin-4-yl)-acrylic acid methyl ester (16b). Yield 61% (0.12 g from 0.25 g) as a white solid; mp 134–136 °C; ν_{max} (KBr) 1660 (CO), 1714 (CO_2Me) cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 2.57 (s, 3H, CH_3), 3.56 (s, 3H, CO_2CH_3), 5.81 (s, 1H, =CH), 6.67 (s, 1H, =CH), 7.40–7.47 (m, 2H, ArH), 7.57–7.77 (m, 5H, ArH), 8.04 (d, 1H, J=9.0 Hz, ArH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 24.5, 52.8, 126.2, 129.2, 130.1, 131.1, 131.6, 133.1, 133.8, 134.2, 134.7, 135.3, 136.9, 140.6, 146.3, 155.5, 165.4, 197.2; mass (ES+) m/z 366.2 (M^++1); HR-EIMS calculated for $C_{21}H_{16}ClNO_3$: 365.0819, found: 365.0820.

4.3.3. 2-(7-Benzoyl-6-methyl-[1,3]dioxolo[4,5-*g*]quinolin-8-yl)-acrylic acid methyl ester (16c). Yield 50% (0.26 g from 0.6 g) as a white solid; mp 188–189 °C; ν_{max} (KBr) 1665 (CO), 1723 (CO_2Me) cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 2.51 (s, 3H, CH_3), 3.54 (s, 3H, CO_2CH_3), 5.76 (s, 1H, =CH), 6.12 (s, 2H, OCH_2O), 6.60 (s, 1H, =CH), 6.89 (s, 1H, ArH), 7.39–7.45 (m, 3H, ArH), 7.54–7.60 (m, 1H, ArH), 7.75 (d, 2H, J=7.4 Hz, ArH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 24.1, 52.6, 101.1, 102.3, 106.0, 122.2, 129.0, 130.1, 134.3, 137.4, 140.3, 146.5, 148.6, 151.6, 152.7, 165.7, 198.0; mass (FAB+) m/z 376 (M^++1); HR-EIMS calculated for $C_{22}H_{17}NO_5$: 375.1107, found: 375.1108.

4.3.4. 2-(3-Benzoyl-2-methyl-1,4-dihydro-quinolin-4-yl)-acrylonitrile (17a). Yield 71% (0.12 g from 0.2 g) as a white solid; mp 188–189 °C; ν_{max} (KBr) 1664 (CO), 2226 (CO_2Me) cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 2.51 (s, 3H, CH_3), 5.02 (s, 1H, CH), 5.68 (s, 1H, =CH), 5.73 (s, 1H, =CH), 6.76 (s, 1H, NH exchangeable with D_2O), 6.80–6.83 (m, 1H, ArH), 7.08–7.11 (m, 1H, ArH), 7.20–7.24 (m, 2H, ArH), 7.39–7.44 (m, 3H, ArH), 7.45–7.49 (m, 2H, ArH); mass (ES+) m/z 301.1 (M^++1); Anal. Calcd for $C_{20}H_{16}N_2O$: C, 79.98; H, 5.37; N, 9.33. Found: C, 80.09; H, 5.45; N, 9.55.

4.3.5. 2-(3-Benzoyl-6-chloro-2-methyl-1,4-dihydro-quinolin-4-yl)-acrylonitrile (17b). Yield 65% (0.28 g from 0.5 g) as a yellow solid; mp 192–194 °C; ν_{max} (KBr) 1665 (CO), 2227 (CN) cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 2.50 (s, 3H, CH_3), 4.92 (s, 1H, CH), 5.74 (s, 1H, =CH), 5.83 (s, 1H, =CH), 6.99 (d, 1H, J=9.0 Hz, ArH), 7.27–7.29 (m, 1H, ArH), 7.35 (d, 1H, J=3.0 Hz, ArH), 7.42–7.52 (m, 5H, ArH), 9.62 (s, 1H, NH); ^{13}C NMR ($CDCl_3$+DMSO-d_6, 50 MHz) δ 20.6, 43.2, 102.4, 107.3, 111.6, 117.1, 118.6, 122.8, 125.9, 126.8, 127.9, 128.3, 128.8, 129.6, 131.0, 136.0, 142.7, 149.7, 194.4; mass (ES+) m/z 335.1 (M^++1); Anal. Calcd for $C_{20}H_{15}ClN_2O$: C, 71.75; H, 4.52; N, 8.37. Found: C, 72.01; H, 4.20; N, 8.55.

4.3.6. 2-(7-Benzoyl-6-methyl-[1,3]dioxolo[4,5-*g*]quinolin-8-yl)-acrylonitrile (18c). Yield 64% (0.36 g from 0.65 g) as a yellow solid; mp 188–190 °C; ν_{max} (KBr) 1664 (CO), 2223 (CN) cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 2.53 (s, 3H, CH_3), 6.02 (s, 1H, =CH), 6.17 (s, 2H, OCH_2O), 6.32 (s, 1H, =CH), 7.18 (s, 1H, ArH), 7.42–7.53 (m, 3H, ArH), 7.61–7.64 (m, 1H, ArH), 7.76 (d, 2H, J=9.4 Hz, ArH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 24.0, 100.2, 102.7, 106.4, 116.9, 117.9, 120.4, 129.6, 130.0, 131.2, 135.0, 136.0, 137.0, 139.1, 146.9, 149.4, 152.2, 153.1, 197.1; mass (ES+) m/z 343.3 (M^++1); HR-EIMS calculated for $C_{21}H_{14}N_2O_3$: 342.1004, found: 342.1009.

4.3.7. 4-(1-Methoxycarbonyl-vinyl)-2-methyl-quinoline-3-carboxylic acid ethyl ester (19a). Yield 86% (0.44 g from 0.6 g) as a brown oil; ν_{max} (Neat) 1727 (CO_2Me and CO_2Et) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.34 (t, 3H, J=7.2 Hz, CH_2CH_3), 2.82 (s, 3H, CH_3), 3.73 (s, 3H, CO_2CH_3), 4.34 (q, 2H, J=7.2 Hz, CH_2CH_3), 5.81 (d, 1H, J=0.6 Hz, =CH), 6.81 (s, 1H, =CH), 7.52–7.56 (m, 1H, ArH), 7.70–7.79 (m, 2H, ArH), 8.09 (d, 1H, J=8.2 Hz, ArH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 14.3, 24.6, 52.9, 62.1, 125.3, 126.1, 126.9, 127.1, 129.3, 131.06, 137.2, 143.1, 147.9, 155.7, 166.0, 168.1; mass (ES+) m/z 300.3 (M^++1), 322.0 (M^++Na); HR-EIMS calculated for $C_{17}H_{17}NO_4$: 299.1158, found: 299.1160.

4.3.8. 6-Chloro-4-(1-methoxycarbonyl-vinyl)-2-methyl-quinoline-3-carboxylic acid ethyl ester (19b). Yield 68% (0.36 g from 0.6 g) as a brown solid; mp 124–126 °C; ν_{max} (KBr) 1729 (CO_2Me and CO_2Et) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.33 (t, 3H, J=7.2 Hz, CH_2CH_3), 2.78 (s, 3H, CH_3), 3.75 (s, 3H, CO_2CH_3), 4.35 (q, 2H, J=7.2 Hz, CH_2CH_3), 5.81 (s, 1H, =CH), 6.82 (s, 1H, =CH), 7.65–7.68 (m, 2H, ArH), 7.99 (d, 1H, J=9.6 Hz, ArH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 13.8, 24.1, 52.5, 61.7, 124.4, 125.6, 127.1, 130.6, 131.1, 131.4, 132.5, 136.2, 141.6, 145.9, 155.6, 165.2, 167.3; mass (ES+) m/z 334.1 (M^++1); HR-EIMS calculated for $C_{17}H_{16}ClNO_4$: 333.0768, found: 333.0770.

4.3.9. 8-(1-Methoxycarbonyl-vinyl)-6-methyl-[1,3]dioxolo[4,5-*g*]quinoline-7-carboxylic acid ethyl ester (19c). Yield 68% (0.36 g from 0.6 g) as a pale yellow oil; ν_{max} (Neat) 1727 (CO_2Me and CO_2Et); ^1H NMR ($CDCl_3$, 200 MHz) δ 1.31 (t, 3H, J=7.0 Hz, CH_2CH_3), 2.73 (s, 3H, CH_3), 3.74 (s, 3H, CO_2CH_3), 4.31 (q, 2H, J=7.0 Hz, CH_2CH_3), 5.76 (s, 1H, =CH), 6.10 (s, 2H, CH_2), 6.74 (s, 1H, =CH), 6.95 (s, 1H, ArH), 7.33 (s, 1H, ArH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 14.3, 24.3, 52.9, 61.9, 101.4, 102.4, 105.9, 122.1, 130.7, 137.8, 144.4, 148.5, 152.0, 153.7, 166.1; mass (ES+) m/z 344.1 (M^++1); Anal. Calcd for $C_{18}H_{17}NO_6$: C, 67.30; H, 4.76; N, 6.20. Found: C, 67.03; H, 4.95; N, 5.95.

4.3.10. 4-(1-Cyano-vinyl)-2-methyl-quinoline-3-carboxylic acid ethyl ester (20a). Yield 65% (0.35 g from 0.65 g) as a brown oil; ν_{max} (Neat) 1728 (CO_2Me), 2228 (CN) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.43 (t, 3H, J=7.2 Hz, CH_2CH_3), 2.80 (s, 3H, CH_3), 4.45 (q, 2H, J=7.2 Hz, CH_2CH_3), 6.13 (s, 1H, =CH), 6.55 (s, 1H, =CH), 7.59–7.66 (m, 1H, ArH), 7.70–7.85 (m, 1H, ArH), 7.93 (d, 1H, J=8.4 Hz, ArH), 8.09 (d, 1H, J=8.4 Hz, ArH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 14.4, 24.4, 30.1, 62.7, 116.9, 118.6, 123.5, 125.0, 128.1, 129.8, 131.6, 137.3, 148.1, 155.7, 165.6; mass (ES+) m/z 267.2 (M^++1), 289.0 (M^++Na); HR-EIMS calculated for $C_{16}H_{14}N_2O_2$: 266.1105, found: 266.1107.

4.3.11. 8-(1-Cyano-vinyl)-6-methyl-[1,3]dioxolo[4,5-*g*]-quinoline-7-carboxylic acid ethyl ester (20c). Yield 50% (0.07 g from 0.18 g) as a pale yellow solid; mp 128–130 °C; ν_{max} (KBr) 1710 (CO_2Et), 2225 (CN) cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 1.43 (t, 3H, J=7.2 Hz, CH_2CH_3), 2.72 (s, 3H, CH_3), 4.42 (q, 2H, J=7.2 Hz, CH_2CH_3), 6.07 (s, 1H, =CH), 6.15 (s, 2H, OCH_2O), 6.49 (s, 1H, =CH), 7.15 (s, 1H, ArH), 7.35 (s, 1H, ArH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 14.3, 24.0, 62.5, 100.3, 102.7, 106.2, 117.0, 119.0, 120.5, 125.3, 136.9, 147.0, 149.3, 152.4, 153.5, 167.8, 183.7; mass (ES+) m/z 311.1 (M^++1); Anal. Calcd for $C_{17}H_{14}N_2O_4$: C, 65.80; H, 4.55; N, 9.03. Found: C, 65.77; H, 4.49; N, 8.81.

4.3.12. 6-Chloro-4-(1-cyano-vinyl)-2-methyl-1,4-dihydro-quinoline-3-carboxylic acid ethyl ester (21b). Yield 60% (0.15 g from 0.3 g) as a white solid; mp 127–129 °C; ν_{max} (KBr) 1717 (CO_2Me), 2218 (CN) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.30 (t, 3H, J=7.2 Hz, CH_2CH_3), 2.44 (s, 3H, CH_3), 4.19 (q, 2H, J=7.2 Hz, CH_2CH_3), 4.85 (s, 1H, CH), 5.69 (s, 1H, =CH), 5.75 (s, 1H, =CH), 6.39 (s, 1H, NH exchangeable with D_2O), 6.68 (d, 1H, J=9.0 Hz, ArH), 7.11–7.15 (m, 2H, ArH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 14.8, 20.9, 43.9, 60.23, 93.4, 116.5, 118.8, 122.2, 127.1, 128.4, 128.6, 128.7, 129.2, 135.5, 150.1, 167.4; mass (ES+) m/z 303.1 (M^++1); Anal. Calcd for $C_{16}H_{15}ClN_2O_2$: C, 63.47; H, 4.99; N, 9.25. Found: C, 63.47; H, 4.99; N, 9.25.

4.3.13. 4-(1-Methoxycarbonyl-vinyl)-2-phenyl-quinoline-3-carboxylic acid ethyl ester (22a). Yield 58% (0.25 g from 0.5 g) as a white solid; mp 107–109 °C; ν_{max} (KBr) 1727 (CO_2Me and CO_2Et) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.05 (t, 3H, J=7.2 Hz, CH_2CH_3), 3.79 (s, 3H, CO_2CH_3), 4.10 (q, 2H, J=7.2 Hz, CH_2CH_3), 5.88 (d, 1H, J=0.6 Hz, =CH), 6.87 (d, 1H, J=0.6 Hz, =CH), 7.43–7.50 (m, 3H, ArH), 7.56–7.62 (m, 1H, ArH), 7.64–7.74 (m, 4H, ArH), 8.09 (d, 1H, J=8.2 Hz, ArH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 14.3, 24.6, 52.9, 62.1, 125.3, 126.1, 126.9, 127.1, 129.3, 131.06, 137.2, 143.1, 147.9, 155.7, 166.0, 168.1; mass (ES+) m/z 362.2 (M^++1); HR-EIMS calculated for $C_{22}H_{19}NO_4$: 361.1314, found: 361.1318.

4.3.14. 4-(1-Methoxycarbonyl-vinyl)-2-oxo-1,2,3,4-tetrahydro-quinoline-3-carboxylic acid ethyl ester (23a). Yield 62% (0.2 g from 0.4 g) as a low melting white solid; ν_{max} (Neat) 1674 (CONH), 1739 (CO_2Et), 3205 (NH) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.15 (t, 3H, J=7.2 Hz, CH_2CH_3), 3.81 (s, 3H, CO_2CH_3), 4.08 (d, 1H, J=6.0 Hz, CH), 4.13–4.20 (q merged with d, 3H, CH_2CH_3 and CHC–C=CH_2), 5.37 (d, 1H, J=0.6 Hz, =CH), 6.40 (d, 1H, J=0.6 Hz, =CH), 7.05–7.12 (m, 1H, ArH), 7.37–7.45 (m, 2H, ArH), 7.51 (d, 1H, J=7.5 Hz, ArH), 8.98 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 12.7, 40.0, 50.8, 51.0, 60.8, 112.8, 121.6, 123.4, 126.7, 127.5, 127.9, 135.7, 136.8, 159.4, 166.0, 166.4; mass (ES+) m/z 304 (M^++1); Anal. Calcd for $C_{16}H_{17}NO_5$: C, 63.36; H, 5.65; N, 4.62. Found: C, 63.52; H, 5.67; N, 4.70.

4.3.15. 4-(1-Cyano-vinyl)-2-oxo-1,2,3,4-tetrahydro-quinoline-3-carboxylic acid ethyl ester (24a). Yield 51% (0.3 g from 0.85 g) as a brown oil; ν_{max} (Neat) 1671 (CONH), 1739 (CO_2Et), 2227 (CN) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.18 (t, 3H, J=7.2 Hz, CH_2CH_3), 3.96 (d, 1H, J=7.5 Hz, $CHCO_2Et$), 4.17 (q, 2H, J=7.1 Hz, CH_2CH_3), 4.28 (d, 1H, J=7.5 Hz, CHC–C=CH_2), 5.73 (d, 1H, J=0.6 Hz, =CH), 6.14 (s, 1H, =CH), 7.18–7.21 (m, 2H, ArH), 7.40–7.44 (m, 1H, ArH), 7.52 (d, 1H, J=8.0 Hz, ArH), 9.21 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 12.7, 42.4, 49.9, 61.3, 113.1, 115.2, 118.3, 119.8, 123.7, 126.5, 128.5, 132.9, 135.4, 158.7, 165.5; mass (ES+) m/z 271.1 (M^++1); Anal. Calcd for $C_{15}H_{14}N_2O_3$: C, 66.66; H, 5.22; N, 10.36. Found: C, 66.68; H, 5.45; N, 10.19.

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Spiroquinazoline support studies: methods for the preparation of imidazoloindolines from oxindoles

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Abstract—Two methods for the annulation of glycine to the 1 and 2 positions of oxindoles are described. The first method involves introduction of an α-azidoacetyl group on the oxindole nitrogen followed by an intramolecular Staudinger reaction to complete the annulation. The second method involves acylation of the oxindole nitrogen with an *N*-Cbz–glycine derivative followed by reduction of the oxindole carbonyl group and subsequent cyclization to provide an imidazoloindoline.

1. Introduction

Imidazoloindolines appear as a substructure in a variety of alkaloids including the tryptoquivalines,[1] asperlicins,[2] and fumiquinazolines.[3] Several years ago we hoped to accomplish the synthesis of structurally unique alkaloid spiroquinazoline (**2**)[4] from the spirooxindole alkaloid alantrypinone (**1**) via reductive annulation of a glycine residue to the 1 and 2 positions of an oxindole (Fig. 1).[5,6] In support of this idea we developed several methods for accomplishing this task. This paper describes the results of these studies and introduces *p*-nitrophenyl 2-azidoacetate as a new reagent for the N-acylation of amides.

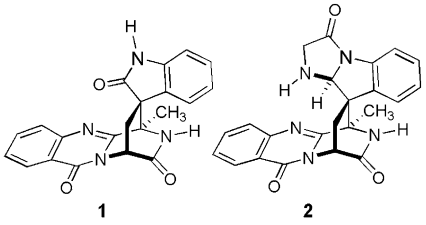

Figure 1. Alantrypinone (**1**) and Spiroquinazoline (**2**).

2. Results and discussion

Our initial studies focused on the transformation of 3,3-dimethyloxindole (**3**) to imidazoloindoline **7** and involved an intramolecular Staudinger reaction largely developed by Eguchi for use in heterocycle synthesis (Scheme 1).[6,7] Thus, the anion derived from deprotonation of known oxindole **3**[8] was acylated using α-chloroacetyl chloride to provide **4** in 90% yield. Treatment of **4** with sodium azide in DMSO gave **5** in 70% yield along with 10% of oxindole **3**. Thus imide N-deacylation is a minor problem in this reaction. Treatment of **5** with triphenylphosphine provided the expected intramolecular Staudinger product **6** in 92% yield. Borch reduction of **6** with sodium cyanoborohydride completed the desired four-step annulation and provided **7** in quantitative yield.[9]

An alternative method for converting **3** into **7** involved initial acylation of the anion derived from **3** with *p*-nitrophenyl *N*-Cbz–glycinate[10] to provide imide **8** in 88% yield. It is notable that in structurally related *N*-acyl oxindoles with *gem*-dimethyl substitution at C_α, the *N*-Cbz group readily adds to the oxindole carbonyl group.[11] Compound **8** exists entirely in the acyclic form as shown in Scheme 1. It was speculated that treatment of **8** with triethylsilane in the presence of an appropriate acid might effect sequential cyclization of the glycine derived nitrogen onto the oxindole carbonyl group, ionization of the resulting carbinol, and reduction of the resulting *N*-acyliminium ion to provide **7**.[10] In reality, treatment of **8** with triethylsilane (4 equiv) in dichloromethane/trifluoroacetic acid (10:1) provided only deacylation product **3**. Treatment of **8** with boron trifluoride etherate (8 equiv) and triethylsilane (2 equiv) in dichloromethane at room temperature returned only the starting imide. On the other hand, saturation of a dichloromethane solution of **8** with BF_3 gas at −78 °C in the presence of triethylsilane (2 equiv), followed by gradual addition of another 4 equiv of triethylsilane and an aqueous workup, gave carbinol **9** in 79% yield. Treatment of **9** with *p*-toluenesulfonic acid in benzene provided **10** and hydrogenolysis of the *N*-Cbz group provided **7** in 98% overall yield.

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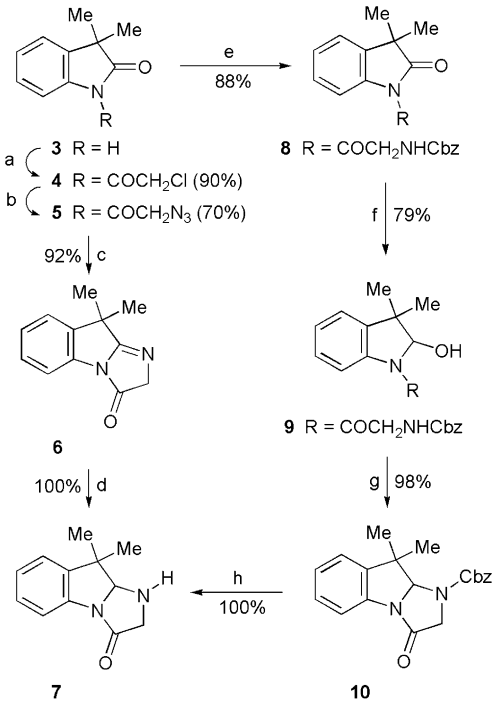
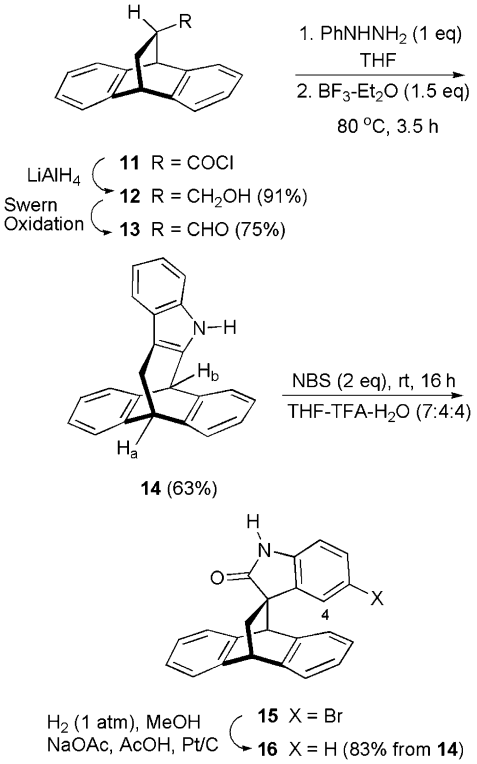


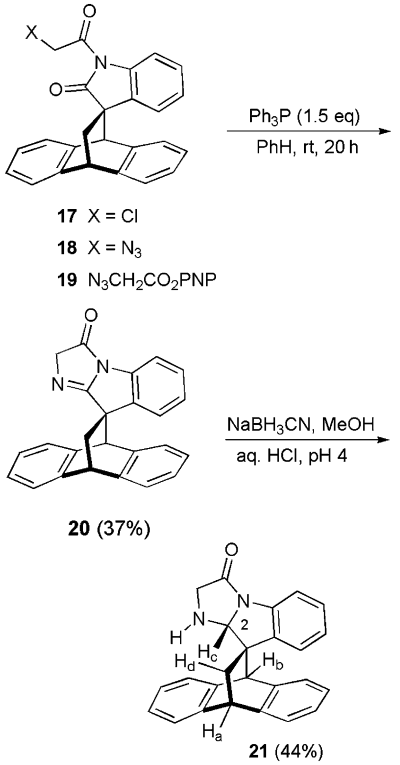
Scheme 1. Two methods for preparing **7** from oxindole **3**: (a) *n*-BuLi, THF, −78 °C; $ClCH_2COCl$ (5 equiv), −78 °C; (b) NaN_3 (4 equiv), DMSO, rt, 20 min; (c) Ph_3P (1.1 equiv), PhH, rt, 2 h; (d) $NaBH_3CN$, MeOH, aq HCl, pH 4, rt; (e) *n*-BuLi, THF, −78 °C; $CbzNHCH_2CO_2PNP$ (1.5 equiv), −78 °C; (f) Et_3SiH (6 equiv), BF_3 (excess), CH_2Cl_2, −78 °C; (g) *p*-TsOH monohydrate, PhH, 80 °C, 30 min and (h) H_2 (1 atm), 10% Pd/C, EtOAc, 3 h.

Our next studies focused on a substrate more closely related to alantrypinone. Thus oxindole **16** was prepared as shown in Scheme 2.[6] The Diels–Alder reaction between acryloyl chloride and anthracene provided acid chloride **11**. Reduction of **11** with lithium aluminum hydride provided alcohol **12** in 91% overall yield from anthracene.[12] Swern oxidation of **12** provided **13** in 75% yield,[13,14] and treatment of **13** with phenylhydrazine in the presence of boron trifluoride etherate gave indole **14** in 63% yield.[15] The regiochemistry of the indole was established using difference NOE experiments. For example irradiation of H_a led to enhancement of the signal for the adjacent methylene and irradiation of H_b led to enhancement of the indole NH. Ring contraction of **14** to oxindole **16** was accomplished using a standard procedure followed by reduction of the intermediate aryl bromide **15** using platinum on carbon.[5,16] It is notable that H_4 of the oxindole aromatic ring in **15** appeared as an upfield singlet (δ 5.0), as one would expect based on its position relative to the dihydroanthracene substructure, and as observed for the analogous proton in 21-*epi*-alantrypinone.[5] The chemical shift of this proton served as an analytical landmark through the remainder of the chemistry described in this paper.

We next examined the intramolecular Staudinger annulation protocol as described in Scheme 3.[6] Acylation of the anion derived from **16** with chloroacetyl chloride provided imide **17** in 40% yield. Treatment of **17** with sodium azide in DMSO, however, failed to provide the desired azide **18**. Only oxindole **16** was obtained in 66% yield. Thus N-deacylation was more problematic with **17** than with substrate **4** (Scheme 1). Introduction of the azido group in the acylating agent circumvented this problem. Rather than using the well known, but hazardous, azidoacetyl chloride as an acylating agent,[17] we decided to examine *p*-nitrophenyl azidoacetate (**19**) as an acylating agent. This new crystalline reagent was prepared by DCC coupling of azidoacetic acid[18] with *p*-nitrophenol in 64% yield. Sequential treatment of



Scheme 2. Preparation of oxindole **16**.



Scheme 3. Preparation of **21**.

oxindole **16** with *n*-BuLi and **19** provided **18** in 40% yield. Treatment of **18** with a slight excess of triphenylphosphine in benzene at room temperature gave a 37% yield of crystalline cyclization product **20** along with 11% of oxindole **16**. Thus, imide deacylation, as with **4** and **17**, was once again a problem. It is also notable that **20** underwent nearly complete conversion to anthracene upon standing in $CDCl_3$ for 2 h. Nonetheless, reduction of **20** using the Borch conditions provided **21** and the corresponding C_2 epimer in 78% yield as a 5:1 mixture, respectively.[9] Pure **21** (43%) and its epimer (4%) were isolated and difference NOE experiments were used to assign stereochemistry at C_2. For example, irradiation of H_c in **21** showed an enhancement of 3.7% at H_d and of 2.5% at the NH. On the other hand, irradiation of H_c in the C_2 diastereomer of **21** showed an enhancement of 3.3% at H_b and of 2.0% at the NH.

Attempts to apply the annulation methods described above for the conversion of alantrypinone to spiroquinazoline have thus far met with failure. For example, the method that relies on the regioselective reduction of imide **8** provides a complex mixture of unidentifiable products when applied to an appropriate alantrypinone derivative. Perhaps this is not surprising given the presence of numerous Lewis basic sites present in such derivatives. Although we were able to prepare appropriate cyclization substrates from alantrypinone, we were not able to accomplish the key aza-Wittig reaction. Instead, the deacylation reaction mentioned above became dominant reaction pathway.

In spite of this disappointment, the research described herein does provide two routes to analogs of spiroquinazoline. The stereochemical course of the reduction of **20** is also notable and suggests that stereochemical problems may accompany attempts to access spiroquinazoline via reduction of an acylimine. In addition, it was found that compound **10** was a weak inhibitor of Substance-P binding to the human NK-1 receptor, the biological activity that has (in part) rendered spiroquinazoline an interesting target for total synthesis.[19] Nonetheless, the quest for other methods (and variations of the methods described herein) for converting alantrypinone to spiroquinazoline and related alkaloids[20] continues in our laboratories.

3. Experimental

3.1. General

All compounds were prepared as racemic mixtures. Melting points are uncorrected. Solvents were dried using standard protocols. All carbon multiplicities (s=C, d=CH, t=CH_2, and q=CH_3) were determined using DEPT techniques. COSY and NOE experiments were used to support NMR peak assignments. Mass spectra were recorded using EI (electron impact) or ESI (electrospray ionization) techniques as indicated.

3.1.1. 1-(Chloroacetyl)-3,3-dimethyl-2-indolinone (4). To a stirred solution of 310 mg (1.90 mmol) of oxindole **3**[8] in 12 mL of dry THF, cooled to −78 °C, was added dropwise 1.66 mL (2.66 mmol) of a 1.6 M solution of *n*-BuLi in hexanes over a period of 3 min. After 10 min at −78 °C, 757 μL (9.50 mmol) of freshly distilled chloroacetyl chloride was added in one portion. The reaction mixture was stirred at −78 °C for 1 h, and then partitioned between 120 mL of ethyl acetate and 30 mL of water. The organic layer was sequentially washed with two 40-mL portions of saturated aqueous $NaHCO_3$, 30 mL of water, dried ($MgSO_4$), and concentrated in vacuo. The residue was recrystallized from a mixture of CH_2Cl_2 and hexanes to give 260 mg (58%) of **4** as a white crystalline solid. The mother liquor was concentrated and the residue was flash chromatographed over 10 g of silica gel (EtOAc/hexanes, 1:3) to give additional 140 mg (32%) of **4**: mp 139.0–140.0 °C (recrystallized from CH_2Cl_2/hexanes); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.46 (s, 6H, $2CH_3$), 4.86 (s, 2H, CH_2Cl), 7.23–7.26 (m, 2H, ArH), 7.28–7.37 (m, 1H, ArH), 8.24 (ddd, J=8.1, 0.9, 0.9 Hz, 1H, H7).

3.1.2. 1-(Azidoacetyl)-3,3-dimethyl-2-indolinone (5). To a vigorously stirred solution of 435 mg (1.68 mmol) of **4** in 8 mL of DMSO was added 438 mg (6.73 mmol) of NaN_3 in one portion. The reaction mixture was stirred at room temperature for 20 min, then poured into 30 mL of water, and extracted with 150 mL of ethyl acetate. The organic solution was washed with four 30-mL portions of water, dried ($MgSO_4$), and concentrated in vacuo to give an orange liquid, which solidified under high vacuum (1 mm of Hg). The solid was flash chromatographed over 15 g of silica gel (Et_2O/hexanes, 1:3, then 1:2) to provide 380 mg (85%) of azide **5** as a white solid in addition to 43 mg (10%) of oxindole **3**. An analytically pure sample of **5** as a white crystalline solid (300 mg or about 80% recovery) was obtained by recrystallization from a mixture of Et_2O and hexanes: mp 101–102 °C; IR (KBr) 2172, 2110, 1757, 1712, 1606 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.45 (s, 6H, $2CH_3$), 4.62 (s, 2H, $COCH_2$), 7.23–7.27 (m, 2H, ArH), 7.28–7.38 (m, 1H, ArH), 8.28 (ddd, J=8.1, 0.9, 0.9 Hz, 1H, H7); ^{13}C NMR (acetone-d_6, 75.5 MHz) δ 25.5 (q), 45.2 (s), 55.1 (t), 117.0 (d), 123.5 (d), 126.5 (d), 128.9 (d), 136.5 (s), 139.4 (s), 170.1 (s), 182.7 (s); mass-spectrum (EI), *m*/*z* (relative intensity) 244 (M^+, 16), 161 (100); Anal. calcd for $C_{12}H_{12}N_4O_2$: C, 59.05; H, 4.96. Found: C, 59.18; H, 4.90.

3.1.3. 2,9-Dihydro-9,9-dimethyl-3*H*-imidazo[1,2-*a*]-indole-3-one (6). To a stirred solution of 220 mg (0.90 mmol) of azide **5** in 6 mL of benzene was added 260 mg (0.99 mmol) of solid triphenylphosphine in one portion. The reaction mixture was stirred at room temperature for 2 h, after which time TLC (silica gel, EtOAc/hexanes, 1:1) indicated complete consumption of starting material. The reaction mixture was concentrated in vacuo and the residue was flash chromatographed over 50 g of silica gel (EtOAc/hexanes, 2:1) to give 165 mg (92%) of imidazoline **6** as a white solid. Sublimation at 1 mm of Hg and 100 °C provided an analytically pure sample of **6** as a white crystalline solid: mp 138–139 °C (sublimed); IR (KBr) 1731, 1673, 1665, 1610 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.56 (s, 6H, $2CH_3$), 4.53 (s, 2H, $COCH_2$), 7.19 (ddd, J=7.5, 7.5, 1.2 Hz, 1H, ArH), 7.29 (ddd, J=7.5, 1.2, 0.6 Hz, 1H, ArH), 7.31 (ddd, J=7.5, 7.5, 1.4 Hz, 1H, ArH), 7.60 (dm, J=7.8 Hz, 1H, ArH); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 26.3 (q), 40.6 (s), 66.2 (t), 112.8 (d), 123.4 (d), 125.6 (d), 128.5 (d), 134.3 (s), 140.9 (s), 174.8 (s), 176.7 (s); mass-spectrum (EI), *m*/*z* (relative intensity) 200 (M^+, 44), 172 (100); Anal.

calcd for $C_{12}H_{12}N_2O$: C, 72.03; H, 6.05. Found: C, 72.12; H, 6.29.

3.1.4. 1,2,9,9a-Tetrahydro-9,9-dimethyl-3*H*-imidazo[1,2-*a*]indole-3-one (7). From **6**: to a stirred solution of 145 mg (0.725 mmol) of imidazoline **6**, a tiny amount of bromocresol green (just to get a green solution), and 45 mg (0.750 mmol) of $NaBH_3CN$ (addition of hydride changes green solution color to blue) in 10 mL of MeOH was added dropwise 1 N aqueous HCl until the solution became yellow (4–5 drops). Addition of HCl was continued whenever the yellow solution turned blue. The reaction mixture was stirred for 1 h at room temperature, after which time TLC (silica gel, Et_2O/Et_3N, 95:5) still showed the presence of starting material. Additional $NaBH_3CN$ (45 mg, 0.75 mmol) was added until starting material was completely consumed. The reaction mixture was partitioned between 150 mL of EtOAc and 20 mL of saturated aqueous $NaHCO_3$. The organic layer was washed with 15 mL of water, dried ($MgSO_4$), and concentrated in vacuo. The residue was chromatographed over 14 g of flash silica gel (Et_2O/Et_3N, 95:5) to give 146 mg (100%) of **7** as a white solid. From **10**: to a solution of 18 mg (0.054 mmol) of **10** in 5 mL of ethyl acetate was added 20 mg of 10% Pd/C. The mixture was stirred at room temperature under 1 atm of H_2 for 3 h. Then the reaction mixture was passed through a 1-cm pad of Celite and concentrated in vacuo to give 11 mg (100%) of amine **7** as a white solid. The hydrochloride of **7** was readily obtained as a white crystalline solid by passing dry HCl through a solution of **7** in ether. Hydrochloride: mp 203–204 °C (dec) (Et_2O). Free base: mp 91.5–92.5 °C; IR (KBr) 3304, 3253, 1708, 1693, 1604 cm^{-1}; ^1H NMR (MeCN-d_3, 300 MHz) δ 1.02 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 2.71 (br s, 1H, NH), 3.55 (d, J=15.7 Hz, 1H, CHH), 3.94 (d, J=15.7 Hz, 1H, CHH), 5.21 (s, 1H, NHCH), 7.12 (tm, J=7.8 Hz, 1H, ArH), 7.2–7.26 (m, 2H, ArH), 7.38 (d, J=7.8 Hz, 1H, ArH); ^{13}C NMR (C_6D_6, 75.5 MHz) δ 23.7 (q), 24.9 (q), 44.6 (s), 54.5 (t), 88.5 (d), 116.2 (d), 123.4 (d), 125.4 (d), 128.4 (d), 138.0 (s), 143.6 (s), 170.9 (s); mass-spectrum (EI), m/z (relative intensity) 202 (M^+, 100); Anal. calcd for $C_{12}H_{15}ClN_2O$ (hydrochloride): C, 60.54; H, 6.35. Found: C, 60.30; H, 6.26.

3.1.5. Benzyl [[(3,3-dimethyl-2-oxo-1-indolinyl)carbonyl]-methyl]carbamate (8). To a stirred solution of 175 mg (1.09 mmol) of oxindole **3** in 5 mL of dry THF, cooled to −78 °C, was added dropwise 885 μL of a 1.6 M solution of *n*-BuLi in hexanes over a period of 2 min. After 10 min at −78 °C, a solution of 538 mg (1.63 mmol) of *p*-nitrophenyl *N*-Cbz–glycinate (**10**) in 3 mL of THF was added by cannula. The reaction mixture was stirred at −78 °C for 20 min, and then left to warm to room temperature. After 4 h at room temperature, the reaction mixture was dissolved in 150 mL of ethyl acetate and washed with two 20-mL portions of 1 M aqueous sodium carbonate. The organic layer was dried ($MgSO_4$), concentrated in vacuo, and the residue was flash chromatographed over 14 g of silica gel (EtOAc/hexanes, 1:3, then 1:2) to give 336 mg (88%) of imide **8** as a white crystalline solid: mp 118.5–119.0 °C; IR (KBr) 3434, 1756, 1724, 1699 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.45 (s, 6H, $2CH_3$), 4.70 (d, J=5.6 Hz, 2H, CH_2NH), 5.17 (s, 2H, OCH_2), 5.52 (br s, 1H, NH), 7.23–7.26 (m, 2H, ArH), 7.27–7.38 (m, 6H, ArH), 8.23 (ddd, J=8.1, 0.9, 0.9 Hz, 1H, H7); ^{13}C NMR (DMSO-d_6, 75.5 MHz) δ 25.4 (q), 44.7 (s), 47.4 (t), 67.2 (t), 116.7 (d), 122.3 (d), 125.8 (d), 128.2 (d), 128.4 (d), 128.6 (d), 135.2 (s), 136.5 (s), 138.2 (s), 156.6 (s), 170.4 (s), 182.1 (s) (one doublet was not seen due to overlap with other peaks); mass-spectrum (EI), m/z (relative intensity) 352 (M^+, 0.15), 161 (100); Anal. calcd for $C_{20}H_{20}N_2O_4$: C, 68.22; H, 5.72. Found: C, 68.05; H, 5.71.

3.1.6. Benzyl [[(2-hydroxy-3,3-dimethyl-1-indolinyl)carbonyl]-methyl]carbamate (9). A solution of 81 mg (0.23 mmol) of imide **8** and 80 μL (0.49 mmol) of Et_3SiH in 8 mL of CH_2Cl_2, cooled to −78 °C, was saturated with gaseous BF_3. The resulting mixture was stirred at −78 °C for 2 h. TLC (EtOAc/hexanes, 1:1) indicated the presence of starting material. Two 20-μL (0.12 mmol) portions of Et_3SiH were added over a period of 1 h to achieve complete consumption of starting material. The reaction mixture was poured into 100 mL of EtOAc and washed with two 30-mL portions of saturated aqueous $NaHCO_3$. The organic layer was dried ($MgSO_4$) and concentrated in vacuo to give 85 mg of colorless amorphous residue. The residue was flash chromatographed over 12 g of silica gel (EtOAc/hexanes, 1:2, then 1:1) to give 65 mg (79%) of **9** as a white solid (**9** solidifies very slowly): mp 136–137 °C: IR (KBr) 3395, 3385, 1699, 1673, 1598 cm^{-1}; ^1H NMR (Me_2CO-d_6, 300 MHz) δ 1.21 (s, 3H, CH_3), 1.44 (s, 3H, CH_3), 4.36 (dd, J=17.1, 5.6 Hz, 1H, COCHNH), 4.44 (dd, J=17.1, 5.6 Hz, 1H, COCHHNH), 5.12 (s, 2H, OCH_2), 5.50 (d, J=8.3 Hz, 1H, CHOH, exchangeable), 5.56 (d, J=8.3 Hz, 1H, CHOH), 6.46 (dt, J=5.6 Hz, 1H, NH), 7.06 (ddd, J=7.4, 7.4, 1.1 Hz, 1H, ArH), 7.19 (ddd, J=7.7, 7.7, 1.4 Hz, 1H, ArH), 7.23 (dm, J=7.4 Hz, 1H, ArH), 7.27–7.42 (m, 5H, ArH), 8.08 (br d, J=7.2 Hz, 1H, ArH); ^{13}C NMR (Me_2CO-d_6, 75.5 MHz) δ 20.0 (q), 29.8 (q), 44.4 (t), 45.8 (s), 66.9 (t), 91.9 (d), 117.1 (d), 123.2 (d), 124.9 (d), 128.2 (d), 128.7 (d), 129.2 (d), 138.2 (s), 140.1 (s), 141.2 (s), 157.6 (s), 169.4 (s), one doublet was not seen due to overlap with other signals; mass-spectrum (EI), m/z (relative intensity) 354 (M^+, 3.5), 91 (100); Anal. calcd for $C_{20}H_{22}N_2O_4$: C, 67.83; H, 6.26. Found: C, 67.57; H, 6.37.

3.1.7. Benzyl 2,3,9,9a-tetrahydro-9,9-dimethyl-3-oxo-1*H*-imidazo[1,2-*a*]indole-3-carboxylate (10). To a solution of 194 mg (0.54 mmol) of **9** in 30 mL of benzene was added 10 mg (10 mol %) of *p*-toluenesulfonic acid monohydrate. The resulting mixture was refluxed for 30 min after which time TLC (EtOAc/hexanes, 1:1) indicated complete consumption of starting material. The reaction mixture was cooled to room temperature, passed through a 1-cm pad of basic alumina (Brockman activity II), and concentrated in vacuo to give 180 mg (98%) of imidazolidine **10** as a thick colorless liquid slowly solidifying into a white solid: mp 67–68 °C; IR (KBr) 1725, 1712, 1604 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz, 60 °C) δ 1.02 (s, 3H, CH_3), 1.58 (br s, 3H, CH_3), 4.18 (dd, J=16.4, 1.8 Hz, 1H, COCHNH), 4.45 (d, J=16.4 Hz, 1H, COCHHN), 5.22 (1/2 of AB quartet, J=12.2 Hz, 1H, OCHH), 5.27 (1/2 of AB quartet, J=12.2 Hz, 1H, OCHH), 5.62 (d, J=1.8 Hz, 1H, NCHN), 7.14–7.20 (m, 2H, ArH), 7.26 (ddd, J=7.6, 5.5, 3.6 Hz, 1H, ArH), 7.34–7.41 (m, 5H, ArH), 7.51 (ddd, J=7.6, 0.9, 0.9 Hz, 1H, ArH); ^{13}C NMR ($CDCl_3$, 75.5 MHz, 60 °C) δ 23.3 (q), 24.5 (q), 45.9 (s), 52.9 (t), 68.1 (t), 87.1 (d), 116.7 (d),

123.2 (d), 126.3 (d), 128.1 (d), 128.4 (d), 128.7 (d), 128.8 (d), 136.1 (s), 136.5 (s), 142.8 (s), 154.5 (s), 167.3 (s); mass-spectrum (EI), *m/z* (relative intensity) 336 (M^+, 7), 91 (100); Anal. calcd for $C_{20}H_{20}N_2O_3$: C, 71.46; H, 6.00. Found: C, 71.27; H, 5.99.

3.1.8. Indole 14. To a stirred solution of 9.0 g (38.5 mmol) of aldehyde **13** in 100 mL of tetrahydrofuran was added 3.78 mL (4.16 g, 38.5 mmol) of phenylhydrazine. The reaction mixture was stirred for 10 min after which 6.82 mL (7.64 g, 53.85 mmol) of $BF_3 \cdot Et_2O$ was added dropwise over a period of 10 min. The reaction mixture was heated at 80 °C with stirring for 3.5 h. The reaction mixture was partitioned between 150 mL of chloroform and 35 mL of saturated aqueous sodium bicarbonate. The organic layer was washed with two 50-mL portions of saturated aqueous sodium bicarbonate and two 50-mL portions of water. The combined aqueous layers were extracted with 70 mL of chloroform. The combined organic phases were dried ($MgSO_4$) and concentrated under reduced pressure to afford 11 g of a dark orange solid. This solid was purified by column chromatography over 400 g of silica gel (sample loaded in CH_2Cl_2 and eluted with hexanes/CH_2Cl_2, 99:1; then hexanes/CH_2Cl_2, 2:1; then EtOAc) to give 7.44 g (63%) of indole **14** as a white solid: mp 295–296.5 °C; IR (KBr) 3388 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 3.22 (d, J=3.7 Hz, 2H, CH_2), 4.47 (t, J=3.6 Hz, 1H, $CHCH_2$), 4.78 (s, 1H, CH), 6.98 (t, J=9.0 Hz, 1H, ArH), 7.05 (t, J=9.0 Hz, 1H, ArH), 7.13 (t, J=8 Hz, 2H, ArH), 7.15 (t, J=8.5 Hz, 2H, ArH), 7.21 (d, J=7 Hz, 1H, ArH), 7.28 (m, 3H, ArH), 7.46 (d, J=6 Hz, 2H, ArH), 7.96 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 31.1 (t), 46.4 (d), 47.4 (d), 104.4 (s), 111.0 (d), 117.8 (d), 119.9 (d), 121.6 (d), 124.4 (d), 126.5 (d), 127.0 (d), 127.2 (d), 130.1 (s), 134.5 (s), 136.5 (s), 141.6 (s), 144.7 (s); exact mass (ESI) calcd for $C_{23}H_{17}NNa^+$: *m/z* 330.1259, observed: *m/z* 330.1253. Anal. calcd for $C_{23}H_{17}N$: C, 89.86; H, 5.58; N, 4.56. Found: C, 89.71; H, 5.82; N, 4.61.

3.1.9. Bromooxindole 15. To a stirred solution of 107.5 mg (0.35 mmol) of indole **14** in 15 mL of a mixture of THF/TFA/H_2O (7:4:4) cooled to 3 °C was added 62 mg (0.35 mmol) of *N*-bromosuccinimide. The reaction was allowed to stir for 1.5 h at 2–3 °C after which another 62 mg (0.35 mmol) of *N*-bromosuccinimide was added. The reaction was allowed to stir for a total of 7.5 h at 2–3 °C. The reaction mixture was partitioned between 50 mL of saturated aqueous sodium bicarbonate and 200 mL of ethyl acetate. The organic layer was washed with two 50-mL portions of water, dried ($MgSO_4$), and concentrated under reduced pressure to afford 121 mg of a beige solid. This material was normally used in subsequent reactions without further purification. For characterization purposes this solid was purified by column chromatography over 15 g of flash silica (eluted with hexanes/ethyl acetate 9:1, then 4:1) to give 57 mg (53%) of bromospirooxindole **15** as a white solid: mp 208–211 °C; IR (KBr) 1716, 1614 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 1.84 (dd, J=11.2, 3.0 Hz, 1H, CH_2), 2.03 (dd, J=11.2, 3.0 Hz, 1H, CH_2), 4.08 (s, 1H, CH), 4.60 (t, J=8.2 Hz, 1H, $CHCH_2$), 5.01 (d, J=2.1 Hz, 1H, ArH), 6.75 (d, J=8.1 Hz, 1H, ArH), 7.08 (m, 4H, ArH), 7.19 (dd, J=10.2, 2.0 Hz, 1H, ArH), 7.25 (m, 2H, ArH), 7.34 (d, J=1.2 Hz, 1H, ArH), 7.50 (d, J=7.0 Hz, 1H, ArH), 10.4 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 62 MHz) δ 39.9 (t), 43.5 (d), 51.8 (s), 52.0 (d), 111.1 (d), 112.7 (s), 123.0 (d), 123.9 (d), 125.41 (d), 125.49 (d), 126.0 (d), 126.5 (d), 126.8 (d), 127.1 (d), 130.5 (d), 136.6 (s), 139.6 (s), 141.14 (s), 141.16 (s), 144.1 (s), 144.2 (s), 179.2 (s) (one aromatic CH obscured by solvent); exact mass (ESI) calcd for $C_{23}H_{16}N^{79}BrNa^+$: *m/z* 424.0307, observed; *m/z* 424.0329.

3.1.10. Oxindole 16. To a stirred solution of 1.0 g (3.26 mmol) of indole **14** in 100 mL of a mixture of THF/TFA/H_2O (60:20:20) cooled to 3 °C was added 1.16 g (6.51 mmol) of *N*-bromosuccinimide. The reaction was allowed to reach room temperature and stirred for 16 h after which it was filtered. The filtrate was partitioned between 150 mL of saturated aqueous sodium bicarbonate and 200 mL of ethyl acetate. The organic layer was washed with two 50-mL portions of saturated aqueous sodium bicarbonate and two 40-mL portions of water, dried ($MgSO_4$), and concentrated under reduced pressure to afford 1.17 g of bromooxindole **15** as a beige solid. To a stirred solution of 1.15 g (2.71 mmol) of bromooxindole **15** in 770 mL of methanol was added 4.64 g (56.6 mmol) sodium acetate, 9.3 mL (9.76 g, 162.6 mmol) of acetic acid and 0.9 g of 5% platinum on carbon. The system was placed under a hydrogen atmosphere for 72 h. The reaction mixture was filtered through a short pad of Celite 545 and the filtrate was concentrated to give a white solid. The solid was partitioned between 200 mL of ethyl acetate and 50 mL of water. The organic layer was washed with five 40-mL portions of water, dried ($MgSO_4$) and concentrated under reduced pressure to afford 0.99 g (83%) of oxindole **16** as a white solid: mp 215–216 °C; IR (KBr) 3231, 1718 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 1.83 (dd, J=13.1, 2.0 Hz, 1H, CH_2), 2.05 (dd, J=13.1, 2.0 Hz, 1H, CH_2), 4.04 (s, 1H, CH), 4.58 (t, J=5.2 Hz, 1H, $CHCH_2$), 5.05 (d, J=8.0 Hz, 1H, ArH), 6.55 (t, J=7.0 Hz, 1H, ArH), 6.78 (d, J=7.8 Hz, 1H, ArH), 6.98 (d, J=7.0 Hz, 1H, ArH), 7.08 (m, 4H, ArH), 7.18 (d, J=6.0 Hz, 1H, ArH), 7.23 (td, J=8.1, 1.0 Hz, 1H, ArH), 7.33 (d, J=6.0 Hz, 1H, ArH), 7.48 (d, J=8.0 Hz, 1H, ArH), 10.25 (br s, 1H, ArH); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 39.6 (t), 43.3 (d), 51.0 (s), 51.8 (d), 108.9 (d), 120.4 (d), 122.6 (d), 123.3 (d), 123.6 (d), 124.9 (d), 125.0 (d), 125.5 (d), 126.0 (d), 126.2 (d), 126.3 (d), 127.5 (d), 133.9 (s), 139.7 (s), 141.0 (s), 141.4 (s), 143.8 (s), 144.0 (s), 179.4 (s); exact mass (ESI) calcd for $C_{23}H_{17}NONa^+$: *m/z* 346.1202, found: *m/z* 346.1200.

3.1.11. *p*-Nitrophenyl α-azidoacetate (19). To a solution of 0.92 g (9.12 mmol) of azidoacetic acid in 10 mL of dichloromethane was added 1.15 g (8.29 mmol) of *p*-nitrophenol and 50 mg (0.415 mmol) of 4-dimethylaminopyridine. The solution was stirred for 5 min and 1.80 g (9.12 mmol) of DCC in 5 mL of dichloromethane was added dropwise over a period of 30 min. The mixture was stirred for 2 h at room temperature. The resulting mixture was filtered and the filtrate was concentrated to give a yellow solid. The residue was purified by recrystallization from 90 mL of hexanes/ether (2:1) to give 1.17 g (64%) of *p*-nitrophenyl α-azidoacetate (**19**) as a white solid: mp 81–82.5 °C; IR (KBr) 3111, 2115, 1763, 1616 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 4.19 (s, 2H, $COCH_2$), 7.33–7.38 (m, 2H, ArH), 8.27–8.33 (m, 2H, ArH); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 50.4 (t), 122.3 (d), 125.4 (d), 145.8 (s), 154.7 (s), 166.3 (s); mass-spectrum

(EI), *m*/*z* (relative intensity) 222 (M^+, 2), 109 (100); Anal. calcd for $C_8H_6N_4O_4$: C, 43.28; H, 2.72. Found: C, 43.37; H, 2.69.

3.1.12. Imide 18. To a stirred solution of 76 mg (0.24 mmol) of spirooxindole **16** in 4 mL of tetrahydrofuran cooled to −70 °C was added 195 μL (0.27 mmol) of *n*-butyllithium (1.3 M in hexanes). The solution was stirred for 20 min and then 68 mg (0.31 mmol) of *p*-nitrophenyl α-azidoacetate (**19**) in 2 mL of tetrahydrofuran was added. The reaction mixture was stirred at −70 °C for 30 min, then at room temperature for 2 h. The reaction mixture was partitioned between 60 mL of ethyl acetate and 20 mL of water. The organic layer was washed with three 20-mL portions of water, dried ($MgSO_4$), and concentrated under reduced pressure to afford 150 mg of solid. This solid was purified by column chromatography over 5 g of silica (eluted with EtOAc/hexanes, 1:8, then 1:4) to give 33 mg (40%) of imide **18** as a white solid: mp 169–171 °C (dec); IR (KBr) 2120, 1753, 1716 cm^{-1}; ^1H NMR ($CDCl_3$, 250 MHz) δ 1.94 (dd, J=12.6, 2.8 Hz, 1H, CH_2), 2.26 (dd, J=12.6, 2.8 Hz, 1H, CH_2), 3.96 (s, 1H, $CHAr_2$), 4.39 (s, 2H, CH_2N_3), 4.47 (t, J=2.3 Hz, 1H, $CHCH_2$), 5.25 (d, J=7.6 Hz, 1H, ArH), 6.78 (d, J=7.3 Hz, 1H, ArH), 6.85 (d, J=8.3 Hz, 1H, ArH), 7.12 (m, 6H, ArH), 7.35 (m, 2H, ArH), 8.13 (d, J=8.1 Hz, 1H, ArH); ^{13}C NMR ($CDCl_3$, 62 MHz) δ 41.1 (t), 44.2 (d), 52.4 (s), 53.9 (d), 54.6 (t), 115.8 (d), 123.1 (d), 123.5 (d), 124.0 (d), 125.0 (d), 125.7 (d), 125.9 (d), 126.8 (d), 126.9 (d), 127.1 (d), 128.3 (d), 132.7 (s), 138.1 (s), 138.4 (s), 139.1 (s), 143.3 (s), 143.8 (s), 168.4 (s), 179.2 (s) (one aromatic CH obscured by solvent); exact mass (ESI) calcd for $C_{25}H_{18}N_4O_2Na^+$: *m*/*z* 429.1321, found: *m*/*z* 429.1302.

3.1.13. *N*-Acylamidine 20. To a stirred solution of 129 mg (0.32 mmol) of azide **18** in 4 mL of benzene was added 127 mg (0.47 mmol) of triphenylphosphine in 2 mL of benzene. The mixture was stirred for 20 h. The resulting precipitate was collected by suction filtration to give 35 mg (32%) of imidazolinone **20** as a white solid. The filtrate was concentrated in vacuo to give a mixture of oxindole **16**, triphenylphosphine oxide and *N*-acylamidine **20**. The mixture was purified by column chromatography over 10 g of silica (eluted with EtOAc/Hexanes, 1:2) to give 13 mg (11%) of oxindole **16** and 5.9 mg (5%) of *N*-acylamidine **20** as a white solid: mp 202–203 °C (dec); IR (KBr) 1737, 1654 cm^{-1}; ^1H NMR (C_6D_6, 400 MHz) δ 1.85 (dd, J=12.6, 2.8 Hz, 1H, CH_2CH), 2.32 (dd, J=12.6, 2.8 Hz, 1H, CH_2CH), 3.60 (s, 1H, $CHAr_2$), 3.92 (d, J=22 Hz, 1H, $CH_2C{=}O$), 4.05 (d, J=22 Hz, 1H, $CH_2C{=}O$), 4.13 (t, J=2.6 Hz, 1H, $CHCH_2$), 5.44 (d, J=8.1 Hz, 1H, ArH), 6.62 (d, J=7.3 Hz, 1H, ArH), 6.83 (d, J=8.1 Hz, 1H, ArH), 6.97 (m, 2H, ArH), 7.08 (m, 4H, ArH), 7.17 (s, 1H, ArH), 7.76 (d, J=7.8 Hz, 1H, ArH), the remaining ArH was obscured by the benzene; ^{13}C NMR (C_6D_6, 125 MHz) δ 42.9 (t), 44.7 (d), 48.0 (s), 53.6 (d), 65.7 (t), 112.1 (d), 123.4 (d), 123.6 (d), 124.2 (d), 125.2 (d), 125.7 (d), 125.8 (d), 126.7 (d), 127.0 (d), 126.98 (d), 135.7 (s), 138.8 (s), 139.4 (s), 140.5 (s), 143.9 (s), 144.5 (s), 172.3 (s), 174.5 (s), the remaining two aromatic carbons (CH) were obscured by the benzene; exact mass (ESI) calcd for $C_{25}H_{18}N_2O^+$: *m*/*z* 362.1413, found: *m*/*z* 362.1445.

3.1.14. Imidazoloindolines 21 and 2-*epi*-21. To a stirred solution of 57 mg (157 μmol) of imidazolinone **20** in 22 mL of methanol, was added four drops of bromocresol green indicator solution (0.04 g of the indicator in 100 mL of 95% EtOH and 0.1 M aqueous NaOH until blue) and 21 mg (315 μmol) of sodium cyanoborohydride. The reaction was kept at pH 4, maintaining the yellow color by the dropwise addition of 3 N aqueous hydrochloric acid. The reaction was stirred at room temperature for 5.5 h. The reaction mixture was partitioned between 200 mL of ethyl acetate and 60 mL of saturated aqueous sodium bicarbonate. The organic layer was washed with two 50-mL portions of saturated aqueous sodium bicarbonate and 30 mL of water, dried ($MgSO_4$), and concentrated under reduced pressure. The residue was chromatographed over 30 g of silica (loaded in EtOAc and eluted with EtOAc/hexanes, 1:3) to give 24.8 mg (43%) of imidazoloindoline **21** as a white solid, 18.2 mg of a 7:4:1 mixture of imidazoloindoline **21**, the C_2 epimers of **21** and oxindole **16**, respectively, and 2 mg (4%) of the C_2 epimer of **21** as a white solid. Imidazoloindoline **21**: mp 259.5–260 °C (dec); IR 3432, 1708 cm^{-1}; ^1H NMR (C_6D_6, 500 MHz) δ 0.43 (br s, 1H, NH), 1.70 (dd, J=12.9, 2.1 Hz, 1H, CH_2), 2.10 (dd, J=12.9, 2.1 Hz, 1H, CH_2), 3.13 (d, J=14.3 Hz, 1H, CH_2N), 3.41 (d, J=14.3 Hz, 1H, CH_2N), 4.01 (t, J=2.3 Hz, 1H, $CHCH_2$), 4.14 (s, 1H, $CHAr_2$), 4.52 (s, 1H, CHNH), 5.22 (d, J=7.5 Hz, 1H, ArH), 6.53 (td, J=7.7, 0.8 Hz, 1H, ArH), 6.62 (d, J=7.3 Hz, 1H, ArH), 6.88 (t, J=7.8 Hz, 1H, ArH), 6.95 (m, 5H, ArH), 7.09 (d, J=7.2 Hz, 2H, ArH), 7.87 (d, J=7.7 Hz, 1H, ArH); ^{13}C NMR (C_6D_6, 125 MHz) δ 45.0 (d), 45.8 (t), 50.3 (d), 52.3 (s), 54.2 (t), 88.6 (d), 114.3 (d), 122.9 (d), 123.3 (d), 123.7 (d), 125.2 (d), 125.5 (d), 125.6 (d), 125.8 (d), 126.3 (d), 127.2 (d), 137.5 (s), 141.3 (s), 142.0 (s), 142.5 (s), 145.1 (s), 145.6 (s), 168.5 (s), the remaining two aromatic carbons (CH) were obscured by the benzene; exact mass (ESI) calcd for $C_{25}H_{20}N_2ONa^+$: *m*/*z* 387.1473, found: *m*/*z* 387.1547. Anal. calcd for $C_{25}H_{20}N_2O$: C, 82.38; H, 5.54; N, 7.69. Found: C, 82.32; H, 5.82; N, 7.67. C_2 epimer of **21**: mp 265–267 °C (dec); ^1H NMR (C_6D_6, 400 MHz) δ 0.40 (br s, 1H, NH), 1.24 (dd, J=12.5, 2.2 Hz, 1H, CH_2), 2.98 (dd, J=12.5, 3.2 Hz, 1H, CH_2), 3.06 (d, J=1.2 Hz, 2H, CH_2), 3.75 (s, 1H, CH), 3.94 (t, J=2.6 Hz, 1H, $CHCH_2$), 4.82 (br s, 1H, CHNH), 6.13 (d, J=7.6 Hz, 1H, ArH), 6.65 (td, J=7.6, 1.1 Hz, 1H, ArH), 6.80 (m, 2H, ArH), 6.93 (m, 2H, ArH), 7.05 (m, 5H, ArH), 7.85 (d, J=8.0 Hz, 1H, ArH); ^{13}C NMR (C_6D_6, 125 MHz) δ 39.4 (t), 44.9 (d), 52.9 (t), 53.2 (s), 54.5 (d), 85.3 (d), 114.9 (d), 123.2 (d), 124.3 (d), 124.8 (d), 125.0 (d), 125.5 (d), 125.7 (d), 126.3 (d), 126.5 (d), 126.7 (d), 127.5 (d), 138.4 (s), 140.4 (s), 140.9 (s), 141.2 (s), 144.7 (s), 146.3 (s), 168.6 (s), the remaining aromatic carbon was obscured by the benzene; exact mass (ESI) calcd for $C_{25}H_{20}N_2ONa^+$: *m*/*z* 387.1473, found: *m*/*z* 387.1461.

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Supplementary data

Experimental procedures for the preparation of **12** and **13**, and ^1H and ^{13}C NMR spectra of most compounds are

available as supplementary material. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2006.06.103.

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Cyclization reactions of *N*-acryloyl-2-aminobenzaldehyde derivatives: formal total synthesis of martinellic acid

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Abstract—*N*-Alkyl acryloylamides derived from *o*-aminobenzaldehyde derivatives react with *N*-alkyl glycine derivatives to provide cis-fused pyrrole[3,2-*c*]quinolones in moderate yield and high diastereoselectivity. These same substrates engage in a tandem Michael–Mannich pathway on treatment with a secondary amine, providing corresponding quinolone derivatives. The elaboration of a pyrroloquinolone derivative via addition of an in situ generated functionalized copper acetylide to an in situ generated iminium ion provided the C2-substituted derivative. Global deprotection and reduction of the alkyne afford the tricyclic triamine core (as the HCl salt) found in martinellic acid.

1. Introduction

The *Martinella* alkaloids, martinelline (**1**) and martinellic acid (**2**), were isolated from the South American medicinal plant *Martinella iquitosensis* by Witherup and co-workers at Merck Laboratories in 1995 in the course of a natural products screening program.[1,2] These alkaloids were among the first potent, naturally occurring (non-peptide) bradykinin (BK) receptor antagonists to be reported.[3] In addition to their biological activity, these alkaloids contain a partially reduced pyrrolo[3,2-*c*]quinoline ring system, which had not been observed in a natural product prior to the isolation of **1** and **2**, although the parent aromatic heterocycle was well known.[2] The novelty of the ring system found in martinelline and martinellic acid combined with the BK antagonistic behavior of these two natural products has elicited significant interest from the synthetic community,[2,4–17] which in turn has led to the development of a number of strategies for the assembly of the heterocyclic core and most recently to several total syntheses of **1** and **2**.[7,10,14,16,17] Our approach to these targets, which is illustrated below in a retrosynthetic manner (Fig. 1), involves the obvious disconnection of the guanidine groups and functional group manipulations to provide the tricyclic core **3**. Further, disconnection of the C2–C10 bond then provides the key tricyclic intermediate **4** as shown in Figure 1, which not only should function as an useful intermediate *en route* to the natural products, but also may be useful for the eventual preparation of a diverse library of analogs for application in chemical biology studies. It was envisioned that

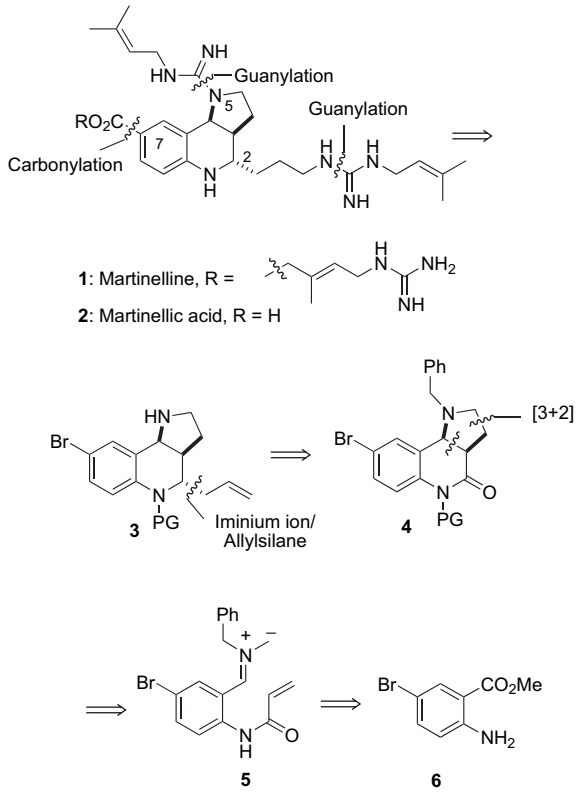

Figure 1. Retrosynthetic analysis of **1** and **2**.

the carbonyl group in the C2-position could be utilized for the stereoselective incorporation of the three-carbon side chain found in the natural products, presumably by the Lewis

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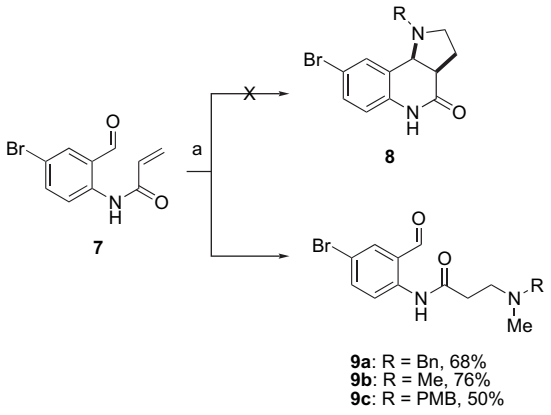
acid-mediated nucleophilic addition of an allylsilane to an iminium ion. The pyrroloquinolone core **4** would be accessed in turn via an intramolecular [3+2] cycloaddition reaction between a non-stabilized azomethine ylide and an electron deficient alkene (Fig. 1, **5**→**4**).[18,19] Related azomethine ylide-based strategies for construction of the key pyrrolo-[3,2-*c*]quinoline skeleton have not only been investigated by our group,[9] but also by Snider et al.[10] and by Nyerges et al. (intermolecular variant with stabilized ylides).[13]

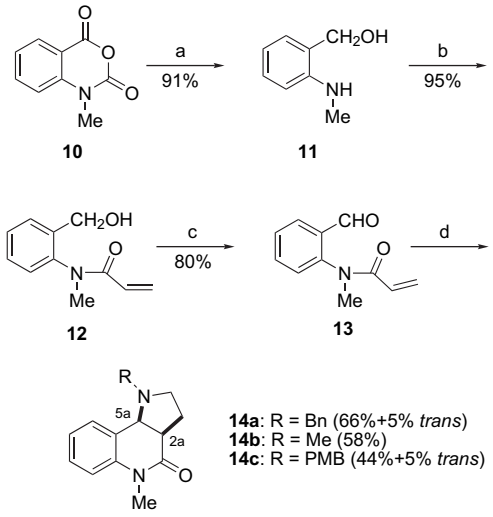
Herein, we report the full details of our investigation of the azomethine ylide cycloaddition reactions of the *N*-acryloyl-2-aminobenzaldehyde derivatives for the construction of pyrroloquinolones **4**, and subsequent conversion of one of these cycloadducts into an advanced precursor for the total synthesis of the *Martinella* alkaloids.[9] In addition to the formal total synthesis, the observation of some initially unanticipated, but nonetheless interesting, events during the course of the reactions of *N*-acryloyl-2-aminobenzaldehyde substrates with *N*-alkyl glycine derivatives, i.e., a domino Michael–Mannich reaction, are described.

Our initial approach to the construction of the key heterocyclic core of these natural products involved the reaction of acrylamide derivative **7** with various *N*-alkyl glycine derivatives.[20,21] However, the expected product **8** was not obtained, but adducts **9a–c** were obtained from a net decarboxylative Michael addition (Scheme 1).[22] Extensive control studies indicated that the requisite azomethine ylide **5** (Fig. 1) was being formed, but underwent protonation and hydrolysis, leading to formation of the amine, followed by conjugate addition to the acryloyl moiety. Our interpretation of this observation centered on low population of the reactive rotamer.[23,24] Therefore to address this possibility, we turned our attention to the use of *N*-alkyl acrylamide derivatives.[25]



Scheme 1. Reagents and conditions: (a) $RNHCH_2CO_2H$ (R=Bn, PMB as HCl salt), Et_3N, DMF, reflux.

Initially attempts were made to alkylate **7** with MeI directly, however, these experiments were unsuccessful, and therefore, *N*-methyl isatoic anhydride (**10**) was employed as the starting material. Reduction with $LiAlH_4$ provided the known amino alcohol **11**, which was then acylated chemoselectively with acryloyl chloride according to the protocol of Heaney and co-workers,[26] providing **12** in an excellent yield (Scheme 2). Subsequent MnO_2 oxidation provided the required cyclization precursor, acrylamide **13**, however, the yield of this reaction was found to be scale dependent (0.3 mmol scale, 88%; 8.0 mmol scale, 55%). PCC oxidation only afforded the product in a moderate 40% yield. Ultimately, the hypervalent IBX (*o*-iodoxybenzoic acid) reagent[27] was found to give the most satisfactory result providing **13** in an excellent yield, regardless of the reaction scale.

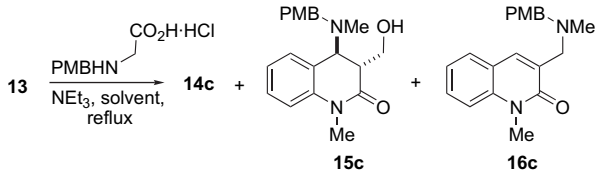


Scheme 2. Reagents and conditions: (a) $LiAlH_4$, THF; (b) acryloyl chloride, CH_2Cl_2, $NaHCO_3$; (c) IBX, DMSO; (d) $RNHCH_2CO_2H{\cdot}HCl$, Et_3N, toluene, reflux.

When **13** was subjected to reaction with *N*-benzylglycine·HCl in refluxing toluene, we were delighted to find that it had undergone the desired cycloaddition reaction to provide the cis-fused pyrroloquinolone **14a** in 66% yield. In addition to the cis-fused adduct ($J_{2a,5a}$=5.5 Hz), a small quantity of the *trans*-adduct ($J_{2a,5a}$=13.8 Hz) ca. 5% was isolated.[9c] In similar cycloaddition reactions with sarcosine and PMB-glycine·HCl, *cis*-pyrroloquinolones, **14b** and **14c** were obtained in 58 and 44% (the latter in DMF, vide infra) yields, respectively. The corresponding *trans*-isomers were also produced in these reactions, but in less than 5% yield based on the analysis of the ^1H NMR spectra of the crude reaction mixture. However, isolation of this minor adduct only proved possible with the *N*-PMB-protected derivative.

It was observed in initial experiments conducted with **13** and PMB-glycine·HCl that the expected cycloaddition product **14c** was isolated in relatively poor yield (<20%, Scheme 3). This outcome struck us as unusual as our prior experience suggested that this protected glycine derivative should behave similarly to the benzyl analog. It was determined that this was due to the formation of two byproducts **15c** and **16c**, while similar reactions with sarcosine or benzylglycine·HCl appear to afford only the cycloaddition products in reasonable yields. Analysis of the NMR data suggested that the structure of byproduct **15c** was a 1,2,3,4-tetrahydroquinolin-2-one derivative, with *trans*-substituents at C3 and C4 ($J_{3,4}$=12.4 Hz), and byproduct **16c** was established to be the 1,2-dihydroquinolin-2-one derivative. The initial cycloaddition reaction with PMB-glycine·HCl was performed in toluene at reflux for 10 h, giving **14c** (16%), **15c** (11%), and **16c** (20%) (entry 1, Table 1). It was subsequently determined that these experiments were conducted with an older sample of *N*-PMB-glycine·HCl, which presumably had picked up water from the atmosphere. Employing more

recently prepared material led to an improvement in the amount of **14c** produced, but byproducts **15c** and **16c** were still obtained in reasonable amounts (entry 2, Table 1). Interestingly, extending the reaction time to 20 h saw a reduction in the amount of **15c** formed, providing only **14c** and **16c**, whereas, adding water to the reaction increased the yield of byproduct **15c**. In contrast, when the reaction was carried out in DMF at reflux for 1 h, only byproduct **15c** was obtained, along with the expected cycloadduct **14c**.



Scheme 3.

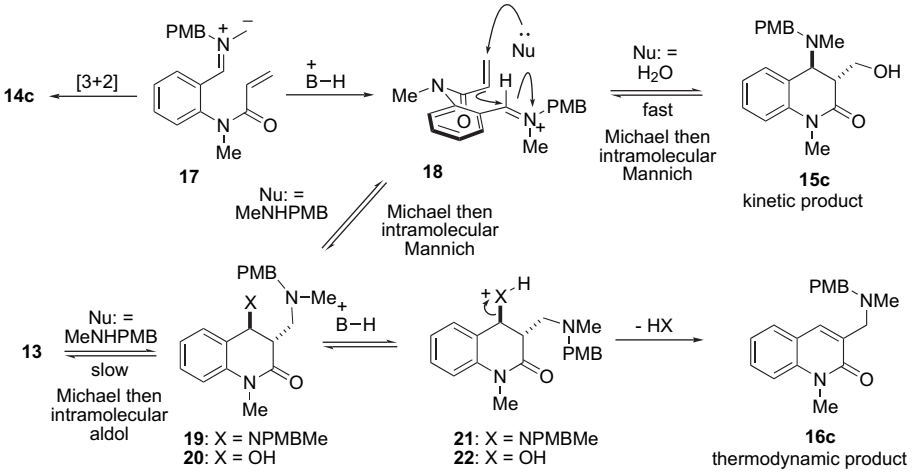
Table 1. Product distribution from reaction of PMB-glycine with aldehyde **13**

Entry	Solvent	Time (h)	**14c** (%)	**15c** (%)	**16c** (%)
1	Toluene	10	16	11	20
2	Toluene	8	38	20	18
3	Toluene	20	40	0	48
4	DMF	1	44	10	0

Our interpretation of the results obtained in these reactions involves two competing pathways, the 'normal' [3+2] pathway (**17**→**14c**) and a domino Michael–Mannich reaction sequence (**17**→**15c** and **16c**) and are illustrated in Scheme 4.[28] The studies on the decarboxylative Michael addition described previously (Scheme 9)[9c] have already shown that the azomethine ylide can be protonated under the reaction conditions to form an iminium ion **18**. In the presence of nucleophiles (H_2O or MeNHR), **18** undergoes an intermolecular Michael reaction and subsequent intramolecular Mannich reaction to furnish the cycloadduct 1,2,3,4-tetrahydroquinolin-2-one derivatives **15c** or **16c**, the latter after an elimination reaction. The cycloaddition appears to be stereoselective, providing the *trans*-substituted product, presumably as a result of the large iminium moiety occupying the sterically more favorable pseudoequatorial position in the putative transition state **18** (Scheme 4). It has also been demonstrated in the studies described above that the PMB methylamine is formed under these reaction conditions. Thus, when PMB methylamine functions as the nucleophile, **19** and/or **20** would be formed,[29] elimination then affords the more stable conjugated 1,2-dihydroquinolin-2-one **16c**.[14b,30]

The time variation in byproduct distribution suggests that **15c** is the kinetic product and **16c** is the thermodynamic product. It is assumed that the initial concentration of H_2O in the reaction mixture would be higher than NHMePMB as the formation of the latter nucleophile requires additional steps, and thus an induction period for the concentration to build up. However, once it accumulates it competes effectively with H_2O leading to the formation of **16c**, which is thermodynamically more stable due to its extended conjugation. This is consistent with the experimental observation that extending the reaction time leads to the exclusive formation of **16c**. The isolation of these byproducts in the case of reactions involving an *N*-PMB moiety and not with an *N*-Bn or *N*-Me warrants further discussion. Several factors may contribute to the competitive formation of the byproducts and the desired pyrroloquinolone. (a) The PMB moiety is electron donating; therefore, the azomethine ylide is rendered more electron rich and as a result is rapidly protonated (Scheme 4, **17**→**18**), and thus cannot participate in cycloaddition. (b) The thus produced iminium ion is more electron rich and thus is less susceptible to hydrolysis, therefore, it accumulates and will lead to an increase in intermolecular reactions (with water or amine). (c) *p*-Methoxybenzylmethylamine is more nucleophilic than methylbenzylamine and dimethylamine, leading to an increase in the rate of Michael addition processes.[31]

In order to provide support for the proposed mechanism, some additional experiments were performed. For example, aldehyde **13** was heated at reflux with NHMeBn in the presence of Et_3N under various conditions, the results of which are listed in Table 2 (Scheme 5). As can be seen, in refluxing toluene, both analogs **15a** and **16a** were obtained in 38 and 13%, respectively. Adding water to the reaction mixture led to the formation of **15a** in an excellent 85% yield plus a small amount (12%) of **16a**. In refluxing DMF and

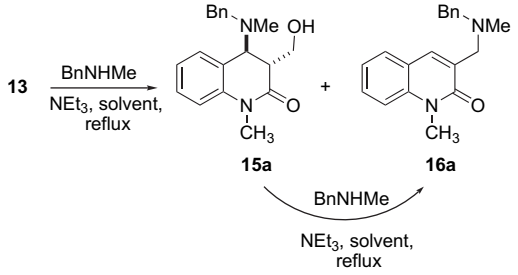


Scheme 4.

Table 2. Yields and conditions for the Michael–Mannich reaction of **13**

Entry	Solvent	Time (h)	**15a** (%)	**16a** (%)
1	Toluene	3	38	13
2	Toluene, H_2O (5 equiv)	2	85	12
3	DMF	1.5	70	28
4	DMF	2	60	38
5	DMF	8	0	90
6	DMF, H_2O	1.5	90	0

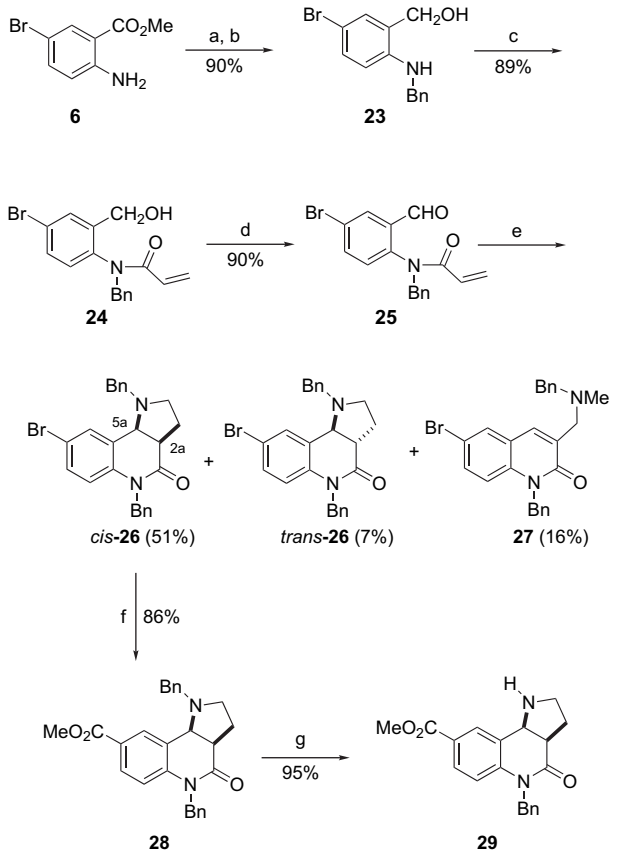
extending the reaction time led to the formation of more of the thermodynamic product **16a** and less of the kinetic product **15a**. Heating at reflux in DMF for 8 h led to the exclusive formation of **16a** in an excellent 90% yield. Not surprisingly, adding water to the reaction and heating at reflux for a short time (1.5 h) gave the **15a** exclusively. These results provide compelling evidence that compound **15a** is the kinetic product and **16a** is formed as the thermodynamic product. This was further supported by control experiments in which **15a** was heated at reflux with only Et_3N, no reaction was observed but when NHMeBn was added, **15a** converted to **16a** quantitatively after refluxing for 18 h. Thus by controlling the reaction conditions, either cycloadduct (**15a** or **16a**) can be obtained in an excellent yield. The fact that MeNHBn participates in the tandem Mannich–Michael sequence strongly suggests that the corresponding azomethine ylide undergoes cycloaddition faster than protonation.



Scheme 5.

Although the *N*-methyl derivatives were useful for assessing the influence of *N*-substituents in the azomethine ylide cycloaddition reaction, as far as the total synthesis was concerned, a more appropriate substrate had to be evaluated which contained, inter alia, a readily removable N1-protecting group and a halo moiety at C7 for introduction of the carboxyl group. Thus, the *N*-benzyl acrylamide derivative **25** was identified as being appropriate and was constructed through a largely analogous sequence of reactions to those previously employed for the synthesis of **13**. Thus, benzoylation of anthranilate derivative **6**[32] and reduction with $LiAlH_4$ gave the *N*-benzyl alcohol **23** (Scheme 6). Subsequent acroylation and IBX-oxidation afforded the cyclization substrate **25**.

Gratifyingly, when **25** was subjected to the cycloaddition reaction using *N*-benzylglycine in toluene at reflux, the desired pyrroloquinolone, *cis*-**26** was obtained in 51% yield (Scheme 6). The H_{5a} benzylic proton appeared as a doublet in the ^1H NMR spectrum at δ=3.40 ppm, with an associated coupling constant of 5.0 Hz, which suggested that the ring fusion was cis (the magnitude of the *J*-value is consistent with our previous results).[9] This assignment was subsequently confirmed through an X-ray structure determination



Scheme 6. Reagents and conditions: (a) BzCl, $NaHCO_3$, CH_2Cl_2, 0 °C→rt; (b) $LiAlH_4$, THF, −9 °C→ rt; (c) acryloyl chloride, $NaHCO_3$, CH_2Cl_2; (d) IBX, DMSO; (e) $BnNHCH_2CO_2H\cdot HCl$, PhMe, Et_3N, reflux; (f) $Pd(OAc)_2$, PPh_3, CO (60 psi), MeOH, *i*-Pr_2NEt, DMF, 100 °C; (g) 20% $Pd(OH)_2/C$, HCl, H_2, MeOH.

on this adduct (Fig. 2), which unequivocally demonstrated the cis fusion of the pyrrole/quinolone ring system. In addition to the major *cis*-adduct, a small quantity (7%) of the diastereoisomer, *trans*-**26** ($J_{2a,5a}$=13.8 Hz) was isolated.[9] This stereochemical assignment was confirmed independently

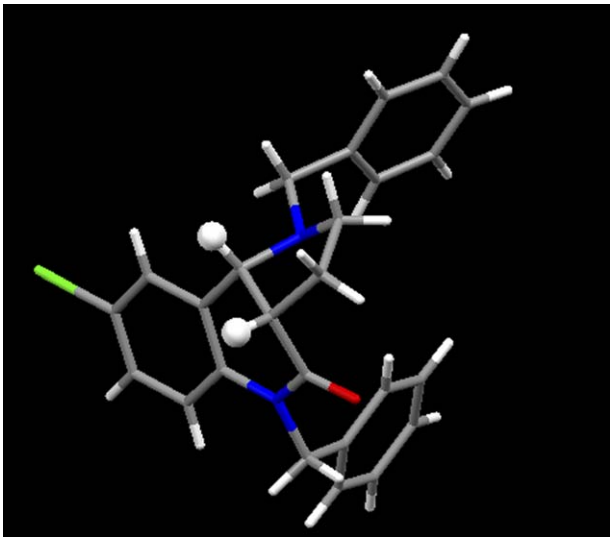


Figure 2. X-ray structure of *cis*-**26** (bridge head hydrogens picked out for emphasis).

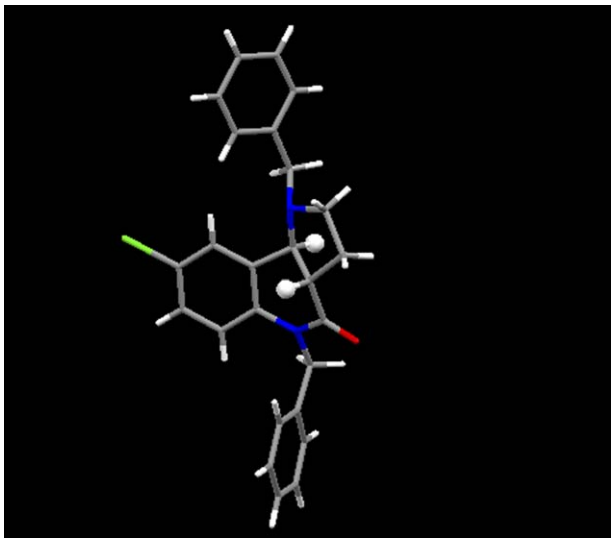


Figure 3. X-ray structure of *trans*-**26** (bridge head hydrogens picked out for emphasis).

through X-ray crystallography (Fig. 3). The formation of the *cis*/*trans*-isomers appears to be under kinetic control, since subjection of either isomer to the reaction conditions did not result in their interconversion. In addition to *cis*-**26** and *trans*-**26**, 16% of a polar byproduct was also isolated. The structure of compound was assigned as **27** and this is based on the NMR data, which is similar to **16a**, and presumably arises through a similar pathway to the formation of **16b** in the course of the cycloaddition reactions with PMB-protected glycine. Interestingly, however, the *trans*-disubstituted analog of **15a** was not observed in these reactions.

With *cis*-**26** in hand, methods for the introduction of the C7-carboxyl group were evaluated. In our earlier studies, Pd-catalyzed carbonylations were employed, and thus we gravitated toward these protocols.[9] However, unlike the carbonylation reaction (conditions=$Pd(OAc)_2$, PPh_3, CO, NaOAc) employed previously in the pyrroloquinoline series,[9a,b] it was found that the yield of the ester was substantially lower ca. 62% versus >90%. This was due to the formation of **30** (32%, Fig. 4) via a base-induced net retro Michael reaction. It was found that the ratio of the desired product and byproduct was dependent on the concentration of the base (NaOAc). Decreasing the amount of the NaOAc (0.8 equiv) led to the increased formation of **28** in 76%, however, further reduction in the amount of base (0.6 equiv) employed led to a reduction in the yield of **28** (44%). Other bases were screened and it was determined that Hunig's base (diisopropylethylamine) provided satisfactory results affording an excellent 86% yield of **28**, and a small quantity of **30** (12%). Preliminary scouting experiments were performed in an attempt to remove both of the benzyl protecting groups, interestingly with Pearlman's catalyst, the *N*5-benzyl group was removed chemoselectively to provide a polar product, **29**, in 95% (Scheme 6). At this point, no further attempts were made to remove the quinolinoyl benzyl group, or to find conditions to remove both simultaneously, rather we moved onto developing approaches for the incorporation of the C2-substitutent.

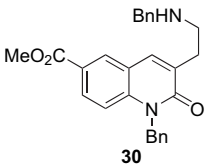


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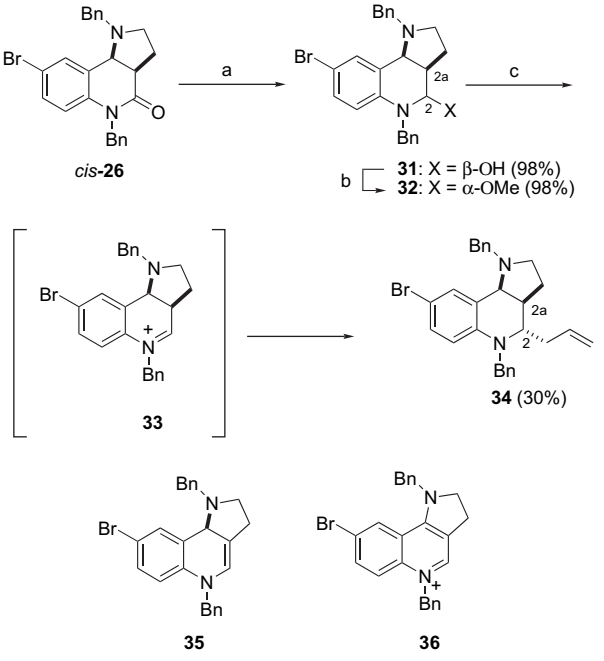
Figure 4. Byproduct from the Pd-catalyzed carbonylation.

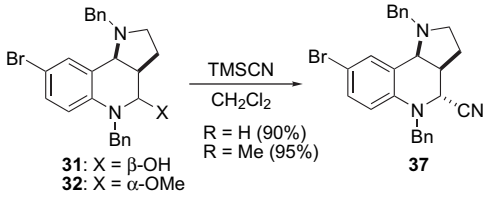
As described above, it was our intent to incorporate the C2-substituent via the formation of the iminium ion, and subsequently trap it through reaction with a nucleophilic C3-synthon. Although the formation of *N*-acyliminium (and other electron withdrawing groups on nitrogen) ions is well precedented,[33] the generation and trapping of iminium ions from simple *N*-alkyl lactams, are much less common. Overman et al. have demonstrated that reduction of an *N*-benzyl lactam to the α-hydroxybenzylamine using DIBAL as the reducing agent, followed by acid catalyzed elimination, will provide the corresponding *N*-benzyl iminium ion salt,[34] which exhibits very good diastereoselectivity in the nucleophilic addition reaction of Grignard reagents. Attempts to employ similar procedures with *cis*-**26**, involving the preparation of iminium salt and subsequent nucleophilic addition of allylmagnesium bromide were unsuccessful. Given this failure, we decided to investigate the preparation and utility of the corresponding α-methoxyamine as an iminium ion precursor. Accordingly, the *N*-benzyl aminol **31** was prepared by reduction of *cis*-**26** using DIBAL at −78 °C providing a 1:0.15 mixture of two diastereomers (for the major isomer, $J_{2,2a}$=3.2 Hz) (Scheme 7).[35] Without further purification, the mixture was converted to the corresponding α-methoxyamine **32** in 98% yield as a single diastereomer ($J_{2,2a}$=1.8 Hz) by simply refluxing in $CHCl_3$/MeOH. Compound **32** was then treated with allyltrimethylsilane in the presence of a Lewis acid, $TiCl_4$. The putative iminium ion intermediate **33**, generated in situ underwent nucleophilic addition at C2, presumably from the



Scheme 7. Reagents and conditions: (a) DIBAL-H, THF, −78 °C; (b) $CHCl_3$, MeOH, reflux; (c) allyltrimethylsilane, $TiCl_4$, CH_2Cl_2, −78 °C.

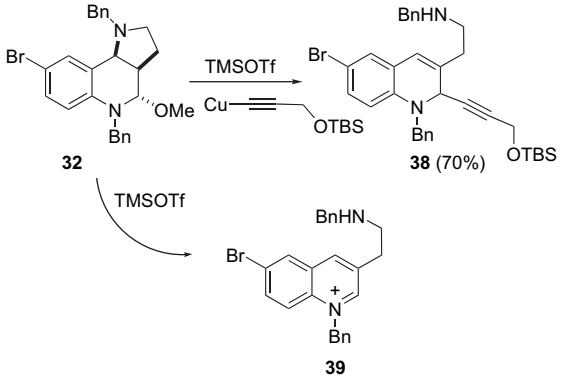
less hindered face, i.e., the opposite side to the cis-fused pyrrolidine ring, leading to the formation of major diastereoisomer **34** in 30% yield (dr=7:1 crude reaction mixture, in the major isomer, relative stereochemistry was assigned through an NOESY experiment). The low yield of the desired product was due to the formation of the β-elimination product **35** (ca. 50%), which was relatively unstable and further converted to the fully conjugated product **36**. Other Lewis acids were evaluated in this reaction, but with generally disappointing results. For example $Ti(OPr\text{-}i)_4$ was unreactive, whereas $MeAlCl_2$ gave the β-elimination product exclusively, $BF_3 \cdot Et_2O$, on the other hand, led to complete decomposition of the substrate. We attempted to elaborate **34** via hydroboration and an oxidative work-up, a product was isolated from this treatment that displayed spectroscopic properties consistent with the desired primary alcohol. However, the efficiency was very low ~30%, and the isolated material was not very pure, therefore, we sought an alternative and more efficient means to incorporate the C2-side chain.

Although the desired allylation product was not obtained from the majority of these reactions, the formation of enamine **35** was consistent with the formation of the desired reactive intermediate, iminium ion **33**. Presumably allyltrimethylsilane is insufficiently nucleophilic to trap the incipient iminium ion, which then results in alternative fates. Bearing this in mind, we wished to establish an idea of the general reactivity patterns of **33** and whether nucleophiles could be identified that would react faster than competing elimination, and so TMSCN was evaluated in this capacity. We were delighted to find that when the α-methoxyaminol **32** was treated with 4 equiv of TMSCN at room temperature, the desired cyano-substituted adduct **37** was obtained in an excellent 95% yield within couple of hours (Scheme 8). The reaction provided a single stereoisomer based on analysis of the NMR spectrum of the crude reaction mixture. It was found that reaction proceeds with essentially the same facility with the aminol **31** under similar reaction conditions, providing the same product in 90% (Scheme 8). In both cases, the stereochemistry of the adduct appears to be *exo* (based on an NOESY experiment), i.e., that required for an approach to the natural product.[36]



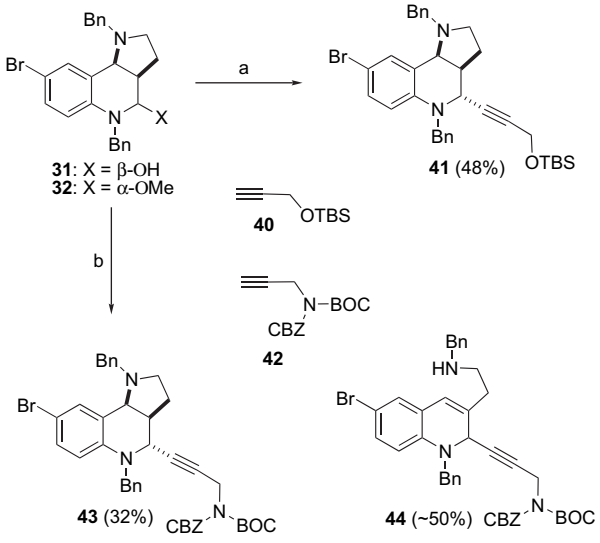
Scheme 8.

Encouraged by the fact that this silicon-based Lewis acid successfully mediated the formation of the iminium ion, which was then captured by CN^-, the use of TMSOTf in combination with a three-carbon copper acetylide (prepared from **40** and $CuCl/Et_3N$) as nucleophile was investigated.[37] As can be seen in Scheme 9, the addition took place smoothly, but was complicated by a fragmentation of the pyrrolidine ring to provide **38** in an unoptimized 70% yield (Scheme 9). Apparently the combination of relatively low electrophilicity of the iminium ion coupled with the aromatization driving force (**32**→**39**, Scheme 9) thwarted addition prior to fragmentation.[38] Indeed simply treating **32** with TMSOTf led to the efficient formation of the quinolinium ion (**39**, Scheme 9), a similar type of fragmentation has been observed previously in related systems.[8c,13a] Significant effort was expended in order to identify conditions that permitted the introduction of the side chain via acetylide chemistry using numerous modes of additions, a variety of metal acetylides (Cu, Zn, Mg) and Lewis acids, all without success. The use of **37** in the presence of $AgBF_4$, as an alternative iminium ion precursor, was evaluated with various metal acetylides, but met with no success.[39]



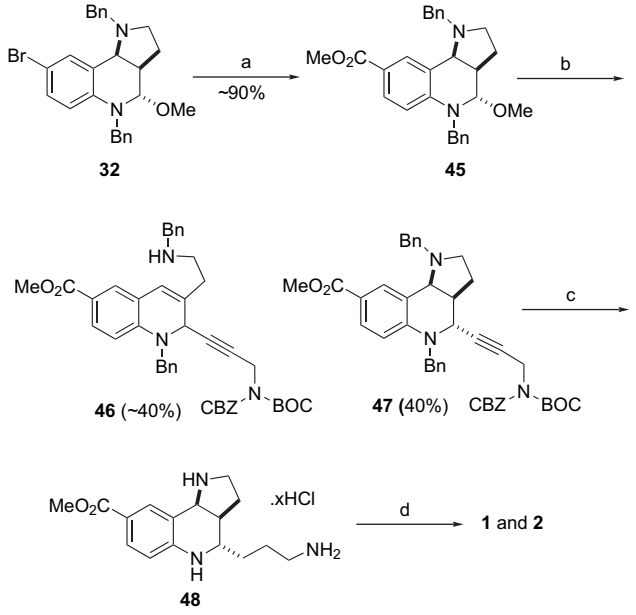
Scheme 9.

Several options were open to us at this point, none of which were particularly attractive until we became aware of Li's work, describing the in situ preparation of a copper acetylide (terminal alkyne/CuBr) under aqueous conditions with sonication in the absence of a Lewis acid (or other externally added promoter).[40] Presumably the heterogeneous conditions keep the solution concentrations of all of the reagents low thus minimizing side reactions, and the acid that is produced on reaction of the acetylene with CuBr is sufficient to ionize the α-methoxyamine **32**. Gratifyingly, when Li's protocol was applied to **32** and the silyl-protected propargyl alcohol derivative **40**, the desired adduct **41** was obtained in 48% yield, along with 40% of the fragmentation product **38** (cf. Scheme 9), it was also found that the aminol **31** would react analogously (Scheme 10).



Scheme 10. Reagents and conditions: (a) CuBr, H_2O, **40**, ultrasound; (b) CuBr, **42**, H_2O, ultrasound.

Encouraged by this result, it was decided to evaluate a propargyl amine derivative, thereby incorporating all of the remaining carbon and the nitrogen atoms in one step. When the doubly protected amine derivative **42**[41] was employed under the Li's conditions with **31**, it was found that addition took place, providing the required adduct **43** in 32% yield along with the corresponding fragmentation product **44** (~50%). We were unable to isolate and fully characterize the fragmentation product **44** as it co-eluted with unreacted **42** during column chromatography. Presumably, the two carbamate protecting groups attenuate the nucleophilicity of the acetylide and thus the fragmentation pathway becomes more dominant, thus lowering the yield of the desired adduct. Rather than spending time trying to optimize this reaction, however, it was decided to introduce the carboxymethyl group in the aryl ring and then evaluate this as a substrate. The rationale behind this approach rests on the electron withdrawing effect of the methyl ester, which should increase the electrophilicity of the iminium ion (in effect a doubly vinylogous carbamate), and additionally it should reduce the propensity of the system to fragment since it would destabilize the developing positive charge at the benzylic position. It was hoped that this modification would lead to an increase in the proportion of the simple addition product with respect to the fragmentation–addition product. Incorporation of the methyl ester was readily achieved by bromine/lithium exchange with **32**, followed by quenching of the organolithium with dimethyl carbonate, which gave the desired product (**32**→**45**, Scheme 11). Gratifyingly, when this material was treated with **42** under Li's conditions, the addition product **47** was obtained in 40%, along with the fragmentation product in a comparable amount (~40%, purification was again problematic due to co-elution of the acetylene) estimated from the integration data obtained from the ^{1}H NMR spectrum of the crude product. Reductive debenzylation, reduction of the alkyne, and acid hydrolysis of the BOC group were achieved simultaneously by treatment of **47** with $Pd(OH)_2$ and H_2 in the presence of HCl, providing the tricyclic triamine **48** in 95% (ca. 90% purity) as the hydrochloride salt, which we and others have converted into martinellic acid and martinelline.[10c,17a,b,42]



Scheme 11. Reagents and conditions: (a) *n*-BuLi, THF, −78 °C then $CO(OMe)_2$, −78→0 °C; (b) CuBr, **42**, H_2O, ultrasound; (c) 20% $Pd(OH)_2$/C, MeOH, H_2; (d) lit.[7b,10c,17a]

In conclusion, an efficient approach for the synthesis and elaboration of the pyrrolo[3,2-*c*]quinolone, the tricyclic core of the *Martinella* alkaloids, have been developed via a stereoselective intramolecular [3+2] azomethine ylide/alkene cycloaddition approach. Conformational effects play an important role in this and related intramolecular cycloaddition processes investigated in this study. The presence of an *N*-alkyl substituent is critical to the success of this and related cycloaddition processes. In addition to the desired pyrroloquinolones, various 1,2,3,4-tetrahydroquinolin-2-one and 1,2-dihydroquinolin-2-one derivatives have also been synthesized via an interesting domino Michael–Mannich reaction of *N*-alkylated substrates, whereas the corresponding N–H substrates undergo only the Michael portion of the sequence. An advanced precursor **47** for the total synthesis of the *Martinella* alkaloids was obtained through an in situ acid mediated addition of a functionalized copper acetylide to an iminium ion, though the efficiency needs to be further improved. Global deprotection with concomitant alkyne reduction provided the tricyclic triamine **48** in good yield as the HCl salt.

2. Experimental

2.1. General

All chemicals and solvents were purchased from commercial vendors and were used as received unless indicated otherwise. All reactions involving air- or water-sensitive compounds were conducted in oven-dried glassware under an atmosphere of dry argon or nitrogen. A Pure-Solv 400 solvent purification system from Innovative Technology Inc. was also used to obtain anhydrous CH_3CN, THF, CH_2Cl_2, benzene, and toluene. NMR spectra were obtained on a JEOL Eclipse+ 500 MHz; ^{1}H NMR spectra were recorded in deuteriochloroform (unless otherwise indicated) at a spectrometer frequency of 500.16 MHz, residual protiochloroform was used as internal reference; ^{13}C NMR spectra were obtained in deuteriochloroform (unless otherwise indicated) at 125.79 MHz using $^{13}CDCl_3$ (δ=77.0 ppm) as internal reference. Infrared (IR) spectra were obtained on a Bruker Vector 22 FT-IR spectrometer, using KBr pressed pellets for solids or neat films on NaCl plate for liquids and oils, and were reported in cm^{-1}. Electron impact mass spectra (EIMS) were recorded in-house on a Bear Instruments, Kodiak 1200 spectrometer (at 70 eV) and electrospray ionization mass spectra (ESIMS) and LC/MS were recorded on an Agilent 1100 LC/MS system controlled by Chemstation version 8.3. (the ESI condition is 4.5 kV spray voltage with 10 μL min^{-1} infusion) at HT Laboratories Inc. High-resolution mass spectra (HRMS) were recorded using electrospray ionization (ESI) time-of-flight reflectron experiments performed on an Agilent ESI-TOF mass spectrometer at the Center for Mass Spectrometry at the Scripps Research Institute, La Jolla, California. Elemental analyses were performed in-house on a Perkin–Elmer 2400 CHN Elemental Analyzer or at Quantitative Technologies Inc. Melting points were recorded on a Thomas Hoover Scientific capillary tube

melting point apparatus and were uncorrected. Analytical thin-layer chromatography (TLC) was performed on silica gel 60 F_{254} aluminum backed precoated plates (layer thickness=200 μm). A Fisher Scientific sonicator (Model FS20H, 3 qt., 120 V, 50/60 Hz, 1 A, 143 W, with heater) was used in this work.

2.1.1. *N*-(2-Hydroxymethylphenyl)-*N*-methylacrylamide (12). Acryloyl chloride (0.33 mL, 4.04 mmol) was added dropwise to a cooled suspension of the amino alcohol **11** (553 mg, 4.04 mmol) and sodium bicarbonate (400 mg, 4.76 mmol) in anhydrous CH_2Cl_2 (5 mL) and stirred for 1 h. The reaction mixture was then diluted with CH_2Cl_2 (5 mL) and washed with water (2×10 mL) and brine (10 mL), dried (Na_2SO_4), and concentrated. The crude oil was re-dissolved in CH_2Cl_2 and hexane and concentrated again. The process was repeated until a solid product was obtained. Trituration of the solid with Et_2O several times provided pure **12** (686 mg, 89%) as a colorless solid. The combined Et_2O washings were concentrated and purification of the resulting oil by chromatography (hexane/ethyl acetate, 4:1) gave additional *N*-acylated product (46 mg, 6%). Mp: 64–66 °C. ^{1}H NMR: δ=7.60 (d, J=7.3 Hz, 1H), 7.41 (dd, J=7.6, 7.3 Hz, 1H), 7.36 (dd, J=7.8, 7.6 Hz, 1H), 7.13 (d, J=7.8 Hz, 1H), 6.34 (dd, J=16.8, 1.8 Hz, 1H), 5.90 (dd, J=16.8, 10.3 Hz, 1H), 5.48 (dd, J=10.3, 1.8 Hz, 1H), 4.60 (m, 2H), 3.28 (s, 3H), 2.30 (br s, 1H); ^{13}C NMR: δ=166.1, 140.8, 138.5, 129.1, 129.03, 128.97, 128.3, 128.2, 127.9, 60.8, 37.1; IR (KBr, cm^{-1}): 3401, 2873, 1650, 1613; EIMS (*m*/*z*): 191.1 (M^+, 18), 173.3 (19), 135.2 (40), 159.2 (100), 117.1 (84), 89.9 (34), 53.7 (32). Anal. Calcd for $C_{11}H_{13}NO_2$: C, 69.09; H, 6.85; N, 7.32. Found: C, 69.36; H, 7.09; N, 7.32.

2.1.2. *N*-(2-Formylphenyl)-*N*-methylacrylamide (13). Alcohol **12** (150 mg, 0.78 mmol, 1 equiv) was dissolved in DMSO (3 mL) and then IBX (330 mg, 1.18 mmol, 1.5 equiv) was added and the reaction mixture was stirred at room temperature for 1 h. The mixture was diluted with H_2O. The resulting precipitate was filtered and rinsed with H_2O. The aqueous layer was extracted with CH_2Cl_2 (3×). The combined organic extracts were washed with brine (2×), dried (Na_2SO_4), after concentration the crude product was passed through a plug of SiO_2 (CH_2Cl_2) and concentrated to afford pure **13** as a thick oil (119 mg, 80%). ^{1}H NMR: δ=10.07 (s, 1H), 7.99 (dd, J=7.8, 1.4 Hz, 1H), 7.70 (ddd, J=7.8, 7.3, 1.4 Hz, 1H), 7.54 (dd, J=7.8, 7.3 Hz, 1H), 7.29 (d, J=7.8 Hz, 1H), 6.40 (dd, J=16.7, 1.8 Hz, 1H), 5.87 (dd, J=16.7, 10.5 Hz, 1H), 5.54 (dd, J=10.5, 1.8 Hz, 1H), 3.39 (s, 3H); ^{13}C NMR: δ=189.2, 166.0, 145.4, 135.7, 132.9, 130.0, 129.4, 129.1, 129.0, 127.7, 38.4; IR (neat, cm^{-1}): 3055, 2918, 2850, 1695, 1656, 1596, 1265; EIMS (*m*/*z*): 189.3 (M^+, 45), 159.2 (100), 133.0 (88), 105.0 (70), 75.9 (41), 53.7 (56). HRMS (*m*/*z*): calcd for MH^+ $C_{11}H_{12}NO_2$ 190.0863, found 190.0868.

2.2. General procedure for azomethine ylide cycloaddition reactions

2.2.1. (3a*R,9b*S**)-1-Benzyl-5-methyl-2,3,3a,4,5,9b-hexahydro-1*H*-pyrrolo[3,2-*c*]quinolin-4-one *cis*-(14a).** Compound **13** (60 mg, 0.32 mmol) and *N*-benzylglycine·HCl (116 mg, 0.57 mmol, 1.8 equiv) were mixed in anhydrous toluene (10 mL), then Et_3N (0.13 mL, 0.95 mmol, 3 equiv) was added. The resulting mixture was heated under reflux for 2 h, at which time TLC analysis indicated the completion of the reaction. The mixture was cooled to room temperature, diluted with EtOAc (10 mL), and washed with H_2O (2×15 mL) and brine (15 mL), dried (Na_2SO_4), and then concentrated. The crude product was purified by chromatography (hexane/ethyl acetate, 2:1) to give the title compound as a colorless solid (61 mg, 66%). Mp: 80–82 °C. ^{1}H NMR: δ=7.35 (m, 1H), 7.12–7.26 (m, 6H), 7.03–7.06 (m, 2H), 4.02 (d, J=13.1 Hz, 1H), 3.44 (s, 3H), 3.38 (d, J=5.5 Hz, 1H), 3.14 (d, J=13.1 Hz, 1H), 2.98 (m, 2H), 2.63 (m, 1H), 2.16 (m, 2H); ^{13}C NMR: δ=171.7, 140.3, 139.5, 130.9, 129.2, 128.3, 128.1, 126.8, 122.8, 122.2, 114.8, 64.9, 57.4, 51.3, 43.6, 29.9, 26.2; IR: 3025, 2914, 2788, 1665, 1602; EIMS (*m*/*z*): 292.4 (M^+, 4), 291.1 (M^+-1, 64), 200.0 (33), 185.0 (62), 158.9 (70). Anal. Calcd for $C_{19}H_{20}N_2O$: C, 78.05; H, 6.89; N, 9.58. Found: C, 78.43; H, 7.12; N, 9.41.

2.2.2. (3a*S,9b*S**)-1-Benzyl-5-methyl-2,3,3a,4,5,9b-hexahydro-1*H*-pyrrolo[3,2-*c*]quinolin-4-one *trans*-(14a).** Eluted out right after the *cis*-adduct as brown oil (5 mg, 5%). ^{1}H NMR: δ=7.49 (d, J=7.3 Hz, 2H), 7.46 (d, J=7.3 Hz, 1H), 7.37 (dd, J=7.8, 7.3 Hz, 2H), 7.28–7.32 (m, 2H), 7.11 (dd, J=7.3, 7.3 Hz, 1H), 7.07 (d, J=7.8 Hz, 2H), 4.48 (d, J=14.2 Hz, 1H), 3.65 (d, J=13.8 Hz, 1H), 3.49 (d, J=14.2 Hz, 1H), 3.39 (s, 3H), 3.36 (ddd, J=10.5, 8.3, 8.3 Hz, 1H), 2.76 (ddd, J=14.0, 10.8, 8.3 Hz, 1H), 2.67 (ddd, J=10.5, 10.1, 3.8 Hz, 1H), 2.01–2.12 (m, 2H); ^{13}C NMR: δ=171.8, 140.6, 139.2, 131.3, 128.6, 128.4, 127.4, 127.2, 123.3, 122.3, 115.4, 64.4, 61.2, 55.2, 47.4, 29.8, 26.7; IR (neat, cm^{-1}): 3062, 3030, 2926, 1683, 1648, 1604; EIMS (*m*/*z*): 290.7 (M^+-1, 74), 200.0 (76), 104.1 (30), 89.7 (100).

2.2.3. (3a*R,9b*S**)-1,5-Dimethyl-2,3,3a,4,5,9b-hexahydro-1*H*-pyrrolo[3,2-*c*]quinolin-4-one *cis*-(14b).** Compound **13** (210 mg, 1.11 mmol) and sarcosine (198 mg, 2.22 mmol, 2 equiv) were mixed in toluene (37 mL), then Et_3N (0.46 mL, 3.33 mmol, 3 equiv) was added. The mixture was then heated under reflux for 5 h. After work-up (see synthesis of **14a**), chromatography (ethyl acetate/MeOH, 4:0.2) gave the title compound as a colorless solid (140 mg, 58%). Mp: 116–117 °C. ^{1}H NMR: δ=7.35 (ddd, J=8.3, 7.3, 1.6 Hz, 1H), 7.17 (dd, J=7.3, 1.6 Hz, 1H), 7.06 (ddd, J=7.3, 7.3, 0.9 Hz, 1H), 7.02 (d, J=8.3 Hz), 3.38 (s, 3H), 3.16 (m, 1H), 3.05 (d, J=6.0 Hz, 1H), 2.98 (m, 1H), 2.66 (m, 1H), 1.98–2.27 (m, 2H), 2.24 (s, 3H); ^{13}C NMR: δ=171.6, 140.1, 130.6, 129.2, 122.2, 122.0, 114.8, 66.3, 54.5, 43.6, 40.0, 30.0, 26.5; IR (KBr, cm^{-1}): 2945, 2803, 1664, 1602; EIMS (*m*/*z*): 216.3 (M^+, 100), 199.2 (22), 186.1 (35), 173.0 (10). Anal. Calcd for $C_{13}H_{16}N_2O$: C, 72.19; H, 7.46; N, 12.95. Found: C, 71.93; H, 7.50; N, 12.77.

2.3. Synthesis of 14c, 15c, and 16c

Compound **13** (60 mg, 0.32 mmol) and *N*-*p*-methoxybenzylglycine hydrochloride (116 mg, 0.57 mmol, 1.8 equiv) were mixed in anhydrous toluene (11 mL), then Et_3N (0.13 mL, 0.95 mmol, 3 equiv) was added. The mixture was heated under reflux for 10 h, at which time TLC analysis indicated the completion of the reaction. The mixture was cooled to room temperature, diluted with EtOAc (10 mL), and washed with

H_2O (2×15 mL) and brine (15 mL), dried (Na_2SO_4), and then concentrated. The crude oil was purified by chromatography (hexane/ethyl acetate, 2:1) to give *cis*-**14c** (16 mg, 16%), *trans*-**14c** (3 mg, 3%), byproduct **15c** (12 mg, 11%), and byproduct **16c** (20 mg, 20%) in the order of elution from the column. Following the above procedure, when anhydrous DMF (11 mL) was used instead of toluene and the reaction was refluxed for 1 h instead of 10 h, after chromatography, *cis*-**14c** (45 mg, 44%), *trans*-**14c** (5 mg, 5%), and byproduct **15c** (11 mg, 10%) were obtained.

2.3.1. (3a*R,9b*S**)-1-(4-Methoxybenzyl)-5-methyl-2,3,3a,4,5,9b-hexahydro-1*H*-pyrrolo[3,2-*c*]quinolin-4-one *cis*-(14c).** A colorless solid. Mp: 94–95 °C. ^{1}H NMR: δ=7.35 (ddd, *J*=7.8, 7.8, 1.4 Hz, 1H), 7.23 (dd, *J*=7.3, 1.4 Hz, 1H), 7.03–7.06 (m, 4H), 6.74 (d, *J*=8.7 Hz, 2H), 3.95 (d, *J*=12.8 Hz, 1H), 3.75 (s, 3H), 3.43 (s, 3H), 3.36 (d, *J*=5.5 Hz, 1H), 3.07 (d, *J*=12.8 Hz, 1H), 2.92–2.99 (m, 2H), 2.59–2.62 (m, 1H), 2.11–2.19 (m, 2H); ^{13}C NMR: δ=171.7, 158.5, 140.3, 131.5, 130.9, 129.4, 129.1, 122.8, 122.2, 114.8, 113.5, 64.8, 56.8, 55.3, 51.2, 43.6, 29.9, 26.2; IR (KBr, cm^{-1}): 3039, 3006, 2924, 1662, 1604; EIMS (*m*/*z*): 322.1 (M$^+$, 35), 279.1 (28), 201.2 (21), 167.1 (30), 149.1 (98), 136.3 (40), 121.2 (100). Anal. Calcd for $C_{20}H_{22}N_2O_2$: C, 74.51; H, 6.88; N, 8.69. Found: C, 74.45; H, 6.84; N, 8.96.

2.3.2. (3a*S,9b*S**)-1-(4-Methoxybenzyl)-5-methyl-2,3,3a,4,5,9b-hexahydro-1*H*-pyrrolo[3,2-*c*]quinolin-4-one *trans*-(14c).** Colorless oil. ^{1}H NMR: δ=7.46 (d, *J*=7.3 Hz, 1H), 7.39 (d, *J*=8.7 Hz, 2H), 7.30 (dd, *J*=7.3, 7.3 Hz, 1H), 7.11 (dd, *J*=7.8, 7.3 Hz, 1H), 7.06 (d, *J*=7.8 Hz, 1H), 6.90 (d, *J*=8.7 Hz, 2H), 4.40 (d, *J*=14.2 Hz, 1H), 3.82 (s, 3H), 3.62 (d, *J*=13.8 Hz, 1H), 3.42 (d, *J*=14.2 Hz, 1H), 3.39 (s, 3H), 3.31 (ddd, *J*=10.5, 8.3, 8.3 Hz, 1H), 2.74 (ddd, *J*=13.8, 10.5, 8.3 Hz, 1H), 2.67 (ddd, *J*=10.5, 10.1, 4.1 Hz, 1H), 2.00–2.12 (m, 2H); ^{13}C NMR: δ=171.8, 158.9, 140.6, 131.3, 131.1, 129.6, 127.4, 123.3, 122.4, 115.4, 113.9, 64.3, 60.5, 55.4, 55.0, 47.4, 29.9, 22.6; IR (neat, cm^{-1}): 3032, 2962, 2923, 2853, 2839, 1683, 1605. EIMS (*m*/*z*): 322.0 (M$^+$, 31), 201.1 (42), 179.1 (59), 121.1 (100).

2.3.3. (3*R,4*R**)-3-Hydroxymethyl-4-[(4-methoxybenzyl)methylamino]-1-methyl-3,4-dihydro-1*H*-quinolin-2-one (15c).** Brown oil. ^{1}H NMR: δ=7.61 (d, *J*=7.8 Hz, 1H), 7.29 (dd, *J*=7.8, 7.3 Hz, 1H), 7.24 (d, *J*=8.7 Hz, 2H), 7.16 (dd, *J*=7.3, 7.3 Hz, 1H), 6.96 (d, *J*=7.3 Hz, 1H), 6.87 (d, *J*=8.7 Hz, 2H), 4.82 (d, *J*=12.4 Hz, 1H), 3.79 (s, 3H), 3.70 (d, *J*=12.8 Hz, 1H), 3.41 (d, *J*=12.8 Hz, 1H), 3.31 (s, 3H), 3.23 (dd, *J*=12.8, 3.7 Hz, 1H), 3.03 (dd, *J*=12.8, 11.9 Hz, 1H), 2.80 (ddd, *J*=12.4, 11.9, 3.7 Hz, 1H), 2.28 (s, 3H); ^{13}C NMR: δ=169.1, 159.1, 137.7, 130.6, 129.9, 129.3, 128.0, 124.2, 123.7, 114.3, 114.0, 70.9, 62.4, 58.2, 55.3, 42.5, 42.0, 29.8; IR (neat, cm^{-1}): 2965, 2923, 2856, 1669, 1604; EIMS (*m*/*z*): 340.2 (M$^+$, 8), 219.2 (19), 201.2 (59), 172.2 (21), 150.2 (100), 121.1 (68), 84.0 (16).

2.3.4. 3-{[(4-Methoxybenzyl)methylamino]methyl}-1-methyl-1*H*-quinolin-2-one (16c). Pale yellow oil. ^{1}H NMR: δ=7.88 (s, 1H), 7.60 (d, *J*=7.8 Hz, 1H), 7.58 (dd, *J*=8.3, 7.3 Hz, 1H), 7.33 (d, *J*=8.3 Hz, 1H), 7.31 (d, *J*=8.7 Hz, 2H), 7.23 (dd, *J*=7.8, 7.3 Hz, 1H), 6.86 (d, *J*=8.7 Hz, 2H), 3.78 (s, 3H), 3.73 (s, 3H), 3.59 (s, 2H), 3.58 (s, 2H), 2.28 (s, 3H); ^{13}C NMR: δ=162.3, 158.7, 139.1, 135.8, 131.3, 130.8, 130.1, 129.8, 128.6, 122.1, 120.8, 114.0, 113.7, 61.9, 55.8, 55.3, 42.7, 29.7; IR (neat, cm^{-1}): 3031, 2934, 2834, 1649, 1597; EIMS (*m*/*z*): 323.8 (M$^+$+1, 9), 201.4 (95), 174.7 (30), 149.7 (100), 130.1 (20), 79.8 (12). HRMS (*m*/*z*): calcd for MH$^+$ $C_{20}H_{23}N_2O_2$ 323.1754, found 323.1756.

2.4. Control experiments

Compound **13** (150 mg, 0.79 mmol) and HNMeBn (0.20 mL, 1.55 mmol, 2 equiv) were mixed in anhydrous DMF (27 mL), then Et_3N (0.33 mL, 2.37 mmol, 3 equiv) was added followed by addition of H_2O (71 mg, 3.95 mmol). The mixture was heated at 150 °C for 1.5 h. The solvent was completely removed by vacuum distillation and the residue was dissolved in EtOAc (30 mL) and washed with H_2O (2×15 mL) and brine (15 mL), dried (Na_2SO_4), and then concentrated. The crude product was purified by chromatography (hexane/ethyl acetate, 1:1) to give **15a** (220 mg, 90%). When the same reaction was conducted in the absence of water and heated at 150 °C for 8 h, **16a** (206 mg, 90%) was obtained after chromatography (hexane/ethyl acetate, 1:1).

2.4.1. (3*R,4*R**)-4-(Benzylmethylamino)-3-hydroxymethyl-1-methyl-3,4-dihydro-1*H*-quinolin-2-one (15a).** A colorless solid. Mp: 94–95.5 °C. ^{1}H NMR: δ=7.86 (br s, 1H), 7.62 (d, *J*=7.3 Hz, 1H), 7.26–7.36 (m, 5H), 7.16 (dd, *J*=7.8, 7.3 Hz, 1H), 6.96 (d, *J*=7.8 Hz, 1H), 4.84 (d, *J*=12.1 Hz, 1H), 3.76 (d, *J*=12.8 Hz, 1H), 3.48 (d, *J*=12.8 Hz, 1H), 3.32 (s, 3H), 3.27 (dd, *J*=13.1, 3.4 Hz, 1H), 3.04 (d, *J*=13.1, 11.9 Hz, 1H), 2.81 (ddd, *J*=12.1, 11.9, 3.4 Hz, 1H), 2.29 (s, 3H); ^{13}C NMR: δ=169.1, 137.7, 137.3, 129.9, 129.3, 128.7, 128.1, 127.7, 124.3, 123.7, 114.3, 70.9, 63.1, 58.5, 42.5, 29.9; IR (neat, cm^{-1}): 3062, 3029, 2951, 2854, 2801, 1669, 1605; EIMS (*m*/*z*): 310.9 (M$^+$, 22), 220.0 (95), 201.5 (13), 176.8 (100), 159.6 (15), 134.4 (28), 120.3 (9), 91.9 (12). Anal. Calcd for $C_{19}H_{22}N_2O_2$: C, 73.52; H, 7.14; N, 9.03. Found: C, 73.51; H, 6.96, N, 9.36.

2.4.2. 3-[(Benzylmethylamino)methyl]-1-methyl-1*H*-quinolin-2-one (16a). Brown oil. ^{1}H NMR: δ=7.93 (s, 1H), 7.60 (d, *J*=7.8 Hz, 1H), 7.55 (dd, *J*=8.3, 7.3 Hz, 1H), 7.39–7.43 (m, 2H), 7.30–7.38 (m, 3H), 7.23–7.26 (m, 2H), 3.74 (s, 3H), 3.68 (s, 2H), 3.63 (s, 2H), 2.31 (s, 3H); ^{13}C NMR: δ=162.3, 139.1, 135.9, 130.5, 129.9, 129.0, 128.6, 128.4, 127.1, 122.1, 120.8, 113.9, 62.5, 56.0, 42.7, 29.7; IR (neat, cm^{-1}): 3062, 3028, 2938, 2841, 2783, 1649, 1597; EIMS (*m*/*z*): 293.8 (M$^+$+1, 21), 202.0 (100), 187.9 (10), 173.1 (50), 120.7 (50). HRMS (*m*/*z*): calcd for MH$^+$ $C_{19}H_{21}N_2O$ 293.1648, found 293.1647.

2.4.3. (2-Benzylamino-5-bromophenyl)methanol (23). Benzoyl chloride (0.28 mL, 2.39 mmol, 1.1 equiv) was added dropwise to a cooled suspension of the methyl 5-amino-2-bromobenzoate (**6**) (500 mg, 2.17 mmol, 1 equiv) and sodium bicarbonate (365 mg, 4.35 mmol, 2 equiv) in CH_2Cl_2 (4 mL) at 0 °C. The reaction mixture was allowed to warm up to room temperature and stirred for 5 h then diluted with CH_2Cl_2 (10 mL) and washed with water (2×15 mL) and brine (15 mL), dried (Na_2SO_4), and

evaporated to dryness. Purification of the crude product by trituration with Et_2O gave benzamide[32] (706 mg, 97%) as a colorless solid. Mp: 130–132 °C. ^{1}H NMR: δ=11.97 (br s, 1H), 8.87 (d, J=9.2 Hz, 1H), 8.02 (d, J=2.8 Hz, 2H), 7.69 (dd, J=9.2, 2.8 Hz, 1H), 7.50–7.56 (m, 3H), 3.98 (s, 3H); ^{13}C NMR: δ=168.0, 165.8, 141.0, 137.6, 134.6, 133.6, 132.3, 129.0, 127.5, 122.2, 116.8, 115.1, 52.9.

A solution of the benzamide (650 mg, 1.95 mmol) in THF (5 mL) was added dropwise at −9 °C (ice/methanol) to a suspension of $LiAlH_4$ (406 mg, 10.7 mmol) in THF (12 mL). The reaction mixture was then allowed to warm up to room temperature and continued stirring overnight. Reaction was then quenched by H_2O (1 mL), NaOH (15%, 1 mL), and Rochelle's salt (20%, 5 mL). The resulting white slurry was filtered and then the filtrate was concentrated under vacuum. The filter cake was washed thoroughly by EtOAc (4×5 mL). The extract was combined with concentrated filtrate and washed with brine. After drying (Na_2SO_4), concentrating under vacuum, the residue was purified by flash chromatography (hexane/ethyl acetate, 4:1) to provide the title compound **23** (530 mg, 93%) as colorless oil. ^{1}H NMR: δ=7.32–7.36 (m, 4H), 7.25–7.29 (m, 1H), 7.23 (dd, J=8.7, 2.3 Hz, 1H), 7.19 (d, J=2.3 Hz, 1H), 6.50 (d, J=8.7 Hz, 1H), 5.20 (br s, 1H), 4.64 (s, 2H), 4.36 (s, 2H), 1.56 (br s, 1H); ^{13}C NMR: δ=146.4, 139.0, 132.1, 131.6, 128.8, 127.4, 127.3, 126.2, 112.7, 108.3, 64.3, 47.8; IR (neat, cm^{-1}): 3555, 3407, 3062, 3029, 2870, 1598; EIMS (*m*/*z*): 293.0 (M^++1, 49), 291.1 (M^+−1, 44), 213.2 (100), 194.2 (78).

2.4.4. *N*-Benzyl-*N*-(4-bromo-2-hydroxymethylphenyl)-acrylamide (24). Following the general procedure for N-acrylation described for compound **12**, amino alcohol **23** (1.03 g, 3.53 mmol) was used, and then purification of the crude oil by chromatography (hexane/ethyl acetate, 3:2) gave the *N*-acylated product **24** (1.09 g, 89%). Mp: 76–77 °C. ^{1}H NMR: δ=7.71 (d, J=2.3 Hz, 1H), 7.40 (dd, J=8.3, 2.3 Hz, 1H), 7.26–7.28 (m, 3H), 7.21–7.18 (m, 2H), 6.78 (d, J=8.3 Hz, 1H), 6.44 (dd, J=17.0, 1.8 Hz, 1H), 5.84 (dd, J=17.0, 10.1 Hz, 1H), 5.56 (dd, J=10.1, 1.8 Hz, 1H), 4.92 (d, J=14.2 Hz, 1H), 4.85 (d, J=14.2 Hz, 1H), 4.28 (dd, J=13.8, 6.4 Hz, 1H), 4.14 (dd, J=13.8, 5.5 Hz, 1H), 1.30 (dd, J=6.4, 5.5 Hz, 1H); ^{13}C NMR: δ=165.5, 141.4, 137.6, 136.6, 132.2, 131.9, 130.8, 129.6, 129.4, 128.7, 128.0, 127.7, 123.0, 60.2, 52.8; IR (KBr, cm^{-1}): 3321, 3094, 3055, 3023, 1642, 1608; EIMS (*m*/*z*): 347.0 (M^++2, 40), 345.8 (M^+, 28), 329.2 (100), 273.1 (34), 193.1 (21), 117.1 (11), 91.0 (92), 55.0 (37). Anal. Calcd for $C_{17}H_{16}BrNO_2$: C, 58.97; H, 4.66; N, 4.05. Found: C, 59.15; H, 4.90; N, 3.96.

2.4.5. *N*-Benzyl-*N*-(4-bromo-2-formylphenyl)acrylamide (25). Following the general procedure for the IBX-oxidation described for compound **13**, alcohol **24** (4.00 g, 11.5 mmol) provided aldehyde **25** (3.57 g, 90%). Mp: 112–113 °C. ^{1}H NMR: δ=9.45 (s, 1H), 8.00 (d, J=2.3 Hz, 1H), 7.73 (dd, J=8.4, 2.3 Hz, 1H), 7.26–7.28 (m, 3H), 7.14–7.16 (m, 2H), 7.00 (d, J=8.4 Hz, 1H), 6.45 (dd, J=16.5, 1.8 Hz, 1H), 5.80 (dd, J=16.5, 10.1 Hz, 1H), 5.60 (dd, J=10.1, 1.8 Hz, 1H), 5.10 (d, J=13.8 Hz, 1H), 4.87 (d, J=13.8 Hz, 1H); ^{13}C NMR: δ=187.4, 165.3, 141.3, 138.2, 135.4, 134.5, 132.2, 131.7, 130.4, 129.6, 128.9, 128.4, 127.6, 123.3, 54.0; IR (KBr, cm^{-1}): 3083, 2871, 1938, 1690, 1653, 1616, 1582; EIMS (*m*/*z*): 345.1 (M^++2, 11), 343.0 (M^+, 11), 290.1 (100), 288.1 (92), 209.2 (27), 179.9 (28). Anal. Calcd for $C_{17}H_{14}BrNO_2$: C, 59.32; H, 4.10; N, 4.07. Found: C, 59.60; H, 4.21; N, 3.95.

2.4.6. (3a*R,9b*S**)-8-Bromo-1,5-dibenzyl-2,3,3a,4,5,9b-hexahydro-1*H*-pyrrolo[3,2-*c*]quinolin-4-one *cis*-(26).** Benzaldehyde **25** (800 mg, 2.32 mmol) and *N*-benzylglycine·HCl (844 mg, 4.18 mmol) were dissolved in anhydrous toluene (80 mL), then Et_3N (0.97 mL, 6.97 mmol) was added to the mixture. The resulting mixture was stirred under reflux for 2 h. After this period the mixture was cooled to room temperature, then diluted with EtOAc (10 mL). The organic phase was washed with H_2O (2×10 mL) and brine (10 mL), dried (Na_2SO_4), and then concentrated by rotary evaporation. The crude oil, which solidified after coevaporation with CH_2Cl_2/hexane, was triturated with Et_2O several times to provide pure solid product *cis*-**26** (363 mg, 35%). The Et_2O washings were concentrated and purified by chromatography (hexane/ethyl acetate, 5:1) to give an additional batch of *cis*-**26** as a white solid (166 mg, 16%) Total yield: 51%. Mp: 139–140 °C. ^{1}H NMR: δ=7.32 (d, J=2.3 Hz, 1H), 7.16–7.30 (m, 11H), 6.80 (d, J=8.7 Hz, 1H), 5.59 (d, J=16.5 Hz, 1H), 4.98 (d, J=16.5 Hz, 1H), 4.01 (d, J=12.8 Hz, 1H), 3.40 (d, J=5.0 Hz, 1H), 3.19 (d, J=12.8 Hz, 1H), 3.04–3.12 (m, 2H), 2.72–2.78 (m, 1H), 2.16–2.28 (m, 2H); ^{13}C NMR: δ=171.4, 139.1, 138.2, 136.5, 133.5, 131.9, 128.8, 128.3, 128.2, 127.2, 127.0, 126.6, 125.2, 117.5, 114.9, 64.8, 57.6, 51.2, 45.4, 43.7, 26.0; IR (KBr, cm^{-1}): 2802, 1669; EIMS (*m*/*z*): 448.2 (M^++2, 24), 446.1 (M^+, 28), 356.9 (14), 354.9 (13), 314.0 (16), 201.2 (12), 150.2 (14), 133.2 (28), 91.1 (100), 65.0 (15), 35.9 (17). Anal. Calcd for $C_{25}H_{23}BrN_2O$: C, 67.12; H, 5.18; N, 6.26. Found: C, 67.44; H, 5.23; N, 6.10.

2.4.7. (3a*S,9b*S**)-8-Bromo-1,5-dibenzyl-2,3,3a,4,5,9b-hexahydro-1*H*-pyrrolo[3,2-*c*]quinolin-4-one *trans*-(26).** The *trans*-diastereoisomer eluted out immediately after the *cis*-cycloadduct as a colorless solid (73 mg, 7%). Mp: 185–186 °C. ^{1}H NMR: δ=7.52 (d, J=2.3 Hz, 1H), 7.48–7.50 (m, 2H), 7.1–7.38 (m, 9H), 7.30–7.33 (m, 3H), 7.23–7.26 (m, 2H), 7.19–7.20 (m, 2H), 6.83 (d, J=8.7 Hz, 1H), 5.24 (d, J=16.3 Hz, 1H), 5.11 (d, J=16.3 Hz, 1H), 4.41 (d, J=14.0 Hz, 1H), 3.70 (d, J=13.8 Hz, 1H), 3.53 (d, J=14.0 Hz, 1H), 3.37 (ddd, J=10.5, 8.3, 8.3 Hz, 1H), 2.90 (ddd, J=14.0, 10.8, 8.0 Hz, 1H), 2.72 (ddd, J=10.5, 10.5, 3.4 Hz, 1H), 2.05–2.20 (m, 2H); ^{13}C NMR: δ=171.5, 138.8, 138.7, 136.7, 133.7, 130.2, 128.9, 128.7, 128.4, 127.4, 126.6, 125.7, 117.9, 116.5, 64.4, 61.2, 55.2, 47.4, 46.1, 22.7; IR (KBr, cm^{-1}): 3029, 2880, 1689; EIMS (*m*/*z*): 448.2 (M^++3, 25), 447.2 (M^++2, 27), 446.1 (M^++1, 26), 445.0 (M^+, 28), 357.1 (87), 354.9 (100), 329.0 (12), 326.9 (15), 277.1 (17), 91.1 (73), 65.0 (12). Anal. Calcd for $C_{25}H_{23}BrN_2O$: C, 67.12; H, 5.18; N, 6.26. Found: C, 67.34; H, 5.22; N, 6.19.

2.4.8. 1-Benzyl-3-[(benzylmethylamino)methyl]-6-bromo-1*H*-quinolin-2-one (27). Compound **27** eluted out immediately after *trans*-**26** as brown oil (166 mg, 16%). ^{1}H NMR: δ=7.91 (s, 1H), 7.74 (d, J=2.3 Hz, 1H), 7.44 (dd, J=9.2, 2.3 Hz, 1H), 7.42 (d, J=7.3 Hz, 2H), 7.33–7.36 (m, 3H), 7.23–7.31 (m, 4H), 7.17 (d, J=7.3 Hz, 2H), 7.11 (d, J=9.2 Hz, 1H), 5.53 (br s, 2H), 3.70 (s, 2H), 3.67

(s, 2H), 2.34 (s, 3H); ^{13}C NMR: δ=162.1, 139.3, 137.4, 136.2, 134.9, 132.5, 132.3, 130.8, 129.0, 128.9, 128.4, 127.5, 127.2, 126.7, 122.6, 116.6, 115.0, 62.6, 55.9, 46.3, 42.9; IR (neat, cm^{-1}): 3062, 3029, 2943, 2840, 2787, 1650, 1620, 1590; EIMS (*m*/*z*): 446.3 (M$^+$, 7), 357.1 (100), 355.0 (86), 238.0 (8), 235.9 (12), 235.9 (11), 120.1 (60), 91.0 (98). Anal. Calcd for $C_{25}H_{23}BrN_2O$: C, 67.12; H, 5.18; N, 6.26. Found: C, 67.20; H, 4.92; N, 6.10.

2.4.9. (3a*R,9b*S**)-1,5-Dibenzyl-2,3,3a,4,5,9b-hexahydro-1*H*-pyrrolo[3,2-*c*]quinolin-4-one-8-carboxylic acid methyl ester (28).** *cis*-**26** (100 mg, 0.22 mmol), $Pd(OAc)_2$ (16 mg, 0.07 mmol, 0.3 equiv), PPh_3 (19 mg, 0.07 mmol, 0.33 equiv), *N*,*N*-diisopropylethylamine (23 mg, 0.18 mmol, 0.8 equiv), anhydrous MeOH (1.5 mL), and anhydrous DMF (1.5 mL) were added to a high pressure reaction tube, which was connected to a Teflon screw cap fitted with a pressure gauge. The reaction mixture was pressurized with CO, purged 4–6 times with CO, and then heated to 100 °C with stirring for 24 h at 75 psi of CO. After cooling down to the room temperature, the mixture was diluted with CH_2Cl_2 and filtered through Celite and the Celite pad was washed several times with CH_2Cl_2. The combined filtrates were washed 3–4 times with water. The organic phase was dried and evaporated to dryness. The residue was purified by chromatography (hexane/ethyl acetate, 4:1) to give the ester **28** (81 mg, 86%) and the byproduct **30** (12 mg, 13%). Data for **28**: a colorless solid. Mp: 157–158 °C. ^{1}H NMR: δ=7.90 (d, *J*=2.0 Hz, 1H), 7.86 (dd, *J*=8.3, 2.0 Hz, 1H), 7.26–7.30 (m, 4H), 7.20–7.24 (m, 3H), 7.14–7.18 (m, 3H), 6.98 (d, *J*=8.3 Hz, 1H), 5.66 (d, *J*=16.6 Hz, 1H), 5.02 (d, *J*=16.6 Hz, 1H), 3.99 (d, *J*=12.7 Hz, 1H), 3.87 (s, 3H), 3.50 (d, *J*=4.9 Hz, 1H), 3.20 (d, *J*=12.7 Hz, 1H), 3.14 (m, 1H), 3.07 (m, 1H), 2.78 (m, 1H), 2.22 (m, 2H); ^{13}C NMR: δ=171.8, 166.5, 143.0, 139.1, 136.4, 132.3, 130.9, 128.8, 128.3, 128.2, 127.2, 126.9, 126.6, 123.9, 122.9, 115.5, 64.9, 57.5, 52.1, 51.2, 45.5, 43.8, 26.0; IR (KBr, cm^{-1}): 3029, 2951, 2804, 1717, 1678, 1612; EIMS (*m*/*z*): 426.1 (M$^+$, 100), 335.2 (25), 320.2 (25), 294.2 (20), 133.2 (58), 91.1 (49). Anal. Calcd for $C_{27}H_{26}N_2O_3$: C, 76.03; H, 6.14; N, 6.57. Found: C, 75.94; H, 6.30; N, 6.45.

2.4.10. 1-Benzyl-3-(2-benzylaminoethyl)-1*H*-quinolin-2-one-6-carboxylic acid methyl ester (30). Brown oil. ^{1}H NMR: δ=8.21 (d, *J*=1.8 Hz, 1H), 8.00 (dd, *J*=8.8, 1.8 Hz, 1H), 7.64–7.67 (m, 2H), 7.46 (m, 1H), 7.21–7.34 (m, 7H), 7.18 (d, *J*=7.3 Hz, 2H), 5.57 (br s, 2H), 3.91 (s, 3H), 3.85 (s, 2H), 3.01 (t, *J*=6.9 Hz, 2H), 2.92 (t, *J*=6.9 Hz, 2H); ^{13}C NMR: δ=166.4, 162.8, 141.7, 140.5, 136.6, 136.1, 132.8, 130.4, 130.3, 130.0, 128.5, 128.3, 127.5, 127.0, 126.7, 124.0, 120.4, 114.8, 53.9, 52.3, 47.9, 46.6, 31.8; IR (neat, cm^{-1}): 1718, 1650, 1601; EIMS (*m*/*z*): 426.1 (M$^+$, 6), 424.1 (8), 335.5 (22), 319.0 (13), 277.9 (100), 229.5 (14), 217.1 (34), 183.4 (24), 119.8 (53), 90.1 (50).

2.4.11. (3a*R,9b*S**)-5-Benzyl-2,3,3a,4,5,9b-hexahydro-1*H*-pyrrolo[3,2-*c*]quinolin-4-one-8-carboxylic acid methyl ester (29).** Compound **28** (50 mg, 0.12 mmol) was dissolved in methanol (4 mL) under nitrogen. $Pd(OH)_2$/C (20%) (8 mg, 0.01 mmol, 10 mol %.) and four drops of concd HCl (ca. 0.04 mL, 0.44 mmol) were added to it. The reaction flask was purged with H_2 and then stirred under a hydrogen balloon for 24 h. The mixture was diluted with MeOH and filtered through Celite and the Celite pad was washed several times with MeOH. The combined filtrates were concentrated and the residue was dissolved in saturated $NaHCO_3$ solution (8 mL), extracted with CH_2Cl_2 three times. The organic extract was dried and evaporated. The residue was purified through a short silica gel plug (ethyl acetate/MeOH, 4:0.2) to give **29** as a colorless solid (37 mg, 95%). Mp: 127–129 °C. ^{1}H NMR: δ=8.05 (d, *J*=2.3 Hz, 1H), 7.82 (dd, *J*=8.7, 2.3 Hz, 1H), 7.17–7.31 (m, 5H), 6.90 (d, *J*=8.7 Hz, 1H), 5.33 (d, *J*=16.5 Hz, 1H), 5.15 (d, *J*=16.5 Hz, 1H), 4.27 (d, *J*=6.0 Hz, 1H), 3.86 (s, 3H), 3.14 (ddd, *J*=8.7, 6.4, 4.1 Hz, 1H), 3.05 (m, 2H), 2.56 (m, 1H), 2.35 (m, 1H), 1.90 (br s, 1H); ^{13}C NMR: δ=171.4, 166.5, 142.4, 136.4, 130.9, 130.6, 128.9, 127.3, 126.3, 124.6, 124.4, 115.5, 58.4, 52.1, 46.4, 44.3, 44.2, 29.7; IR (KBr, cm^{-1}): 2950, 1715, 1675, 1610; EIMS (*m*/*z*): 336.0, 294.2 (100) (M$^+$, 85), 216.2 (39), 128.2 (24), 91.1 (86). Anal. Calcd for $C_{20}H_{20}N_2O_3$: C, 71.41; H, 5.99; N, 8.33. Found: C, 71.28; H, 6.02; N, 8.26.

2.4.12. (3a*R,4*R**,9b*S**)-8-Bromo-1,5-dibenzyl-2,3,3a,4,5,9b-hexahydro-4-hydroxy-1*H*-pyrrolo[3,2-*c*]quinoline (31).** DIBAL-H (1 M in hexanes, 2.68 mL, 2.68 mmol) was added dropwise to a solution of lactam *cis*-**26** (600 mg, 1.34 mmol) in anhydrous THF (6.2 mL) at −78 °C and stirred for 4 h at −78 °C. The reaction was then quenched by dropwise addition of MeOH (2.4 mL) at the same temperature. Rochelle's salt (20%, 6 mL) and water (6 mL) were added and mixture was warmed up to room temperature. The aqueous solution was extracted with CH_2Cl_2 (3×15 mL). The organic layers were dried and evaporated to provide the pure product **26** (592 mg, 98%, a 87:13 mixture of two diastereomers) as a colorless solid after drying under vacuum overnight, which was stored at −20 °C. Data for the major *endo* isomer: mp: 133–135 °C. ^{1}H NMR: δ=7.19–7.34 (m, 12H), 6.91 (d, *J*=9.9 Hz, 1H), 6.49 (d, *J*=8.9 Hz, 1H), 4.72 (dd, *J*=9.9, 3.2 Hz, 1H), 4.71 (d, *J*=17.4 Hz, 1H), 4.66 (d, *J*=17.4 Hz, 1H), 4.43 (d, *J*=12.1 Hz, 1H), 3.39 (d, *J*=4.4 Hz, 1H), 3.07 (d, *J*=12.1 Hz, 1H), 2.90 (m, 1H), 2.69 (m, 1H), 2.18 (m, 1H), 2.02–2.09 (m, 2H); ^{13}C NMR: δ=142.3, 138.6, 138.3, 134.6, 132.4, 128.8, 128.60, 128.57, 127.3, 127.1, 126.5, 120.1, 114.9, 108.5, 84.3, 64.4, 57.8, 54.3, 51.2, 38.9, 24.2; IR (neat, cm^{-1}): 3028, 2949, 2806, 1594; EIMS (*m*/*z*): 450.0 (M$^+$+2, 7), 448.1 (M$^+$, 9), 432.0 (11), 430.0 (11), 358.8 (79), 356.9 (71), 312.9 (100), 310.9 (94).

2.4.13. (3a*R,4*S**,9b*S**)-8-Bromo-1,5-dibenzyl-2,3,3a,4,5,9b-hexahydro-4-methoxy-1*H*-pyrrolo[3,2-*c*]quinoline (32).** The diastereomeric mixture of aminals **31** (459 mg, 1.02 mmol) directly from last step was dissolved in $CHCl_3$ (4.5 mL) and MeOH (18 mL) and then heated to reflux under N_2 for 4 h. The solvents were completely removed and the crude product was further dried under high vacuum to provide the pure methoxyaminal **32** (463 mg, 98%) as a light brown semi-solid, which was stored at −20 °C and used without any further purification. ^{1}H NMR: δ=7.40–7.39 (m, 2H), 7.28–7.34 (m, 2H), 7.22–7.26 (m, 2H), 7.11 (dd, *J*=8.7, 2.3 Hz, 1H), 6.48 (d, *J*=8.7 Hz, 1H), 4.82 (d, *J*=16.3 Hz, 1H), 4.51 (d, *J*=16.3 Hz, 1H), 4.36 (d, *J*=16.3 Hz, 1H), 4.36 (d, *J*=2.1 Hz, 1H), 4.04 (d, *J*=12.8 Hz, 1H), 3.62 (d, *J*=8.5 Hz, 1H), 3.38 (d, *J*=12.8 Hz, 1H), 3.26 (s, 3H), 2.87–2.96 (m, 2H), 2.31 (ddd, *J*=9.4,

9.4, 6.9 Hz, 1H), 2.06 (m, 1H), 1.83 (m, 1H); ^{13}C NMR: δ=142.4, 140.0, 138.4, 132.52, 132.46, 130.8, 129.0, 128.8, 128.7, 128.3, 127.2, 127.0, 115.5, 110.9, 95.1, 61.7, 58.9, 55.3, 55.0, 52.0, 43.1, 28.4; IR (neat, cm^{-1}): 3027, 2928, 2806, 1592; EIMS (*m*/*z*): 464.2 (M^++2, 3), 462.2 (M^+, 24), 373.0 (100), 371.0 (99), 312.9 (78), 310.9 (59), 106.2 (45), 105.2 (33), 86.0 (32), 84.0 (50).

2.4.14. (3a*R,4*S**,9b*S**)-4-Allyl-8-bromo-1,5-dibenzyl-2,3,3a,4,5,9b-hexahydro-1*H*-pyrrolo[3,2-*c*]quinoline (34).** A solution of $TiCl_4$ (0.38 mL, 3.43 mmol) in anhydrous CH_2Cl_2 (3.4 mL) was added dropwise to a stirred solution of methoxyaminal **32** (265 mg, 0.57 mmol) and allyltrimethylsilane (1.09 mL, 6.86 mmol) in anhydrous CH_2Cl_2 (12 mL) at −78 °C. The reaction mixture was allowed to warm up to room temperature slowly and stirred overnight. An NaOH solution (1 N, 20 mL) was added carefully to the reaction mixture and the organic phase was separated. The aqueous layer was further extracted with CH_2Cl_2 (2×). The combined organic layers were dried (Na_2SO_4) and concentrated. An ^{1}H NMR spectrum of the crude reaction mixture showed the formation of **34** and **35** in a ratio of ca. 0.6:1. The residue was purified by chromatography (hexane/ethyl acetate, 8:1) to provide the major isomer **34** as pale yellow oil (80 mg, 30%). Compound **35** was not isolated from the column due to its instability. Data for **34**: ^{1}H NMR: δ=7.28–7.38 (m, 8H), 7.21–7.26 (m, 2H), 7.24 (d, *J*=2.5 Hz, 1H), 7.07 (dd, *J*=8.7, 2.5 Hz, 1H), 6.35 (d, *J*=8.7 Hz, 1H), 5.69–5.78 (m, 1H), 4.99–5.06 (m, 2H), 4.56 (d, *J*=16.0 Hz, 1H), 4.23 (d, *J*=16.0 Hz, 1H), 4.01 (d, *J*=12.8 Hz, 1H), 3.64 (d, *J*=8.5 Hz, 1H), 3.54 (d, *J*=12.8 Hz, 1H), 3.21 (ddd, *J*=8.7, 5.3, 2.3 Hz, 1H), 2.93 (ddd, *J*=9.6, 7.6, 3.7 Hz, 1H), 2.67 (ddd, *J*=17.1, 8.7, 2.3 Hz, 1H), 2.36 (ddd, *J*=9.6, 8.9, 7.1 Hz, 1H), 2.28–2.33 (m, 1H), 2.06–2.12 (m, 1H), 1.90–1.98 (m, 2H); ^{13}C NMR: δ=144.3, 140.0, 138.6, 135.4, 132.5, 130.6, 129.0, 128.7, 128.4, 128.2, 127.2, 127.1, 127.0, 117.7, 114.9, 109.4, 63.1, 60.6, 59.4, 54.5, 52.3, 41.1, 35.9, 29.6; IR (neat, cm^{-1}): 3062, 3028, 2928, 2795, 1591; EIMS (*m*/*z*): 474.1 (M^++2, 12.5), 472.0 (M^+, 12), 432.5 (100), 282.8 (22). HRMS (*m*/*z*): calcd for MH^+ $C_{28}H_{30}{}^{79}BrN_2$ 473.1587, found 473.1571; calcd for MH^+ $C_{28}H_{30}{}^{81}BrN_2$ 475.1566, found 475.1558.

2.4.15. 1,5-Dibenzyl-8-bromo-2,3,5,9b-tetrahydro-1*H*-pyrrolo[3,2-*c*]quinoline (35). Following the above representative procedure, methoxyaminal **32** (72 mg, 0.16 mmol), allyltrimethylsilane (0.25 mL, 1.56 mmol), $MeAlCl_2$ (1 M in hexanes, 0.62 mL, 0.62 mmol), and anhydrous CH_3CN (3.1 mL) were used. After work-up, **35** (~71 mg, >85% based on ^{1}H NMR) was obtained, which was not very stable and after trituration with Et_2O and exposure to air, slowly converted to **36**. Data for **35**: a brown semi-solid. ^{1}H NMR: δ=7.36–7.88 (m, 10H), 7.10 (d, *J*=2.3 Hz, 1H), 7.04 (dd, *J*=8.7, 2.3 Hz, 1H), 6.39 (d, *J*=8.7 Hz, 1H), 6.31 (s, 1H), 5.13 (s, 1H), 4.70 (m, 2H), 3.68 (m, 2H), 3.09 (m, 1H), 2.75 (m, 1H), 2.58 (m, 2H); ^{13}C NMR: δ=142.5, 139.8, 137.6, 137.5, 130.4, 129.0, 128.6, 128.5, 128.4, 128.3, 127.1, 127.0, 126.8, 125.1, 118.3, 114.4, 109.6, 80.6, 58.6, 50.4, 49.3, 29.3.

2.4.16. 1,5-Dibenzyl-8-bromo-2,3-dihydro-1*H*-pyrrolo[3,2-*c*]quinolinium chloride (36). A brown semi-solid. ^{1}H NMR: δ=9.35 (s, 1H), 8.07 (s, 1H), 7.72 (d, *J*=9.3 Hz, 1H), 7.68 (d, *J*=9.3 Hz, 1H), 7.21–7.46 (m, 11H), 5.94 (s, 2H), 5.14 (s, 2H), 4.30 (t, *J*=8.8 Hz, 2H), 3.59 (t, *J*=8.8 Hz, 2H), 1.85 (br s, 1H); ^{13}C NMR: δ=158.4, 141.5, 139.0, 137.3, 134.2, 133.6, 129.9, 129.4, 128.9, 128.7, 127.9, 126.2, 122.1, 120.5, 119.5, 115.7, 58.7, 58.1, 54.1, 24.5; IR (neat, cm^{-1}) 3400, 3031, 2943, 1637, 1609. HRMS (*m*/*z*): calcd for M^+ $C_{25}H_{22}{}^{79}BrN_2^+$ 429.0961, found 429.0959; calcd for M^+ $C_{25}H_{22}{}^{81}BrN_2^+$ 431.0941, found 431.0944.

2.4.17. (3a*R,4*R**,9b*S**)-8-Bromo-4-cyano-1,5-dibenzyl-2,3,3a,4,5,9b-hexahydro-1*H*-pyrrolo[3,2-*c*]quinoline (37).** Methoxyaminal **32** (60 mg, 0.13 mmol) was dissolved in anhydrous CH_2Cl_2 (2.6 mL), and TMSCN (51 mg, 0.07 mL, 0.52 mmol) was added dropwise at room temperature. After stirring at room temperature for 3 h, the reaction mixture was diluted with CH_2Cl_2 and quenched with saturated $NaHCO_3$ (2 mL). The organic layer was separated, washed with H_2O and brine, dried (Na_2SO_4), and concentrated. The crude sample was purified by flash chromatography (hexane/ethyl acetate, 2:1) to provide the title product **37** (58 mg, 98%) as a colorless solid. Mp: 126–128 °C. ^{1}H NMR: δ=7.27–7.37 (m, 9H), 7.20–7.24 (m, 3H), 6.76 (d, *J*=8.8 Hz, 1H), 4.82 (d, *J*=15.2 Hz, 1H), 4.17 (d, *J*=15.2 Hz, 1H), 4.06 (d, *J*=13.0 Hz, 1H), 4.03 (d, *J*=4.6 Hz, 1H), 3.53 (d, *J*=7.8 Hz, 1H), 3.29 (d, *J*=13.0 Hz, 1H), 2.85–2.92 (m, 2H), 2.25 (dd, *J*=17.4, 8.6 Hz, 1H), 2.02–2.10 (m, 1H), 1.75–1.81 (m, 1H); ^{13}C NMR: δ=144.1, 139.2, 136.2, 133.6, 131.7, 129.0, 128.6, 128.3, 128.04, 128.00, 127.1, 126.6, 118.2, 116.2, 112.2, 61.9, 57.4, 53.9, 53.8, 51.4, 42.3, 28.1; IR (neat, cm^{-1}): 3067, 3028, 2970, 2939, 2797. HRMS (*m*/*z*): calcd for MH^+ $C_{26}H_{25}{}^{79}BrN_3$ 458.1226, found 458.1213; calcd for MH^+ $C_{26}H_{25}{}^{81}BrN_3$ 460.1206, found 460.1198.

2.4.18. 1-Benzyl-6-bromo-2-[3-(*tert*-butyldimethylsilanyloxy)-prop-1-ynyl]-3-(2-benzylaminoethyl)-1,2-dihydroquinoline (38). Methoxyaminal **32** (20 mg, 0.043 mmol) was dissolved in anhydrous CH_2Cl_2 (0.5 mL), and TMSOTf (8 μL, 0.04 mmol) was added dropwise at −78 °C. After stirring at −78 °C for 5 min, a copper acetylide solution (1.3 mL, 0.13 mmol), which was prepared in situ by stirring the TBS-protected propargyl alcohol **40** (22 mg, 0.13 mmol), CuCl (13 mg, 0.13 mmol), and NEt_3 (18 μL, 0.13 mmol) in anhydrous CH_2Cl_2 (1.3 mL) at room temperature for 20 min, was added. The reaction mixture was allowed to warm up slowly from −78 °C to room temperature. After stirring overnight, the reaction mixture was diluted with CH_2Cl_2 and washed with saturated $NaHCO_3$, H_2O, and brine, dried (Na_2SO_4), and concentrated. The crude product was purified by flash chromatography (hexane/ethyl acetate, 2:1) to provide **38** (17 mg, 70%) as brown oil. ^{1}H NMR: δ=7.23–7.35 (m, 10H), 7.12 (dd, *J*=8.8, 2.4 Hz, 1H), 7.06 (d, *J*=2.4 Hz, 1H), 6.49 (d, *J*=8.8 Hz, 1H), 6.18 (s, 1H), 4.61 (d, *J*=14.7 Hz, 1H), 4.45 (t, *J*=1.5 Hz, 1H), 4.22 (d, *J*=1.5 Hz, 2H), 4.19 (d, *J*=14.6 Hz, 1H), 3.76 (d, *J*=2.4 Hz, 2H), 2.69–2.79 (m, 2H), 2.35–2.42 (m, 2H), 0.85 (s, 9H), 0.02 (s, 6H); ^{13}C NMR: δ=144.8, 140.3, 136.8, 134.6, 130.6, 129.0, 128.8, 128.5, 128.2, 127.9, 127.7, 127.1, 125.4, 122.0, 114.0, 110.6, 83.5, 81.3, 54.0, 52.2, 51.8, 51.5, 46.7, 34.5, 25.8, 18.3, −5.1; IR (neat, cm^{-1}): 3375, 3031, 2957, 2928, 2856, 1651, 1590. HRMS (*m*/*z*): calcd for MH^+ $C_{34}H_{42}{}^{79}BrN_2OSi$ 601.2244, found

601.2236; calcd for MH^+ $C_{34}H_{42}{}^{81}BrN_2OSi$ 603.2224, found 603.2225.

2.4.19. 1-Benzyl-3-(2-benzylaminoethyl)-6-bromoquinolinium triflate (39). To a stirred solution of methoxyaminal **32** (20 mg, 0.044 mmol) in anhydrous CH_2Cl_2 (0.9 mL) was added dropwise a solution of TMSOTf (16 μL, 0.089 mmol) in anhydrous at −78 °C. The reaction mixture was allowed to warm to room temperature slowly and stirred for 5 h. Then it was diluted with CH_2Cl_2, washed by saturated $NaHCO_3$ and brine (2×), dried ($CaCl_2$), and concentrated to provide the title compound **38** (~18 mg, ca. 90%) as pale yellow oil. ^{1}H NMR: δ=10.29 (s, 1H), 8.85 (s, 1H), 8.25 (s, 1H), 8.14 (d, J=9.5 Hz, 1H), 7.95 (d, J=9.5 Hz, 1H), 7.39–7.42 (m, 2H), 7.20–7.38 (m, 8H), 6.35 (s, 1H), 3.93 (s, 2H), 3.48 (m, 2H), 3.25 (m, 2H); ^{13}C NMR: δ=152.5, 146.4, 138.2, 135.7, 135.0, 133.8, 132.6, 132.3, 131.1, 129.9, 129.6, 129.5, 128.8, 128.5, 127.5, 124.5, 120.6, 61.8, 52.4, 47.0, 30.5, 29.9.

2.5. General procedure for CuBr mediated reactions with sonication

2.5.1. (3a*R,4*R**,9b*S**)-8-Bromo-1,5-dibenzyl-2,3,3a,4,5,9b-hexahydro-4-[3-(*tert*-butyldimethylsilyloxy)-prop-1-ynyl]-1*H*-pyrrolo[3,2-*c*]quinoline (41).** Aminal **31** (50 mg, 0.11 mmol) and the TBS-protected propargyl alcohol **40** (57 mg, 0.33 mmol) were mixed efficiently in a test tube. Then CuBr (48 mg, 0.33 mmol) was added followed by addition of water (1 mL) and mixed by efficient magnetic stirring. Then the test tube was sonicated for 3 h under N_2 protection in a darkened hood. During the course of the reaction, the water bath was warmed up to ~40–45 °C under sonication. After cooling to room temperature, the reaction mixture was partitioned between CH_2Cl_2 and 10% aqueous NH_3 solution. The organic layer was separated and further washed with 10% NH_3 solution, H_2O, and brine, dried (Na_2SO_4), and concentrated. The crude oil was purified by column chromatography (hexanes/ethyl acetate, gradient elution, 10:1 to 1:1) to provide product **41** (32 mg, 48%) as a colorless oil and fragmentation product **38** (27 mg, 40%) as a brown oil. ^{1}H NMR: δ=7.22–7.35 (m, 10H), 7.21 (d, J=2.2 Hz, 1H), 7.14 (dd, J=8.8, 2.2 Hz, 1H), 6.54 (d, J=8.8 Hz, 1H), 4.78 (d, J=16.1 Hz, 1H), 4.42 (d, J=16.1 Hz, 1H), 4.23 (d, J=1.5 Hz, 2H), 4.16 (d, J=13.0 Hz, 1H), 4.12 (dt, J=6.6, 1.5 Hz, 1H), 3.45 (d, J=6.6 Hz, 1H), 3.29 (d, J=13.0 Hz, 1H), 2.90 (ddd, J=9.0, 8.8, 3.4 Hz), 2.62 (m, 1H), 2.24 (ddd, J=8.8, 8.8, 8.8 Hz, 1H), 2.04 (m, 1H), 1.88 (m, 1H), 0.87 (s, 9H), 0.03 (s, 6H); ^{13}C NMR: δ=144.7, 139.9, 138.3, 133.5, 131.1, 128.65, 128.61, 128.2, 127.3, 127.1, 126.9, 125.3, 115.4, 109.4, 83.7, 82.9, 62.5, 57.8, 53.6, 53.4, 51.8, 51.3, 42.7, 27.8, 25.9, 18.3, −5.1; IR (neat, cm^{-1}): 3064, 3030, 2958, 2929, 2857. HRMS (*m*/*z*): calcd for MH^+ $C_{34}H_{42}{}^{79}BrN_2OSi$ 601.2244, found 601.2227; calcd for MH^+ $C_{34}H_{42}{}^{81}BrN_2OSi$ 603.2224, found 603.2222.

2.5.2. *N*-(Benzyloxycarbonyl)-*N*-(*tert*-butyloxycarbonyl)-propargylamine (42). To a stirred solution of *N*-(benzyloxycarbonyloxy)succinimide (3.00 g, 12.0 mmol) in anhydrous CH_2Cl_2 (30 mL) was added dropwise propargylamine (0.80 mL, 12.5 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min and then overnight at room temperature. On completion of the reaction, the mixture was washed with 2 N HCl (10 mL), water (10 mL), and brine (10 mL). The organic layer was separated, dried (Na_2SO_4), and concentrated. The crude product, which can be directly used in the next step without further purification, was purified by flash chromatography (hexane/ethyl acetate, 3:1) to provide the corresponding *N*-(benzyloxycarbonyl)propargylamine (1.64 g, 72%) as a colorless solid. Mp: 39–41 °C. (lit.[41] mp: 35–36 °C). ^{1}H NMR: δ=7.30–7.37 (m, 5H), 5.12 (s, 2H), 5.08 (br s, 1H), 3.98 (d, J=2.4 Hz, 2H), 2.25 (t, J=2.4 Hz, 1H); ^{13}C NMR: δ=156.0, 136.3, 128.7, 128.34, 128.30, 79.8, 71.7, 67.2, 30.9. *N*-(Benzyloxycarbonyl)propargylamine (500 mg, 2.64 mmol) and di-*tert*-butyl-dicarbonate (700 mg, 3.20 mmol) were dissolved in anhydrous CH_3CN (5 mL) at room temperature, then DMAP (20 mg, 0.16 mmol) was added. After stirring at room temperature for 4 h, the reaction mixture was diluted with CH_2Cl_2, washed with saturated $NaHCO_3$ solution, H_2O, and brine, dried (Na_2SO_4), and concentrated. The crude sample was purified by chromatography (hexane/ethyl acetate, 2:1) to provide the title product **42** (688 mg, 90%) as a colorless oil. ^{1}H NMR: δ=7.38–7.41 (m, 2H), 7.30–7.37 (m, 3H), 5.25 (s, 2H), 4.41 (d, J=2.4 Hz, 2H), 2.20 (t, J=2.4 Hz, 1H), 1.48 (s, 9H); ^{13}C NMR: δ=153.1, 151.1, 135.4, 128.6, 128.5, 128.3, 83.7, 79.3, 71.2, 68.8, 36.1, 28.0; IR (neat, cm^{-1}): 3309, 3067, 3036, 2982, 2935, 1797, 1762, 1729, 1696. ESIMS (*m*/*z*): 328 ($M+K^+$, 2), 312 ($M+Na^+$, 100), 256 (15), 212 (16).

2.5.3. (3a*R,4*R**,9b*S**)-4-[3-(Benzyloxycarbonyl-*tert*-butyloxycarbonylamino)prop-1-ynyl]-8-bromo-1,5-dibenzyl-2,3,3a,4,5,9b-hexahydro-1*H*-pyrrolo[3,2-*c*]quinoline (43).** Following the general sonication procedure, aminal **31** (50 mg, 0.11 mmol), propargylamine **42** (95 mg, 0.33 mmol) and CuBr (48 mg, 0.33 mmol) were used. ^{1}H NMR of the crude reaction mixture indicated that the required adduct and the fragmentation product were formed in the ratio of 0.6:1. Chromatographic purification (hexane/ethyl acetate, 6:1 to 1:1) of the crude product provided the title compound **43** (25 mg, 32%) as colorless oil. The pure byproduct **44** was not obtained from the column due to the contamination with unreacted **42**. ^{1}H NMR: δ=7.21–7.38 (m, 15H), 7.18 (d, J=2.4 Hz, 1H), 7.12 (dd, J=8.8, 2.4 Hz, 1H), 6.52 (d, J=8.8 Hz, 1H), 5.18 (s, 2H), 4.75 (d, J=16.1 Hz, 1H), 4.40 (d, J=16.1 Hz, 1H), 4.35 (d, J=1.7 Hz, 2H), 4.15 (d, J=13.0 Hz, 1H), 4.09 (dt, J=7.1, 1.7 Hz, 1H), 3.38 (d, J=6.3 Hz, 1H), 3.25 (d, J=13.0 Hz, 1H), 2.87 (ddd, J=9.0, 9.0, 3.7 Hz), 2.52 (m, 1H), 2.21 (ddd, J=8.8, 8.8, 8.3 Hz, 1H), 1.99 (m, 1H), 1.81 (m, 1H), 1.44 (s, 9H); ^{13}C NMR: δ=153.0, 151.0, 144.6, 139.9, 138.2, 135.5, 133.6, 131.1, 128.65, 128.62, 128.55, 128.4, 128.2, 127.3, 127.1, 126.9, 124.9, 115.4, 109.3, 83.5, 81.7, 79.9, 68.6, 62.5, 57.6, 53.3, 53.1, 51.2, 42.4, 36.4, 28.0, 27.6; IR (neat, cm^{-1}): 3089, 3065, 3032, 2982, 2934, 2885, 2850, 2801, 1796, 1757, 1725, 1697. HRMS (*m*/*z*): calcd for MH^+ $C_{41}H_{43}{}^{79}BrN_3O_4$ 720.2431, found 720.2424; calcd for MH^+ $C_{41}H_{43}{}^{81}BrN_3O_4$ 722.2411, found 720.2422.

2.5.4. Methyl (3a*R,4*S**,9b*S**)-1,5-dibenzyl-4-methoxy-2,3,3a,4,5,9b-hexahydro-1*H*-pyrrolo[3,2-*c*]quinoline-8-carboxylate (45).** To Methoxyaminal **32** (200 mg, 0.43 mmol) in anhydrous THF (4.5 mL) at −78 °C was added *n*-BuLi (1.6 M solution in hexane, 0.28 mL, 0.45 mmol) and stirred for 15 min at −78 °C. Dimethyl

carbonate (0.09 mL, 1.08 mmol) was added dropwise and stirred for 3 h while the reaction temperature was allowed to warm up slowly to 0 °C. The reaction was quenched by careful addition of saturated NH_4Cl solution (15 mL) and the aqueous layer was extracted with CH_2Cl_2 (3×). The combined organics were dried (Na_2SO_4) and concentrated. Ether was added to the crude oily product, removed by rotary evaporation, and then dried under high vacuum overnight to provide **45** (189 mg, greater than 90% pure by ^1H NMR analysis) as a brown semi-solid, which was directly used in the next step without further purification. ^1H NMR: δ=7.89 (d, J=2.2 Hz, 1H), 7.76 (dd, J=8.6, 2.2 Hz, 1H), 7.39–7.41 (m, 2H), 7.20–7.34 (m, 8H), 6.65 (d, J=8.6 Hz, 1H), 4.92 (d, J=16.4 Hz, 1H), 4.60 (d, J=16.4 Hz, 1H), 4.47 (d, J=2.9 Hz, 1H), 4.08 (d, J=13.0 Hz, 1H), 3.83 (s, 3H), 3.66 (d, J=8.1 Hz, 1H), 3.37 (d, J=13.0 Hz, 1H), 3.27 (s, 3H), 2.95 (m, 1H), 2.90 (ddd, J=16.1, 8.1, 2.7 Hz), 2.32 (ddd, J=9.3, 9.0, 7.6 Hz), 2.08 (m, 1H), 1.83 (ddd, J=16.1, 12.2, 8.1 Hz); ^{13}C NMR: δ=167.4, 147.9, 140.1, 138.0, 131.7, 130.4, 128.7, 128.2, 127.2, 127.0, 126.9, 125.4, 119.7, 113.0, 94.3, 62.0, 58.6, 54.9, 54.0, 51.79, 51.71, 51.67, 42.4, 28.2; IR (neat, cm^{-1}): 3062, 3028, 2963, 2930, 2850, 2824, 1711, 1609. ESIMS (m/z): 465 ($M+Na^+$, 1), 443 ($M+H^+$, 5), 411 (100), 353 (15).

2.5.5. Methyl (3a*R,4*R**,9b*S**)-4-[3-(benzyloxycarbonyl-*tert*-butyloxycarbonylamino)-prop-1-ynyl]-1,5-dibenzyl-2,3,3a,4,5,9b-hexahydro-1*H*-pyrrolo[3,2-*c*]quinoline-8-carboxylate (47).** Following the general sonication procedure, crude methyl ester **45** obtained directly from last step (100 mg, ~0.22 mmol), **42** (195 mg, 0.66 mmol) and CuBr (97 mg, 0.66 mmol) were used. Chromatography (hexanes/EtOAc, 6:1 to 1:1) of the crude oil provided product **47** (63 mg, 40% for two steps) as colorless oil. The pure fragmentation byproduct was not obtained from the column due to the contamination with unreacted **42**. ^1H NMR: δ=7.78 (d, J=2.0 Hz, 1H), 7.74 (dd, J=8.8, 2.0 Hz, 1H), 7.26–7.37 (m, 8H), 7.18–7.25 (m, 7H), 6.67 (d, J=8.8 Hz, 1H), 5.18 (s, 2H), 4.93 (d, J=16.9 Hz, 1H), 4.69 (d, J=16.9 Hz, 1H), 4.37 (d, J=1.5 Hz, 2H), 4.28 (dt, J=8.8, 1.5 Hz, 1H), 4.21 (d, J=13.0 Hz, 1H), 3.83 (s, 3H), 3.36 (d, J=5.1 Hz, 1H), 3.20 (d, J=13.0 Hz, 1H), 2.89 (ddd, J=9.0, 9.0, 3.7 Hz), 2.45 (m, 1H), 2.19 (ddd, J=9.8, 9.5, 7.1 Hz, 1H), 2.04 (m, 1H), 1.78 (m, 1H), 1.45 (s, 9H); ^{13}C NMR: δ=167.3, 153.0, 151.1, 149.0, 140.0, 137.8, 135.4, 133.6, 130.8, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 127.1, 127.0, 126.8, 120.4, 117.9, 112.6, 83.6, 81.6, 80.6, 68.7, 63.0, 57.0, 53.0, 52.3, 51.7, 50.8, 41.5, 36.4, 28.0, 27.1; IR (neat, cm^{-1}): 3062, 3006, 3030, 2979, 2945, 2796, 1794, 1756, 1711, 1610. HRMS (m/z): calcd for MH^+ $C_{43}H_{46}N_3O_6$ 700.3381, found 700.3380.

2.5.6. Methyl (3a*R,4*S**,9b*S**)-4-(3-aminopropyl)-2,3,3a,4,5,9b-hexahydro-1*H*-pyrrolo[3,2-*c*]quinoline-8-carboxylate, hydrochloride salt (48).** A slurry of 20% $Pd(OH)_2$ on carbon (51 mg, 0.073 mmol) and **46** (30 mg, 0.043 mmol) in MeOH (6 mL) and concd HCl (0.30 mL) was stirred under H_2 balloon for 28 h at room temperature. The catalyst was then removed by filtration and rinsed with MeOH (2×6 mL). The filtrate was concentrated, and then more MeOH was added and concentrated again to co-evaporate water. This procedure was repeated several times until an oily compound was obtained, which was triturated with Et_2O to provide the oily compound **48** as the hydrochloride salt (15 mg, >90% pure by ^1H NMR analysis).[43] ^1H NMR (CD_3OD): δ=7.98 (s, 1H), 7.74 (d, J=8.0 Hz, 1H), 6.83 (d, J=8.0 Hz, 1H), 4.65 (br s, 1H), 3.81 (s, 3H), 3.34–3.42 (br s, 2H), 3.10 (br s, 1H), 2.99 (br s, 2H), 2.39–2.50 (br s, 2H), 2.12–2.20 (br s, 1H), 1.80–1.95 (br s, 3H), 1.70 (br s, 1H); ^{13}C NMR (CD_3OD): δ=168.3, 151.0, 133.9, 132.6, 119.2, 115.7, 113.3, 59.3, 52.3, 50.8, 43.9, 41.1, 39.4, 30.5, 28.2, 24.0; ESIMS (m/z): 290 ($M+H^+$, 100), 273 (75), 256 (22).

2.6. X-ray data collection, solution, and refinement of the structures *cis*-26 and *trans*-26

A suitable crystal was selected and attached to a glass fiber using 5 min epoxy-glue and immediately placed in the path of the X-ray beam. Data collections for compounds *cis*-**26** and *trans*-**26** were carried out on a Siemens P4 diffractometer equipped with a LT-2A device for low-temperature work and graphite monochromated Mo Kα radiation (λ=0.710 73 Å). Data were collected at the room temperature. All the structures were solved and refined using the Bruker SHELXTL software package. Compound *cis*-**26** crystallizes in the triclinic $P\bar{1}$ space group with two chemically similar, but crystallographically different molecules in the asymmetric unit. *trans*-**26** Crystallizes in the monoclinic $P2(1)/n$ space group. Crystallographic data (cif files) for *cis*-**26** and *trans*-**26** have been deposited at the Cambridge Crystallographic Data Center, CCDS nos. 287965 and 287966. Copies of this information may be obtained free of charge from the Director, Cambridge Crystallographic Data Centre (CCDC), 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk).

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A homologous series of eunicellin-based diterpenes from *Acalycigorgia* sp. characterised by tandem mass spectrometry

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Abstract—The discovery and structure determination of a homologous series of eunicellin-based diterpenes from the gorgonian *Acalycigorgia* sp. is described. Extensive use was made of 1D and 2D NMR data to determine the structure of the diterpene skeleton. The relative stereochemistry was confirmed via the use of NOE data in conjunction with molecular modelling. A series of homologues were identified using a combination of product and precursor ion scanning modes in tandem mass spectrometry. This powerful technique afforded excellent clarification to aid the analysis of the complex mass spectral data.

1. Introduction

Species of the class Alcyonaria produce a wealth of bioactive diterpenes, amongst which are the promising anti-cancer leads, the sarcodictyins and eleutherobin. The Mediterranean gorgonian *Eunicella singularis* (=*Eunicella stricta*) was the source of the unique marine diterpenoid eunicellin first reported by Djerassi's group in 1968.[1] Since then, many other species from the genera *Eunicella*,[2] *Cladiella*,[3] *Briareum*,[4] *Sarcodictyon*,[5] *Eleutherobia*,[6] *Solenopodium*,[7] *Sclerophytum*[8] and *Alcyonium*[9] have resulted in a very large number of marine diterpenoids based on the eunicellin skeleton. It appears that eunicellin-based diterpenoids are a very pronounced feature of the class Alcyonaria since the species that produce them have been collected from many locations around the world (Mediterranean, Atlantic, Indian and Pacific Oceans). Even though their exact ecological significance is still not certain, several ecological and agrochemical related activities have been reported for these diterpenes, including molluscidal and mollusc repellant activity,[10,11] hemolytic activity,[12] inhibition of cell division in fertilised starfish eggs[13] and insect growth inhibitory activity.[14] The structure, source and biological activities of the many known members of the cladiellins, briarellins, asbestinins and sarcodictyins have been reviewed by Paquette et al.[14] Pharmacologically, there is a significant relationship between the structure and activity of these diterpenoids; both sarcodictyins A and B and eleutherobin (the 'eleutheside' family of microtubule-stabilising compounds) are characterised by an activity profile different from that of the anti-cancer agent paclitaxel (Taxol™).[15] In particular, they are active against paclitaxel resistant tumour cell-lines. Theoretical pharmacophore studies on the various features of microtubule stabilising compounds taxol, epothilones, eleutherobin/sarcodictyin and laulimalides have led to the proposal of the hypothesis that the core ring systems and side chains present in these compounds might bind to common binding sites in β-tubulin, resulting in a similarity in their biological functions.[16,17] The apparent lack of activity amongst several eunicellin-based diterpenoids might only be due to the absence of appropriate side chain functional groups, which bind efficiently to sites in β-tubulin.

In this study we present the structures of a new homologous series of eunicellin-based diterpenoids from *Acalycigorgia* sp. The structures of these compounds were elucidated using 1D and 2D NMR and MS techniques. We extended the analysis of the homologous series by tandem mass spectrometry using precursor ion scans.

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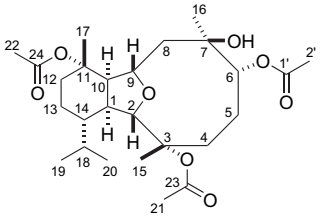
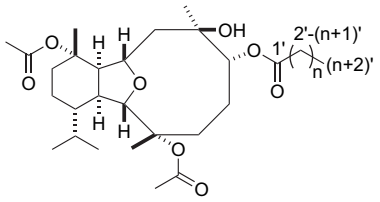
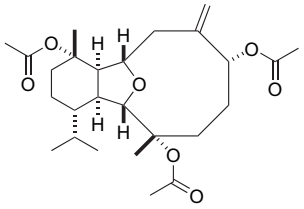


1



2a-t
n = 4-20



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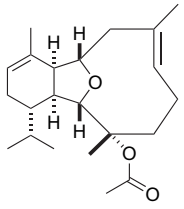

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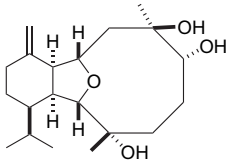


5

2. Results and discussion

The US National Cancer Institute's Open Repository Program provides our laboratory with crude extracts of marine invertebrates, which are screened for differential cytotoxicity at the Ford Cancer Center, Detroit, USA. As a part of this collaboration we had the opportunity to study the gorgonian *Acalycigorgia* sp. The sample of *Acalycigorgia* sp. was collected by the Australian Institute of Marine Science scientists in Thailand in June 1990 at 15 m. Solvent partitioning, followed by size exclusion, low- and high-pressure liquid column chromatographic procedures afforded two new eunicellin-based diterpenoids and an intractable mixture of homologues.

The ^{1}H NMR spectrum of compound **1** was characterised by eight methyl signals made up of three acetyl methyls at δ_H 2.04 (s, 3H), 1.99 (s, 3H), 1.95 (s, 3H), three vinyl methyls or methyls attached to oxygenated quaternary carbons at δ_H 1.45 (s, 3H), 1.33 (s, 3H), 1.20 (s, 3H) and an isopropyl group with two methyls at δ_H 0.94 (d, 3H, J=6.8 Hz) and 0.80 (d, 3H, J=6.8 Hz). The ^{13}C NMR of compound **1** was made up of 26 ^{13}C resonances in total. These data suggested that **1** was a marine diterpene with three acetate groups attached to the C_{20} skeleton. A database search on MarinLit[18,19] using the genus and information from 1D NMR indicated the likelihood of an eunicellin skeleton being present. Six quaternary carbon atoms confirmed the presence of three ester functionalities (δ_C 172.2, 170.8, 170.8), two acetylated quarternary carbons (δ_C 86.5 and 82.4) and a hydroxylated quaternary carbon (δ_C 75.6) forming part of a ring structure. There were seven methine carbons resonating at δ_C 83.8 (acetylated methine group), δ_C 92.0 and 74.9 (ether group) and δ_C 52.3, 42.7, 42.7, 29.0, which formed part of the ring structure. Also forming a part of this apparent ring structure were five methylene groups at δ_C 48.2, 35.5, 32.4, 29.7, 17.4 and five methyl groups at δ_C 23.9, 22.8, 21.5, 22.1, 14.6, were identified. Three acetyl methyls could clearly be seen resonating at δ_C 21.5, 21.1, 20.4. From this data, the molecular formula was calculated as $[(C)_6+(CH)_7+(CH_2)_5+(CH_3)_8+(O)_7+(OH)=482]$ $C_{26}H_{42}O_8$ indicating the presence of six degrees of unsaturation three of which could be accounted for the three acetate groups initially identified. The LRESIMS gave a peak at m/z 505 $[M+Na]^+$ and m/z 423 $[M-CH_3COO]^+$. The accurate mass measurement of m/z 505 was difficult and did not yield the level of accuracy desired, probably due to the rapid loss of CH_3COONa. However, accurate mass measurement at m/z 423 gave 423.2741, Δ=+0.0 calculated for $C_{24}H_{39}O_6$ $[M-CH_3COO]^+$.

With the aid of an HSQC spectrum all protons were assigned to their directly bonded carbon atoms (Table 1). Five substructures were constructed by interpretation of the ^{1}H–^{1}H COSY spectrum (Fig. 1a). The connection between these substructures were established by interpretation of an HMBC spectrum (Fig. 1b and 1c) and all the spin systems were confirmed by data from an HSQC–TOCSY spectrum (Fig. 1d). The HSQC–TOCSY spectrum proved invaluable by providing correlations, which confirmed the ^{1}H–^{1}H COSY substructures and enabled their extension. HMBC correlations from C-10 to H-17, C-12 to H-17, C-15 to H-2, C-4 to H-15, C-8 to H-16 and C-6 to H-16 confirmed the relative positions of the three methyls C-15, C-16 and C-17 on the skeleton. ^{1}H–^{1}H COSY correlations from H-9 to H-10 and HMBC correlations from C-9 to H-10 confirmed the adjacency of C-10 and C-9. Also, HMBC correlations from C-1 to H-2 and C-3 to H-2 together with ^{1}H–^{1}H COSY correlations between H-1 and H-10 confirm the

Table 1. ^{1}H, ^{13}C and 2D NMR data at 400/100 MHz for the 3,11-diacetylated diterpene skeleton in structures **1** and **2a–t** in CD_3OD

	δ^{13}C/ppm, mult	δ^{1}H/ppm, mult, *J* (Hz)	COSY H→H	HMBC C→H	HSQC–TOCSY C→H
1	42.7, d	2.16 dd, 7.2, 12.0	H-12b, H-13a, H-14	H-2, H-10, H-19, H-20	H-1, H-13a
2	92.0, d	3.53 s	—	H-15	H-2
3	86.5, s	—		H-2, H-15	
4a	35.5, t	2.01 ddd, 14.8, 7.7, 1.8	H-4b	H-6, H-15	H-4a/4b, H-5
4b		2.47 m	H-4a		
5	29.7, t	1.45 m	H-6	—	H-5
6	83.8, d	5.56 d, 4.8	H-5	H-16	H-4a, H-6
7	75.6, s	—	—	H-6, H-16	—
8a	48.2, t	1.79 dd, 4.0, 14.8	H-8a/8b, H-9	H-10, H-16	H-8a/8b
8b		1.99 dd, 4.0, 14.8			
9	74.9, d	4.03 ddd, 4.0, 8.0, 11.6	H-8a/8b, H-10	H-2, H-10	H-8a/8b, H-9
10	52.3, t	3.24 t, 7.2	H-9, H-14	H-2, H-17	H-8a/8b, H-10
11	82.4, s	—	—	H-10, H-17	—
12a	32.4, t	1.41 m	H-12a/12b	H-17	—
12b		2.06 m			
13a	17.4, t	1.09 m	—	—	H-12a, H-13a
13b		1.40 m			
14	42.7, d	1.13 m	H-1, H-10	H-2, H-10, H-19, H-20	H-12a, H-13a, H-14
15	22.1, q	1.33 s		H-2	H-15
16	22.8, q	1.20 s	—	—	H-16
17	23.9, q	1.45 s	—	—	H-17
18	29.0, d	1.75 m	H-19, H-20	H-19, H-20	H-12a, H-18, H-19, H-20
19	21.5, q	0.94 d, 6.8	—	H-20	H-19, H-20
20	14.6, q	0.80 d, 6.8	—	H-19	H-19, H-20
21	21.1, q	2.04 s	—	—	—
22	21.5, q	1.95 s	—	—	—
23	170.8, s	—	—	H-21	—
24	170.8, s	—	—	H-22	—

situation of C-1 relative to C-10 and C-2. HMBC correlations from C-11 to H-17, C-7 to H-16, C-4 to H-6 and C-3 to H-15 show the exact positions of the oxygenated carbons C-3, C-6, C-7 and C-11. The lack of any relevant correlations linking the acetate groups to the eunicellin-based skeleton made it difficult to assign their exact positions on the ring. The same problem was reported by Ortega et al.[20] in assigning the structure of palmonine B (**3**) where the three acetates could not be assigned to their exact positions on the ring. In many cases derivatisation has been used to solve this problem, as in the structure determination of klyxumines A and B.[21] Compound **1** cannot be readily derivatised so another solution must be sought. In compound **4** it was easy to assign the position of the acetate group as there was only one possibility.[22] Oxygenated carbons are easily assigned as in sclerophytin A (**5**),[23] but the attachment of acetates can be problematic. Chemical shift evidence suggested that the C-7 position in **1** was not acetylated, as the ^{13}C NMR chemical shift was more shielded relative to the acetylated carbons C-3, C-6 and C-11. Additional evidence for this hypothesis came from a homologue, compound **2a**.

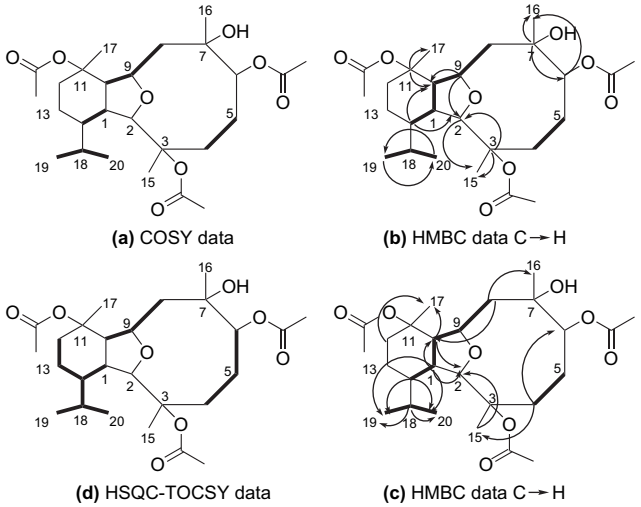


Figure 1. Important 2D NMR correlations for compound **1**.

The ^{1}H NMR of compound **2a** was very similar to that of compound **1**. One of the major differences was the disappearance of one of the acetyl methyls initially observed at δ_H 1.99 (s, 3H) and found from HMBC correlations to be attached to δ_C 172.2 (Table 2). In place of this, a new methylene signal appeared at δ_H 2.26 (t, 2H, J=7.2 Hz). A new methyl signal was also observed at δ_H 0.83 (m, 3H) and two methylene signals were also found at δ_H 1.21 (m, 2H) and 1.24 (m, 2H) (Table 2). The ^{13}C NMR of compound **2a** was made up of 30 ^{13}C signals. The four additional signals compared to compound **1** were found at δ_C 14.1, 22.0, 25.1, 31.8. The main carbon skeleton of **2a** was found to be the same as that of **1**, the new signals arising as a consequence of fatty acid chain incorporation on the acetate group found at δ_C 172.2 in compound **1** and was seen to shift to δ_C 174.2 in compound **2a**. The additional 2D NMR correlations observed for the fatty acid side chain incorporated onto, δ_C 174.2, ester carbonyl group are shown in Figure 2. Most of the 2D NMR data collected for the carbon skeleton of compound **2a** were the same as that shown in Figure 1 for compound **1**. Assigning the exact positions of the acetate groups were a problem in compound **2a** however, the HMBC spectra for a mixture of homologues with the same structure as compound **2a** but with longer fatty acid side chains revealed a correlation from δ_C 174.9 to H-6, which led us to infer that the hexanoyl group was attached at C-6. Another interesting correlation seen in this spectrum was from δ_C 170.8 (attached to the δ_H 2.04, s, 3H) to H-2 suggesting

Table 2. ^{1}H, ^{13}C and 2D NMR data at 400/100 MHz for the acetyl side chains at C-6 in structures **1** in CD_3OD and for **2a** and **2b–f** in $CDCl_3$

	δ^{13}C/ppm, mult	δ^{1}H/ppm, mult, J/Hz	COSY H→H	HMBC C→H	HSQC–TOCSY C→H
Compound ***1***					
1′	172.2, s	—	—	H-2′	—
2′	20.4, q	1.99 s	—	—	H-2′
Compound ***2a***					
1′	174.2, s	—	—	H-2′	—
2′	34.0, t	2.26 t, 7.2	H3′	—	H-2′, H-3′
3′	25.1, t	1.56 m	H2′, ω3-H	H-2′, ω3-H	H-2′, H-3′, ω3-H
ω3	31.8, t	1.21 m	—	ω2-H, ω1-H	—
ω2	22.0, t	1.24 m	H3′	ω1-H	H-2′, ω3-H, ω1-H
ω1	14.1, q	0.83 m	ω3-H	—	ω2-H, ω1-H
Compounds ***2b–f***					
1′	174.9, s	—	—	H-6, H-2′	—
2′	34.8, t	2.17 t, 7.2	H3′	H-3′	H-2′, H-3′, H-4′
3′	25.3, t	1.59 m	H2′	H-2′	H-2′, H-3′, H-4′
$(CH_2)_n$	29.9, t	1.22 s			
ω3	31.9, t	1.24 m	—	ω2-H, ω1-H	ω3-H, ω1-H
ω2	22.9, t	1.27 m	ω4-H	ω1-H	ω4-H, ω2-H, ω1-H
ω1	14.1, q	0.86 m	ω2-H	—	ω4-H, ω3-H, ω2-H, ω1-H

that this acetyl group is substituted on C-3. The remaining acetyl group δ_C 170.8 (attached to the δ_H 1.95, s, 3H) was therefore placed on C-11. From the ^{13}C NMR of compound **2a**, a molecular formula was calculated as $C_{30}H_{50}O_8$ indicating the presence of six degrees of unsaturation. The LRESIMS gave a *m/z* 561, which was measured accurately as 561.3398, Δ=+1.0.

To establish that the relative configuration of **1** was as reported for related compounds **3–5**, the global minimum energy conformation of compound **1** was calculated using a Monte Carlo conformational search (10,000 steps, global minimum shown in Fig. 3).[24] The molecular mechanics calculations indicated H-10 as being cis to H1 (α-position of the ring-downwards). A strong correlation in the T-ROESY spectrum was consistent with this configuration. In the energy-minimised structure H9 was cis to H2 (β-position of the ring-upwards) and no correlations were expected because of the five-membered ring. However, the absence of any T-ROESY correlations between H-9–H-10 and between H-2–H-1 suggested that H-10 and H-1 were in the α-position and H-9 and H-2 in the β-position. The equatorial placement of methyl H-17 in the molecular mechanics calculations was confirmed by the strong correlation in the T-ROESY spectrum between H-17 and the protons H-8′. Strong T-ROESY correlations between isopropyl methyls H-20/H-19 and methyl H-15 confirmed the equatorial positions occupied by these two substituents. Strong correlations were also seen between methyl H-16 and H-8′, which suggested that this methyl substituent was axially placed. The lack of T-ROESY correlations between H-6 and the protons on methyl H-16 corroborated this assumption.

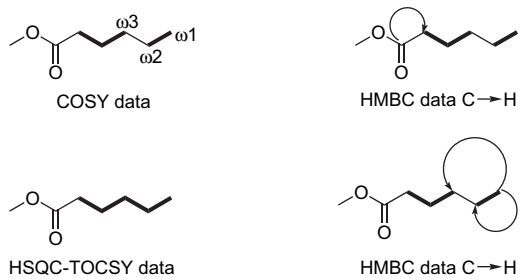


Figure 2. Important 2D NMR correlations for the side chain in compound **2a**.

Analysis of these HPLC fractions by LRESIMS revealed that the parent mixture, which yielded **1** and **2a** was composed of a homologous series of eunicellin-based diterpenoids with the same skeleton as compound **1** but with sequential aliphatic side-chain elongation on one of the ester groups attached to the skeleton (compounds **2a–t**). When the parent mixture was subjected to HPLC effective separation was unachievable, and peaks with the same ^{1}H and ^{13}C NMR were obtained. The mass spectra of these peaks showed them still to be complex mixtures, and therefore the parent mixture was investigated by tandem mass spectrometry (MS/MS). Of the available modes in MS/MS, product ion scanning is the most frequently used. Ions of a particular *m/z* value are selected in the first stage of mass analysis, these precursor ions are fragmented and the product ions resulting from the fragmentation are analysed in a second stage of mass analysis.[25] Less often applied is precursor ion scanning, in which all molecular ions giving rise to the same product ion are identified.

In compounds **2a–t**, two pathways of fragmentation were proposed leading to key product ions at *m/z* 481 (Scheme 1) and *m/z* 423 (Scheme 2). The fragmentation in Scheme 1 was

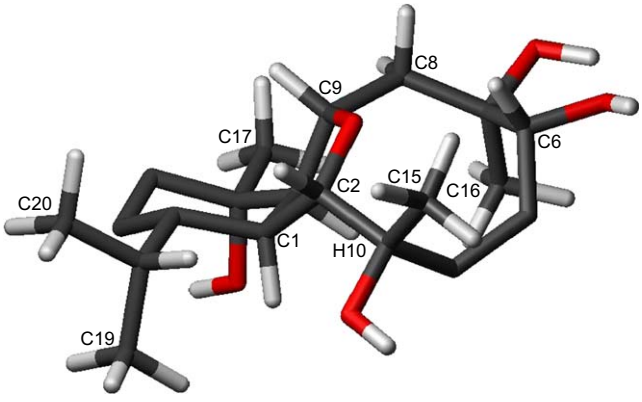
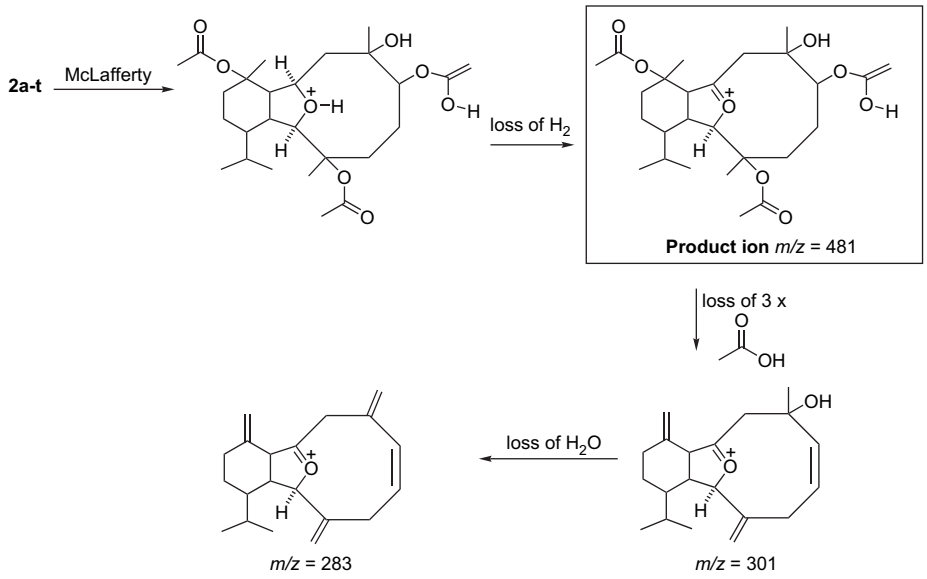


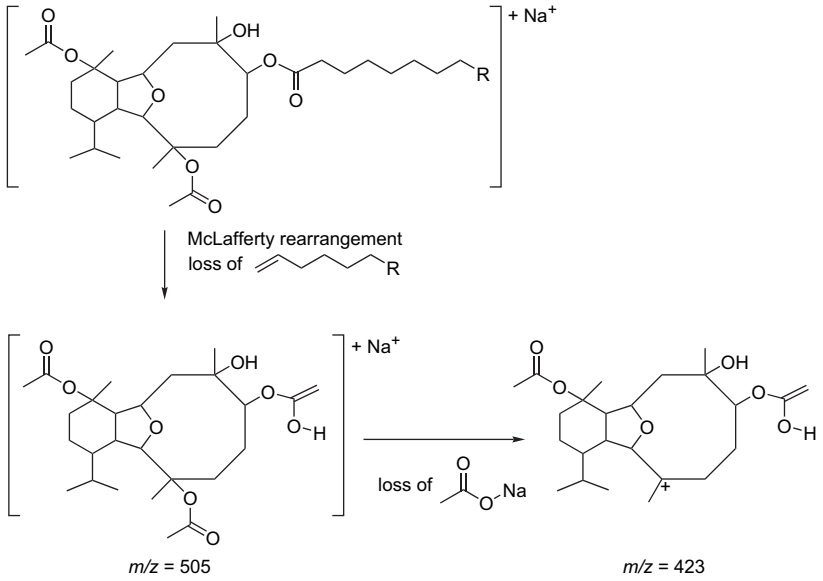
Figure 3. Global energy minimum of compound **1** (for clarity, only key H atoms are shown). Figure was generated using MolMol.[28]



Scheme 1. Positively ionised molecular ions of **2a–t** undergo McLafferty rearrangement with subsequent loss of H_2, which leads to the formation of the onium ion at *m/z* 481. Subsequent fragmentations of *m/z* 481 gives information about its structure.

confirmed by the iterative loss of acetate groups from *m/z* 481 followed by the loss of water as seen in the product ion scan (Fig. 4c). It is evident from Figure 4a, that analysis of the raw MS data would be impossible in this case. The clarification offered by the use of tandem mass spectrometric techniques is invaluable here (Fig. 4b–d). The species at *m/z* 481 (Scheme 1) is the onium ion, which is frequently seen in the spectra of ethers, alcohols and amines. The species at *m/z* 481 loses $3\times CH_3COOH$ to give rise to the species at *m/z* 301, followed by a loss of H_2O giving *m/z* 283. Other fragments of *m/z* 481 can be accounted for as follows: loss of C_2H_2O from the species at *m/z* 421 gives rise to *m/z* 379; loss of C_2H_2O from *m/z* 361 leads to *m/z* 319; loss of H_2O from *m/z* 361 gives the peak at *m/z* 343 and a loss of H_2O from *m/z* 421 gives *m/z* 403.

In Scheme 2 sodiated molecular ions undergo a McLafferty type rearrangement to give a product at *m/z* 505 (Fig. 4b). This species then undergoes a direct cleavage leading to a loss of 82 mass units ($C_2H_3O_2Na$) leaving *m/z* 423. Using the precursor ion scanning strategy on *m/z* 423 we obtained all the precursors, from *m/z* 561 upwards in increments of 14 mass units (Fig. 4b, Table 3). Accurate mass values were obtained for several of these, which agree with the molecular formulae of acetylated compounds **2b–t** with varying lengths of acyl chains (Table 3). In addition it appears that some monounsaturated side chains are also present (e.g., at *m/z* 727 and *m/z* 741). In a similar fashion, we obtained the precursors of *m/z* 481 (Fig. 4d), but the data is less clear than the *m/z* 423 precursor ion scan and not easily interpreted (Fig. 4b).



Scheme 2. Sodiated molecular ions of **2a–t** undergo McLafferty rearrangement with the concomitant loss of the appropriate neutral molecule and formation of the species at *m/z* 505, which subsequently loses a mass of 82 giving rise to the species at *m/z* 423.

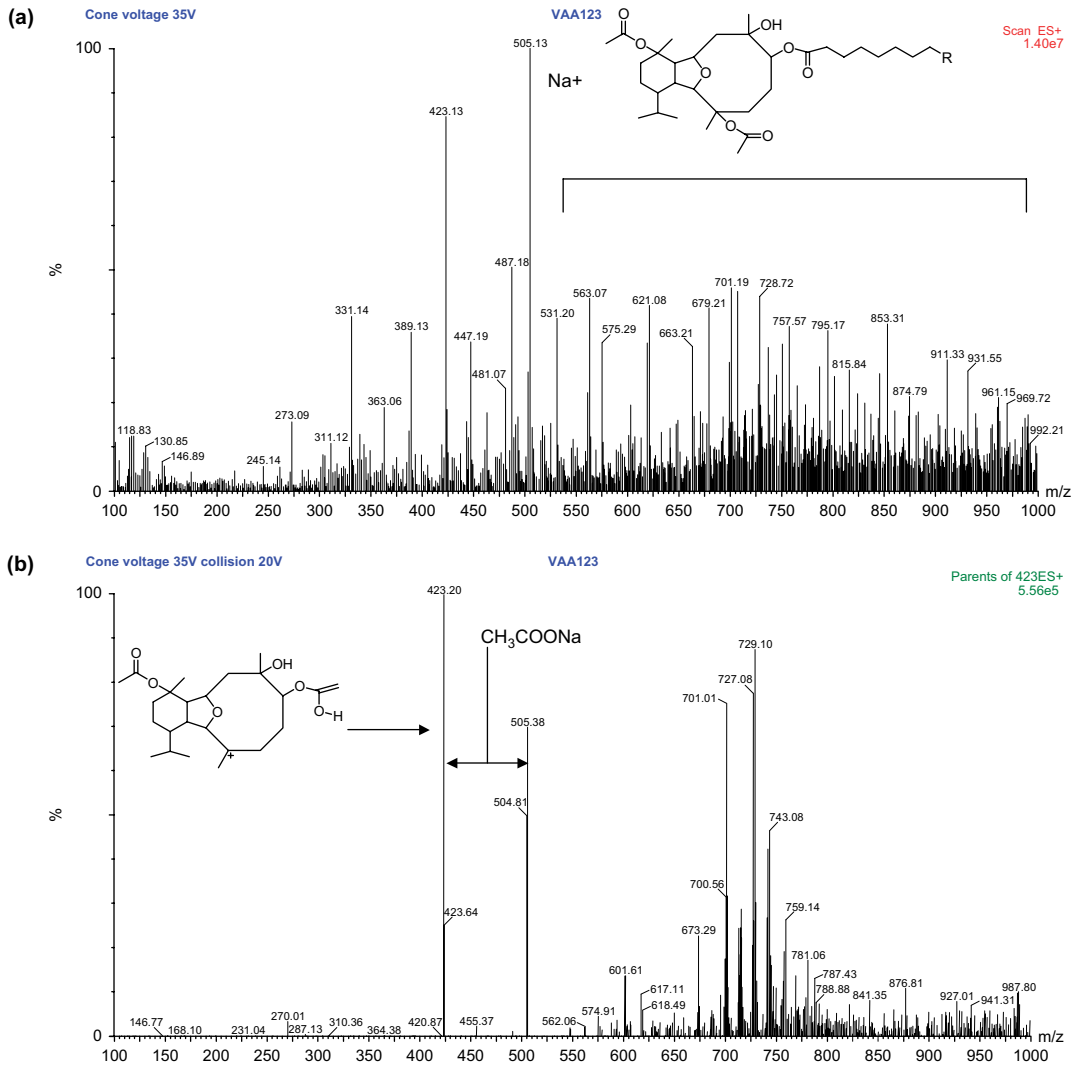


Figure 4. MS and MS/MS spectra of parent fraction of acetylated eunicellin diterpenes **2a–t**: (a) MS spectrum; (b) precursor ion scan of *m/z* 423; (c) product ion scan of *m/z* 481; and (d) precursor ion scan of *m/z* 481.

To confirm our conclusions obtained from the tandem mass spectra, we obtained full 1D and 2D NMR data on the parent mixture analysed by tandem mass spectrometry. The diterpene skeleton was found to be identical to **1** and additional data was obtained for the fatty acid side chains (Table 2). The data for the termini of the fatty acid chain were clear, but the central methylenes of the chain were contained within the CH_2 envelope at δ_H 1.22 and δ_C 29.9. An HMBC correlation from C-1′ to H-6 places the acyl chain at C-6. Table 3 indicates the mass data for the homologous series of long chain eunicellin diterpenes **2a–t**.

2.1. Biological activity data

Compared to their eleutherobin and sarcodictyin counterparts, eunicellin-based diterpenoids exhibit moderate cytotoxicities. Sclerophytin A (**5**) exhibits the most remarkable cytotoxicity profile, active against L1210 leukaemia cell-lines at a concentration of 1 ng/mL.[23] Other examples include **4** reported by Shin et al.[22] to exhibit moderate in vitro cytotoxicity against human tumour cell-lines. The ED_{50} values of this compound were 12.7, 21.3, 11.6 and 13.9 μg/mL against A-549 non-small cell lung cancer, SKOV-3 ovarian cancer, SK-MEL-2 melanoma and HCT-15 colon cancer cell-lines, respectively. The highly acetylated eunicellin-based diterpenoids do not exhibit interesting cytotoxicity profiles with the exception of palmonine B, which was originally reported by Ortega et al.[20] to have activity against P-388 and MEL28 cells with (ED_{50}=5 μg/mL). Compounds **1** and **2a** were evaluated in vitro at the Ford Cancer Center, for their differential cytotoxicity in the soft agar assay.[26,27] They were found to be inactive against the murine colon adenocarcinoma-38 (C-38) cells. The compounds exhibited no murine solid tumour selectivity relative to murine normal cells.

3. Conclusion

The series of compounds identified adds to the growing number of diterpenes of this type isolated from the class Alcyonaria. The difference in the oxygenation pattern is of interest, but it is notable that an entire homologous series appears to be generated biosynthetically, perhaps with a function to allow lipid transport or membrane anchoring of these compounds. These compounds could not have been adequately identified without tandem mass spectrometry techniques,

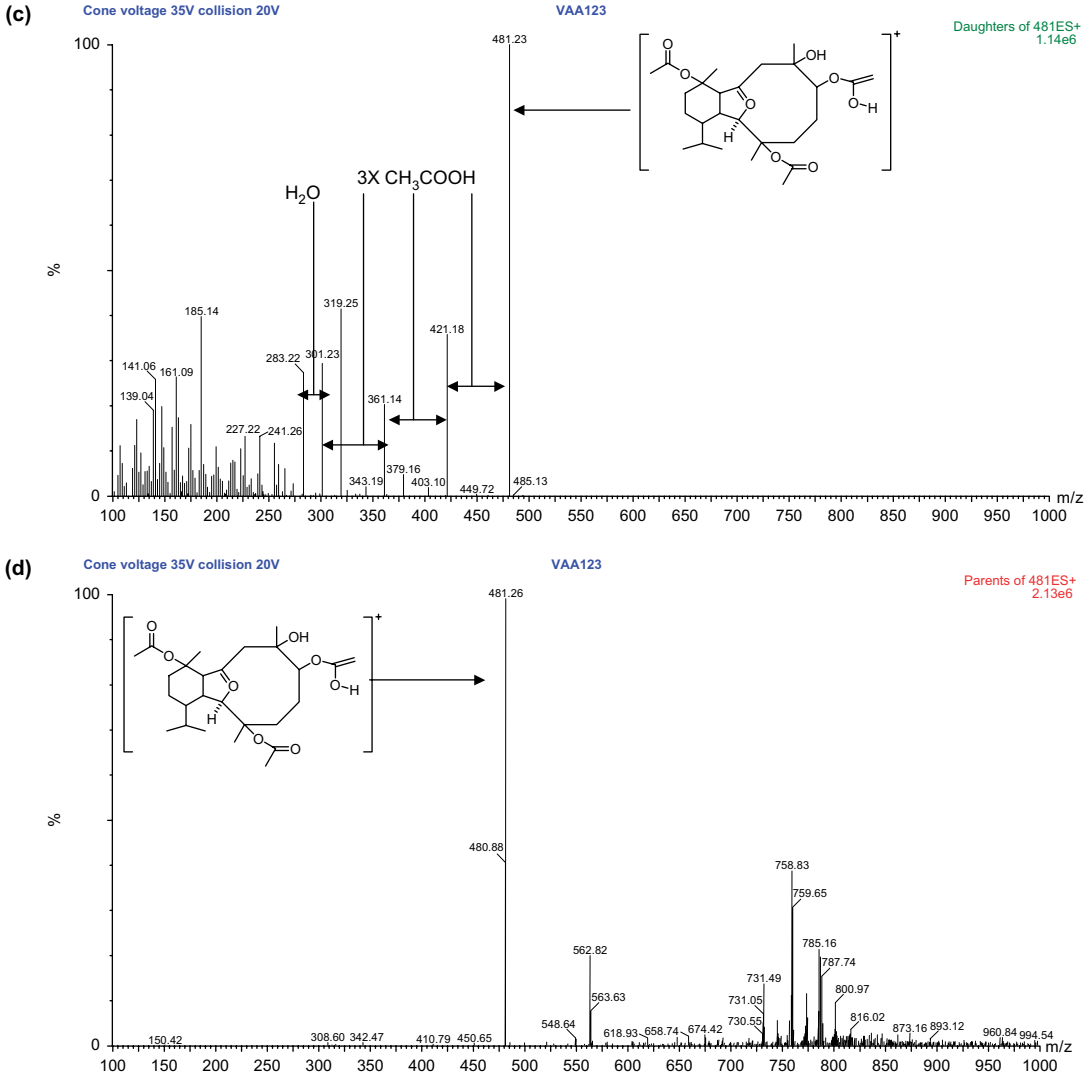


Figure 4. (*continued*)

Table 3. Masses observed for acetyl side chains at C-6 in compounds **2a–t**

Compounds	*n*	$[RCOO]^-$	$[RCOO]^-$ nominal mass	Molecular formula for $[M+H]^+$ or $[M+Na]^+$	Calculated nominal mass	Observed mass (LRESIMS/HRESIMS)	Accurate mass error (ppm)
2a	4	$C_6H_{11}O_2$	115	$C_{30}H_{50}O_8Na$	561	561.3398	+1.0
2b	5	$C_7H_{13}O_2$	129	$C_{31}H_{52}O_8Na$	575	575	
2c	6	$C_8H_{15}O_2$	143	$C_{32}H_{54}O_8Na$	589	589	
2d	7	$C_9H_{17}O_2$	157	$C_{33}H_{56}O_8Na$	603	603	
2e	8	$C_{10}H_{19}O_2$	171	$C_{34}H_{58}O_8Na$	617	617	
2f	9	$C_{11}H_{21}O_2$	185	$C_{35}H_{60}O_8Na$	631	631	
2g	10	$C_{12}H_{23}O_2$	199	$C_{36}H_{62}O_8Na$	645	645	
2h	11	$C_{13}H_{25}O_2$	213	$C_{37}H_{64}O_8Na$	659	659	
2i	12	$C_{14}H_{27}O_2$	227	$C_{38}H_{66}O_8Na$	673	673.4658	+1.2
2j	13	$C_{15}H_{29}O_2$	241	$C_{39}H_{68}O_8Na$	687	687	
2k	14	$C_{16}H_{31}O_2$	255	$C_{40}H_{70}O_8Na$	701	701.4970	+0.9
2l	15	$C_{17}H_{33}O_2$	269	$C_{41}H_{72}O_8Na$	715	715.5102	−2.4
2m	16	$C_{18}H_{35}O_2$	283	$C_{42}H_{74}O_8Na$	729	729.5279	+0.5
2n	17	$C_{19}H_{37}O_2$	297	$C_{43}H_{76}O_8Na$	743	743.5432	+0.8
2o	18	$C_{20}H_{39}O_2$	311	$C_{44}H_{78}O_8Na$	757	757	
2p	19	$C_{21}H_{41}O_2$	325	$C_{45}H_{80}O_8Na$	771	771	
2q	20	$C_{22}H_{43}O_2$	339	$C_{46}H_{82}O_8Na$	785	785	
2r	21	$C_{23}H_{45}O_2$	353	$C_{47}H_{84}O_8Na$	799	799	
2s	22	$C_{24}H_{47}O_2$	367	$C_{48}H_{86}O_8Na$	813	813	
2t	23	$C_{25}H_{49}O_2$	381	$C_{49}H_{88}O_8Na$	827	827	

which are powerful tools for the analysis of a homologous series of compounds. On occasion it proves difficult to achieve effective separation of metabolites by chromatographic methods, and in these instances we must rely on the separating power afforded by tandem mass spectrometric techniques. In this study we have shown the power of the precursor ion scan, in which all parent molecules giving rise to the same product ion are identified. The simplification this technique provides compared to analysing raw spectra is unparalleled. As was shown, the NMR data for this intractable mixture did not provide sufficient data to allow a full analysis of the structures of the homologous series, whereas this was facile using tandem mass spectrometric techniques.

4. Experimental

4.1. General experimental procedures

IR spectra were measured on an Ati Mattson Genesis Series FTIR machine. ^{1}H, ^{13}C and all 2D NMR experiments were recorded on a Varian Unity INOVA 400 MHz spectrometer, in CD_3OD or $CDCl_3$. Mass spectrometry/Mass spectrometry spectra were obtained from a Waters Micromass Quattro Premier XE instrument equipped with a MassLynx 4.0 software. LRESIMS were obtained from a Waters ZQ-4000 low-resolution single quadruple mass spectrometer with API capability; mass range *m/z* 4000; unit resolution. (*Waters-Micromass, Manchester, UK*). All accurate mass measurements were obtained from a Finnigan MAT 900 XLT high resolution double focussing mass spectrometer with tandem Ion Trap and EI, CI, LSIMS, API capability (*Thermo-Finnigan, Bremen, Germany*). HPLC separations were carried out using a Thomsons Scientific Silica-EL5-30784 Silica EXSIL 5 μm×25 cm×10 mm i.d. column and Spectraseries P100 isocratic pump and monitored using a Waters Associates, Inc. (Milford Mass) Differential Refractometer R401.

4.2. Biological material

The sample of *Acalycigorgia* sp. was collected by the Australian Institute of Marine Science in Thailand at 07 41.2 N; 98 46.4 E on 1 June 1990 at 15 m. Taxonomic identification was carried out by Phil Aderslade at the Museum of the Northern Territories and vouchers (COO6609) are kept at both the National History Museum in Washington DC, USA and at the Queensland Museum in Brisbane, Australia. Collected specimens were stored at −20 °C until used.

4.3. Extraction and isolation

The frozen sample was ground into a coarse powder and extracted with H_2O. The ground-up tissue was lyophilised and extracted using a 1:1 mixture of CH_2Cl_2/MeOH. The solvent was removed under reduced pressure leaving 1.47 g of organic extract, which was subjected to a solvent partition. The extract (1.47 g) was partitioned between water and CH_2Cl_2. The solvent was removed from the CH_2Cl_2 layer and the resulting oil was partitioned between *n*-hexane and 10% aqueous MeOH. The 10% aqueous MeOH layer was extracted three times with equal volumes of *n*-hexane. The solvent was removed from the *n*-hexane layer and the resultant extract (0.83 g) was subjected to normal phase flash chromatography with gradient elution. Gradient elution was achieved by first flushing the silica gel column with 100% hexane and collecting all the metabolites that eluted, this was then followed by a 50/50 v/v mixture of hexane/ethylacetate then a 100% solution of ethylacetate before finally flushing all the remaining material off the column with 100% methanol. The gradient elution protocol was designed in order to elute the least polar metabolites first. The resultant fractions listed in order of increasing polarity were FHFS1-1/7 (least polar metabolites=230 mg), FHFS2-8/20 (300 mg), FHFS3-21/48 (200 mg) and FHFS4-49/71 (most polar metabolites=100 mg). Many compounds were isolated from all four fractions but the compounds described herein were obtained from the FHFS1-1/7, which was subsequently subjected to size exclusion chromatography by Sephadex LH-20 gel using a 1:1 mixture of MeOH:CH_2Cl_2. Resulting fractions of interest were further purified by repeated normal phase HPLC using 85/15 and 90/10 v/v mixtures of hexane and ethylacetate as eluents. A total of 6.75 mg of compound **1** and 9.85 mg of compound **2a** was fortuitously collected after several HPLC runs.

4.4. Biological assay

Murine colon adenocarcinoma-38 (C-38) cells and the corresponding murine normal cells CFU (M) were inoculated on different Petri dishes. Circular-shaped filter disks (impregnated with test material at dosages from 50 to 100 μg/disk) were placed at the ends of different Petri dishes inoculated with the two cell types under investigation. The test materials (compounds **1** and **2a**) were solubilised in 100% DMSO before they were impregnated into the filter disks and allowed to dry overnight before use. The plates were incubated for 7–10 days and examined by an inverted stereomicroscope (10×) for the measurement of 'zones of inhibition'. Zones of inhibition were defined by measuring the distance in millimetre from the edge of the filter disk to the beginning of normal-sized colony formation. The assay is designed to determine large differences in the relative sensitivity of leukemias, solid tumours and normal cell for a given sample by comparison of the magnitude of inhibition zones. Generally, high values of inhibition zones are desirable. However, high values of inhibition zones for solid tumour cells were preferred over those for leukaemia cells (solid tumour selective). The diameter of the filter disk, 6.5 mm, is arbitrarily taken as 200 units therefore, 1 mm ≡ 30.8 units. A zone of less than 100 units taken as the extract was of insufficient activity to be of further interest. A difference in zones between solid tumour cells and either normal or leukaemia cells of 250 units defined solid tumour selective compounds.

4.4.1. Compound 1. Colourless oily substance, 6.75 mg; IR ν_{max} 3435 (b), 2930, 1728; NMR data (Table 1); LRESIMS *m/z* 505 $[M+Na]^+$; HRESIMS *m/z* 423.2741 $[M-CH_3COONa]^+$, Δ=+0.0 calculated for $[C_{24}H_{39}O_6]^+$.

4.4.2. Compound 2a. Oily substance, pale yellow when in solution, 9.85 mg; IR ν_{max} 3432 (b), 2925, 1728; NMR data (Table 1); LRESIMS *m/z* 561 $[M+Na]^+$; HRESIMS *m/z* 561.3398 $[M+Na]^+$, Δ=+1.0 calculated for $C_{30}H_{50}O_8Na$.

Acknowledgements

This work was supported by the NCI Open Repository Programme and the Australian Institute of Marine Science by providing the organic extract of *Acalycigorgia* sp. The EPSRC National Mass Spectrometry Centre, University of Wales Swansea, provided LRESIMS and all accurate mass measurements. Prof. Fred Valeriote of the Ford Cancer Center, Detroit, USA is thanked for biological assay data.

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(4*R*)-4-Hydroxy-1-nitroso-L-proline: synthesis, X-ray structure, ab initio and conformational calculations

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Abstract—4-Hydroxy-L-proline, an amino acid, an important component of collagen, was transformed into its *N*-nitroso-derivative, (4*R*)-4-hydroxy-1-nitroso-L-proline, **1** by butylnitrite in the acidic medium. The structure is a cyclic hydroxy-*N*-nitrosoacid with the carboxyl and hydroxyl groups trans to each other. The carboxyl group is in the *syn*-conformation. In the structure, the neutral molecules are connected via classical intermolecular O–H···O hydrogen bonds involving the hydroxyl and carboxyl groups [O···O=2.6251(14) Å], and form chains along the *a*-axis direction. The chains are linked into sheets via O–H···O hydrogen bond, [O···O=2.6813(15) Å] with participation of oxygen atom of nitroso group. Ab initio calculations based on density functional theory at the B3LYP/6-311++G(d, p) level of theory were performed to analyze the influence of 4-hydroxy-L-proline (Hyp) nitrosation on the conformation of the synthesized *N*-nitroso-compound. The geometry optimization of **1** and initial 4-hydroxy-L-proline was carried out in the gas phase and in solution using the polarizable continuum model. The single-point calculation was performed for the crystal structure of **1**. The most stable conformer of **1** is observed in an aqueous solution. In this state, the pyrrolidine ring adopts an envelope conformation, which is also maintained in the gas phase. The twisted conformation of the pyrrolidine ring is present in all states of Hyp and in the crystal structure of **1**. In **1** the interchange of five-membered ring conformation in solution and in the gas phase in comparison with the crystal is accompanied by an increase of the dipole moment of the molecule, which is maximal in solution.

1. Introduction

The functional specificity of proteins and polypeptides depends on the conformational behavior of the initial amino acids, which are extensively studied by theoretical and experimental methods.[1] L-Proline (Pro) and 4(*R*)-hydroxy-L-proline (Hyp) are abundant amino acids in collagen and exceptional among amino acids, as they are the only ones that have the amino group fixed within a pyrrolidine ring, making it rigid and directional in biological systems despite its conformational flexibility. Due to its crucial biological role (it is the most abundant protein in vertebrates) and very peculiar structure, collagen has been deeply investigated, together with several related polypeptides. Collagen is composed of approximately 300 repeats of the sequence $X_{aa}Y_{aa}Gly$, where X_{aa} and Y_{aa} are in most cases Pro and Hyp, respectively. The occurrence of Pro and Hyp in collagen restricts the orientational freedom of the chain in relation to the fiber axis and permits only left-handed helices.[2]

The importance of Pro and Hyp explains the extensive theoretical and structural studies of these amino acids and their derivatives including several di- and polypeptides. Aside from recent electron-density studies and quantum chemical investigations, the study of amino acid clusters in the gas phase has attracted considerable attention. The stereoelectronic effects crucial for the collagen stability have been studied, as well as quantum mechanical calculations for Pro, Hyp, and fluorinated dipeptide analogues in aqueous solution and for the Pro gaseous isomers have been fulfilled.[3] Moreover, in a recent paper, Conticello et al. discuss in detail the stereoelectronic effects in elastin-mimetic polypeptides due to Pro substitution.[4]

The structural studies of vitally important amino acids are fundamentally important. With respect to the metal free Pro and Hyp, so far the crystal structures of L-Pro and DL-Pro,[5,6]

Keywords: Oxyproline; Nitrosation; X-ray structure; Theoretical calculations.
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the monohydrates of L-Pro and DL-Pro[7,8] and numerous other solvates and salts of Pro have been determined. For Hyp and its derivatives, structural data are available for the zwitterion of 4-hydroxy-L-proline itself defined by X-ray analysis[9,10] and precision neutron diffraction,[11] 4-hydroxy-L-proline hydrate,[12] 4-hydroxy-*N*-methylproline,[13] and *N*,*N*′-dimethylated 4-hydroxy-D- and 4-hydroxy-L-proline chloride.[14] The acyclic peptides, ALB*-ALB-PRO* (three residues) and PRO*-PRO* were also described.[15,16]

As the only amino acids with the imino group, Pro and Hyp were extensively studied to elucidate the conditions responsible for the generation of carcinogenic *N*-nitroso-derivatives. A dose–response study on the endogenous formation of *N*-nitrosoproline was performed in rats, dogs, and in smokers and non-smokers. Solutions containing nitrite, proline, and in some cases ascorbic acid (ASC) and/or SCN^- were infused into the stomach and samples taken to determine gastric [nitrite], [NPro], [ASC], [SCN^-], and pH as functions of time. The ability of ASC to inhibit nitrosation (by reaction with nitrite) was shown to be highly dependent on initial [ASC] and on the rate of entry of O_2 into the stomach from blood. The rate of *N*-nitroso-Pro formation in the absence of ASC and SCN^-, the inhibitory effects on nitrosation of ASC, and the catalytic effects of SCN^- were all accurately predicted by the mathematical model.[17]

2. Results and discussion

In continuation of our previous studies devoted to the conditions of mutagenesis of the biologically important molecules to their carcinogenic *N*-nitroso-derivatives[18] and the stereochemistry of *N*-nitroso-compounds,[19] the nitrosation of 4-hydroxy-L-proline (**Hyp**) by butylnitrite[20] in the acidic medium has been fulfilled and the crystal structure of *N*-nitroso-derivative, 4-hydroxy-1-nitrosopyrrolidine-2-carboxylic acid **1**, was studied by X-ray crystallography (Fig. 1). The aim of this article is to study the product of **Hyp** nitrosation analyzing the structural peculiarities of *N*-nitrosooxyproline in the crystalline, liquid, and gaseous phases.

X-ray structural analysis was carried out at 100 K that permits the unambiguous localization of all the hydrogen atoms and to state that the molecule exists in the crystal in its neutral form. In the neutral molecule **1**, the hydroxyl and carboxyl groups are in the trans configuration relative to the ring, as found earlier for the zwitterions of L-Hyp,[9–11] 4-hydroxy-*N*-methylproline[13] and its hydrochloride salt[14] and differs from *cis*-zwitterion of Hyp in its dihydrate.[12] Molecular bond distances and bond angles as well as torsion angles, labeled according to the IUPAC conventions,[21] are given in Tables 1 and 2, respectively. The average C–C length of 1.530(2) Å is in agreement with 1.533(8) Å in Hyp dihydrate. The carboxylic group is in a *syn*-orientation and practically planar with the asymmetrical C(1)–O(2) 1.204(2) and C(1)–O(1) 1.328(2) Å distances which are consistent with the double and single C–O bonds. C(4)–O(3) length of 1.433(2) Å is consistent with 1.435(7) Å obtained by Shamala et al.[12] The *N*-nitroso-group has an ordinal geometry[19] with the equalized O(4)–N(2) 1.266(1) and N(1)–N(2) 1.289(2) Å distances and N–N–O bond angle O(4)–N(2)–N(1) 113.3(1)°. The dihedral angles formed by the carboxyl [O(1)/C(1)/O(2)] and nitroso [N(1)/N(2)/O(4)] groups with the pyrrolidine ring are equal 65.4(1)° and 11.4(1)°, respectively.

Two approaches could be applied to describe the puckering of the pyrrolidine ring. Generalization by Cremer and Pople[22] requires specification of an appropriate mean plane and uses the displacements of each atom from that plane to define puckering coordinates (q is a puckering amplitude and ψ is a phase angle). Geise et al.[23] use endocyclic torsion angles and thus avoid the need to define a mean plane. We use the advantages of both of these approaches and say that the pyrrolidine ring is in a twisted form with C^γ [C(4)] atom lying 0.608(2) Å [0.516 Å in Hyp[11]] below the plane through N, C^α, C^β, and C^δ, and with the latter four atoms coplanar to within 0.0367 Å [0.037(3) Å in Hyp]. The dihedral angle between the planes N–C^α–C^β–C^δ and C^α–C^β–C^γ–C^δ is 39.6(1)° [33.5° in Hyp]. The pyrrolidine ring conformation corresponds to the C^γ-*endo* or down puckering. The Cremer and Pople puckering ring parameters for **1** are gathered in Table 3 in comparison with those for Hyp and Pro derivatives. The closeness of the conformations for **1**, Hyp, and Pro monohydrates is evident.

To compare the puckering of the pyrrolidine ring in **1** and some derivatives of Hyp we use the data available in the Cambridge Structural Database.[24] Compound **1** and some structurally related molecules were fitted by the same atoms in the pyrrolidine ring skeleton. Figure 2 reveals the overlap

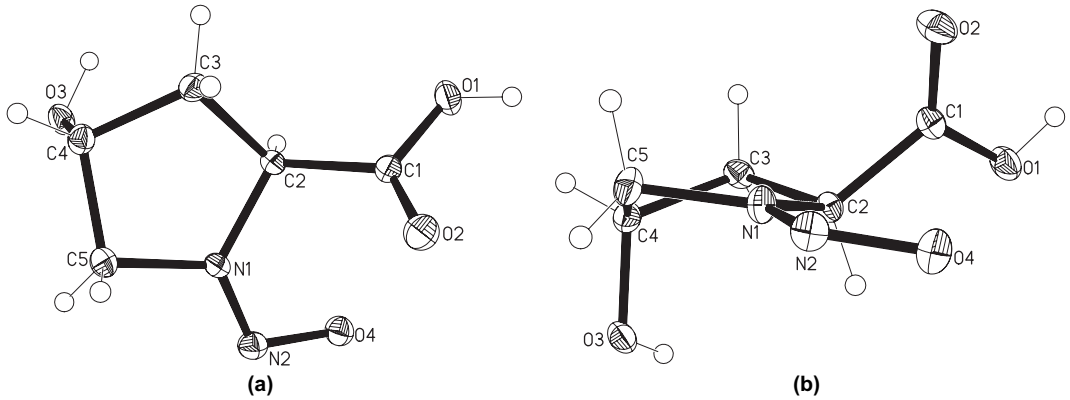

Figure 1. ORTEP (a) top and (b) side view of **1**. Thermal ellipsoids are drawn with the 50% probability level.

Table 1. Bond lengths (Å) and angles (degree) for **1** in crystal, in the gas phase (ε=1), and in solution (ε=78.4)

	Crystal	ε=1	ε=78.4
Bond distances			
O(1)–C(1)	1.328(2)	1.349	1.334
O(1)–H(1)	0.83(3)	0.97	0.99
O(2)–C(1)	1.204(2)	1.203	1.211
O(3)–C(4)	1.433(2)	1.426	1.427
O(3)–H(3O)	0.90(2)	0.96	0.98
O(4)–N(2)	1.266(1)	1.226	1.242
N(1)–N(2)	1.289(2)	1.319	1.299
N(1)–C(2)	1.473(2)	1.466	1.472
N(1)–C(5)	1.477(2)	1.465	1.472
C(1)–C(2)	1.529(2)	1.524	1.523
C(2)–C(3)	1.536(2)	1.547	1.548
C(2)–H(2)	0.97(2)	1.09	1.1
C(3)–C(4)	1.532(2)	1.541	1.541
C(3)–H(31)	0.98(2)	1.09	1.09
C(3)–H(32)	0.96(2)	1.09	1.09
C(4)–C(5)	1.522(2)	1.528	1.526
C(4)–H(4)	0.97(2)	1.1	1.1
C(5)–H(51)	0.91(2)	1.09	1.09
C(5)–H(52)	0.96(2)	1.09	1.09
Bond angles			
C(1)–O(1)–H(1)	109(2)	107	109
C(4)–O(3)–H(3O)	109(2)	109	109
N(2)–N(1)–C(2)	124.4(1)	123.4	124.2
N(2)–N(1)–C(5)	121.7(1)	121.6	121.6
C(2)–N(1)–C(5)	113.5(1)	114.2	113.6
O(4)–N(2)–N(1)	113.3(1)	114.4	115.2
O(2)–C(1)–O(1)	125.6(1)	123.9	124.5
O(2)–C(1)–C(2)	124.5(1)	124.8	124.5
O(1)–C(1)–C(2)	109.8(1)	111.2	111.0
N(1)–C(2)–C(1)	111.9(1)	111.6	111.9
N(1)–C(2)–C(3)	101.7(1)	102.2	102.4
C(1)–C(2)–C(3)	112.4(1)	112.0	112.4
N(1)–C(2)–H(2)	107(1)	109	109
C(1)–C(2)–H(2)	111(1)	109	109
C(3)–C(2)–H(2)	113(1)	113	112
C(4)–C(3)–C(2)	103.7(1)	104.5	104.4
C(4)–C(3)–H(31)	111(1)	109	109
C(2)–C(3)–H(31)	109(1)	113	110
C(4)–C(3)–H(32)	113(1)	110	112
C(2)–C(3)–H(32)	110(1)	113	112
H(31)–C(3)–H(32)	110(2)	108	109
O(3)–C(4)–C(5)	109.0(1)	107.1	107.5
O(3)–C(4)–C(3)	110.5(1)	112.4	112.1
C(5)–C(4)–C(3)	102.8(1)	103.1	102.9
O(3)–C(4)–H(4)	111(1)	111	111
C(5)–C(4)–H(4)	112(1)	112	112
C(3)–C(4)–H(4)	112(1)	113	111
N(1)–C(5)–C(4)	101.6(1)	102.5	102.4
N(1)–C(5)–H(51)	108(1)	111	110
C(4)–C(5)–H(51)	116(1)	110	113
N(1)–C(5)–H(52)	107(1)	112	110
C(4)–C(5)–H(52)	111(1)	111	111
H(51)–C(5)–H(52)	113(2)	110	110

of two molecules. It is evident that the best fitting was found for the zwitterion of Hyp, where the atoms of the pyrrolidine rings of the two molecules fit within 0.06 Å. Figure 2 also reveals an essential difference in an arrangement of the carboxylic and hydroxyl groups that is evident from the corresponding torsion angles. Torsion angles for Hyp in Table 2 are taken from Ref. 11.

A view of the molecular packing and hydrogen-bonding scheme is shown in Figure 3. The hydrogen-bonding geometry is summarized in Table 4. A classical hydrogen bond between the carboxyl hydrogen and the hydroxyl oxygen links the molecules into chains parallel to the [100] direction that can be described by the first level graph set, C(7).[26] Along the [010] direction the hydroxyl group acts as H-donor, being involved in an O–H···O hydrogen bond with the nitroso group oxygen via again C(7) graph set and the second level graph set, $R_4^4(23)$, thus generating the H-bonded sheet. Between the layers only one type of contact C(3)–H(31)···O(4) 3.152(2) Å deserves to be mentioned.

The rather close crystal packing is observed in Hyp itself,[9–11] where the molecules are also combined in the chains via the same C(7) O–H···$^-$OOC synthon, and the chains being further combined in the layer via NH_2^+(ammonium)···$^-$OOC(carboxyl) interactions analogous to those with nitroso group involvement in **1**.

In the analysis of the conformational and intermolecular characteristics of *N*-nitroso-compounds, it is of basic importance to consider environmental effects, so the ab initio calculations at the density functional theory (DFT) level have been thus fulfilled both in gas phase (ε=1) and in an aqueous solution (ε=78.4). To obtain a more definitive estimate of the conformational flexibility and relative stabilities of **1** in different states, geometry optimization was carried out in the solution using the polarizable continuum model (PCM) and in the gas phase. The single-point calculation was performed for the crystal structure. The computed SCF energies and energy differences for **1** in different states are summarized in Table 5.

The criteria of Cremer and Pople[22] have been used to analyze the non-planar character of the five-membered ring. Thus, a 'pure envelope' conformation with apex at 1 would be such that

$$z_2 = z_5,\ z_3 = z_4, \quad \psi = k \times 36, \tag{1}$$

and a 'pure twist' with axis through 1 would have

$$z_1 = z_2 + z_5 = z_3 + z_4, \quad \psi = k \times 36 + 18, \tag{2}$$

where the values of z_i are the displacements of *i*th atom perpendicular to the least-square plane.

However, it should be noted that for a ring with unequal lengths and angles, the conditions in Eq. 1 do not necessarily imply coplanarity of atoms 2–5. The list of z_i values for **1** in different states is summarized in Table 6.

The most stable conformer of **1** is observed in an aqueous solution. In this state according to the criteria of Cremer and Pople (Tables 3 and 6) the pyrrolidine ring adopts an envelope conformation, which is also maintained in the gas phase. The deviations of the carbon atom C(4) from the best-fit planes [N(1)/C(2)/C(3)/C(5)] in the solution and gas phase are equal to −0.57 and −0.56 Å, respectively. In the crystal structure of **1** the pyrrolidine ring adopts a conformation, which is twisted on C(3)–C(4) bond. Therefore for **1** we performed the conformational search (CS) implemented in Hyperchem 6.03 using the MM+ force field varying the dihedral angles inside the pyrrolidine ring. The envelope conformation of this ring is maintained in the lowest energy

Table 2. Torsion angles (degree) for **1** and L-Hyp[11]

IUPAC designation[21]	Atoms involved		**1**	L-Hyp[11]
ψ^1	O^1–C–C^α–N^1	O(1)–C(1)–C(2)–N(1)	151.2(1)	−3.2(2)
ψ^2	O^2–C–C^α–N	O(2)–C(1)–C(2)–N(1)	−31.6(2)	178.7(1)
χ^1	N^1–C^α–C^β–C^γ	N(1)–C(2)–C(3)–C(4)	−30.0(1)	−18.3(2)
$\chi^{2,1}$	C^α–C^β–C^γ–O^δ	C(2)–C(3)–C(4)–O(3)	−73.6(1)	−87.0(2)
$\chi^{2,2}$	C^α–C^β–C^γ–C^δ	C(2)–C(3)–C(4)–C(5)	41.4(1)	32.0(2)
$\chi^{3,1,1}$	$H^{\delta1}$–O^δ–C^γ–C^β	H(3O)–O(3)–C(4)–C(3)	−30.7(2)	−158.8(2)
$\chi^{3,1,2}$	$H^{\delta1}$–O^δ–C^γ–C^δ	H(3O)–O(3)–C(4)–C(5)	−142.4(2)	87.7(2)
$\chi^{3,2,1}$	O^δ–C^γ–C^δ–N	O(3)–C(4)–C(5)–N(1)	81.7(1)	84.0(1)
$\chi^{3,2,2}$	C^β–C^γ–C^δ–N^1	C(3)–C(4)–C(5)–N(1)	−35.2(1)	−33.5(2)
χ^4	C^γ–C^δ–N^1–C^α	C(4)–C(5)–N(1)–C(2)	17.3(1)	23.1(1)
χ^5	C^δ–N^1–C^α–C^β	C(5)–N(1)–C(2)–C(3)	8.0(1)	−3.1(1)
	O^4–N^2–N^1–C^α	O(4)–N(2)–N(1)–C(2)	4.6(2)	—
	O^4–N^2–N^1–C^δ	O(4)–N(2)–N(1)–C(5)	176.6(1)	—

Table 3. Computed ring puckering parameters for **1**, L-Hyp,[a] and Pro[b]

Compound	Crystal		Solution (ε=78.4)		Gas phase (ε=1)		Conformers **1a**, **1H**	
	q/Å	ψ/°	q/Å	ψ/°	q/Å	ψ/°	q/Å	ψ/°
1	0.403(1)	276.1(2)	0.373	0.363	279.3	281.9	0.330	280.2
L-Hyp	0.236	309.6	0.376	0.371	270.3	314.1	0.384	336.4
DL-Pro	0.403(2)	57.7(2)						
L-Pro	0.404	89.1						
DL-Pro monohydrate	0.4033(5)	308.63(7)						
L-Pro monohydrate	0.395(4)	309.9(6)						

[a] Atomic coordinates for the computing calculations are taken from Cambridge Structural Database (refcode HOPROL).
[b] All cited puckering parameters for Pro are taken from Ref. 6.

conformer **1a** according to the criteria of Cremer and Pople (Tables 3 and 6). In this molecule the deviation of the C^γ [C(4)] carbon atom from the best-fit plane [N(1)/C(2)/C(3)/C(5)] is equal to −0.51 Å and the values of O(2)–C(1)–C(2)–N(1) (−52.0°) and O(3)–N(2)–N(1)–C(2) (11.1°) torsion angles are maximal in comparison with those in other states. For the crystal, gas, and solvent states these values are equal to −31.6(2)°, 4.6(2)°; −41.18°, 6.55°; and −33.67°, 5.86°, respectively. The change of pyrrolidine ring conformation in solution and in gas phase in comparison with the crystal is accompanied by an increase in the dipole moment of the molecule, which is maximal in solution (Table 5).

In order to study the influence of nitrosation on the conformation of pyrrolidine ring, the DFT-based geometry optimizations as well as the CS implemented in Hyperchem 6.03 were carried out for hydroxy-L-proline (Hyp) in solution, gas, and crystal phases. The atomic numbering scheme for hydroxy-L-proline (Hyp) is the same as that of compound **1**. As in the case of **1**, the most stable conformer is observed in solution (Table 5). In the crystal and in solution, the pyrrolidine ring adopts the conformation twisted on the C(4)–C(5) bond. In the gas phase and in the conformer **1H** the pyrrolidine rings are twisted on C(3)–C(4) and N(1)–C(5) bonds, respectively. Thus, the twisted conformation of the pyrrolidine ring is present in all conformers of Hyp and in the crystal structure of **1**. However, due to nitrosation of Hyp, this ring in the crystalline state is twisted on the C(3)–C(4) bond, while in the same state of Hyp the ring is twisted along the C(4)–C(5) bond. In solution and in the gas phase after nitrosation the pyrrolidine ring in **1** adopts an envelope conformation. The optimized geometries of **1** and Hyp in solution and in gas phase as well as bond distances and bond angles in their crystal structures are listed in Table 1.

3. Conclusion

4-Hydroxy-L-proline, an amino acid, an important component of collagen, was transformed into its potentially carcinogenic *N*-nitroso-derivative, 4-hydroxy-1-nitroso-L-pyrrolidine-2-carboxylic acid by butylnitrite in the acidic medium. The X-ray structural analysis and ab initio calculations based on density functional theory at the B3LYP/6-311++G(d, p) level of theory were performed to analyze the influence of the *N*-nitroso-group on conformation of synthesized *N*-nitroso-compound. The most stable conformer of **1** was observed in an aqueous solution. In this state the pyrrolidine ring adopts an envelope conformation, which is maintained in the gas phase. In the crystal structure of **1** this ring adopts a twisted conformation. The change of the five-membered ring conformation in solution and gas phase in comparison with the crystal is accompanied by an increase of the dipole moment of the molecule, which is maximal in solution.

4. Experimental

4.1. General

The initial chemicals were used as received without further purification. IR spectra were recorded on a Specord-80 spectrophotometer as KBr disks. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker DPX-250 instrument (300 MHz ^{1}H; 75 MHz ^{13}C) in dimethylsulfoxide using TMS as an internal reference. Mass spectra were recorded on a MX-1321 device. Mass spectrometer operating at 70 eV. Perkin–Elmer 241 MC polarimeter was used for $[\alpha]_D^{20}$ measurements. Crystallographic measurements were carried out on a PX

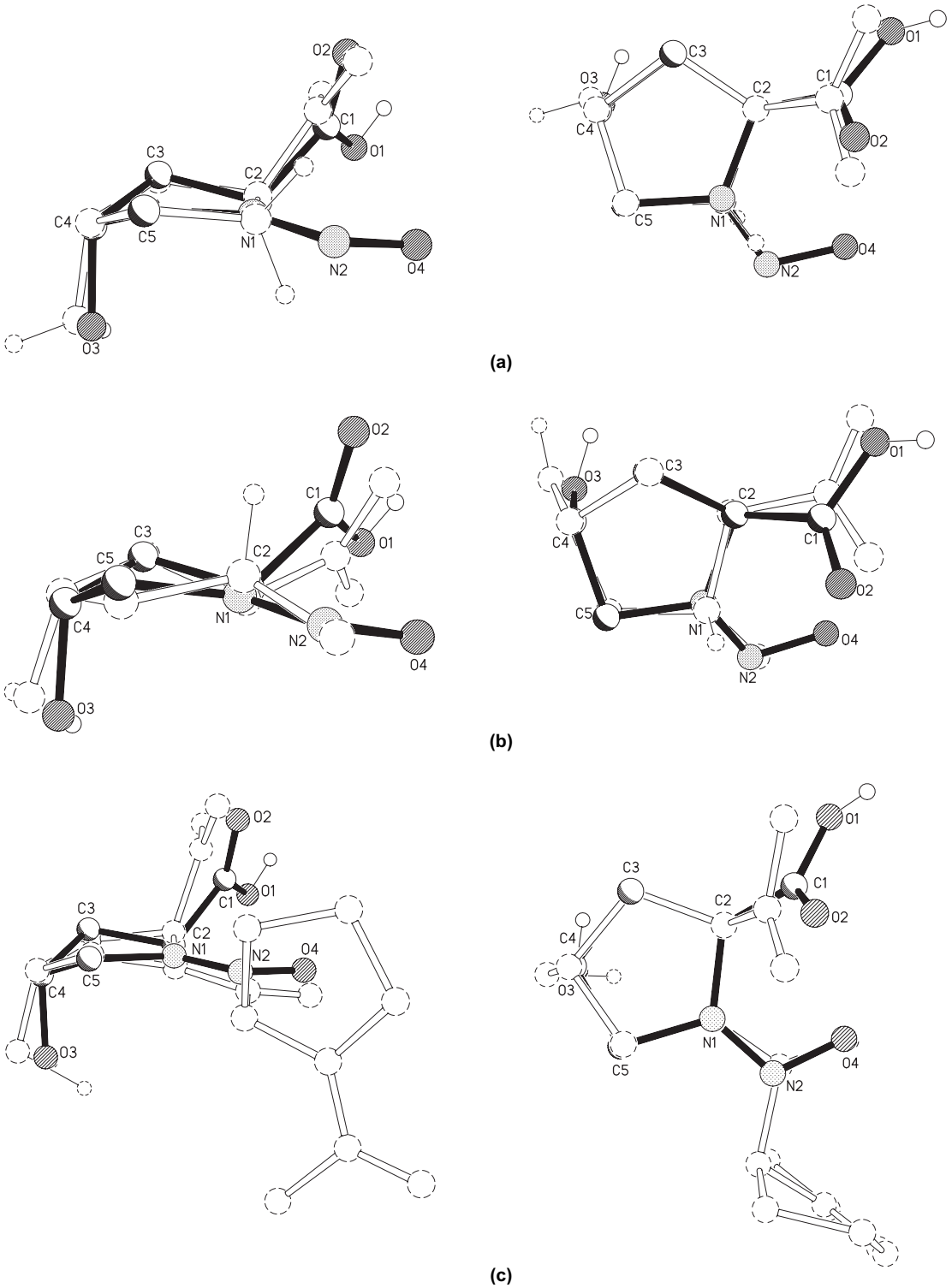

Figure 2. Fitting of the molecules **1** and Hyp derivatives (side and top views). Molecule of **1** is shown by solid lines. C-bound H-atoms are omitted for clarity. (a) **1** and 4-hydroxy-L-proline[11] (refcode HOPROL12 in CSD). Atoms of the pyrrolidine ring fit within 0.062 Å; (b) **1** and 4-hydroxy-*N*-methylproline[13] (refcode UGUHOT in CSD). Atoms of the pyrrolidine ring fit within 0.172 Å; and (c) **1** and *N*-acetyl-L-prolyl-l-4-hydroxyproline[25] (refcode GLHPRC in CSD). Atoms of the pyrrolidine ring fit within 0.168 Å.

kappa-geometry diffractometer with Onyx CCD camera at 100 K. The structure was solved by direct methods and refined by full-matrix least-squares on F^2 using SHELX-97 package.[27] All non-hydrogen atoms were refined anisotropically. Locations of all H-atoms were justified by difference Fourier synthesis and refined isotropically. The absolute configuration was not been defined.

Ab initio calculations were carried out using density functional theory with the Gaussian 03 package at the B3LYP/6-311++G(d, p) level of theory.[28] Polarizable continuum model (PCM) was included in the SCF procedure for the description of aqueous solution. The dielectric constant was set at 78.4. All calculations were carried out using the restricted spin formalism (closed-shell). Crystallographic

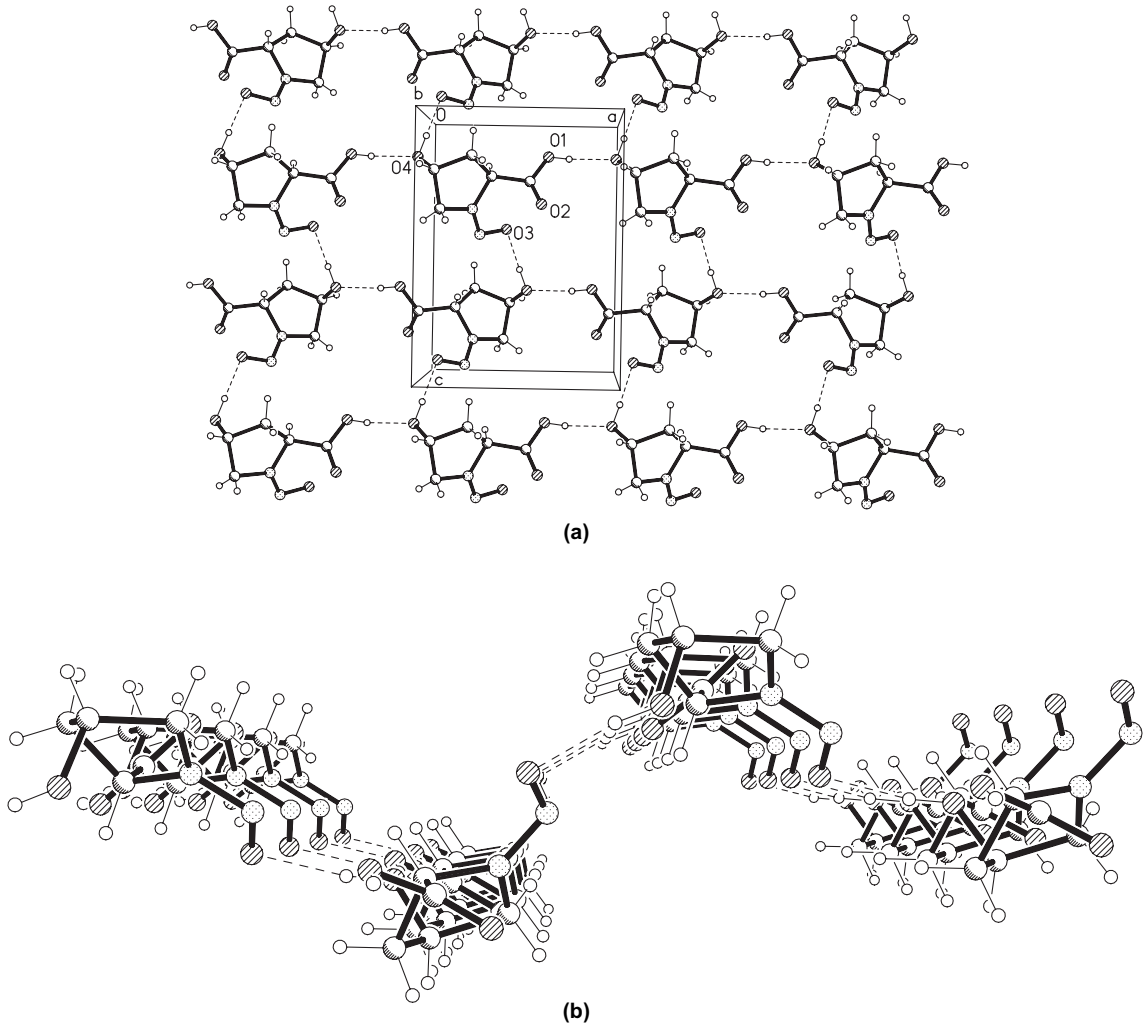

Figure 3. (a) Top and (b) side view of the layer of molecules **1**.

Table 4. Hydrogen-bonding geometry in **1**

D–H···A	*d*(H···A)/Å	*d*(D···A)/Å	∠DHA/°	Symmetry code for acceptor
O(1)–H(1)···O(3)	1.81(3)	2.625(1)	167(3)	*x*+1, *y*, *z*
O(3)–H(3O)···O(4)	1.79(2)	2.681(2)	171(2)	−*x*+1/2, −*y*+1, *z*−1/2
O(3)–H(3O)···N(2)	2.47(2)	3.254(2)	146(2)	−*x*+1/2, −*y*+1, *z*−1/2
C(3)–H(31)···O(4)	2.50(2)	3.152(2)	124(1)	−*x*+1, *y*+1/2, −*z*+1/2

data available for **1** and Hyp provided the initial geometries. The search of conformers was performed using the module conformational search implemented in Hyperchem 6.03[29] for finding low energy conformations of molecular systems by varying user-specified dihedral angles. The method involves random variation of dihedral angles to generate new structures and then energy minimizing of these angles using the MM+ force field. The dihedral angles in a ring are rotated by the 'torsion flexing' motion of Kolossvary and Guida,[30] which effectively leads to new ring conformations while avoiding large atomic displacements that can decrease the efficiency of optimization. The analysis of conformations has been carried out using the PLATON program package.[31]

Table 5. Computed SCF energies, energy differences, and dipole moments for **1** and Hyp compounds in the crystal, gas phase, and in solution and conformers **1a** and **1H**[a]

	1			Hyp		
	E/au	Δ*E*/au	*μ*/D	*E*/au	Δ*E*/au	*μ*/D
Crystal	−605.7354	0	6.3821	−476.3587	0	2.1041
Gas phase	−605.864	0.1286	6.0627	−476.5233	0.1646	1.87
Solution	−605.902	0.1666	9.1683	−476.5532	0.1945	2.4104
1a, **1H**	−605.7952	0.0598	6.0367	−476.4664	0.1077	5.7367

[a] Conformers found by the conformational search implemented in Hyperchem 6.03 for **1** (**1a**) and **Hyp** (**1H**).

Table 6. Displacements of pyrrolidine ring atoms from the least-square plane

	z_1	z_2	z_3	z_4	z_5
I					
Atoms of the pyrrolidine ring	N(1)	C(2)	C(3)	C(4)	C(5)
Crystal	0.027(1)	0.129(1)	−0.2324(14)	0.249(1)	−0.1707(14)
Gas phase	0.037	0.1032	−0.204	0.2269	−0.1631
Solution	0.0485	0.0963	−0.2044	0.2344	−0.1748
1a	0.0385	0.0894	−0.1831	0.2069	−0.1516
Hyp					
Crystal	0.0951	−0.0093	−0.0801	0.1389	−0.1446
Gas phase	0.0014	0.1369	−0.2229	0.2237	−0.1391
Solution	0.1657	−0.0337	−0.1112	0.2136	−0.2344
1H	0.223	−0.123	−0.0237	0.1613	−0.2374

4.1.1. 4-Hydroxy-1-nitroso-L-pyrrolidine-2-carboxylic acid, 1. 4-Hydroxy-L-proline (0.2 g) was stirred with the solution of butylnitrite (0.3 ml) and glacial acetic acid (0.2 ml) at 25 °C for 7 days. The colorless precipitate of 4-hydroxy-1-nitrosopyrrolidine-2-carboxylic acid was filtered off and recrystallized from a 1:1 mixture of *n*-butanol (5 ml) and methanol (5 ml). The yield of 4-hydroxy-1-nitrosopyrrolidine-2-carboxylic acid was 82%, mp=138–140 °C (dec). Anal. Calcd for $C_5H_8N_2O_4$: C, 37.50; H, 5.04; N, 17.49. Found: C, 37.47; H, 5.09; N, 17.53. IR (cm^{-1}, peaks of strong absorption only, Vaseline oil): 3500–3200, 3170, 3150, 3125, 3120, 3110, 3090, 3080, 3050, 3040, 3030, 3020, 3000–2800, 2780–2570, 1730, 1430, 1365, 1310, 1290, 1280, 1260, 1250, 1190, 1130, 1060. ^{1}H NMR δ (ppm) (DMSO-d_6, 300 MHz): 1.61 m (2H, CH_2), 2.78 m (2H, CH_2), 3.35 m (1H, CH). ^{13}C NMR δ (ppm) (DMSO-d_6, 300 MHz): 34.62 (C–CH_2–C), 54.15 (C–CH_2–N), 56.18 (C–CH–COOH), 67.54 (C–CH_2–OH), 172.94 (COOH). MS: *m/z* 160(22) (calcd for $C_5H_8N_2O_4$, 160.128) 130(5), 115(46), 88(20), 86(24), 85(16), 84(22), 68(15), 56(100); $[\alpha]_D^{20}$ −1.47.

Crystal data for (**1**), orthorhombic, $P2_12_12_1$, a=7.644(2), b=8.849(3), c=10.303(3) Å, V=696.9(4) Å^3, Z=4, D_x=1.526 g cm^{-3}, T=100 K, λ(Mo Kα)=0.71073 Å, μ=1.33 cm^{-1}, F(000)=336, GooF=1.097, R indices (all data) $R1$=0.0337, $wR2$=0.0836 for 1369 reflections and 132 parameters and R indices $R1$=0.0309, $wR2$=0.0821 for 1261 reflections obeying $I>2\sigma(I)$ criterion of observability.

Crystallographic data for the structural analysis of compound **1** have been deposited at the Cambridge Crystallographic Data Center, CCDC no. 602946. Copies of this information may be obtained free of charge from The Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44 1233 336 033; e-mail: deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk).

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2006.06.097.

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Condensed 2-pyrrolidinone-1,2-oxazines from lithium enolate of 1-benzyl-5-oxo-3-pyrrolidinecarboxylic acid and β-aryl, β-nitroenamines

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Abstract—The reaction of the lithium enolate of 1-benzyl-5-oxo-3-pyrrolidinecarboxylic acid **3** with a series of β-aryl, β-nitroenamines unexpectedly afforded 6-aryl-2-benzyl-4-oxa-1-oxo-3a-methoxycarbonyl-2,5-diazaindenes **9a–d**, whose structure was determined by analytical and NMR spectroscopical analysis. The structure of **9b** was further confirmed by X-ray analysis. A reasonable mechanism for their formation is given.

1. Introduction

The γ-lactam nucleus (2-pyrrolidinone) characterizes several compounds with biological and pharmaceutical activities.[1] Furthermore, 2-pyrrolidinones are also useful intermediates in organic synthesis.[2] In particular, polyfunctionalized γ-lactams have been synthesized to obtain γ-aminobutyric acid (GABA) analogues by hydrolysis under either acidic or basic conditions.[3] GABA[4] is a major inhibitory neurotransmitter in the mammalian central nervous system (CNS) and several psychiatric and neurological diseases are correlated with a disfunctioning of the GABA system, which acts in opposition to excitatory systems such as glutamate.[5] Many unnatural γ-amino acids have been designed for their promising therapeutic use in the treatment of these diseases, acting either as specific agonists at post-synaptic $GABA_A$ receptors[6] or as inhibitors of the GABA-uptake mechanism.[7] Within the frame of our research in the field of γ-lactams bearing a β-carboxylic group (such as **1**,[8] i.e., the aza analogue of paraconic acid **2**[9]), (Fig. 1) we have examined the reactivity of 1-benzyl-5-oxo-3-pyrrolidinecarboxylic acid (**3**)[8b] with a series of β-aryl, β-nitroenamines (**4a–e**),[10] under strongly basic conditions, with the aim of obtaining new α-functionalized aza paraconic acid derivatives.

As a matter of fact, β-nitroenamines[11] react under strongly basic conditions with ketones, esters, and lactones **5** containing active hydrogens at the α-position to give the corresponding 2-*aci*-nitroalkylidene derivatives **6** as a consequence of a conjugative addition–elimination reaction (Scheme 1).[12]

The fate of these intermediates depends on the structure of both the substrate and the nitroenamine as well as on the type of the final treatment. With ketones,[12a] linear esters,[12b] and lactones the corresponding 1,4-dicarbonyl compounds **7** were isolated (for R^3=Me), as a result of a Nef reaction,[12a,13] while by treatment with a suitable alkylating reagent nitronic

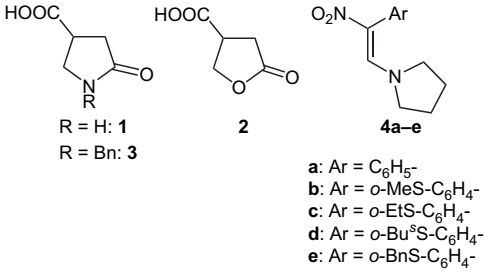

Figure 1. Reactants **3** and **4a–e**.

Keywords: γ-Lactams; β-Nitroenamines; Michael addition; Intramolecular redox process; Electrocyclic reaction; Condensed 1,2-oxazines.
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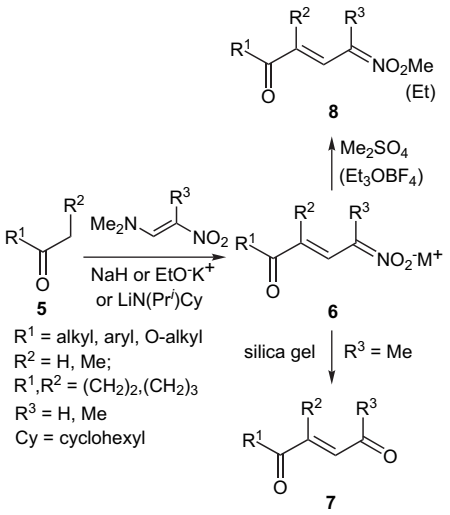
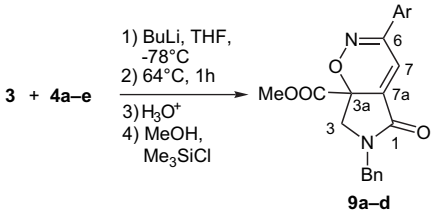


Scheme 1. General reactivity of carbonyl compounds and acidic derivatives with nitroenamines.

alkyl esters of the type **8** were the products, which could undergo further transformations.

2. Results and discussion

Lactamic acid **3** (twofold excess) in THF was treated with LDA (or *n*-butyllithium) (2.2–2.5 molar ratio with respect to **3**) under an inert atmosphere, at −78 °C. The β-nitroenamines **4a–e** were then added and the mixture was heated for 1 h. The crude reaction mixtures were acidified to pH 2 and subsequently esterified with methanol, in the presence of trimethylchlorosilane,[14] in order to separate esters **9a–d** from the methyl ester of substrate **3** (Scheme 2). No other products were detected.



Scheme 2. Formation of the 1,2-oxazine derivatives **9a–d**.

The structures of compounds **9a–d** were attributed on the basis of ^{1}H and ^{13}C NMR spectra, as well as by HRMS data, and definitively confirmed by a single crystal X-ray crystallographic analysis performed on 1,2-oxazine **9b** (Fig. 2).[15]

^{1}H and ^{13}C NMR spectra of compounds **9a–d** show very strict resemblances, in particular for protons and carbon atoms of the heterocyclic skeleton. The variations range within 0.3 ppm for protons and within less than 4 ppm for carbons (Table 1).

In the reaction of chiral racemic β-nitroenamine **4d**, the resulting product **9d** was about 1:1 mixture of two diastereomers, which could not be separated. Strangely enough, the β-nitroenamine **4e**, whose aromatic ring contains the *ortho*-benzylthio group, was found unreactive, probably due to steric reasons, allowing the complete recovery of the reagents.

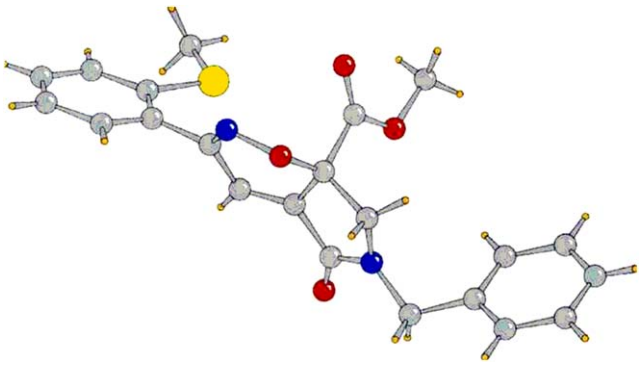


Figure 2. X-ray crystal structure of the 1,2-oxazine derivative **9b**.

Table 1. The main NMR spectroscopic data for compounds **9a–d**

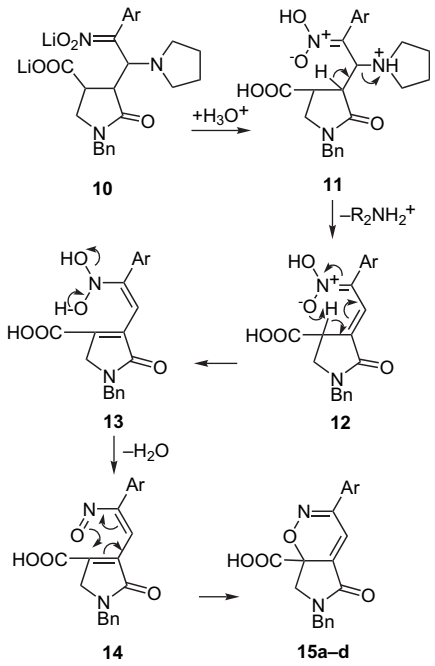
Compd	H-3	H-7	C-3	C-3a	C-6	C-7
9a	3.60, 3.80	7.07	46.8	74.4	155.0	112.0
9b	3.60, 3.82	6.83	46.6	74.4	156.4	115.1
9c	3.60, 3.81	6.81	46.5	74.2	156.7	115.2
9d (two diast.)	3.59, 3.81	6.81, 6.83	46.6	74.3	156.8, 156.9	115.62, 115.57

Compounds **9a–d** are unknown in the literature as yet. In fact, formations of 6*H*-1,2-oxazine derivatives are reported to proceed by a hetero Diels–Alder reaction of suitable alkenes with α-nitroso alkenes.[16,17] α-Nitro alkenes are known to give smoothly 1,2-oxazine-*N*-oxide derivatives in their reactions with enamines,[18] whereas silyl enol ethers, vinyl ethers, and isolated dienes react only in the presence of Lewis acid promoters.[19,20,21]

In order to gain information on the mechanism of the reaction observed, several attempts have been made to isolate at least one of the possible intermediates. To this purpose, the reaction work-up was performed with acids weaker than mineral acids and attempts to trap the expected nitronate intermediate of the type **6** (Scheme 1) with ethyl acrylate[22] and ethylidene malonate[22] were also made, but unsuccessfully. Anyway, on the basis of the expected reactivity of the reagents, the mechanism reported in Scheme 3 is proposed to account for the formation of these unusual bicyclic 1,2-oxazine derivatives **9a–d**.

As a matter of fact, we can assume that the lithium enolate of **3** adds to the β-nitroenamines **4** by the usual 1,4-conjugate Michael addition to give nitronate salt **10**. By acidification to pH 2, i.e., under the conditions of Nef reaction,[13] **10** gives **11**, which converts into **12** via the usual acid-catalyzed elimination of the secondary amine.

The *E*-configuration of the carbon–carbon double bond in **12** is necessary for the subsequent reaction steps. In fact, a fast cascade reaction can conceivably occur due to the simultaneous presence of the allylic proton and oxidizing nitronic acid function,[23] suitably positioned for an intramolecular



Scheme 3. Proposed mechanism of formation of the acidic forms **15a–d** of the 1,2-oxazine derivatives **9a–d**.

redox process. The resulting intermediate **13** would in turn lose a molecule of water from the $-N(OH)_2$ grouping, thus forming **14**. Finally, a 6π-thermal electrocyclization, involving the 1-nitroso-1,3-butadiene system[24,25] would afford the acids **15a–d**, isolated as their respective methyl esters **9a–d**. As to the intermediate **14**, very little is known about aliphatic nitrosodienes.[24] However, recent computational studies carried out on the cyclization pathways of 1-nitroso-1,3-butadiene[25] indicate that, although, 1,5-electrocyclization leading to pyrrole derivative is thermodynamically more favored than the 1,6-electrocyclization giving the 6*H*-1,2-oxazine, formation of this latter system is slightly faster than that of the former one.

3. Conclusions

The peculiar reactivity of the arylated β-nitroenamines **4** with the lithium enolate of the lactamic acid **3** leads to the heterocyclic derivatives **9**, which are to the best of our knowledge, the first examples of aza paraconic acid derivatives condensed with a six-membered heterocyclic ring. The key step in the reaction is likely to be the formation of the intermediate **12** of the proposed mechanism, from which the α-butenolides **13** and **14** are produced, thus allowing the heterodiene with an intramolecular inverse electron demand Diels–Alder reaction to occur.

4. Experimental

4.1. General

Melting points were measured using a Büchi 510 apparatus and were uncorrected. IR spectra were recorded for neat samples and for $CHCl_3$ solutions on a Jasco FTIR 200 spectrophotometer. ^1H and ^{13}C NMR spectra were run on either a 300 MHz Varian Inova 300 instrument or a Jeol EX-400 spectrometer (400 MHz for proton), using deuteriochloroform as the solvent and tetramethylsilane as the internal standard. Coupling constants are given in hertz. Mass spectra were recorded on a VG 7070 (70 eV) spectrometer. HRMS spectra were performed on a Finnigan MAT95XP spectrometer. TLC analyses were performed on Polygram® Sil G/UV_{254} silica gel pre-coated plastic sheets (eluant: dichloromethane–ethyl acetate). Flash chromatography was run on silica gel 230–400 mesh ASTM (Kieselgel 60, Merck). THF was distilled over benzophenone ketyl before each use.

Reagents: 1-benzyl-5-oxo-3-pyrrolidinecarboxylic acid **3** was prepared in accordance with the literature.[26] Analytical and spectroscopic data for (*S*)-(+)-**3** are given in Ref. 8b.

β-Nitroenamines **4a**[10a] and **4b**[10b] were synthesized according to the literature. Compounds **4c–e** were obtained following the synthetic procedure used for **4b**;[10c] the silver salt derived from the ring-opening of 3-nitrobenzo[*b*]thiophene[27] has been treated with a large excess of ethyl iodide, 2-iodobutane, and benzyl bromide, respectively.

4.1.1. (1*E*,3*Z*)-Ethylthio-2-nitro-1-pyrrolidino-1,3-butadiene (4c). Yield 42%; yellow solid; mp 112–113 °C; IR ($CHCl_3$) 1617, 1487, 1457, 1400, 1244 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.32 (1H, t, J=7.5 Hz), 1.82 (4H, br band), 2.71 (2H, br band), 2.93 (2H, q, J=7.5 Hz), 3.66 (2H, br band), 7.12–7.19 (1H, m), 7.24–7.30 (2H, m), 7.32–7.39 (1H, m), 8.67 (1H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.8, 24.3, 25.9, 26.2, 47.9, 55.0, 122.5, 125.8, 129.5, 130.9, 133.5, 140.8, 145.9; ESIMS, *m/z* 301.0 $[M+Na]^+$; MS (70 eV) *m/z* 278 (4), 232 (12), 216 (22), 203 (14), 163 (59), 148 (30), 135 (100), 108 (16), 99 (17); HRMS (EI) calcd for $C_{14}H_{18}N_2O_2S$ (M^+): 278.1084; found: 278.1086.

4.1.2. (1*E*,3*Z*)-*sec*-Butylthio-2-nitro-1-pyrrolidino-1,3-butadiene (4d). Yield 12%; yellow oil; IR (film) 1618, 1487, 1455, 1399, 1256, 1219 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.94–1.04 (m, 3H), 1.28 (3H, d, J=6.6 Hz), 1.46–1.73 (2H, m), 1.81 (4H, br band), 2.67 (2H, br band), 3.25 (1H, m), 3.66 (2H, br band), 7.14–7.40 (4H, m), 8.66 (1H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.3, 20.1, 20.4, 24.2, 25.8, 29.2, 29.3, 43.1, 48.0, 54.9, 122.7, 124.8, 124.9, 127.9, 128.3, 129.28, 129.32, 132.1, 132.3, 133.45, 133.52, 139.8, 140.1, 145.8; ESIMS *m/z* 328.9 $[M+Na]^+$; MS (70 eV) *m/z* 306 (1), 260 (10), 217 (21), 191 (11), 163 (5), 147 (8), 135 (100), 121 (6), 108 (5), 99 (7). HRMS (EI) calcd for $C_{16}H_{22}N_2O_2S$ (M^+): 306.1400; found: 306.1402.

4.1.3. (1*E*,3*Z*)-Benzylthio-2-nitro-1-pyrrolidino-1,3-butadiene (4e). Yield 27%; yellow solid; mp 128–129 °C; IR ($CHCl_3$) 1616, 1487, 1456, 1399, 1256, 1220 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.81 (4H, br band), 2.54 (2H, br band), 3.63 (2H, br band), 4.12 (2H, m), 7.14–7.38 (9H, m), 8.65 (1H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.0, 25.7, 37.0, 47.9, 54.9, 122.2, 124.8, 126.7, 127.0, 128.2, 128.7, 129.3, 131.2, 133.2, 136.8, 140.1, 145.6;

Found: C ESIMS *m/z* 363.0 [M+Na]$^+$; MS (70 eV) *m/z* 294 (11), 225 (22), 134 (14), 91 (100), 65 (13); HRMS (EI) calcd for $C_{19}H_{20}N_2O_2S$ (M^+): 340.1247; found: 340.1247.

4.2. Reactions between lactamic acid 3 and nitroenamines 4a–d

4.2.1. General procedure. A solution of lithium diisopropylamide (2.5 mmol) in THF (3.5 mL) [or a solution of *sec*-butyllithium (2.2 mmol) in cyclohexane] was slowly added to a solution of **3** (1 mmol) in THF (23 mL), at −78 °C, under argon. The reaction mixture was stirred at −78 °C for 3 or 4 h, then it was slowly added to a solution of the appropriate nitroenamine **4** (0.5 mmol) in THF (2 mL) at the same temperature, by means of a double-ended needle. The mixture was gradually warmed to room temperature and then heated to reflux for 1 h. After cooling to room temperature, the mixture was further stirred overnight. The reaction mixture was quenched by the addition of 3 M HCl. The aqueous phase was extracted three times with CH_2Cl_2. The combined organic layers were washed with saturated $NaHCO_3$, the aqueous phase was acidified to pH 2 with 3 M HCl solution, and extracted three times with CH_2Cl_2. The combined organic layers were dried on anhydrous Na_2SO_4 and the solvent was removed to give the crude product (200–220 mg). This was esterified with methanol in trimethylchlorosilane and purified by flash column chromatography (silica gel, eluent: ethyl acetate–dichloromethane, gradient from 0% up to 10%) to afford **9a–d** as white crystalline solids in yields varying from 26 to 47%.

4.2.1.1. 2-Benzyl-3a-methoxycarbonyl-4-oxa-1-oxo-6-phenyl-2,5-diazaindene (9a). Yield 47 mg (26%); mp 156–158 °C; IR ($CHCl_3$): 1730, 1695, 1525 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.60 (1H, d, J=11.0 Hz, H-3), 3.69 (3H, s, OC*H*$_3$), 3.80 (1H, d, J=11.0 Hz, H-3), 4.55 (1H, d, J=15.0 Hz, PhC*H*$_2$N), 4.77 (1H, d, J=15.0 Hz, PhC*H*$_2$N), 7.07 (1H, s, H-7), 7.26–7.48 (10H, m, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$) 46.8 (t, C-3), 53.3 (q, OCH_3), 54.3 (t, CH_2Ph), 74.6 (s, C-3a), 112.0 (d, C-7), 126.5 (d), 128.3 (d), 129.0 (d), 130.9 (s), 133.8 (s), 134.6 (s), 155.2 (s, C-6), 163.4 (s, O–C=O), 169.0 (s, N–C=O); MS (70 eV) *m/z* 362 ($M^{+\bullet}$, 7.5), 332 (M−NO, 22), 303 (M−$CH_3CO_2^{\bullet}$, 17), 200 (14), 156 (7.5), 91 (100); HRMS (EI) calcd for $C_{21}H_{18}N_2O_4$ (M^+): 362.1266; found: 362.1263.

4.2.1.2. 2-Benzyl-6-(2-methylthiophenyl)-3a-methoxycarbonyl-4-oxa-1-oxo-2,5-diazaindene (9b). Yield 92 mg (45%); mp 153–154 °C; IR ($CHCl_3$) 1728, 1697, 1524 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.43 (3H, s, SC*H*$_3$), 3.60 (1H, d, J=11.0 Hz, H-3), 3.73 (3H, s, OC*H*$_3$), 3.82 (1H, d, J=11.0 Hz, H-3), 4.55 (1H, d, J=14.6 Hz, PhC*H*$_2$N), 4.75 (1H, d, J=14.6 Hz, PhC*H*$_2$N), 6.83 (1H, s, H-7), 7.25–7.48 (9H, m, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.1 (q, SCH_3), 46.6 (t, C-3), 53.1 (q, OCH_3), 54.0 (t, CH_2Ph), 74.4 (s, C-3a), 115.1 (d, C-7), 125.7 (d), 127.7 (d), 128.2 (d), 128.3 (2d), 128.9 (2d), 129.4 (d), 130.7 (d), 131.0 (s), 132.8 (s), 134.8 (s), 138.0 (s), 156.4 (s, C-6), 163.3 (s, O–C=O), 169.1 (s, N–C=O); MS (70 eV) *m/z* 408 ($M^{+\bullet}$, 24), 393 (21), 379 (22), 378 (85), 349 (58), 348 (12), 274 (17), 246 (25), 91 (100), 65 (10); HRMS (EI) calcd for $C_{22}H_{20}N_2O_4S$ (M^+): 408.1144; found: 408.1146.

4.2.1.3. 2-Benzyl-6-(2-ethylthiophenyl)-3a-methoxycarbonyl-4-oxa-1-oxo-2,5-diazaindene (9c). Yield 100 mg (47%); mp 147–149 °C; IR ($CHCl_3$) 1730, 1695, 1525 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25 (3H, t, SCH_2CH_3), 2.84 (2H, dq, S*CH*$_2$CH$_3$), 3.60 (1H, d, J=10.6 Hz, H-3), 3.72 (3H, s, OC*H*$_3$), 3.81 (1H, d, J=10.6 Hz, H-3), 4.52 (1H, d, J=14.9 Hz, PhC*H*$_2$N), 4.77 (1H, d, J=14.9 Hz, PhC*H*$_2$N), 6.81 (1H, s, H-7), 7.26–7.50 (9H, m, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3 (q, SCH_2CH_3), 28.6 (t, S*CH*$_2$CH$_3$), 46.5 (t, C-3), 53.0 (q, OCH_3), 54.0 (t, CH_2Ph), 74.2 (s, C-3a), 115.2 (d, C-7), 126.5 (d), 128.0 (2d), 128.1 (2d), 128.8 (d), 129.5 (d), 130.3 (d), 130.4 (s), 130.4 (d), 134.4 (s), 134.8 (s), 135.8 (s), 156.7 (s, C-6), 163.2 (s, O–C=O), 169.0 (s, N–C=O); MS (70 eV) *m/z* 422 ($M^{+\bullet}$, 33), 390 (50), 392 (54), 361 (34), 363 (85), 335 (23), 332 (27), 274 (34), 246 (34), 91 (100), 65 (10); HRMS (EI) calcd for $C_{23}H_{22}N_2O_4S$ (M^+): 422.1300; found: 422.1300.

4.2.1.4. 2-Benzyl-6-(2-(2-methyl)propylthiophenyl)-3a-methoxycarbonyl-4-oxa-1-oxo-2,5-diazaindene (9d). Yield 59 mg (26%), two diastereomers; mp 128–132 °C; IR ($CHCl_3$) 1730, 1697, 1523 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.94 (3H, t, J=7.33 Hz, CH_2CH_3), 0.94 (3H, t, J=7.32 Hz, CH_2CH_3), 1.18 (3H, d, J=6.23 Hz, $CHCH_3$), 1.20 (3H, d, J=6.59 Hz, $CHCH_3$), 1.40–1.53 (2H, m, CH_3CH_2), 1.53–1.68 (2H, m, CH_3CH_2), 3.04–3.17 (1H, m, CH_3CH), 3.59 (1H, d, J=10.6 Hz, H-3), 3.72 (3H, s, OCH_3), 3.81 (0.5H, d, J=10.6 Hz, H-3), 3.80 (0.5H, d, J=11.0 Hz, H-3), 4.51 (0.5H, d, J=15.0 Hz, $PhCH_2N$), 4.52 (0.5H, d, J=15.0 Hz, $PhCH_2N$), 4.77 (1H, d, J=15.0 Hz, $PhCH_2N$), 6.81 (0.5H, s, H-7), 6.83 (0.5H, s, H-7), 7.20–7.51 (9H, m, 2Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.2, 11.3 (2q, CH_3CH), 20.3 (2q, CH_2CH_3), 29.4, 29.5 (2t, CH_2CH_3), 45.4, 45.8 (2d, CH_3CHCH_2), 46.6 (2t, C-3), 52.98, 53.00 (2q, OCH_3), 54.1 (2t, CH_2Ph), 74.3 (2s, C-3a), 115.57, 115.62 (2d, C-7), 127.1, 127.3 (2d), 128.1 (2d), 128.2 (4d), 128.9 (4d), 129.70, 129.75 (2d), 129.8, 129.9 (2s), 130.41, 130.43 (2d), 133.2, 132.7 (2d), 134.8 (2s), 135.0, 135.1 (2s), 135.8, 136.7 (2s), 156.8 (2s, C-6), 156.9 (2s, C-6), 163.4 (2s, O–C=O), 169.1 (2s, N–C=O); MS (70 eV) *m/z* 450 ($M^{+\bullet}$, 20), 420 (12), 391 (23), 393 (48), 395 (18), 361 (34), 334 (23), 335 (100), 275 (18), 245 (13), 246 (32), 249 (15), 215 (15), 200 (12), 91 (86); HRMS (EI) calcd for $C_{25}H_{26}N_2O_4S$ (M^+): 450.1613; found: 450.1611.

4.2.1.5. Crystal data for 9b. Data collected on a rotating anode (Cu Kα 1.54178 Å). Monoclinic, space group $P2_1/n$, a=8.161(2), b=27.296(4), c=9.950(3) Å, β=113.25(2)°, V=2036.4(8) Å^3, Z=4, ρ_{calcd}=1.332 Mg m^{-3}, θ_{max}=64.76°, temp=293(2) K, no. of measured and independent reflections: 21461/3318, no. of parameters=264, R_1=0.0492, wR_2=0.1422, max residual electron density: 0.214 e/Å^3.

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Synthesis of fluorinated indazoles through ANRORC-like rearrangement of 1,2,4-oxadiazoles with hydrazine

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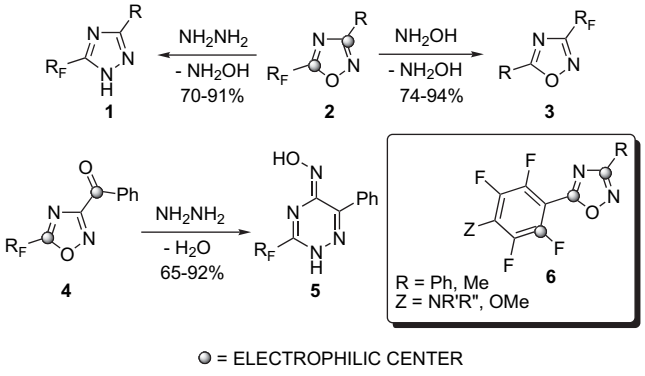
Abstract—A series of 6-substituted fluorinated indazoles has been obtained through an ANRORC-like rearrangement (Addition of Nucleophile, Ring-Opening and Ring-Closure) of 5-tetrafluorophenyl-1,2,4-oxadiazoles with hydrazine. The initial addition of the bidentate nucleophile to the electrophilic C(5) of the 1,2,4-oxadiazole ring, followed by ring opening and ring closure, leads to the formation of fluorinated indazoles in high yield under mild experimental conditions. Functionalization of the C(6) in the final indazole nucleus was preliminarily achieved through a nucleophilic aromatic substitution on the starting 5-pentafluorophenyl-1,2,4-oxadiazole.

1. Introduction

The synthesis of fluorinated heterocycles has generated much recent interest since these compounds have found useful applications in pharmaceuticals, agrochemicals and in materials science.[1,2] In general, the various approaches to achieve this goal include direct introduction of a fluorine or fluoroalkyl group, modification of functional groups, heterocyclization of open-chain fluorinated precursors and ring transformation of a suitable fluorinated heterocycle.[1,2]

For the latter approach, ANRORC-like rearrangements, which consist of the Addition of a Nucleophile followed by Ring-Opening and Ring-Closure steps, represent a very versatile strategy indeed.[3] This reaction pattern is well documented in the azine series,[3,4] but is quite rare in the case of five-membered heterocyclic derivatives.[3,5] In fact, only electron-poor azoles or systems bearing strongly electron-withdrawing groups present such reactivity.[3,5]

In this context, we have recently investigated the reactivity of 5-perfluoroalkyl-1,2,4-oxadiazoles **2** and **4** with bidentate nucleophiles such as hydrazine or hydroxylamine, and reported their ANRORC-like rearrangements into triazoles **1**,[6] 1,2,4-oxadiazole regioisomers **3**[7] or 1,2,4-triazinone oximes **5**[8] (Scheme 1).



Scheme 1.

In these reactions, 1,2,4-oxadiazole **2** reacted as a 1,3-dielectrophilic reagent; in fact, the presence of a strongly electron-withdrawing perfluorinated chain makes the C(5) (of the azole ring) a good electrophilic site, and allows the initial nucleophilic attack and ring-opening steps. The subsequent cyclization, involving the original C(3) of the azole nucleus, is driven by the formation of a more stable heterocyclic system.[6,7]

In 3-benzoyl-5-perfluoroalkyl-1,2,4-oxadiazoles **4**, the presence of a competing electrophilic centre in the side chain, partially changes the reactivity of the system, which is now identified as a 1,4-dielectrophile. The C(5) is still the preferred initial site of attack, while the final cyclization, involving the carbonyl linked at C(3) allowed the synthesis of fluorinated triazines **5** (Scheme 1).[8]

Keywords: 1,2,4-Oxadiazole; Indazole; Fluorinated heterocycles; ANRORC-like rearrangements.
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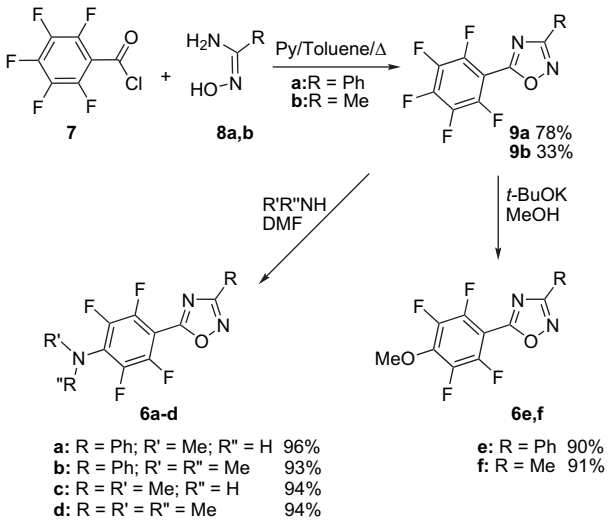
At this point, we became interested in verifying if the electrophilic behaviour of the azole C(5) could also be induced by fluorinated aryl substituents, and studied the reactivity of 5-tetrafluorophenyl-substituted 1,2,4-oxadiazoles **6** (Scheme 1) with hydrazine as a bidentate nucleophile.

2. Results and discussion

Pentafluorophenyl oxadiazoles **9** were prepared through the conventional amidoxime route, by reacting amidoximes **8a**,**b** and pentafluorobenzoyl chloride **7** in the presence of pyridine (Scheme 2).[9,10]

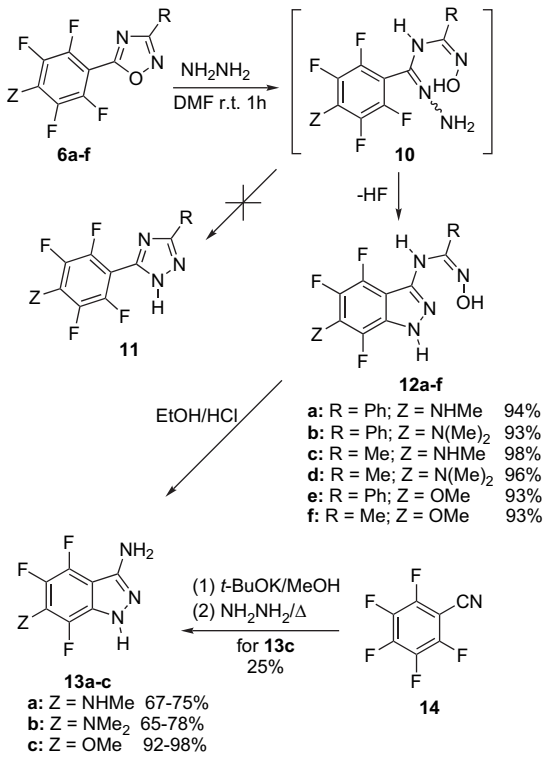


Scheme 2.

On the basis of previous results,[11] one can expect that the 4′-position of the pentafluorophenyl ring may easily undergo a nucleophilic aromatic substitution in the presence of a nucleophile, and this behaviour would interfere with the studied hydrazinolysis reaction.[12] Therefore we decided to preliminarily functionalize the fluorinated ring at its C(4) position by reaction with amines or methanol to obtain tetrafluorophenyl-1,2,4-oxadiazoles **6a–d** or **6e**,**f**, respectively (Scheme 2).

Surprisingly, the reaction of oxadiazoles **6a–f** with hydrazine in DMF at rt did not produce 1,2,4-triazoles **11**, which were expected as a result of an initial nucleophilic attack on the C(5) of the oxadiazole followed by a cyclization at the C(3) position.[6] The final cyclization step, instead, involves the side-chain electrophilic site located on the 2′-position of the fluorinated aromatic ring, and, by substitution of a fluorine atom through an intramolecular S_NAr, produced ring fluorinated 6-substituted *N*-indazolyl-amidoximes **12a–f** (Scheme 3) (yield range: 93–98%).

The structures of **12a–f** were confirmed by analytical and spectroscopic data (^{1}H NMR, ^{19}F NMR, IR and HRMS). Moreover, acidic hydrolysis of **12a–f** yielded the corresponding fluorinated 3-amino-indazoles **13**. For comparison, a sample of **13c** was also prepared, in a *one-pot* reaction, from pentafluorobenzonitrile **14** (Scheme 3).



Scheme 3.

The proposed mechanism considers the attack of the hydrazine onto the C(5) of the oxadiazole ring as the initial step, followed by the ring opening into **10** (the *Z*-configuration of the amidoxime was expected on the basis of preservation along the reaction process of the initial configuration present in the oxadiazole ring).[8] In fact, a possible alternative initial attack on the 2′-position of the fluorinated ring could be excluded since 2′-substitution was never observed in the presence of other nitrogen nucleophiles, under the same conditions, or with stronger nucleophiles such as methoxide anion.

On the other hand, once the open-chain intermediate **10** is obtained, the cyclization into the stable heteroaromatic system **12** constitutes the driving force for the displacement of a fluorine from the C(2) of the fluorinated ring, by nucleophilic attack of the β-nitrogen of the bidentate nucleophile.

It is our opinion that the observed selectivity, between the C(3) of the starting azole and the C(2) of the fluoroaryl ring, for the final cyclization steps is determined by the stability of the reaction products.[13,14]

The proposed mechanism is in agreement with classical methodology reported for the synthesis of indazoles, through the formation of a N(1)–C(7a) bond, by cyclization of *o*-halo-arylhydrazones with the elimination of halogenhydric acid.[15]

3. Conclusions

The title reaction, performed under mild conditions, resulted in excellent yields of the isolated products. This synthetic

protocol allowed the preparation of 6-substituted ring-fluorinated indazoles starting from easily accessible 1,2,4-oxadiazole precursors. This approach represents an interesting synthetic methodology for fluorinated indazoles, also considering that such compounds are present in many pharmaceutically important structures, with a broad range of activities, including antiinflammatory,[16] antitumor,[17] anti-HIV,[18] antimicrobial,[19] contraceptive[20] and as nNOS inhibitors.[21]

The reported results clearly show how ANRORC-like rearrangements of five-membered heterocycles can be considered as a versatile synthetic strategy for the synthesis of fluorinated heterocycles. Such an approach is not restricted to perfluoroalkylated substrates but can also be applied to polyfluoroarylated 1,2,4-oxadiazoles. Moreover, the leaving group ability of the fluorine atom in the C(2) position of the fluoroaryl ring allows the cyclization into benzofused systems.

4. Experimental

4.1. General methods and materials

Melting points were determined on a Reichart-Thermovar hot-stage apparatus and are uncorrected. IR spectra (Nujol) were determined with a Shimadzu FTIR-8300 instrument; ^{1}H NMR spectra were recorded on a BRUKER AC 250 E spectrometer with TMS as an internal standard; ^{19}F NMR spectra were recorded on a BRUKER AVANCE 300 spectrometer with $CFCl_3$ as an internal standard. GC–MS determinations were carried out on a VARIAN STAR 3400 CX/SATURN 2000 system. Flash chromatography was performed by using silica gel (Merck, 0.040–0.063 mm) and mixtures of ethyl acetate and light petroleum (fraction boiling in the range of 40–60 °C) in various ratios. Compound **9a** was prepared in 78% as previously reported.[9]

4.2. Synthesis of 3-methyl-5-pentafluorophenyl-1,2,4-oxadiazole 9b

A mixture of acetamidoxime **8b** (0.74 g; 10 mmol), pyridine (0.9 mL; 11 mmol) and pentafluorobenzoyl chloride **7** (2.54 g, 11 mmol) in anhydrous toluene (100 mL) was refluxed for 4 h. After removal of the solvent, the residue was treated with water (100 mL) and then extracted with EtOAc (3×100 mL). The combined organic layers were dried over Na_2SO_4 and evaporated. Chromatography of the residue gave 1,2,4-oxadiazole **9b** (0.82 g, 33%): mp 41–42 °C (white crystals from EtOAc); ^{1}H NMR ($CDCl_3$) δ 2.55 (s, 3H); MS *m/z* 250 (M^+, 100); IR (Nujol) 1655 cm^{-1}. Anal. Calcd for $C_9H_3F_5N_2O$: C, 43.22; H, 1.21; N, 11.20. Found: C, 43.30; H, 1.20; N, 11.40.

4.3. Reaction of 5-pentafluorophenyl-1,2,4-oxadiazoles 9a,b with amines in DMF. General procedure

To a mixture of 5-pentafluorophenyl-1,2,4-oxadiazole **9** (1 mmol) in dry DMF (2 mL), the appropriate amine (1.1 mmol) was slowly added. After stirring for 3 h at rt, the mixture was diluted with water (50 mL) and the formed precipitate filtered and re-crystallized from an appropriate solvent, giving **6a–d**.

4.3.1. 5-[2,3,5,6-Tetrafluoro-4-(*N*-methylamino)-phenyl]-3-phenyl-1,2,4-oxadiazole 6a. Yield: 0.31 g, 96%, mp 160–163 °C (white crystals from H_2O/EtOH); ^{1}H NMR (acetone-d_6) δ 3.18–3.26 (m, 3H), 6.26 (s, 1H, exch. D_2O), 7.53–7.64 (m, 3H), 8.10–8.17 (m, 2H); ^{19}F NMR (acetone-d_6) δ −144.34 (br d, 2F, J=15.3 Hz), −167.28 (br d, 2F, J=17.1 Hz); MS *m/z* 323 (M^+, 100); IR (Nujol) 3435, 3380, 1690 cm^{-1}. Anal. Calcd for $C_{15}H_9F_4N_3O$: C, 55.73; H, 2.81; N, 13.00. Found: C, 55.70; H, 2.90; N, 12.90.

4.3.2. 5-[2,3,5,6-Tetrafluoro-4-(*N*,*N*-dimethylamino)-phenyl]-3-phenyl-1,2,4-oxadiazole 6b. Yield: 0.31 g, 93%, mp 120–122 °C (white crystals from EtOAc); ^{1}H NMR ($CDCl_3$) δ 3.14 (t, 6H, J_{H-F}=2.7 Hz), 7.50–7.55 (m, 3H), 8.17–8.20 (m, 2H); ^{19}F NMR ($CDCl_3$) δ −138.71 (br d, 2F, J=18.0 Hz), −152.91 (br d, 2F, J=17.1 Hz); MS *m/z* 337 (M^+, 100); IR (Nujol) 1645 cm^{-1}. Anal. Calcd for $C_{16}H_{11}F_4N_3O$: C, 56.98; H, 3.29; N, 12.46. Found: C, 57.00; H, 3.30; N, 12.40.

4.3.3. 5-[2,3,5,6-Tetrafluoro-4-(*N*-methylamino)-phenyl]-3-methyl-1,2,4-oxadiazole 6c. Yield: 0.25 g, 94%, mp 182–184 °C (white crystals from H_2O/EtOH); ^{1}H NMR (DMSO-d_6) δ 2.47 (s, 3H), 3.11–3.14 (m, 3H); 7.06 (s, 1H, exch. D_2O); ^{19}F NMR (DMSO-d_6) δ −132.82 (br d, 2F, J=15.9 Hz), −154.65 (br d, 2F, J=15.9 Hz); MS *m/z* 261 (M^+, 100); IR (Nujol) 3321, 3163, 1653 cm^{-1}. Anal. Calcd for $C_{10}H_7F_4N_3O$: C, 45.99; H, 2.70; N, 16.09. Found: C, 46.10; H, 2.60; N, 16.00.

4.3.4. 5-[2,3,5,6-Tetrafluoro-4-(*N*,*N*-dimethylamino)-phenyl]-3-methyl-1,2,4-oxadiazole 6d. Yield: 0.26 g, 94%, mp 71–74 °C (white crystals from EtOAc); ^{1}H NMR ($CDCl_3$) δ 2.52 (s, 3H), 3.12 (t, 6H, J_{H-F}=2.7 Hz); ^{19}F NMR ($CDCl_3$) δ −139.42 (br d, 2F, J=18.9 Hz), −153.24 (br d, 2F, J=17.7 Hz); MS *m/z* 275 (M^+, 100); IR (Nujol) 1647 cm^{-1}. Anal. Calcd for $C_{11}H_9F_4N_3O$: C, 48.01; H, 3.30; N, 15.27. Found: C, 48.10; H, 3.30; N, 15.30.

4.4. Reaction of 5-pentafluorophenyl-1,2,4-oxadiazoles 9a,b with methanol. General procedure

Oxadiazole **9** (1 mmol) was dissolved in a solution of *t*-BuOK (1.2 mmol) in dry MeOH (20 mL). After stirring for 24 h at rt, the solvent was evaporated, the residue treated with water (100 mL), filtered and crystallized from an appropriate solvent, giving 5-(2,3,5,6-tetrafluoro-4-methoxyphenyl)-1,2,4-oxadiazoles **6e**,**f**.

4.4.1. 5-(2,3,5,6-Tetrafluoro-4-methoxy-phenyl)-3-phenyl-1,2,4-oxadiazole 6e. Yield: 0.29 g, 90%, mp 103–104 °C (white crystals from H_2O/EtOH); ^{1}H NMR ($CDCl_3$) δ 4.25 (t, 3H, J_{H-F}=2.1 Hz), 7.49–7.56 (m, 3H), 8.17–8.21 (m, 2H); ^{19}F NMR ($CDCl_3$) δ −137.32 (br d, 2F, J=19.5 Hz), −157.43 (br d, 2F, J=17.1 Hz); MS *m/z* 324 (M^+, 100); IR (Nujol) 1683 cm^{-1}. Anal. Calcd for $C_{15}H_8F_4N_2O_2$: C, 55.57; H, 2.49; N, 8.64. Found: C, 55.60; H, 2.50; N, 8.60.

4.4.2. 5-(2,3,5,6-Tetrafluoro-4-methoxy-phenyl)-3-methyl-1,2,4-oxadiazole 6f. Yield: 0.24 g, 91%, mp 42–43 °C (white crystals from H_2O/EtOH); ^{1}H NMR ($CDCl_3$)

δ 2.54 (s, 3H), 4.24 (t, 3H, J_{H-F}=2.0 Hz); ^{19}F NMR ($CDCl_3$) δ −137.63 (br d, 2F, J=19.2 Hz), −157.33 (br d, 2F, J=20.7 Hz); MS *m/z* 262 (M^+, 100); IR (Nujol) 1653 cm^{-1}. Anal. Calcd for $C_{10}H_6F_4N_2O_2$: C, 45.81; H, 2.31; N, 10.69. Found: C, 45.70; H, 2.30; N, 10.80.

4.5. Reaction of 5-tetrafluorophenyl-1,2,4-oxadiazoles 6a–f with hydrazine in DMF. General procedure

To a mixture of oxadiazoles **6a–f** (1 mmol) in dry DMF (2 mL), an excess of hydrazine monohydrate (0.25 g, 5 mmol) was slowly added. After stirring for 3 h at rt, the mixture was diluted with 1 M HCl (50 mL) and the formed precipitate filtered. Re-crystallization of the residue from H_2O/EtOH (1:1) gave the corresponding (*Z*)-*N*-(4,5,7-trifluoro-*1H*-indazol-3-yl)-*N′*-hydroxy-amidine **12**.

4.5.1. (*Z*)-*N*-[4,5,7-Trifluoro-6-(*N*-methylamino)-1*H*-indazol-3-yl]-*N′*-hydroxy-benzamidine 12a. Yield: 0.31 g, 94%, mp 190–193 °C (white crystals from H_2O/EtOH); ^{1}H NMR (DMSO-d_6) δ 3.01 (s, 3H), 5.66 (s, 1H, exch. D_2O), 7.28 (br s, 3H), 7.36 (br s, 2H), 8.22 (s, 1H, exch. D_2O), 10.49 (s, 1H, exch. D_2O), 12.64 (s, 1H, exch. D_2O); ^{19}F NMR (DMSO-d_6) δ −153.20 (t, 1F, J=19.8 Hz), −160.44 (d, 1F, J=17.1 Hz), −163.42 (d, 1F, J=21.0 Hz); IR (Nujol) 3404, 3336, 3134, 1670, 1632 cm^{-1}. HRMS calcd for $C_{15}H_{12}F_3N_5O$: 335.0994. Found: 335.0990.

4.5.2. (*Z*)-*N*-[4,5,7-Trifluoro-6-(*N,N*-dimethylamino)-1*H*-indazol-3-yl]-*N′*-hydroxy-benzamidine 12b. Yield: 0.32 g, 93%, mp 196–198 °C (white crystals from H_2O/EtOH); ^{1}H NMR (DMSO-d_6) δ 2.93 (s, 6H), 7.29 (br s, 3H), 7.37 (br s, 2H), 8.33 (s, 1H, exch. D_2O), 10.53 (s, 1H, exch. D_2O), 13.00 (s, 1H, exch. D_2O); ^{19}F NMR (DMSO-d_6) δ −147.68 (d, 1F, J=20.1 Hz), −151.66 (t, 1F, J=20.4 Hz), −156.73 (d, 1F, J=21.3 Hz); IR (Nujol) 3400, 3275, 1657 cm^{-1}. HRMS calcd for $C_{16}H_{14}F_3N_5O$: 349.1150. Found: 349.1143.

4.5.3. (*Z*)-*N*-[4,5,7-Trifluoro-6-(*N*-methylamino)-1*H*-indazol-3-yl]-*N′*-hydroxy-acetamidine 12c. Yield: 0.27 g, 98%, mp 200–202 °C (white crystals from H_2O/EtOH); ^{1}H NMR (DMSO-d_6) δ 2.00 (s, 3H), 3.05 (t, 3H, J=4.27 Hz), 5.74 (s, 1H, exch. D_2O), 7.93 (s, 1H, exch. D_2O), 9.76 (s, 1H, exch. D_2O), 12.77 (s, 1H, exch. D_2O); ^{19}F NMR (DMSO-d_6) δ −155.13 (t, 1F, J=20.7 Hz), −160.21 (d, 1F, J=19.2 Hz), −162.94 (d, 1F, J=13.5 Hz); IR (Nujol) 3386, 3269, 1676, 1647 cm^{-1}. HRMS calcd for $C_{10}H_{10}F_3N_5O$: 273.0837. Found: 273.0838.

4.5.4. (*Z*)-*N*-[4,5,7-Trifluoro-6-(*N,N*-dimethylamino)-1*H*-indazol-3-yl)-*N′*-hydroxy-acetamidine 12d. Yield: 0.27 g, 96%, mp 203–206 °C (white crystals from H_2O/EtOH); ^{1}H NMR (DMSO-d_6) δ 1.98 (s, 3H), 2.97 (s, 6H), 8.04 (s, 1H, exch. D_2O), 9.79 (s, 1H, exch. D_2O), 13.17 (s, 1H, exch. D_2O); ^{19}F NMR (DMSO-d_6) δ −147.46 (d, 1F, J=20.1 Hz), −153.73 (t, 1F, J=20.7 Hz), −156.41 (br s, 1F); IR (Nujol) 3384, 3265, 1675, 1647 cm^{-1}. HRMS calcd for $C_{11}H_{12}F_3N_5O$: 287.0994. Found: 287.0988.

4.5.5. (*Z*)-*N*-(4,5,7-Trifluoro-6-methoxy-1*H*-indazol-3-yl)-*N′*-hydroxy-benzamidine 12e. Yield: 0.31 g, 93%, mp 185–188 °C (white crystals from H_2O/EtOH); ^{1}H NMR (DMSO-d_6) δ 4.07 (s, 3H), 7.25–7.30 (m, 3H), 7.33–7.40 (m, 2H), 8.39 (s, 1H, exch. D_2O), 10.56 (s, 1H, exch. D_2O), 13.21 (s, 1H, exch. D_2O); ^{19}F NMR (DMSO-d_6) δ −143.01 (t, 1F, J=21.0 Hz), −146.79 (d, 1F, J=20.7 Hz), −155.92 (d, 1F, J=21.9 Hz); IR (Nujol) 3385, 3142, 3057, 1670, 1639 cm^{-1}; HRMS calcd for $C_{15}H_{11}F_3N_4O_2$: 336.0834. Found: 336.0835.

4.5.6. (*Z*)-*N*-(4,5,7-Trifluoro-6-methoxy-1*H*-indazol-3-yl)-*N′*-hydroxy-acetamidine 12f. Yield: 0.25 g, 93%, mp 208–211 °C (white crystals from H_2O/EtOH); ^{1}H NMR (DMSO-d_6) δ 1.96 (s, 3H), 4.07 (s, 3H), 8.08 (s, 1H, exch. D_2O), 9.80 (s, 1H, exch. D_2O), 13.37 (s, 1H, exch. D_2O); ^{19}F NMR (DMSO-d_6) δ −144.96 (t, 1F, J=18.6 Hz), −146.52 (d, 1F, J=18.3 Hz), −155.60 (br s, 1F); IR (Nujol) 3389, 3252, 1657 cm^{-1}. HRMS calcd for $C_{10}H_9F_3N_4O_2$: 274.0678. Found: 274.0671.

4.6. Hydrolysis of (*Z*)-*N*-(4,5,7-trifluoro-1*H*-indazol-3-yl)-*N′*-hydroxy-amidines 12a–f. General procedure

To a mixture of **12** (1.0 mmol) in ethanol (10 mL), concd hydrochloric acid (0.5 mL) was added. The solution was refluxed for 24 h. After removal of the solvent, the residue was treated with water, neutralized by the addition of solid $NaHCO_3$ and extracted with EtOAc. The organic layer was dried over Na_2SO_4 and evaporated. Crystallization of the residue from the appropriate solvent gave the corresponding 3-amino-4,5,7-trifluoro-1*H*-indazoles **13a–c**.

4.6.1. 3-Amino-4,5,7-trifluoro-6-(*N*-methylamino)-1*H*-indazole 13a. Yield: 0.15 g, 67% from **12a**; 0.16 g, 75% from **12c**, mp 178–80 °C (white crystals from H_2O/EtOH); ^{1}H NMR (DMSO-d_6) δ 3.01 (t, 3H, J_{H-F}=5 Hz), 5.22 (s, 2H, exch. D_2O), 5.53 (s, 1H, exch. D_2O), 11.74 (s, 1H, exch. D_2O); ^{19}F NMR (DMSO-d_6) δ −145.65 (t, 1F, J=18.9 Hz), −152.28 (d, 1F, J=19.2 Hz), −157.82 (d, 1F, J=20.7 Hz); MS *m/z* 216 (M^+, 100); IR (Nujol) 3471, 3404, 3389, 3333, 3152, 1680 cm^{-1}; Anal. Calcd for $C_8H_7F_3N_4$: C, 44.45; H, 3.26; N, 25.92. Found: C, 44.70; H, 3.20; N, 25.80.

4.6.2. 3-Amino-4,5,7-trifluoro-6-(*N,N*-dimethylamino)-1*H*-indazole 13b. Yield: 0.14 g, 65% from **12b**; 0.17 g, 78% from **12d**, mp 149–150 °C (white crystals from H_2O/EtOH); ^{1}H NMR (DMSO-d_6) δ 2.91 (s, 6H), 5.35 (s, 2H, exch. D_2O), 12.10 (s, 1H, exch. D_2O); ^{19}F NMR (DMSO-d_6) δ −140.11 (d, 1F, J=19.8 Hz), −144.61 (t, 1F, J=20.7 Hz), −151.40 (d, 1F, J=21.9 Hz); MS *m/z* 220 (M^+, 100); IR (Nujol) 3429, 3392, 3342, 3170, 3132, 1670 cm^{-1}; Anal. Calcd for $C_{10}H_9F_3N_4$: C, 46.96; H, 3.94; N, 24.34. Found: C, 47.10; H, 3.80; N, 24.50.

4.6.3. 3-Amino-4,5,7-trifluoro-6-methoxy-1*H*-indazole 13c. Yield: 0.20 g, 92% from **12e**; 0.21 g, 98% from **12f**, mp 209–212 °C (white crystals from H_2O/EtOH); ^{1}H NMR (DMSO-d_6) δ 4.05 (s, 3H), 5.42 (s, 2H, exch. D_2O), 12.31 (S, 1H, exch. D_2O); ^{19}F NMR (DMSO-d_6) δ −143.42 (t, 1F, J=19.2 Hz), −147.27 (d, 1F, J=18.3 Hz), −158.85 (d, 1F, J=21.6 Hz); MS *m/z* 217 (M^+, 100); IR (Nujol) 3429, 3392, 3338, 3136, 3082, 1672 cm^{-1}; Anal. Calcd for $C_8H_6F_3N_3O$: C, 44.25; H, 2.79; N, 19.35. Found: C, 44.30; H, 2.80; N, 19.50.

4.7. Synthesis of 3-amino-4,5,7-trifluoro-6-methoxy-1*H*-indazole 13c

Pentafluorobenzonitrile **14** (1.93 g, 10 mmol) was added to a solution of *t*-BuOK (1.23 g, 11 mmol) in methanol (20 mL). The solution was stirred at rt for 4 h and, after addition of hydrazine monohydrate (0.5 g, 20 mmol), refluxed for 12 h. The solvent was then evaporated under reduced pressure and the residue chromatographed to yield 3-amino-4,5,7-trifluoro-6-methoxy-1*H*-indazole **13c** (0.5 g, 23%).

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Synthesis of 2-amino-2,3-dihydrobenzofurans and fully substituted furans from modified Baylis–Hillman adducts

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Abstract—Syntheses of 2-amino-2,3-dihydrobenzofuran derivatives **3a–g** and fully substituted furans **5a–f** were achieved starting from the Baylis–Hillman adducts. We prepared 2-amino-2,3-dihydrobenzofurans from the Baylis–Hillman adducts of methyl and ethyl acrylates and fully substituted furans from the Baylis–Hillman adducts of alkyl vinyl ketones.

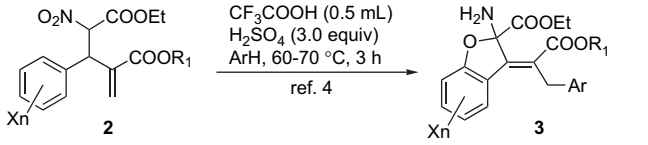
1. Introduction

The Baylis–Hillman reaction is a carbon–carbon bond-forming reaction between activated vinyls and electrophiles like aldehydes and imines with the aid of tertiary amine or phosphine.[1] The Baylis–Hillman adducts have versatile functionality and, as a result, the chemical transformations using the Baylis–Hillman adducts have been investigated extensively by us and other groups.[1–4]

Regioselective introduction of various nucleophiles either at the primary and secondary position of the Baylis–Hillman adducts can be carried out easily.[3] Recently, we introduced ethyl nitroacetate at the secondary position of Baylis–Hillman adduct of methyl and ethyl acrylates to prepare **2** (vide infra, Scheme 2).[3,4] We observed unusual formation of 2-amino-2,3-dihydrobenzofuran derivatives **3a–g** from **2** under the influence of H_2SO_4 and CF_3COOH (TFA) in arene solvent at elevated temperature as shown in Scheme 1.[4]

The structures of **3a** and **3e** were confirmed unequivocally by their X-ray crystal structures.[4] The mechanism for the formation of **3** was proposed as (i) protonation at the nitro group of **2**, (ii) intramolecular transfer of oxygen atom from nitrogen to carbon of benzene moiety, (iii) successive 1,3-H shift, (iv) intermolecular Friedel–Crafts type reaction with arene, and finally (v) formation of cyclic aminal derivative **3**.[4]

Cyclic α,α-disubstituted α-amino acids represent a unique class of sterically constrained amino acids, which have been used to modify the conformation and/or stability of a biologically active peptide.[5] In these respects, the synthesis of highly sterically constrained amino acids has been studied extensively.[5] However, there have been reported only a few examples of cyclic α-amino acid precursors having heteroatom-containing substituent as one of the α-substituents.[6]



3a: R_1 = Me, Xn = H, Ar = C_6H_5- (55%)
3b: R_1 = Me, Xn = H, Ar = 2,5-$Me_2C_6H_3$- (51%)
3c: R_1 = Me, Xn = H, Ar = 2,5-$(MeO)_2C_6H_3$- (38%)
3d: R_1 = Et, Xn = H, Ar = C_6H_5- (56%)
3e: R_1 = Et, Xn = H, Ar = 2,5-$Me_2C_6H_3$- (52%)
3f: R_1 = Me, Xn = *p*-Me, Ar = C_6H_5- (39%)
3g: R_1 = Me, Xn = *p*-Cl, Ar = C_6H_5- (36%)

Scheme 1.

2. Results and discussion

In these respects of the importance of cyclic α-amino acids and the unusual reaction mechanism for the formation of compounds **3a–g**, we examined the reaction with similar compounds, namely, ethyl 4-acetyl-2-nitro-3-phenylpent-4-enoic acid ethyl ester (**4a**) and 2-benzylidene-4-nitropentanedioic acid 5-ethyl ester 1-methyl ester (**6a**). We observed completely different products in these cases and wish to report herein the results.

The starting materials **2a–d**, **4a–d**, and **6a–c** were synthesized from the reaction of cinnamyl bromide derivative **1**, which was synthesized from Baylis–Hillman adduct and

Keywords: 2-Amino-2,3-dihydrobenzofurans; Baylis–Hillman adducts; Ethyl nitroacetate; Furans.
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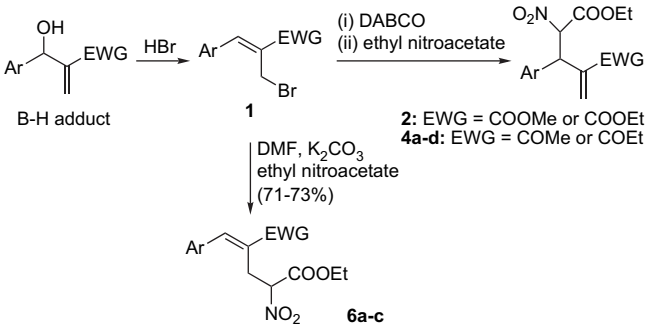
HBr, and ethyl nitroacetate as shown in Scheme 2 (see Section 3). With this compound **4a** (Ar=Ph, EWG=COMe) in our hands, we examined initially the reaction of **4a** in benzene under the influence of H_2SO_4 and CF_3COOH at 50–60 °C. We could not obtain the corresponding 2-amino-2,3-dihydrobenzofuran derivative. Instead, we could isolate fully substituted furan **5a** in 44% yield (Scheme 3 and entry 1 in Table 1).[7,8] The reaction of **4a** with *p*-xylene and mesitylene showed similar results (entries 2 and 3). In addition, the reaction of **4b–d** and benzene showed same pattern of reactivity (entries 4–6). In all cases, we could not obtain 2-amino-2,3-dihydrobenzofuran derivatives. The mechanism for the formation of **5a** could be postulated tentatively as shown in Scheme 3: (i) protonation at the nitro group, (ii) intramolecular attack of carbonyl group[2k,l] toward protonated nitro group to generate the allylic carbocation intermediate, (iii) intermolecular Friedel–Crafts reaction with benzene, and (iv) the final aromatization process by the elimination of *N*-hydroxy hydroxylamine species[9] gave the furan **5a**. As shown in the column of conditions in Table 1, we found that TFA was not critical in the reactions. However, somewhat elevated temperature was needed when we did not use TFA (entries 3–6) in order to obtain similar yields of products.



Scheme 2.

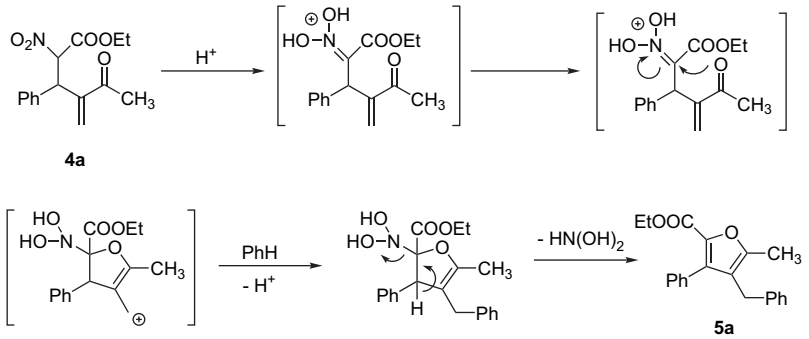
Table 1. Synthesis of tetrasubstituted furans

Entry	Substrate[a]	Conditions	Product (%)
1		Benzene, H_2SO_4 (3.0 equiv), TFA (0.5 mL), 50–60 °C, 2 h	**5a** (44)
2	**4a**	*p*-Xylene, H_2SO_4 (3.0 equiv), TFA (0.5 mL), 50–60 °C, 2 h	**5b** (45)
3		Mesitylene, H_2SO_4 (2.0 equiv), 70–80 °C, 4 h	**5c** (48)
4	**4b**	Benzene, H_2SO_4 (3.0 equiv), 70–80 °C, 5 h	**5d** (43)
5	**4c**	Benzene, H_2SO_4 (2.0 equiv), 70–80 °C, 4 h	**5e** (45)
6	**4d**	Benzene, H_2SO_4 (2.0 equiv), 70–80 °C, 4 h	**5f** (49)

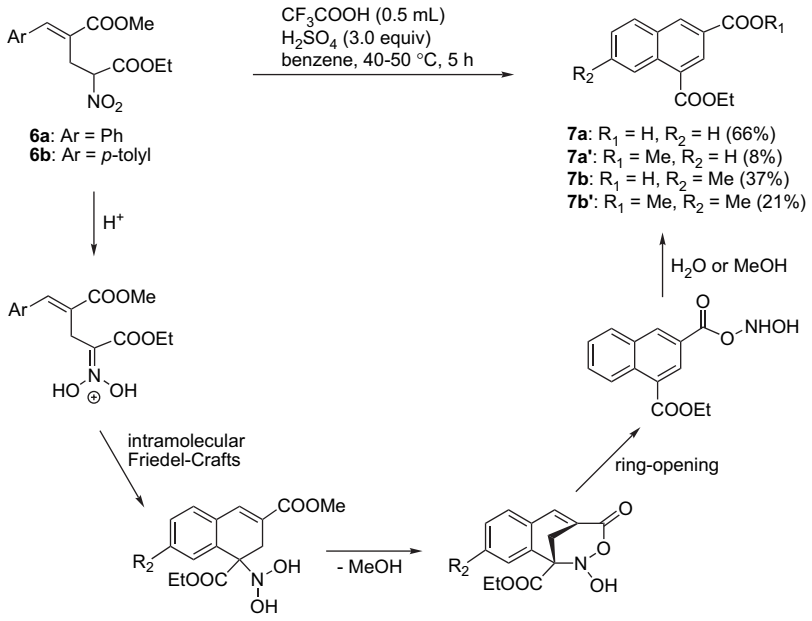
[a] Starting materials **4a–d** were obtained as a *syn*/*anti* mixtures (1:1 in all cases) and used without separation.

As a next trial, we examined the reaction of **6a** under the same reaction conditions. But, we obtained a mixture of **7a** (carboxylic acid form) and **7a′** (ester form) in 66% and 8%, respectively (Scheme 4). We were astonished by the formation of naphthalenic acid **7a** as the major product. The ester group at the 1-position of naphthalene ring was intact while that of the 3-position was hydrolyzed in part. The reaction with **6b** showed similar results although the ratio was different between the acid form **7b** (37%) and the ester form **7b′** (21%). Based on the experimental results, we could propose the reaction mechanism as in Scheme 4: (i) protonation at the nitro group, (ii) intramolecular Friedel–Crafts cyclization, (iii) formation of tricyclic oxazinone intermediate by loss of MeOH, (iv) ring-opening to naphthalene carboxylic acid derivative, (v) reaction with water or MeOH to produce **7a–b** and **7a′–b′** as a mixture. The reaction of **6c** and benzene under the similar conditions gave **7c** by following the similar mechanism (Scheme 5).

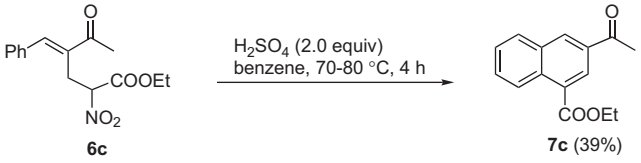
In summary, we disclosed the first synthesis of unusual 2-amino-2,3-dihydrobenzofurans[4] and fully substituted furans starting from the Baylis–Hillman adducts. Depending



Scheme 3.



Scheme 4.



Scheme 5.

upon the substituents on the modified Baylis–Hillman adducts, the major reaction pathway was changed to give different products although the yields were moderate. Further studies on the reaction mechanism and synthetic applications will be examined.

3. Experimental

3.1. General procedure

^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded in $CDCl_3$. The signal positions are reported in parts per million relative to TMS (δ scale) used as an internal standard. IR spectra are reported in cm^{-1}. Mass spectra were obtained from the Korea Basic Science Institute (Gwangju branch). Melting points are uncorrected. The elemental analyses were carried out at Korea Research Institute of Chemical Technology, Taejon, Korea. All reagents were purchased from commercial sources and used without further treatment. The separations were carried out by flash column chromatography over silica gel (230–400 mesh ASTM). Organic extracts were dried over anhydrous $MgSO_4$ and the solvents were evaporated on a rotary evaporator under water aspirator pressure.

3.2. Spectroscopic data of 2-amino-2,3-dihydrobenzofurans

The spectroscopic data of **3a** and **3e** were published in the previous paper.[4] The spectroscopic data of **3b–d**, **3f**, and **3g** are summarized as follows.

3.2.1. Compound 3b. Yield 51%; white solid, mp 177–178 °C; IR (film) 3410, 3336, 1751, 1720, 1200 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.28 (t, J=7.2 Hz, 3H), 2.21 (s, 3H), 2.34 (s, 3H), 2.87 (br s, 2H), 3.64 (s, 3H), 3.95 (d, J=16.8 Hz, 1H), 4.11 (d, J=16.8 Hz, 1H), 4.13–4.22 (m, 1H), 4.33–4.45 (m, 1H), 6.78–7.29 (m, 7H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 14.32, 19.47, 21.43, 33.85, 51.81, 62.14, 98.56, 110.99, 121.64, 124.23, 125.86, 126.46, 127.41 (2C), 130.35, 132.37, 133.53, 135.15, 135.68, 145.30, 161.26, 167.54, 169.54; ESIMS m/z 396 (M^++H). Anal. Calcd for $C_{23}H_{25}NO_5$: C, 69.86; H, 6.37; N, 3.54. Found: C, 69.97; H, 6.53; N, 3.45.

3.2.2. Compound 3c. Yield 38%; white solid, mp 126–127 °C; IR (film) 3417, 3332, 1747, 1720, 1281, 1219 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.26 (t, J=7.2 Hz, 3H), 2.26 (br s, 2H), 3.66 (s, 6H), 3.85 (s, 3H), 3.97 (d, J=17.1 Hz, 1H), 4.17 (d, J=17.1 Hz, 1H), 4.11–4.22 (m, 1H), 4.29–4.40 (m, 1H), 6.67–6.71 (m, 1H), 6.78–6.84 (m, 4H), 7.22–7.28 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 13.83, 30.28, 51.59, 55.50, 55.89, 62.03, 98.31, 110.54, 110.65, 110.71, 115.29, 121.38, 123.95, 125.81, 125.97, 126.74, 132.09, 145.38, 151.76, 153.75, 161.05, 167.36, 169.41; ESIMS m/z 428 (M^++H). Anal. Calcd for $C_{23}H_{25}NO_7$: C, 64.63; H, 5.90; N, 3.28. Found: C, 64.68; H, 6.02; N, 3.19.

3.2.3. Compound 3d. Yield 56%; white solid, mp 124–125 °C; IR (film) 3417, 3340, 1751, 1716 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.18 (t, J=7.2 Hz, 3H), 1.24 (t, J=7.2 Hz, 3H), 2.88 (br s, 2H), 4.00–4.41 (m, 6H), 6.81–6.87 (m, 2H), 7.16–7.30 (m, 6H), 7.39 (d, J=8.1 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.95, 14.15, 35.82, 61.20, 61.98, 98.39, 111.09, 121.58, 124.25, 125.82, 126.55, 126.65, 128.12, 128.80, 132.38, 137.37, 144.96, 161.29, 167.70, 168.93; ESIMS m/z 382 (M^++H). Anal. Calcd for $C_{22}H_{23}NO_5$: C, 69.28; H, 6.08; N, 3.67. Found: C, 69.35; H, 6.00; N, 3.63.

3.2.4. Compound 3f. Yield 39%; white solid, mp 128–129 °C; IR (KBr) 3421, 3332, 1751, 1716, 1203 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.24 (t, J=6.9 Hz, 3H), 2.19 (s, 3H), 2.70 (br s, 2H), 3.62 (s, 3H), 4.14 (d, J=16.2 Hz, 1H), 4.08–4.15 (m, 1H), 4.21 (d, J=16.2 Hz, 1H), 4.31–4.42 (m, 1H), 6.75 (d, J=8.4 Hz, 1H), 7.09 (dd, J=8.4 and 1.2 Hz, 1H), 7.19–7.31 (m, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 13.98, 21.02, 35.57, 51.54, 61.81, 98.29, 110.46, 123.81, 125.68, 125.93, 126.47, 127.95, 128.63, 130.61, 133.11, 137.26, 145.04, 159.22, 167.37, 169.37; ESIMS m/z 382 (M^++H). Anal. Calcd for $C_{22}H_{23}NO_5$: C, 69.28; H, 6.08; N, 3.67. Found: C, 69.22; H, 6.33; N, 3.55.

3.2.5. Compound 3g. Yield 36%; white solid, mp 139–141 °C; IR (KBr) 3421, 3336, 1751, 1720 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.24 (t, J=7.2 Hz, 3H), 2.84 (br s, 2H), 3.63 (s, 3H), 4.14 (s, 2H), 4.07–4.18 (m, 1H), 4.33–4.42 (m, 1H), 6.77 (d, J=8.1 Hz, 1H), 7.20–7.35 (m, 7H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 13.95, 35.60, 51.66, 62.01, 99.08, 111.77, 125.07, 125.38, 126.29, 126.73, 127.83, 128.01, 128.78, 131.92, 136.43, 143.07, 159.45, 166.83, 169.02; ESIMS m/z 402 (M^++H). Anal. Calcd for $C_{21}H_{20}ClNO_5$: C, 62.77; H, 5.02; N, 3.49. Found: C, 62.59; H, 5.28; N, 3.41.

3.3. Synthesis of starting materials 4a–d and 6a–c

Cinnamyl bromide derivatives **1** were prepared from the corresponding Baylis–Hillman adducts by the treatment with HBr as reported.[10] Synthesis of **4a–d** was carried out by using the DABCO salt concept as reported.[3,4] We obtained **4a–d** as *syn*/*anti* mixtures (almost 1:1). The *syn*/*anti* mixtures were used without separation. Synthesis of **6a–c** was carried out by the simple S_N2-type reaction of **1** with ethyl nitroacetate in the presence of K_2CO_3 in DMF as reported.[2b] The spectroscopic data of **6c** were published in the previous paper[2h] and the spectroscopic data of **2a–d**,[4] **4a–d** and **6a–c** are as follows.

3.3.1. Compound 2a (compound 3a in Ref. 4: X_n=H, R_1=Me in Scheme 1). Yield 75% (*syn*/*anti*=1:1); colorless oil; IR (film) 1751, 1724, 1562 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.97 (t, J=7.2 Hz, 3H), 1.27 (t, J=7.2 Hz, 3H), 3.70 (s, 3H), 3.72 (s, 3H), 3.93–4.04 (m, 2H), 4.25 (q, J=7.2 Hz, 2H), 4.89 (d, J=12.0 Hz, 1H), 4.95 (d, J=12.0 Hz, 1H), 5.81 (s, 1H), 5.86 (s, 1H), 5.87 (d, J=12.0 Hz, 1H), 6.06 (d, J=12.0 Hz, 1H), 6.34 (s, 1H), 6.37 (s, 1H), 7.21–7.34 (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.42, 13.73, 48.16, 48.55, 52.23 (2C), 62.93, 63.21, 89.56, 90.13, 125.46, 127.31, 127.89, 128.06, 128.20, 128.70, 128.80, 128.87, 134.78, 136.08, 138.33, 138.41, 162.98, 163.18, 165.58, 165.65; ESIMS m/z 308 (M^++H).

3.3.2. Compound 2b (compound 3b in Ref. 4: X_n=H, R_1=Et in Scheme 1). Yield 80% (*syn*/*anti*=1:1); colorless oil; IR (film) 1751, 1716, 1562 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.96 (t, J=7.2 Hz, 3H), 1.22 (t, J=7.2 Hz, 3H), 1.25 (t, J=7.2 Hz, 3H), 1.27 (t, J=7.2 Hz, 3H), 3.91–4.07 (m, 2H), 4.09–4.20 (m, 4H), 4.26 (q, J=7.2 Hz, 2H), 4.89 (d, J=12.0 Hz, 1H), 4.94 (d, J=12.0 Hz, 1H), 5.79 (s, 1H), 5.83 (s, 1H), 5.87 (d, J=12.0 Hz, 1H), 6.06 (d, J=12.0 Hz, 1H), 6.34 (s, 1H), 6.38 (s, 1H), 7.22–7.33 (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.42, 13.74, 13.93, 13.97, 48.16, 48.56, 61.28 (2C), 62.91, 63.18, 89.60, 90.18, 125.15, 127.02, 127.92, 128.01, 128.15, 128.74 (2C), 128.83, 134.89, 136.15, 138.59, 138.65, 163.04, 163.21, 165.11, 165.17; ESIMS m/z 322 (M^++H).

3.3.3. Compound 2c (compound 3c in Ref. 4: X_n=*p*-Me, R_1=Me in Scheme 1). Yield 84% (*syn*/*anti*=1:1); colorless oil; IR (film) 1751, 1724, 1562 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.99 (t, J=7.2 Hz, 3H), 1.26 (t, J=7.2 Hz, 3H), 2.29 (s, 3H), 2.30 (s, 3H), 3.70 (s, 3H), 3.72 (s, 3H), 3.96–4.04 (m, 2H), 4.25 (q, J=7.2 Hz, 2H), 4.85 (d, J=12.0 Hz, 1H), 4.91 (d, J=12.0 Hz, 1H), 5.79 (s, 1H), 5.83 (s, 1H), 5.85 (d, J=12.0 Hz, 1H), 6.03 (d, J=12.0 Hz, 1H), 6.32 (s, 1H), 6.35 (s, 1H), 7.08–7.21 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.43, 13.72, 20.99 (2C), 47.77, 48.20, 51.18 (2C), 62.89, 63.14, 89.64, 90.24, 125.20, 127.01, 127.74, 128.52, 129.47, 129.56, 131.69, 133.03, 137.83, 137.99, 138.49, 138.57, 162.98, 163.22, 165.63, 165.69; ESIMS m/z 322 (M^++H).

3.3.4. Compound 2d (compound 3d in Ref. 4: X_n=*p*-Cl, R_1=Me in Scheme 1). Yield 83% (*syn*/*anti*=1:1); colorless oil; IR (film) 1751, 1724, 1566 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.03 (t, J=7.2 Hz, 3H), 1.27 (t, J=7.2 Hz, 3H), 3.71 (s, 3H), 3.73 (s, 3H), 3.99–4.07 (m, 2H), 4.26 (q, J=7.2 Hz, 2H), 4.85 (d, J=12.0 Hz, 1H), 4.92 (d, J=12.0 Hz, 1H), 5.82 (s, 1H), 5.86 (s, 1H), 5.87 (d, J=12.0 Hz, 1H), 6.03 (d, J=12.0 Hz, 1H), 6.35 (s, 1H), 6.38 (s, 1H), 7.22–7.31 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.46, 13.70, 47.64, 48.10, 52.28 (2C), 63.11, 63.30, 89.20, 89.82, 125.95, 127.55, 128.96, 129.04, 129.32, 130.07, 133.44, 134.04, 134.18, 134.61, 137.93, 138.00, 162.75, 162.93, 165.39, 165.44; ESIMS m/z 342 (M^++H).

3.3.5. Compound 4a. Yield 62% (*syn*/*anti*=1:1); colorless oil; IR (film) 1751, 1682, 1562 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.97 (t, J=7.2 Hz, 3H), 1.25 (t, J=7.2 Hz, 3H), 2.28 (s, 3H), 2.29 (s, 3H), 3.94–4.05 (m, 2H), 4.22 (q, J=7.2 Hz, 2H), 4.95 (d, J=12.0 Hz, 1H), 5.01 (d, J=12.0 Hz, 1H), 5.89 (d, J=12.0 Hz, 1H), 5.99 (s, 1H), 6.06 (s, 1H), 6.07 (d, J=12.0 Hz, 1H), 6.19 (s, 1H), 6.21 (s, 1H), 7.19–7.34 (m, 10H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 13.41, 13.74, 26.10 (2C), 47.05, 47.46, 62.87, 63.10, 89.61, 90.24, 125.53, 127.60, 127.88 (2C), 128.00, 128.66, 128.74, 128.86, 135.22, 136.63, 146.36, 146.59, 163.08, 163.24, 197.58, 197.83; ESIMS m/z 292 (M^++H).

3.3.6. Compound 4b. Yield 71% (*syn*/*anti*=1:1); colorless oil; IR (film) 1751, 1682, 1562 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.97 (t, J=7.2 Hz, 3H), 1.01 (t, J=7.2 Hz, 3H), 1.03 (t, J=7.2 Hz, 3H), 1.25 (t, J=7.2 Hz, 3H), 2.64 (qd, J=7.2 and 1.2 Hz, 2H), 2.65 (q, J=7.2 Hz, 2H), 3.95–4.04 (m, 2H), 4.22 (qd, J=7.2 and 1.2 Hz, 2H), 4.94 (d, J=12.0 Hz, 1H), 5.00 (d, J=12.0 Hz, 1H), 5.89 (d, J=12.0 Hz, 1H), 5.94 (s, 1H), 6.01 (s, 1H), 6.08 (d, J=12.0 Hz, 1H), 6.17 (s, 1H), 6.19 (s, 1H), 7.22–7.30 (m, 10H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 7.94, 8.00, 13.43, 13.76, 31.21 (2C), 47.45, 47.87, 62.87, 63.07, 89.65, 90.30, 124.11, 126.12, 127.86, 127.90, 127.98, 128.66, 128.74, 128.86, 135.30, 136.66, 145.92, 146.17, 163.12, 163.27, 200.41, 200.63; ESIMS m/z 306 (M^++H).

3.3.7. Compound 4c. Yield 60% (*syn*/*anti*=1:1); colorless oil; IR (film) 1751, 1682, 1562 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.04 (t, J=7.2 Hz, 3H), 1.26 (t, J=7.2 Hz, 3H), 2.29 (s, 3H), 2.30 (s, 3H), 3.99–4.10 (m, 2H), 4.23 (qd, J=7.2 and 0.9 Hz, 2H), 4.89 (d, J=12.0 Hz, 1H), 4.96 (d, J=12.0 Hz, 1H), 5.87 (d, J=12.0 Hz, 1H), 5.99 (s, 1H), 6.04 (d, J=12.0 Hz, 1H), 6.06 (s, 1H), 6.19 (s, 1H), 6.21 (s, 1H), 7.18–7.29 (m, 8H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 13.54, 13.78, 26.14 (2C), 46.73, 47.22, 63.12, 63.24, 89.28, 89.95, 126.12, 127.83, 128.94, 129.08, 129.37, 130.07, 133.90, 133.92, 134.04, 135.12, 146.06, 146.31, 162.86, 163.03, 197.54, 197.78; ESIMS *m*/*z* 326 (M^++H).

3.3.8. Compound 4d. Yield 69% (*syn*/*anti*=1:1); colorless oil; IR (film) 1751, 1682, 1562 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.01 (t, J=7.2 Hz, 3H), 1.26 (t, J=7.2 Hz, 3H), 2.28 (s, 6H), 2.29 (s, 6H), 3.97–4.06 (m, 2H), 4.22 (qd, J=7.2 and 1.2 Hz, 2H), 4.90 (d, J=12.0 Hz, 1H), 4.97 (d, J=12.0 Hz, 1H), 5.86 (d, J=12.0 Hz, 1H), 5.97 (s, 1H), 6.03 (s, 1H), 6.04 (d, J=12.0 Hz, 1H), 6.17 (s, 1H), 6.18 (s, 1H), 7.07–7.19 (m, 8H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 13.48, 13.78, 20.99 (2C), 26.18 (2C), 46.74, 47.20, 62.88, 63.07, 89.71, 90.37, 125.34, 127.34, 127.77, 128.51, 129.45, 129.59, 132.16, 133.60, 137.70, 137.83, 146.56, 146.81, 163.09, 163.31, 197.64, 197.91; ESIMS *m*/*z* 306 (M^++H).

3.3.9. Compound 6a. Yield 71%; colorless oil; IR (film) 1753, 1709, 1566, 1371, 1265, 1219 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.21 (t, J=7.2 Hz, 3H), 3.41 (dd, J=14.4 and 6.6 Hz, 1H), 3.55 (dd, J=14.4 and 8.7 Hz, 1H), 3.84 (s, 3H), 4.14–4.22 (m, 2H), 5.59 (dd, J=8.7 and 6.6 Hz, 1H), 7.28–7.42 (m, 5H), 7.92 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 13.61, 28.15, 52.27, 62.94, 85.81, 125.19, 128.67, 128.73, 129.03, 134.14, 144.33, 163.96, 167.18; ESIMS *m*/*z* 308 (M^++H).

3.3.10. Compound 6b. Yield 71%; colorless oil; IR (film) 1753, 1707, 1562, 1373, 1263, 1219 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.22 (t, J=7.2 Hz, 3H), 2.37 (s, 3H), 3.42 (dd, J=14.7 and 6.6 Hz, 1H), 3.57 (dd, J=14.7 and 8.7 Hz, 1H), 3.83 (s, 3H), 4.13–4.24 (m, 2H), 5.58 (dd, J=8.7 and 6.6 Hz, 1H), 7.18–7.26 (m, 4H), 7.88 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 13.61, 21.19, 28.19, 52.19, 62.90, 85.82, 124.24, 128.89, 129.40, 131.22, 139.38, 144.30, 164.02, 167.34; ESIMS *m*/*z* 322 (M^++H).

3.4. Synthesis of furan derivatives 5a–f

A mixture of **4a** (291 mg, 1.0 mmol), H_2SO_4 (295 mg, 3.0 mmol), and TFA (0.5 mL) in benzene (3 mL) was heated to 50–60 °C for 2 h. After cooling to room temperature, the reaction mixture was poured into cold water and extracted with ether. After removal of the solvent and column chromatographic purification process (hexanes/ether, 98:2) we obtained **5a** as a colorless oil, 141 mg (44%). The other compounds were synthesized analogously and the spectroscopic data of **5a–f** are as follows.

3.4.1. Compound 5a. Yield 44%; colorless oil; IR (film) 1713, 1315, 1176 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.15 (t, J=7.2 Hz, 3H), 2.31 (s, 3H), 3.64 (s, 2H), 4.19 (q, J=7.2 Hz, 2H), 6.91–6.95 (m, 2H), 7.11–7.33 (m, 8H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 12.51, 14.02, 29.02, 60.35, 120.98, 125.99, 127.68, 127.70, 128.00, 128.31, 129.61, 132.00, 136.17, 137.88, 139.73, 153.02, 159.08; ESIMS *m*/*z* 321 (M^++H). Anal. Calcd for $C_{21}H_{20}O_3$: C, 78.73; H, 6.29. Found: C, 78.61; H, 6.37.

3.4.2. Compound 5b. Yield 45%; colorless oil; IR (film) 1716, 1308, 1176 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.16 (t, J=7.2 Hz, 3H), 2.07 (s, 3H), 2.20 (s, 3H), 2.23 (s, 3H), 3.52 (s, 2H), 4.20 (q, J=7.2 Hz, 2H), 6.93 (s, 1H), 6.87–6.98 (m, 2H), 7.13–7.17 (m, 2H), 7.25–7.30 (m, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 12.52, 14.04, 19.05, 21.03, 26.69, 60.40, 120.30, 126.72, 127.64, 127.66, 128.79, 129.50, 129.81, 132.02, 132.78, 135.27, 136.47, 137.22, 137.87, 153.14, 159.16; ESIMS *m*/*z* 349 (M^++H). Anal. Calcd for $C_{23}H_{24}O_3$: C, 79.28; H, 6.94. Found: C, 79.44; H, 6.91.

3.4.3. Compound 5c. Yield 48%; colorless oil; IR (film) 1716, 1261, 1180 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.12 (t, J=7.2 Hz, 3H), 1.73 (s, 3H), 2.10 (s, 6H), 2.24 (s, 3H), 3.53 (s, 2H), 4.17 (q, J=7.2 Hz, 2H), 6.78 (s, 2H), 7.24–7.42 (m, 5H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 12.06, 14.00, 20.06, 20.81, 24.69, 60.28, 119.34, 127.64, 127.79, 128.78, 129.52, 132.20, 132.38, 135.73, 136.44, 136.69, 137.46, 151.86, 159.10; ESIMS *m*/*z* 363 (M^++H). Anal. Calcd for $C_{24}H_{26}O_3$: C, 79.53; H, 7.23. Found: C, 79.78; H, 7.49.

3.4.4. Compound 5d. Yield 43%; colorless oil; IR (film) 1713, 1315, 1176 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.13 (t, J=7.2 Hz, 3H), 1.22 (t, J=7.5 Hz, 3H), 2.66 (q, J=7.5 Hz, 2H), 3.65 (s, 2H), 4.18 (q, J=7.2 Hz, 2H), 6.93–6.95 (m, 2H), 7.10–7.33 (m, 8H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 12.45, 14.03, 20.25, 28.89, 60.32, 120.10, 125.97, 127.62, 127.70, 128.02, 128.28, 129.63, 132.15, 136.10, 137.95, 140.00, 157.88, 159.18; ESIMS *m*/*z* 335 (M^++H). Anal. Calcd for $C_{22}H_{22}O_3$: C, 79.02; H, 6.63. Found: C, 79.11; H, 6.77.

3.4.5. Compound 5e. Yield 45%; colorless oil; IR (film) 1713, 1319, 1176 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.18 (t, J=7.2 Hz, 3H), 2.23 (s, 3H), 3.62 (s, 2H), 4.21 (q, J=7.2 Hz, 2H), 6.91–6.94 (m, 2H), 7.06–7.30 (m, 7H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 12.48, 14.10, 29.01, 60.52, 120.83, 126.16, 127.95, 127.99, 128.42, 130.49, 131.02, 133.77, 134.93, 138.00, 139.48, 153.21, 158.92; ESIMS *m*/*z* 355 (M^++H). Anal. Calcd for $C_{21}H_{19}ClO_3$: C, 71.08; H, 5.40. Found: C, 70.94; H, 5.53.

3.4.6. Compound 5f. Yield 49%; colorless oil; IR (film) 1713, 1315, 1176 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.19 (t, J=7.2 Hz, 3H), 2.29 (s, 3H), 2.35 (s, 3H), 3.64 (s, 2H), 4.21 (q, J=7.2 Hz, 2H), 6.94–6.97 (m, 2H), 7.06–7.25 (m, 7H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 12.53, 14.12, 21.26, 28.98, 60.32, 120.95, 125.97, 127.99, 128.32, 128.46, 128.84, 129.51, 136.35, 137.41, 137.79, 139.83, 152.98, 159.08; ESIMS *m*/*z* 335 (M^++H). Anal. Calcd for $C_{22}H_{22}O_3$: C, 79.02; H, 6.63. Found: C, 79.10; H, 6.87.

3.5. Synthesis of naphthalene derivatives

A mixture of **6a** (307 mg, 1.0 mmol), H_2SO_4 (295 mg, 3.0 mmol), and TFA (0.5 mL) in benzene (3 mL) was heated to 40–50 °C for 5 h. After cooling to room temperature, the reaction mixture was poured into cold water and extracted

with ether. After removal of the solvent and column chromatographic purification process (hexanes/ether, 10:1 for **7a′** and hexanes/EA, 4:1 for **7a**) we obtained **7a** (162 mg, 66%) and **7a′** (21 mg, 8%). The other compounds were synthesized analogously and the spectroscopic data of **7a–c**, **7a′**, and **7b′** are as follows.

3.5.1. Compound 7a. Yield 66%; white solid, mp 153–154 °C; IR (KBr) 3412, 1715, 1690 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.50 (t, J=7.2 Hz, 3H), 4.52 (q, J=7.2 Hz, 2H), 7.60–7.78 (m, 2H), 8.04 (d, J=8.7 Hz, 1H), 8.80 (d, J=1.8 Hz, 1H), 8.85 (s, 1H), 8.97 (d, J=8.7 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 14.60, 61.68, 126.26, 127.37, 128.46, 129.66, 130.42 (2C), 130.69, 133.37, 133.94, 136.80, 167.10, 171.22; ESIMS m/z 245 (M^++H). Anal. Calcd for $C_{14}H_{12}O_4$: C, 68.85; H, 4.95. Found: C, 68.94; H, 5.03.

3.5.2. Compound 7a′. Yield 8%; white solid, mp 49–50 °C; IR (KBr) 1723, 1306, 1233 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.48 (t, J=7.2 Hz, 3H), 4.01 (s, 3H), 4.50 (q, J=7.2 Hz, 2H), 7.56–7.75 (m, 2H), 7.99 (d, J=8.4 Hz, 1H), 8.73 (d, J=1.5 Hz, 1H), 8.74 (d, J=1.5 Hz, 1H), 8.94 (d, J=8.4 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 14.36, 52.42, 61.35, 125.92, 126.23, 126.96, 128.00, 129.24, 129.97, 129.98, 133.15, 133.25, 135.56, 166.42, 166.96; ESIMS m/z 259 (M^++H).

3.5.3. Compound 7b. Yield 37%; white solid, mp 185–186 °C; IR (KBr) 3394, 1714, 1692 cm^{-1}; ^1H NMR ($CDCl_3$+CD_3OD, 300 MHz) δ 1.48 (t, J=7.2 Hz, 3H), 2.59 (s, 3H), 4.49 (q, J=7.2 Hz, 2H), 7.44 (dd, J=8.4 and 1.5 Hz, 1H), 7.90 (d, J=8.4 Hz, 1H), 8.70–8.72 (m, 3H); ^{13}C NMR ($CDCl_3$+CD_3OD, 75 MHz) δ 14.10, 22.23, 61.19, 124.69, 125.48, 126.89, 129.01, 129.57, 129.69, 131.33, 133.36, 135.57, 140.42, 167.32, 168.12; ESIMS m/z 259 (M^++H). Anal. Calcd for $C_{15}H_{14}O_4$: C, 69.76; H, 5.46. Found: C, 69.66; H, 5.74.

3.5.4. Compound 7b′. Yield 21%; white solid, mp 54–55 °C; IR (KBr) 1719, 1627, 1307, 1234 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.48 (t, J=7.2 Hz, 3H), 2.58 (s, 3H), 3.99 (s, 3H), 4.49 (q, J=7.2 Hz, 2H), 7.42 (dd, J=8.4 and 1.5 Hz, 1H), 7.87 (d, J=8.4 Hz, 1H), 8.68–8.73 (m, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 14.36, 22.45, 52.32, 61.22, 124.94, 125.28, 127.13, 129.17, 129.35, 129.75, 131.40, 133.50, 135.30, 140.53, 166.52, 167.07; ESIMS m/z 273 (M^++H).

3.5.5. Compound 7c. Yield 39%; colorless oil; IR (KBr) 1715, 1684 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.48 (t, J=7.2 Hz, 3H), 2.76 (s, 3H), 4.51 (q, J=7.2 Hz, 2H), 7.58–7.76 (m, 2H), 8.02 (d, J=8.4 Hz, 1H), 8.61 (d, J=1.8 Hz, 1H), 8.70 (d, J=1.8 Hz, 1H), 8.93 (d, J=8.4 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 14.38, 26.65, 61.41, 125.99, 127.10, 127.94, 128.31, 130.18, 130.19, 133.14, 133.19, 133.26, 134.43, 167.06, 197.13; ESIMS m/z 243 (M^++H). Anal. Calcd for $C_{15}H_{14}O_3$: C, 74.36; H, 5.82. Found: C, 74.59; H, 5.93.

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Enantio-differential approach to identify the target cell for glucosyl jasmonate-type leaf-closing factor, by using fluorescence-labeled probe compounds

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Abstract—Potassium β-D-glucopyranosyl 12-hydroxyjasmonate (**1**) is a leaf-closing factor of *Albizzia* plants that induces nyctinastic leaf closure. In this paper, we synthesized probe **3** and its congener **4** by using a pair of enantiomerically pure methyl jasmonate that was prepared by using optical resolution, and carried out fluorescence studies using **3** and **4** to identify the target cell of **1**. The probe **3** bound to the motor cells of two *Albizzia* plants, whereas it could not bind to the motor cells of plants belonging to other genus. On the other hand, probe **4** did not bind to the motor cell at all. These results suggested that a specific receptor for **1** is involved in the motor cell of *Albizzia* plants.

1. Introduction

Most leguminous plants close their leaves in the evening, as if to sleep, and open them in the morning according to the circadian rhythm controlled by a biological clock. Charles Darwin, well known for his theory of evolution, carried out the pioneering study in this field.[1] And in the 1970s, physiological studies by Satter using *Albizzia saman* revealed that nyctinastic leaf movement is induced by the swelling and shrinking of motor cells in the pulvini, a small organ located in the joint of the leaf to the stem.[2] The flux of potassium ions through a potassium channel on the plasma membranes of the motor cells is followed by water flux into the cell, which results in swelling and shrinking of these cells. Since then, most of the physiological studies on nyctinasty were carried out using plants belonging to the genus *Albizzia*. Recently, channel proteins, which would be concerned with volume change in motor cell, were identified by a genetic approach.[3] We found that a pair of leaf-movement factors, leaf-opening and leaf-closing factors, controlled nyctinasty.[4] Each nyctinastic plant has a pair of leaf-movement factors whose bioactivities are specific to the plant genus.[5] These factors would be involved in the regulation of potassium channels concerning nyctinasty. Considering the difference of leaf-movement factors between plant genuses,[5] bioorganic studies of nyctinasty using *Albizzia* plants, especially *A. saman*, would be important for the coordination of results between bioorganic and physiological studies. Potassium β-D-glucopyranosyl 12-hydroxyjasmonate (**1**)[6] and *cis-p*-coumaroylagmatine (**2**)[7] were isolated as a leaf-closing and leaf-opening factor of the genus *Albizzia*, respectively. Leaf-movement factors **1** and **2** were not effective for plants belonging to other genuses, such as *Cassia mimosoides*, *Phyllanthus urinaria*, and *Mimosa pudica*.[6,7] We have already revealed that the target cell of **2** is a motor cell by using fluorescence-labeled **2**.[8] And we reported the synthesis of a diastereo-mixture of fluorescent probes (**3** and **4**) from racemic methyl jasmonate, and fluorescence studies using them.[9]

However, the use of a molecular probe in the bioorganic study requires serious attention. It was often discussed whether molecular probes containing a larger and less polar functional unit such as fluorescence dye can bind to the genuine binding protein of a natural product in living organisms, because the addition of a larger and less polar functional unit to a natural product increases affinity with the membrane or some abundant proteins in the cell, which resulted in the observation of nonspecific bindings. Thus, in the study using probes, use of a 'negative' probe, which is synthesized from the biologically inactive analog of a bioactive natural product is highly important, because a biologically inactive 'negative' probe gave only nonspecific bindings due to a larger and less polar functional unit.[10] Comparison of the results using a biologically active 'positive' probe and a biologically inactive 'negative' probe would give exact results (Fig. 1). The 'negative' probe should have a highly similar nature with the 'positive' probe besides the affinity to its binding protein. Then, the enantiomer of a biologically active natural product can be used as an ideal 'negative' probe because all physical properties except optical rotation and affinity to

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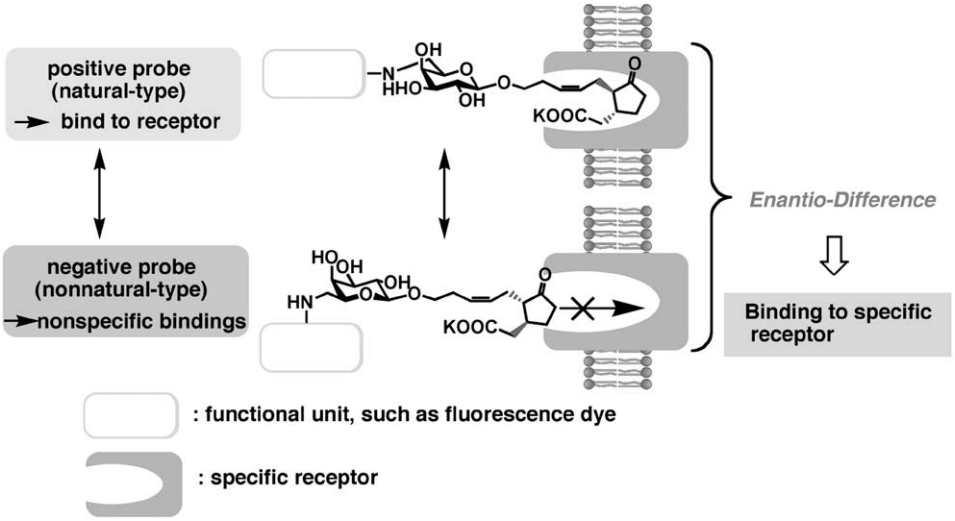
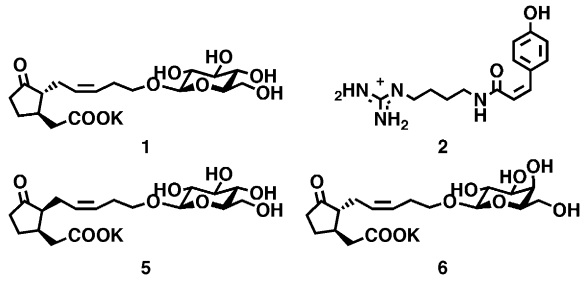


Figure 1. Enantio-differential approach using an enantio-pair of molecular probes.

binding proteins are identical between a pair of enantiomers (Fig. 1). Thus, enantio-differential approach that compares the results using a pair of probes that was prepared from a pair of enantiomers would be an ideal method in the bioorganic study using a molecular probe.

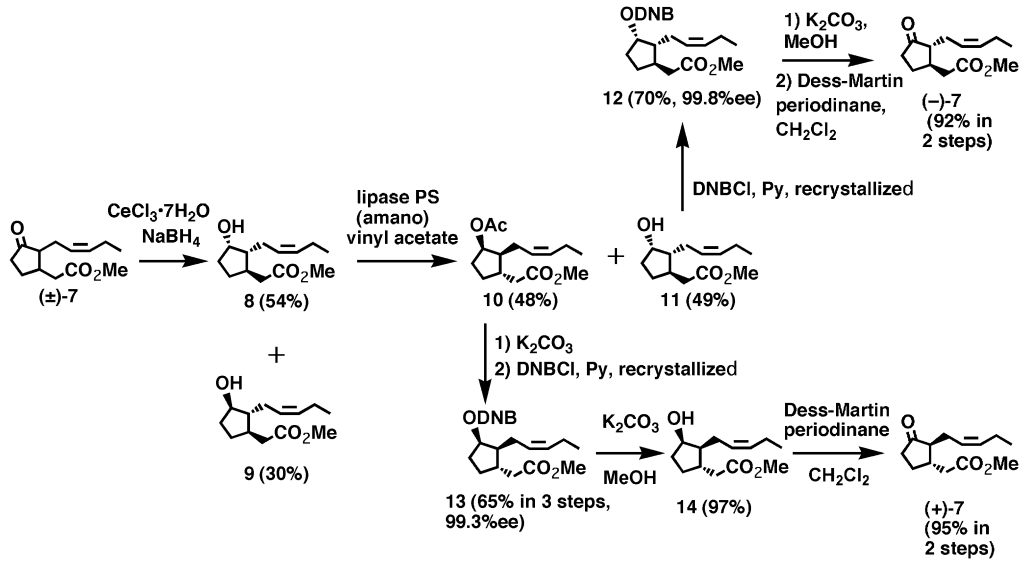
In this paper, we synthesized probe **3** and its congener (**4**) by using a pair of enantiomerically pure methyl jasmonate that was prepared by using optical resolution,[11] and carried out fluorescence studies using **3** and **4** to identify the target cell of **1**.



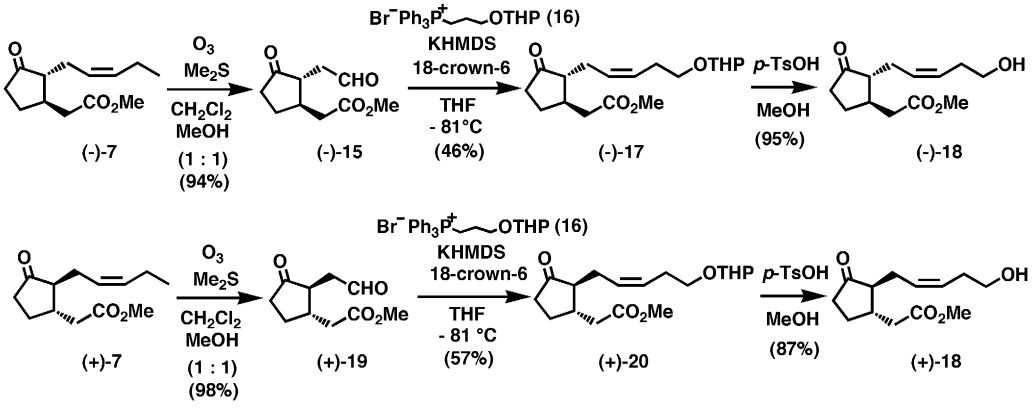
The molecular design of probes **3** and **4** was based on the following results on a structure–activity relationship study: potassium β-D-glucopyranosyl tuberonate (**5**),[12] which is a cis-isomer of **1** on the cyclopentanone ring, had no leaf-closing activity for the *Albizzia* plant,[6] although β-D-galacto-pyranosyl 12-hydroxyjasmonate (**6**)[9] showed leaf-closing activity for *Albizzia julibrissin* at 1×10^{-5} M. These results strongly suggested that an aglycon moiety of **1** would be important for leaf-closing activity, whereas the leaf-closing activity of **1** would not be affected by the structure modification in the sugar moiety similar to other glycoside-type leaf-movement factors.[13,14] Thus, we developed a pair of 'positive' and 'negative' probes from D-galactopyranosyl bromide[13,14] and both enantiomers of aglycon in **1**, which can be prepared from optically pure methyl jasmonate. Introduction of FITC into **1** was designed according to the molecular design of previously developed probes.[13,14] FITC was introduced to the amino group on the 6′-position of the sugar moiety through a glycylglycylglycyl linker by an amide linkage. We synthesized diastereomers **3** and **4** from optically pure enantiomers of aglycon, (+)-**18** and (−)-**18**.

Optically pure aglycons, (+)-**18** and (−)-**18**, were prepared from commercially available (±)-methyl jasmonate ((±)-**7**) (Scheme 1). Optical resolution of racemic **7** was carried out according to the method by Kiyota.[11] Racemic **7** was reduced by using $NaBH_4$–$CeCl_3$,[15] and then one of the resulting diastereomers (**8**) was acetylated by Lipase PS with vinyl acetate to give unnatural-type acetate **10** and natural-type alcohol **11**. The resulting **10** and **11** were converted to 3,5-dinitrobenzyl (DNB) ester (**12** and **13**), respectively, and purified by recrystallization. Optical purities were determined by HPLC analyses of DNB esters **12** and **13**.[16] After deprotection, DMP oxidation of the resulting alcohols gave optically pure (−)-**7** of natural stereochemistry and (+)-**7** of unnatural stereochemistry, respectively. The resulting (−)-**7** (>99.8% ee, $[\alpha]_D$ −93.9) and (+)-**7** (>99.3% ee, $[\alpha]_D$ +91.4) were used in the following syntheses, respectively. Scheme 2 shows an example for the synthesis of natural-type aglycon (−)-**18** from (−)-**7**.[17] Ozonolysis of (−)-**7** gave aldehyde **15**. Then, the Wittig reaction of **15** with ylide from **16** gave THP-protected aglycon (**17**). Wittig reaction by using 2 equiv of Wittig reagent was carried out under salt-free condition to give the (*Z*)-isomer predominantly. The resulting **17** was deprotected by *p*-TsOH to give (−)-methyl 12-hydroxyjasmonate ((−)-**18**), which was used in the glycosidation reaction with the sugar moiety. The enantiomer, (+)-methyl 12-hydroxyjasmonate ((+)-**18**), was also synthesized in the same way.

The resulting optically pure enantiomers (−)-**18** and (+)-**18** were used for the synthesis of positive and negative probes. Positive probe **3** with natural stereochemistry was synthesized from (−)-**18** and Fmoc-protected 6′-aminogalactosyl bromide (**21**)[18] as shown in Scheme 3. Under Königs–Knorr conditions, coupling product **22** was obtained in 37% yield (Scheme 3) with 27% of acetyl (−)-**18**. Careful addition of AgOTf to keep the reaction temperature low improved the yield of glycosilation.[9] Acetyl (−)-**18** can be easily hydrolyzed by K_2CO_3 to give (−)-**18**. Then, all protective groups in **22** were deprotected simultaneously by the treatment with

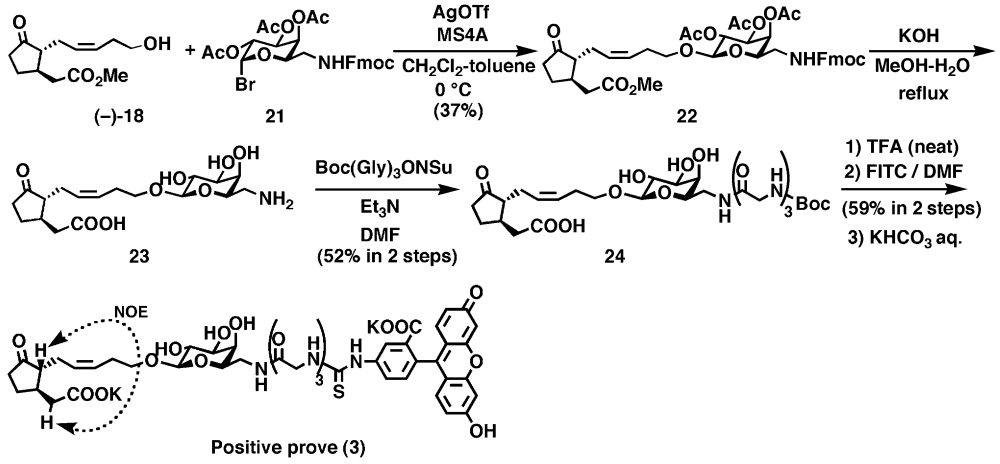


Scheme 1. Optical resolution of racemic methyl jasmonate.

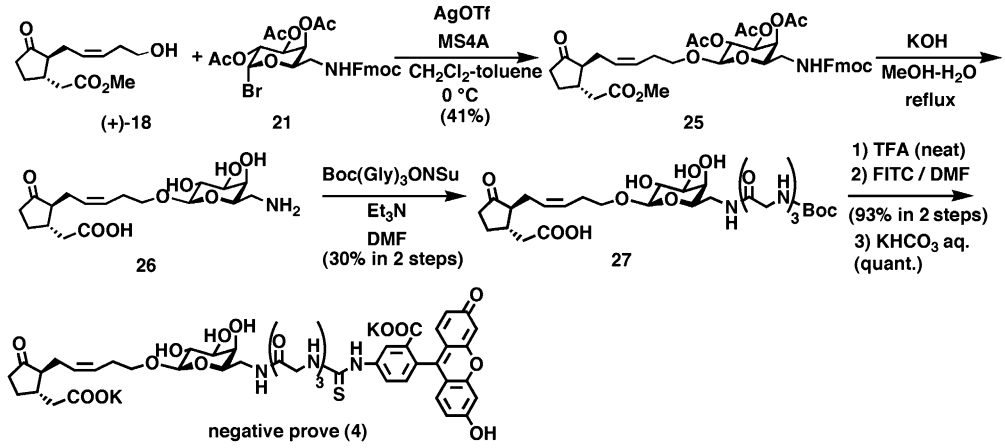


Scheme 2. Synthesis of enantiomerically pure aglycons.

KOH to give **23**, which was coupled with the *N*-hydroxysuccinimide ester of Boc-glycylglycylglycine. The resulting **24** was purified by HPLC under acidic condition, deprotected by TFA, and coupled with FITC to give fluorescence-labeled probe (**3**). According to the same procedure as shown in Scheme 3, negative probe **4** was synthesized from (+)-**18** (Scheme 4). The trans-relationship of the two side chains in **3** and **4** was determined by NOE experiment shown in



Scheme 3. Synthesis of positive probe **3**.



Scheme 4. Synthesis of negative probe **4**.

Scheme 3. The positive probe **3** was effective for the leaf closing of *A. saman* at 5×10^{-4} M, whereas the negative probe **4** was not effective at the same concentration.[19]

We have synthesized enantio-pair-type probes **3** and **4**. By the comparison of the results using them, we can obtain exact biological results because the enantiomer of a bioactive natural product cannot be recognized by the genuine receptor for a natural product that is involved in a biological event. For this purpose, binding experiment using plant sections to seek the target cell for **1** was carried out using a pair of probes **3** and **4**. A leaf of *A. saman* was cut to a thickness of 30 μm. Then, the sections containing a motor cell were incubated in a 0.1 M phosphate buffer (pH 7) containing 1×10^{-4} M of positive probe **3**. After staining, the stained sections were washed with 0.1 M phosphate buffer (pH 7) to remove excess fluorescence probes. Then, the stained sections were monitored by a fluorescent microscope with an appropriate filter for FITC. The use of an antifadant reagent was essential to prevent photo-bleaching (fading of fluorescence). Figure 2 shows photographs of plant pulvini, which contains a motor cell, under a fluorescence microscope. The staining pattern for the yellowish-green fluorescence of probe **3** was observed on the surface of the motor cell in the plant section (Fig. 2). No staining was observed in the competitive binding experiment using 1×10^{-4} M of probe **3** and excess amount (1×10^{-1} M) of non-labeled natural product (**1**). And when the plant section was incubated in a 0.1 M phosphate buffer (pH 7) containing 1×10^{-4} M of negative probe **4**, no staining was observed in the motor cell.

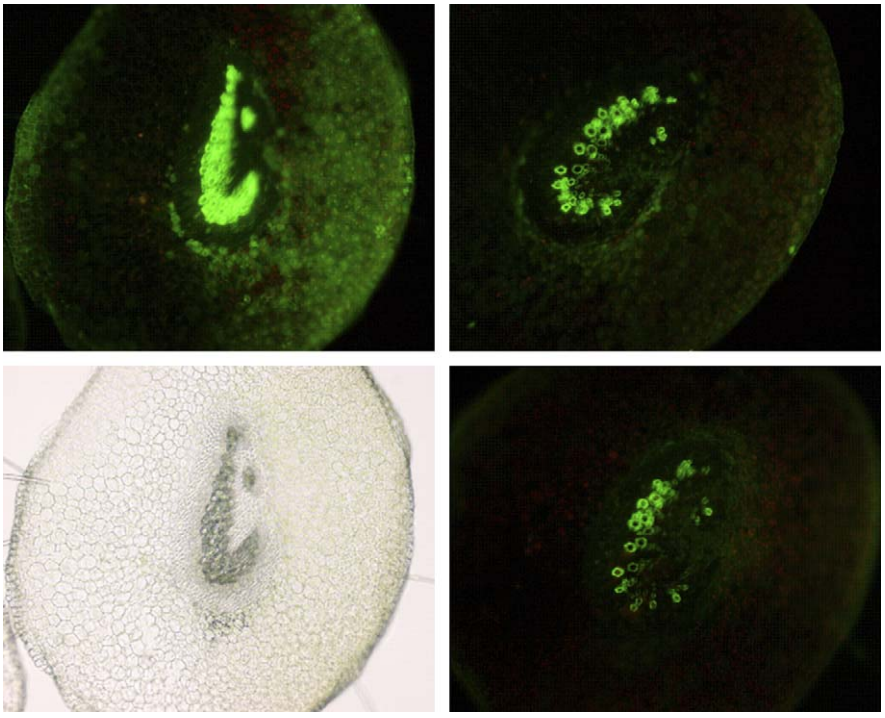


Figure 2. Binding experiment using **3** and **4** with plant section of *A. saman* containing motor cell (upper left: fluorescence image of plant section treated with 1×10^{-4} M of **3** [excitation: 450–490 nm], upper right: fluorescence image of plant section treated with 1×10^{-4} M of **4** [excitation: 450–490 nm], down left: Nomarskii image of the plant section containing motor cell, down right: fluorescence image of plant section treated with 1×10^{-4} M of **3** and 1×10^{-1} M of **1** [excitation: 450–490 nm]).

Comparing the results by probes **3** and **4**, it was clearly shown that strong fluorescence in the xylem (center of the plant section), which was observed in both cases, would be attributed to some nonspecific binding of the probes. These results strongly suggested that the genuine target cell for leaf-closing factor **1** is a motor cell, and a specific receptor for **1**, which recognizes the stereochemistry of aglycon in **1**, is involved in the motor cell of the genus *Albizzia*. The results using an enantio-pair-type probes **3** and **4** showed the effectiveness of an enantio-differential approach using molecular probes in bioorganic studies.

Next, we examined genus specificity in binding of **3** to the plant section. We revealed that **1** is a common leaf-closing substance among three *Albizzia* plants, containing *A. julibrissin* and *Albizzia lebbeck*.[6] And **1** was not effective for the plants belonging to other plant genus. These results strongly suggested that all *Albizzia* plants have specific receptor for **3**. And this indicated that genus-specific biological activity of leaf-movement factors would be attributed to the existence of a receptor for the leaf-movement factor that is common among the same genus.

First, we examined the specificity in the bioactivity of probe **3**. The probe **3** did not show leaf-closing activity against leaves of *C. mimosoides* L., *P. urinaria*, and *Leucaena leucocephala* at 5×10^{-4} M, whereas it was effective at the same concentration for the leaf closing of two *Albizzia* plants: at 1×10^{-4} M for *A. julibrissin* and at 5×10^{-4} M for *A. saman*. From these results, the binding of **3** is expected to be specific to the section of plants belonging to the genus *Albizzia*, and no binding would be observed in the experiment using the section of other plants. Then, we used probe **3** for the binding experiment with the sections of *C. mimosoides*, *P. urinaria*, and *L. leucocephala* together with those of *A. saman* and *A. julibrissin*. The binding experiments were carried out according to the same procedure used in the precedent using *A. saman*. Thus, it was revealed that the sections of *A. juribrissin* and *A. saman* gave a fluorescence image resulting from **3** and no other sections gave the image (Fig. 3). Red stains seen in the fluorescence images are due to the porphyrin in the plant tissue. It was already revealed that the binding of probe **3** to the motor cell of *A. saman* is closely related to the recognition of stereochemistry in probe **3**. Thus, these results showed that the binding of probe **3** with a motor cell is specific to the genus *Albizzia* and strongly suggested that a genus-specific receptor molecule for the genus-specific leaf-movement factor on a motor cell would be involved in nyctinasty.

From these results, we have shown that *Albizzia* plants have a receptor for **1** in the motor cells. Together with the former result of the specific binding of a leaf-opening substance to the motor cell of the genus *Albizzia*,[8] it was strongly suggested that the *Albizzia* plant would have a pair of receptors corresponding to leaf-opening and leaf-closing factors, and both of them are common among the same genus. This result showed that the genus-specific receptor for the leaf-movement factor would be involved in the nyctinasty. And, it was estimated that each plant genus would have a genus-specific combination of leaf-movement factors and receptor molecules for them. Genus-specific recognition of the ligand by a specific receptor for the leaf-movement factor strongly suggests that the membrane receptor concerning nyctinasty would be differentiated in the comparatively later process of evolution in the plant kingdom when the legumes differentiated to various genuses. Also, our results showed that an enantio-differential approach using molecular probes can distinguish the binding of some ligand to its specific receptor from the noise due to the nonspecific bindings, which is one of the most important problem in the experiment using molecular probes; thus an enantio-differential approach would be highly useful in the bioorganic studies of biological phenomena.

2. Experimental

2.1. General procedures

NMR spectra were recorded on a Jeol JNM-A600 spectrometer [^{1}H (600 MHz) and ^{13}C (150 MHz)] and JNM AL300

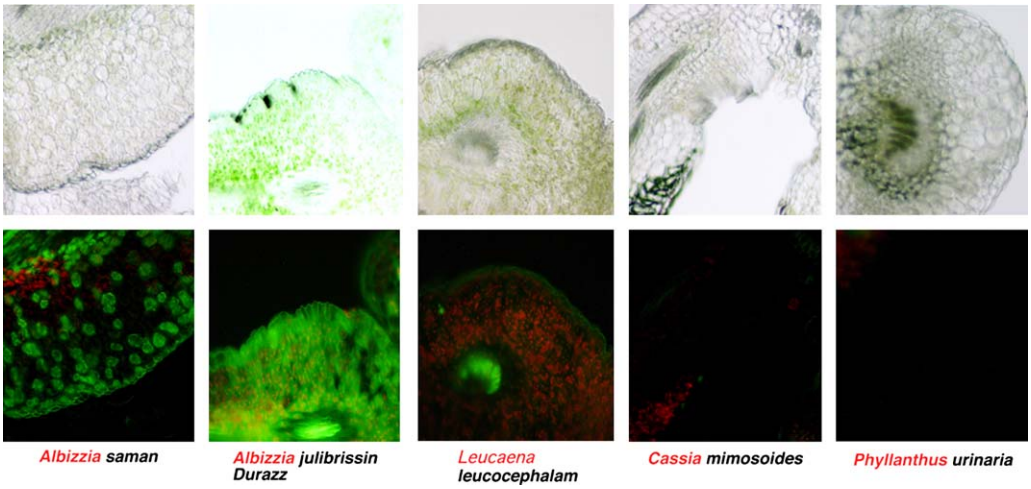


Figure 3. Photographs of plant sections in the binding experiments, which show specific binding of probe **3** with the motor cell of *Albizzia* plants (from the left, *A. saman*, *A. julibrissin*, *Leucaena leucocephala*, *Cassia mimosoides*, and *Phyllanthus urinaria*; upper: Nomarskii image of plant section, lower: fluorescent image of plant section after treatment with 1×10^{-4} M of probe **3** [excitation: 450–490 nm]).

[^{1}H (300 MHz) and ^{13}C (75 MHz)], using TMS in $CDCl_3$, CD_2HOD in CD_3OD (^{1}H; 3.33 ppm, ^{13}C; 49.8 ppm), or *t*-BuOH (^{1}H; 1.24 ppm, ^{13}C; 30.3 ppm) in D_2O as internal standards at various temperatures. The HR ESI-MS spectra were recorded on a Bruker APEX-III. The IR spectra were recorded on a JASCO FT/IR-410. The specific rotations were measured by JASCO DIP-360 polarimeter. The HPLC purification was carried out with a Shimadzu LC-6A pump equipped with SPD-6A detector using COSMOCIL $5C_{18}$-AR column (∅20×250 mm) (Nakalai Tesque Co. Ltd). The solvents used for HPLC were available from Kanto Chemical Co. and were filtered through a Toyo Roshi membrane filter (cellulose acetate of 0.45 mm pore size, 47 mm dia.) before use. Silica gel column chromatography was performed on silica gel 60 K070 (Katayama Chemical Co., Ltd) or silica gel 60N (Kanto Chemical Co., Ltd). TLC was performed on silica gel F_{254} (0.25 or 0.5 mm, MERCK) or RP-$18F_{254S}$ (0.25 mm, MERCK).

2.1.1. Synthesis of positive probe 3.

2.1.1.1. Methyl (1*S*,2*S*)-3-oxo-2-(formylmethyl)cyclopentaneacetate (−)-15. Ozone was bubbled through a solution of (−)-**7** (325 mg, 1.45 mmol) in CH_2Cl_2 (10 mL) and MeOH (10 mL) at −78 °C for more than 50 min. The excess amount of ozone was removed by Ar gas, then dimethyl sulfide (210 μL) was added dropwise at rt. The mixture was stirred overnight and concentrated in vacuo. The residue was chromatographed on silica gel (*n*-hexane/EtOAc=2:1) to afford (−)-**15** (281 mg, 1.42 mmol, 98%) as a colorless oil: $[\alpha]_D^{23}$ −86.8 (*c* 0.67, $CHCl_3$). IR (film) *ν*: 3422, 2856, 1732, 1717, 1437, 1387, 1340, 1212, 1167 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$, 22 °C) *δ* 9.73 (1H, s), 3.66 (3H, s), 2.89 (1H, dd, *J*=3.9, 18.9 Hz), 2.70 (1H, dd, *J*=3.9, 18.9 Hz), 2.58–2.18 (7H, m), 1.55 (1H, m); ^{13}C NMR (75 MHz, $CDCl_3$, 22 °C) *δ* 217.6, 199.7, 172.5, 51.6, 49.1, 42.3, 38.6, 38.3, 36.9, 27.6; ESI-HRMS (positive): [M+Na]$^+$ found *m*/*z* 221.0784, $C_{10}H_{14}O_4Na$ requires *m*/*z* 221.0784.

2.1.1.2. Methyl (1*S*,2*R*,2′*Z*)-3-oxo-2-[5′-(tetrahydropyran-2″-yl)oxy-2′-pentenyl]-cyclopentaneacetate (−)-17. To a mixture of 3-tetrahydropyranyloxypropyltriphenylphosphonium bromide **16** (240 mg, 0.495 mmol) and 18-crown-6 (130 mg, 0.495 mmol) in THF (5 mL) potassium hexamethyldisilazide (0.482 mmol, 0.96 mL as 0.5 mol/L solution of toluene) was slowly added at rt under Ar atmosphere. The mixture was stirred for 10 min at rt. During this period, the mixture developed a bright orange color indicating formation of the ylide. To a solution of **15** (49 mg, 0.247 mmol) in THF (0.8 mL) was slowly added a solution of the ylide (4 mL) at −81 °C for 21 h. The mixture was stirred for 1 h at −78 °C, and then allowed to stand to rt and stirred overnight whereby the orange color disappeared and a whitish suspension was formed. The mixture was concentrated and the residue was chromatographed on silica gel (*n*-hexane/EtOAc=4:1) to afford (−)-**17** (37 mg, 0.114 mmol, 46%) as a colorless oil: $[\alpha]_D^{23}$ −44.9 (*c* 2.0, MeOH). IR (film) *ν*: 2949, 2870, 1740, 1439, 1200, 1032 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$, 23 °C) *δ* 5.48 (1H, dt, *J*=10.8, 7.2 Hz), 5.37 (1H, dt, *J*=10.8, 7.2 Hz), 4.56 (1H, dd, *J*=2.4, 4.2 Hz), 3.81 (1H, ddd, *J*=11.4, 7.2, 3.6 Hz), 3.75–3.69 (4H, m), 3.47 (1H, dt, *J*=11.4, 4.8 Hz), 3.42–3.33 (1H, m), 2.68 (1H, dd, *J*=19.2, 8.4 Hz), 2.38–2.17 (8H, m), 2.08 (1H, ddd, *J*=18.6, 11.1, 8.4 Hz), 1.92–1.85 (1H, m), 1.83–1.75 (1H, m), 1.69 (1H, dt, *J*=12.3, 2.7 Hz), 1.57–1.40 (5H, m); ^{13}C NMR (75 MHz, $CDCl_3$, 22 °C) *δ* 218.7, 172.4, 128.2, 127.6, 98.7, 66.8, 62.2, 53.8, 51.5, 38.7, 37.9, 37.6, 30.6, 28.0, 27.1, 25.6, 25.4, 19.5; ESI-HRMS (positive): [M+Na]$^+$ found *m*/*z* 347.1829, $C_{18}H_{28}O_5Na$ requires *m*/*z* 347.1829.

2.1.1.3. Methyl (1*S*,2*R*,2′*Z*)-2-(5-hydroxy-2-pentenyl)-3-oxo-cyclopentaneacetate ((−)methyl 12-hydroxyjasmonate) (−)-18. To a solution of (−)-**17** (40 mg, 0.12 mmol) in MeOH (1.0 mL) was added catalytic amount of *p*-TsOH·H_2O at 0 °C under Ar atmosphere. After stirring for 1 h at rt, the reaction mixture was diluted with satd $NaHCO_3$ aq and concentrated in vacuo. The residue was extracted with EtOAc, washed with brine, and dried over Na_2SO_4. Purification by silica gel column chromatography (*n*-hexane/EtOAc=2:1) gave (−)-**18** (24 mg, 102 μmol, 85%) as a colorless oil: $[\alpha]_D^{24}$ −67.9 (*c* 0.5, MeOH). ^{1}H NMR (300 MHz, $CDCl_3$, 22 °C) *δ* 5.50 (1H, dt, *J*=10.8, 6.3 Hz), 5.46 (1H, dt, *J*=10.8, 6.3 Hz), 3.70 (3H, s), 3.66 (t, *J*=6.3 Hz), 2.70 (1H, dd, *J*=8.1, 18.9 Hz), 2.48–1.90 (10H, m), 1.50 (1H, m); ^{13}C NMR (75 MHz, $CDCl_3$, 22 °C) *δ* 219.1, 172.7, 128.5, 128.3, 62.0, 53.9, 51.7, 38.8, 37.8, 37.7, 30.9, 27.2, 25.3; ESI-HRMS (positive): [M+Na]$^+$ found *m*/*z* 263.1255, $C_{13}H_{18}O_4Na$ requires *m*/*z* 263.1254.

2.1.1.4. Methyl (1*S*,2*R*,2′*Z*)-2-[5′-(2″,3″,4″-tri-*O*-acetyl-6″-(*N*-9*H*-fluoren-9-ylmethoxycarbonylamino)-β-D-glucopyranosyloxy)-2′-pentenyl]-3-oxo-cyclopentaneacetate 22. A solution of (−)-**18** (17 mg, 70.8 μmol), **21** (63 mg, 107 μmol), and dried molecular sieves of 4 Å (300 mg) in anhydrous CH_2Cl_2 (1.0 mL) was slowly added toluene solution (1.0 mL) of AgOTf (27 mg, 106 mmol) at 0 °C under Ar atmosphere in dark. This reaction mixture was stirred overnight at 0 °C. Then, the reaction mixture was diluted with $CHCl_3$ and filtered through Celite. The filtrate was washed with brine and satd aq $NaHCO_3$. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc=1:1) and preparative TLC (*n*-hexane/EtOAc=1:1) to afford **22** (19.4 mg, 26 mmol, 37%) as a colorless viscous oil: $[\alpha]_D^{18}$ −19.7 (*c* 0.5, MeOH). IR (film) *ν*: 3369, 2953, 1747, 1529, 1439, 1369, 1223, 1167, 1072, 760 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$, 23 °C) *δ* 7.76 (2H, d, *J*=7.2 Hz), 7.57 (2H, d, *J*=7.2 Hz), 7.40 (2H, t, *J*=7.2 Hz), 7.31 (1H, t, *J*=7.5 Hz), 7.30 (1H, t, *J*=7.5 Hz), 5.44–5.35 (3H, m), 5.20 (1H, dd, *J*=10.5, 7.8 Hz), 5.02 (1H, dd, *J*=10.5, 3.0 Hz), 4.47 (1H, d, *J*=7.8 Hz), 4.39 (2H, d, *J*=6.6 Hz), 4.21 (1H, t, *J*=6.6 Hz), 3.88 (1H, dt, *J*=9.0, 6.9 Hz), 3.76 (1H, t, *J*=6.6 Hz), 3.68 (3H, s), 3.53–3.35 (2H, m), 3.26 (1H, dd, *J*=13.2, 6.6 Hz), 2.65 (1H, dd, *J*=18.9, 8.4 Hz), 2.42–2.09 (9H, m), 2.17 (3H, m), 1.90 (1H, m), 1.47 (1H, m); ^{13}C NMR (75 MHz, $CDCl_3$, 23 °C) *δ* 218.9, 172.5, 170.8, 170.0, 169.5, 156.4, 143.8, 141.3, 128.0, 127.7, 127.4, 127.0, 125.0, 120.0, 101.2, 71.5, 70.9, 69.5, 69.0, 68.1, 53.9, 51.6, 47.2, 40.6, 38.7, 37.8, 37.7, 27.8, 27.2, 25.4, 20.8, 20.7, 20.6; ESI-HRMS (positive): [M+Na]$^+$ found *m*/*z* 772.2940, $C_{40}H_{47}NO_{13}Na$ requires *m*/*z* 772.2940.

2.1.1.5. (1*S*,2*R*,2′*Z*)-2-[5′-(6″-(*N*-*tert*-Butoxycarbonylglycylglycylglycylamino)-β-D-glucopyranosyloxy)-2′-pentenyl]-3-oxo-cyclopentaneacetic acid 24. Glycoside **22**

(17 mg, 22.7 μmol) in MeOH/H_2O=3:1 (4 mL) and 1 M KOH aq (114 μmol, 114 μL) was refluxed for 4 h at 85 °C. After the solution was neutralized with 1 N HCl aq and concentrated in vacuo, the residue was purified by ODS TLC (RP-18, H_2O/MeOH=2:3 containing 5% AcOH) to afford an acetate of the resulting amine **23**. To the solution of amine **23** in DMF (2 mL) with Et_3N (12 μL) was added *O*-Boc-glycylglycylglycyl *N*-hydroxysuccinimide (12 mg, 30 μmol). After stirring overnight at rt, the reaction mixture was evaporated to dryness. The residue was purified by ODS TLC (RP-18, H_2O/MeCN=3:1 containing 0.5% AcOH) to afford **24** (6.9 mg, 10.5 μmol, 52% in two steps) as a colorless viscous oil: $[\alpha]_D^{22}$ −13.8 (*c* 0.25, MeOH). IR (film) *ν*: 3310, 2924, 1668, 1653, 1539, 1368, 1252, 1167, 1058, 1033 cm^{-1}. ^1H NMR (300 MHz, CD_3OD, 21 °C) *δ* 5.53 (1H, dt, *J*=10.8, 6.9 Hz), 5.40 (1H, dt, *J*=10.8, 7.5 Hz), 4.21 (1H, d, *J*=6.9 Hz), 3.88–3.82 (5H, m), 3.74–3.72 (3H, m), 3.59–3.51 (3H, m), 3.48–3.39 (3H, m), 2.68 (1H, dd, *J*=8.1, 18.9 Hz), 2.44–1.98 (10H, m), 1.59–1.49 (1H, m), 1.45 (9H, s); ^{13}C NMR (75 MHz, CD_3OD, 22 °C) *δ* 221.9, 173.8, 172.2, 158.8, 129.1, 128.9, 104.8, 81.0, 74.7, 73.9, 72.4, 70.3, 70.2, 55.1, 44.9, 43.9, 43.5, 41.0, 40.0, 39.2, 38.7, 30.8, 29.1, 28.7, 28.2, 26.4; ESI-HRMS (positive): $[M+Na]^+$ found *m/z* 681.2950, $C_{29}H_{46}N_4O_{13}Na$ requires *m/z* 681.2954.

2.1.1.6. (1*S*,2*R*,2′*Z*)-2-[5′-(6″-(*N*-Fluorescein-4-isothiocyanatoglycylglycylglycylamino)-β-D-glucopyranosyloxy)-2′-pentenyl]-3-oxo-cyclopentaneacetic acid (positive probe) 3. Compound **24** (3.9 mg, 5.9 μmol) in TFA (0.3 mL) was mixed for 2 min at rt. The solution was evaporated to give a TFA salt of resulting amine. The TFA salt was dissolved in DMF (0.4 mL) with TEA (10 μL), and then FITC (2.8 mg, 7.2 μmol) was added to this solution at 0 °C. After stirring overnight at rt, the reaction mixture was evaporated to dryness. The residue was purified by ODS TLC (RP-18W, H_2O/MeCN=2:1 containing 0.5% TFA) to afford **3** (3.3 mg, 3.5 μmol, 59% in two steps) as yellow viscous oil. Neutralization of the resulting **3** with 0.1 mM $KHCO_3$ aq (70 μL, 7.0 μmol) gave **3** (3.6 mg as a potassium salt, quant) as an orange crystal: $[\alpha]_D^{20}$ +3.65 (*c* 0.2, MeOH). IR (film) *ν*: 3279, 1681, 1581, 1466, 1333, 1268, 1132, 915, 850, 802, 724 cm^{-1}. ^1H NMR (600 MHz, D_2O, 27 °C) *δ* 7.75 (1H, s), 7.66 (1H, s), 7.37 (1H, d, *J*=7.8 Hz), 7.25 (2H, d, *J*=9.6 Hz), 6.65–6.62 (4H, m), 5.43 (1H, dt, *J*=10.8, 6.6 Hz), 5.37 (1H, dt, *J*=10.8, 7.2 Hz), 4.32 (2H, s), 4.30 (1H, d, *J*=8.4 Hz), 4.00 (2H, s), 3.95 (1H, d, *J*=16.2 Hz), 3.91 (1H, d, *J*=16.2 Hz), 3.83–3.78 (2H, m), 3.69–3.66 (1H, m), 3.60–3.55 (2H, m), 3.49–3.38 (3H, m), 2.50 (1H, dd, *J*=4.8, 13.8 Hz), 2.34–2.20 (6H, m), 2.16–2.09 (3H, m), 1.97–1.94 (1H, m), 1.47–1.46 (1H, m); ^{13}C NMR (150 MHz, D_2O, 22 °C) *δ* 226.9, 227.4, 181.7, 180.8, 176.1, 174.8, 173.2, 163.0, 159.0, 158.8, 158.7, 131.6, 128.5, 127.6, 126.6, 124.9, 123.6, 112.6, 103.7, 102.8, 72.8, 70.8, 69.8, 68.5, 54.5, 47.7, 43.3, 42.6, 42.4, 39.9, 38.7, 38.6, 36.7, 27.4, 26.9, 25.3; ESI-HRMS (negative): $[M-2K+H]^-$ found *m/z* 946.2830, $C_{45}H_{48}N_5O_{16}S$ requires *m/z* 946.2822.

2.1.2. Synthesis of negative probe 4.

2.1.2.1. Methyl (1*R*,2*R*)-3-oxo-2-(formylmethyl)cyclopentaneacetate (+)-19. Ozone was bubbled through a solution of (+)-**7** (185 mg, 0.825 mmol) in CH_2Cl_2 (8 mL) and MeOH (8 mL) at −78 °C for more than 25 min. The excess ozone was removed by Ar gas, then dimethyl sulfide (97 μL) was added dropwise at rt. The mixture was stirred overnight and concentrated in vacuo. The residue was chromatographed on silica gel (*n*-hexane/EtOAc=2:1) to afford (+)-**19** (160 mg, 0.808 mmol, 98%) as a colorless oil: $[\alpha]_D^{22}$ +94.1 (*c* 0.67, $CHCl_3$). IR (film) *ν*: 3422, 2856, 1732, 1717, 1437, 1387, 1340, 1212, 1167 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, 22 °C) *δ* 9.73 (1H, s), 3.66 (3H, s), 2.89 (1H, dd, *J*=3.9, 18.9 Hz), 2.70 (1H, dd, *J*=3.9, 18.9 Hz), 2.58–2.18 (7H, m), 1.55 (1H, m); ^{13}C NMR (75 MHz, $CDCl_3$, 22 °C) *δ* 217.6, 199.7, 172.5, 51.6, 49.1, 42.3, 38.6, 38.3, 36.9, 27.6; ESI-HRMS (positive): $[M+Na]^+$ found *m/z* 221.0784, $C_{10}H_{14}O_4Na$ requires *m/z* 221.0784.

2.1.2.2. Methyl (1*R*,2*S*,2′*Z*)-3-oxo-2-[5′-(tetrahydropyran-2″-yl)oxy-2′-pentenyl]-cyclopentaneacetate (+)-20. To a mixture of 3-tetrahydropyranyloxypropyltriphenylphosphonium bromide **16** (69 mg, 0.14 mmol) and 18-crown-6 (40 mg, 0.15 mmol) in THF (1.4 mL) was slowly added potassium hexamethyldisilazide (0.14 mmol, 275 μL as 0.5 mol/L solution in toluene) at rt under Ar atmosphere. The mixture was stirred for 10 min at rt. During this period, the mixture developed a bright orange color indicating the formation of the ylide. To a solution of **19** (14 mg, 0.07 mmol) in THF (0.5 mL) was added a solution of the ylide (1.5 mL) at −81 °C during 20 min. The mixture was stirred for 1 h at −78 °C, and then the mixture allowed to stand to rt and stirred overnight whereby the orange color disappeared and a whitish suspension was formed. The mixture was concentrated and the residue was chromatographed on a silica gel (hexane/EtOAc=4:1) to afford (+)-**20** (13 mg, 0.04 mmol, 57%) as a colorless oil: $[\alpha]_D^{22}$ +54.2 (*c* 2.68, MeOH). IR (film) *ν*: 2949, 2870, 1740, 1437, 1200, 1032 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, 22 °C) *δ* 5.48 (1H, dt, *J*=10.5, 7.5 Hz), 5.37 (1H, dt, *J*=10.5, 7.5 Hz), 4.56 (1H, br s), 3.83 (1H, m), 3.75–3.66 (4H, m), 3.47 (1H, m), 3.36 (1H, dd, *J*=15.6, 7.5 Hz), 2.68 (1H, dd, *J*=18.9, 8.1 Hz), 2.37–2.02 (9H, m), 1.90–1.67 (3H, m), 1.55–1.36 (5H, m); ^{13}C NMR (75 MHz, $CDCl_3$, 22 °C) *δ* 218.8, 172.4, 128.2, 127.5, 98.7, 98.7, 66.7, 62.2, 53.8, 51.5, 38.6, 38.0, 37.9, 37.6, 30.6, 27.9, 27.1, 25.6, 25.4, 19.5; ESI-HRMS (positive): $[M+Na]^+$ found *m/z* 347.1830, $C_{18}H_{28}O_5Na$ requires *m/z* 347.1829.

2.1.2.3. Methyl (1*R*,2*S*,2′*Z*)-2-(5-hydroxy-2-pentenyl)-3-oxo-cyclopentaneacetate ((+)-*ent*-methyl 12-hydroxyjasmonate) (+)-18. To a solution of (+)-**20** (11 mg, 33.3 μmol) in MeOH (1.0 mL) was added a catalytic amount of *p*-TsOH·H_2O at 0 °C under Ar atmosphere. After stirring for 1 h at rt, the reaction mixture was diluted with satd $NaHCO_3$ aq and concentrated in vacuo. The residue was extracted with EtOAc, washed with brine, and dried over Na_2SO_4. Purification by silica gel column chromatography (*n*-hexane/EtOAc=2:1) gave (+)-**18** (7 mg, 29.1 μmol, 87%) as a colorless oil: $[\alpha]_D^{22}$ +72.1 (*c* 0.5, MeOH). ^1H NMR (300 MHz, $CDCl_3$, 22 °C) *δ* 5.50 (1H, dt, *J*=10.8, 6.3 Hz), 5.46 (1H, dt, *J*=10.8, 6.3 Hz), 3.70 (3H, s), 3.66 (t, *J*=6.3 Hz), 2.70 (1H, dd, *J*=8.1, 18.9 Hz), 2.48–1.90 (10H, m), 1.50 (1H, m); ^{13}C NMR (75 MHz, $CDCl_3$, 22 °C) *δ* 219.1, 172.7, 128.5, 128.3, 62.0, 53.9, 51.7, 38.8, 37.8, 37.7, 30.9, 27.2, 25.3; ESI-HRMS (positive): $[M+Na]^+$ found *m/z* 263.1255, $C_{13}H_{18}O_4Na$ requires *m/z* 263.1254.

2.1.2.4. Methyl (1*R*,2*S*,2′*Z*)-2-[5′-(2″,3″,4″-tri-*O*-acetyl-6″-(*N*-9*H*-fluoren-9-ylmethoxycarbonylamino)-β-D-glucopyranosyloxy)-2′-pentenyl]-3-oxo-cyclopentaneacetate 25. To a solution of (+)-**18** (40 mg, 166 μmol), **21** (150 mg, 250 μmol), and dried molecular sieves of 4 Å (300 mg) in anhydrous CH_2Cl_2 (2.2 mL) was slowly added AgOTf (64 mg, 250 mmol) in toluene (2.23 mL) at 0 °C under Ar atmosphere in the dark. This reaction mixture was stirred for 3.5 h at 0 °C. Then, the reaction mixture was diluted with $CHCl_3$ and filtered through Celite. The filtrate was washed with brine and satd $NaHCO_3$ aq. The organic layer was dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc=2:3) and preparative TLC (*n*-hexane/EtOAc=1:1) to afford **25** (51 mg, 68 mmol, 41%) as a colorless viscous oil: $[\alpha]_D^{21}$ +22.3 (*c* 1.46, MeOH). IR (film) *ν*: 3369, 2953, 1749, 1521, 1508, 1437, 1369, 1225, 1165, 1070, 745 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, 22 °C) *δ* 7.76 (2H, d, *J*=7.5 Hz), 7.58 (2H, d, *J*=7.5 Hz), 7.41 (2H, t, *J*=7.5 Hz), 7.31 (2H, br t, *J*=7.5 Hz), 5.47–5.35 (3H, m), 5.20 (1H, dd, *J*=10.2, 8.7 Hz), 5.02 (1H, dd, *J*=10.2, 2.7 Hz), 4.47–4.36 (3H, m), 4.22 (1H, t, *J*=6.3 Hz), 3.88 (1H, dd, *J*=15.6, 6.9 Hz), 3.78–3.69 (4H, m), 3.50 (1H, dd, *J*=15.6, 6.9 Hz), 3.40 (1H, dd, *J*=13.8, 6.6 Hz), 3.26 (1H, dt, *J*=13.8, 6.6 Hz), 2.66 (1H, dd, *J*=18.9, 8.4 Hz), 2.44–2.00 (18H, m), 1.90 (1H, m), 1.47 (1H, m); ^{13}C NMR (75 MHz, $CDCl_3$, 23 °C) *δ* 218.8, 172.5, 170.8, 170.0, 169.4, 156.4, 143.8, 143.8, 141.3, 127.9, 127.7, 127.4, 127.0, 125.0, 120.0, 101.3, 71.6, 70.9, 69.6, 69.0, 68.1, 66.8, 53.9, 51.6, 47.1, 40.7, 38.7, 37.8, 37.7, 27.8, 27.2, 25.4, 20.8, 20.7, 20.6; ESI-HRMS (positive): $[M+Na]^+$ found *m/z* 772.2937, $C_{40}H_{47}NO_{13}Na$ requires *m/z* 772.2940.

2.1.2.5. (1*R*,2*S*,2′*Z*)-2-[5′-(6″-(*N*-*tert*-Butoxycarbonylglycylglycylglycylamino)-β-D-glucopyranosyloxy)-2′-pentenyl]-3-oxo-cyclopentaneacetic acid 27. Glycoside **25** (46 mg, 61.3 μmol) in MeOH/H_2O=3:1 (4 mL) was refluxed with 1 M KOH aq (307 μmol, 307 μL) for 5 h at 87 °C. After, the reaction mixture was neutralized with 1 N HCl aq and concentrated in vacuo. The residue was purified by ODS TLC (RP-18, H_2O/MeOH=2:3 containing 5% AcOH) to afford an acetate of the resulting amine **26**. To a solution of amine in DMF (0.5 mL) was Et_3N (12 μL) was added *O*-Boc-glycylglycylglycyl *N*-hydroxysuccinimide (47 mg, 123 μmol). After stirring overnight at rt, the reaction mixture was evaporated to dryness. The residue was purified by ODS TLC (RP-18, H_2O/MeCN=4:1, 0.5% AcOH) and HPLC with COSMOSIL 5C_{18}-AR column (∅20.0×250 mm, H_2O/CH_3CN=3:1) to afford **27** (12 mg, 18.2 μmol, 30% in two steps) as a colorless viscous oil: $[\alpha]_D^{22}$ +33.4 (*c* 0.92, MeOH). IR (film) *ν*: 3317, 2934, 1650, 1534, 1369, 1254, 1168, 1225, 1073, 1030 cm^{-1}. ^1H NMR (300 MHz, CD_3OD, 23 °C) *δ* 5.52 (1H, dt, *J*=10.8, 6.9 Hz), 5.41 (1H, dt, *J*=10.8, 6.9 Hz), 4.20 (1H, d, *J*=7.2 Hz), 3.88–3.82 (5H, m), 3.75–3.72 (3H, m), 3.58–3.39 (6H, m), 2.66 (1H, dd, *J*=8.4, 19.5 Hz), 2.44–1.90 (10H, m), 1.49 (1H, m), 1.44 (9H, m); ^{13}C NMR (75 MHz, CD_3OD, 22 °C) *δ* 222.0, 173.8, 172.3, 172.1, 158.8, 129.1, 128.9, 104.9, 81.0, 74.7, 73.9, 72.4, 70.3, 70.2, 55.2, 44.9, 43.9, 43.5, 41.0, 40.6, 39.4, 38.7, 29.1, 28.7, 28.3, 26.4; ESI-HRMS (positive): $[M+Na]^+$ found *m/z* 681.2950, $C_{29}H_{46}N_4O_{13}Na$ requires *m/z* 681.2954.

2.1.2.6. (1*R*,2*S*,2′*Z*)-2-[5′-(6″-(*N*-Fluorescein-4-isothiocyanatoglycylglycylglycylamino)-β-D-glucopyranosyloxy)-2′-pentenyl]-3-oxo-cyclopentaneacetic acid (negative probe) 4. Compound **27** (9 mg, 13.7 μmol) in TFA (0.4 mL) was mixed for 3 min at rt. This solution was evaporated to give a TFA salt of resulting amine. The TFA salt was dissolved in DMF (0.5 mL) with TEA (7 μL), and then FITC (6.4 mg, 16.4 μmol) was added to the solution at 0 °C. After stirring overnight at rt, the reaction mixture was evaporated to dryness. The residue was purified by ODS TLC (RP-18, H_2O/MeCN=4:1 containing 0.5% AcOH) to afford **4** (12 mg, 12.7 μmol, 93% in two steps) as yellow viscous oil. Neutralization with 0.1 mM $KHCO_3$ aq (254 μL, 25.4 μmol) gave **4** (13 mg as a potassium salt, quant) as an orange crystal: $[\alpha]_D^{27}$ +14.4 (*c* 0.12, MeOH). IR (film) *ν*: 3293, 2930, 1637, 1576, 1559, 1465, 1394, 1331, 1111 cm^{-1}. ^1H NMR (600 MHz, D_2O, 23 °C) *δ* 7.79 (1H, br d), 7.64 (1H, d, *J*=7.7 Hz), 7.21 (2H, m), 7.16 (1H, m), 6.67 (2H, d, *J*=7.7 Hz), 6.63 (2H, m), 5.43 (1H, dt, *J*=10.9, 6.8 Hz), 5.37 (1H, dt, *J*=10.9, 6.8 Hz), 4.30 (3H, m), 3.99 (2H, s), 3.94 (1H, d, *J*=16.8 Hz), 3.89 (1H, d, *J*=16.8 Hz), 3.83–3.79 (2H, m), 3.67 (1H, dd, *J*=5.5, 7.8 Hz), 3.59–3.55 (2H, m), 3.46–3.38 (3H, m), 2.52 (1H, dd, *J*=4.9, 13.9 Hz), 2.36–2.21 (6H, m), 2.16–2.09 (3H, m), 1.97 (1H, m), 1.48 (1H, m); ^{13}C NMR (150 MHz, D_2O, 22 °C) *δ* 227.4, 181.9, 181.7, 177.0, 173.1, 172.4, 171.5, 158.2, 140.8, 148.8, 131.7, 131.0, 129.5, 128.5, 127.7, 125.9, 124.8, 121.9, 114.2, 103.6, 102.8, 72.8, 70.8, 69.8, 69.0, 54.5, 47.7, 43.0, 42.6, 39.9, 38.7, 38.1, 27.5, 27.0, 26.8, 25.1; ESI-HRMS (negative): $[M-2K+H]^-$ found *m/z* 946.2830, $C_{45}H_{48}N_5O_{16}S$ requires *m/z* 946.2822.

2.2. Bioassay

The young leaves of the plant *A. saman*, which was grown in the biotron (Nippon Medical & Chemical Instrumentals Co., Ltd) of Tohoku University, were used for bioassay. The young leaves were detached from the stem of *A. saman* with a sharp razor blade. One leaf was placed in H_2O (ca. 300 μL) using a 20-mL glass tube in the biotron kept at 32 °C and allowed to stand overnight. The leaves which opened again in the morning were used for the bioassay. Each test solution was carefully poured into test tubes with a micropipette around 10:30 a.m. Then, the leaves immersed in the sample solution was put in a bell funnel, and decompressed by an aspirator. The sample solution was pumped up through the vessel to the stroma by compulsion. The bioactive fraction made the leaves close within a few minutes in this bioassay. Other nyctinastic plants, *A. julibrissin*, *C. mimosoides*, *P. urinaria*, and *Leucaena leucocephala*, which were used in the bioassay, were also grown in the biotron of Tohoku University.

2.3. Fluorescence study using a fluorescence microscope

The leaf of *A. saman* opening in the morning was cut in an appropriate size and fixed in agar. The agar was sliced perpendicular to the petiole by a microslicer (DSK-1000, Dousaka EM Co., Ltd) to a thickness of 30 μm and the sections containing the pulvini were floated on distilled water. The sections were immersed in a solution containing the various concentration of fluorescence-labeled probe

compounds **3** and **4**, respectively, and allowed to stand for staining under shielded condition at rt for 4.5 h. After staining, the sections were washed by incubation with 0.1 M phosphate buffer (pH 7) for 10 min. This section was placed on a slide glass and covered by a cover glass after adding a drop of antifade reagent (Slow Fade™ Gold Antifade Reagent, Molecular Probes Inc.). The observation of these sections was carried out by using ECLIPSE E800 microscope (Nikon, Co., Ltd) equipped with VFM fluorescence instrument. The B-2A filter (Nikon CO., Ltd; excitation wavelength 450–490 nm) was used for FITC. The plant sections of other nyctinastic plants *A. julibrissin*, *C. mimosoides*, *P. urinaria*, and *L. leucocephala* were prepared similarly and treated with fluorescence-labeled probe compounds in the same procedure.

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Carbenoid rearrangement of *gem*-dihalogenospiropentanes

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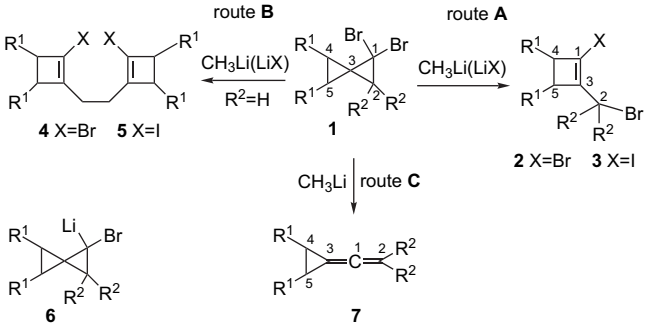
Abstract—A skeletal rearrangement of dihalogenospiropentanes in the presence of alkyllithium reagents has been systematically studied using a number of *gem*-dibromospiropentanes. The scope and limitations of this carbenoid rearrangement are outlined and its mechanism is discussed.

1. Introduction

In 1992 we reported the first example of the skeletal rearrangement of dibromospiranes of type **1** in the presence of MeLi.[1] The general pathways of this new rearrangement are shown in Scheme 1 (routes **A**, **B**). To the best of our knowledge, three short publications appeared afterward, where the products of the same rearrangement were documented.[2,3] The mechanism of this rearrangement was unclear and it was not discussed. The scope and limitations of this rearrangement remained unknown as well.



Scheme 1.

In principle, the reaction of dibromides **1** with MeLi proceeds to give the corresponding allenes **7** (route **C**, Scheme 1) as major products. However, lowering the reaction temperature up −55 °C leads to the rearrangement giving either cyclobutenes **2** (route **A**, Scheme 1) or compounds of type **4** (route **B**, Scheme 1) depending on the substituents in the cyclopropane ring. If starting methyllithium had been prepared from methyl iodide, the corresponding iodides **3** and/or **5** were also isolated. It was supposed that 'monomeric' rearranged products of types **2**, **3** are the intermediates in the formation of 'dimeric' products of types **4**, **5**.[1,2]

The main unusual feature of this rearrangement is to be emphasized: while the transformation **1**→**2** resembles rather *carbocationic* rearrangement (note also the formation of iodides **3**, **5** in the presence of LiI), the reaction conditions used suppose the intermediate formation of Li–C–Br carbenoid (**6**). Generally carbenoids have an *anionic* nature.[4,5] In other words, we deal with a formally cationic rearrangement in Li-carbenoid **6**, which undergoes nucleophilic attack during this process.

Being still not generally recognized, this controversial situation was nevertheless documented in the literature (e.g., "it was and still is indeed remarkable that… carbenoids (anions!) are electrophilic enough to react with rather weakly nucleophilic bonds"[5c]). Such electrophilic ability of different carbenoids has been evidently disclosed and carefully reviewed.[5] Especially noteworthy is that carbenoids of Li–C–Br type can react with such nucleophiles as C–H, C=C, and C–C bonds; the last reaction, which is still a rather rare case, represents the skeletal rearrangements in carbenoids.[5–8]

In this paper, we study the above mentioned carbenoid rearrangement systematically, using a number of substituted spiranes of type **1** to demonstrate the general character of this process, to trace the position of substituents in starting versus final materials, and to put forward some general conclusions about mechanism.

Keywords: *gem*-Dihalogenospiropentanes; Carbenoid rearrangement; Alkyllithium reagents; Bromocyclobutenes.
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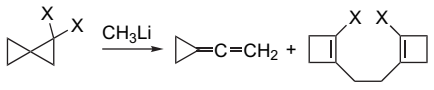
2. Results

In previous works it was found[1,2] that lowering the temperature up to −55 °C favors the formation of the rearrangement products **2** or **4** (Scheme 1) and disfavors the formation of allenes **7** (route **C**, Scheme 1). Also, the reaction of dibromospiropentanes **1** with MeLi contaminated with LiI at elevated temperatures favors both the formation of allene **7** as well as *iodides* **3**, **5** and simultaneously the yields of the rearranged products decrease.[1] For example, we isolated the rearranged iodide **5** [R^1,R^2=H, R^1,R^1=–$(CH_2)_5$–] in the reaction of 10,10-dibromotricyclo-[7.1.0.$0^{1,3}$]decane [**1**, R^1,R^2=H, R^1,R^1=–$(CH_2)_5$–] at −5 °C in only 3% yield, the corresponding bicyclo[7.1.0]deca-1,2-diene being the major product (30% yield).[2]

Other reactions also proceeded accordingly (Scheme 1): the dibromide **8a** reacts with MeLi (LiI) at −55 °C to give allene **9** (10%), rearranged dimeric dibromide **10a** (64%), and rearranged diiodide **10b** (5%). Increasing the temperature of the same reaction up to 0–5 °C gave the same products but in the ratio **9**:**10a**:**10b**=30%:4%:17%. Thus, at higher temperatures the yields of dibromide **10a** were lowered and the yield of allene **9** and diiodide **10b** was increased substantially.



8a, X=Br
8b, X=I

9

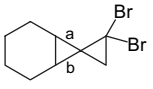
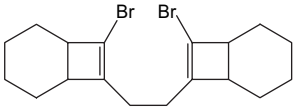
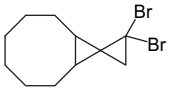
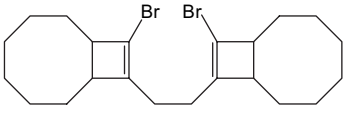
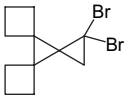
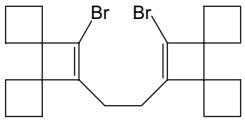
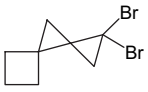
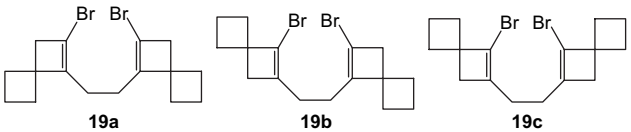
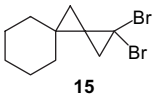
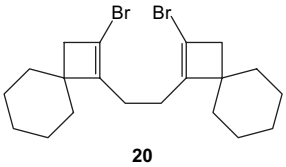
10a, X=Br
10b, X=I

It is of interest that a dramatic increase of the content of LiI in MeLi up to 5 equiv in the reaction run at −55 °C gave only unchanged starting dibromide **8a**. This result is in accordance with the data about lower reactivity of MeLi·LiI complex.[9] On the other hand, the reaction of diiodide **8b** with pure MeLi, not contaminated by LiI, gave rearranged diiodide **10b** in a good yield (45%).

Because the separation of bromides and iodides (in pairs **2/4** and **3/5**) is a difficult task, we used in this work the commercial MeLi, which was not contaminated with LiI or LiBr. All further reactions of dibromospiropentanes (**1**) were performed at −55 °C because at this temperature the ratio of corresponding allenes **7** to rearranged products (**2**, **4**) is minimal. The products composition in the reactions of some dibromides **1** with MeLi, *n*-BuLi, or *t*-BuLi was approximately the same. Allenes were not isolated as individual compounds but their yields were clearly determined by NMR spectroscopy. All products of rearrangement were isolated by preparative column chromatography and characterized by ^{1}H and ^{13}C NMR, mass spectra, and elemental analysis.

The results obtained with substituted dibromospiranes of type **1** (**11**–**15**) where R^2=H are summarized in Table 1. As expected, the reactions of **11**–**15** with MeLi proceeded to give mixtures of the corresponding allenes (10–25% yields) and the 'dimeric' products of rearrangement (**16**–**20**) (see Table 1). For dibromides **11**–**13**, having one symmetrically substituted cyclopropane ring (in a sense that

Table 1. Results of the reactions of dibromides **11**–**15** with MeLi

Starting dibromide	Products of rearrangement	Yield (%)
 **11**	 **16**	44
 **12**	 **17**	45
 **13**	 **18**	76
 **14**	 **19a** **19b** **19c**	50
 **15**	 **20**	75

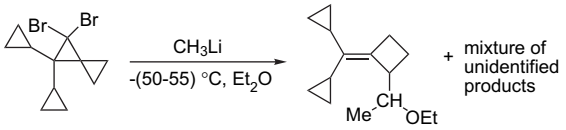
bond **a** is equivalent to bond **b**, see **11**), we isolated single rearranged products **16**–**18**, correspondingly. This is supported by the presence of signals of only two disubstituted C=C atoms in ^{13}C NMR at δ 109–117 and δ 143–155 ppm.

For dibromides **14** and **15** possessing non-equivalent bonds **a** and **b**, which may undergo migration one may expect the formation of three 'dimeric' compounds (two 'homodimeric' and one 'heterodimeric'). In principle, the most substituted bond has to be the most nucleophilic. Previously, we isolated the rearranged iodide **5** [R^1,R^2=H, R^1,R^1=–$(CH_2)_5$–] in the reaction of 10,10-dibromotricyclo[7.1.0.0^{1,3}]decane [**1**, R^1,R^2=H, R^1,R^1=–$(CH_2)_5$–], which supports migration of the monosubstituted bond.[2] However, the yield of the rearrangement product in this case is extremely small (3%) and this example cannot be generalized.

As can seen from Table 1, for the case of dibromide **14** we have observed exactly three rearranged products **19a**–**c**. Contrary to that for the case of dibromide **15** we isolated the single rearranged product **20** due to bond **a** migration. Thus, the data about the migration ability of bonds **a** versus **b** are still contradictory.

Next, dibromides of type **1**, which contain internal dibromospirane framework (**1**, where $R^2 \neq H$) (e.g., **21**) were studied. We have investigated first the tetracyclic dibromide **21** in the reaction with MeLi. The dibromide **21** contains two bonds, **a** and **b**, which presumably are able to migrate, being incorporated in spirosystem of type **1**. We have found that the rearrangement proceeds to give the only 'monomeric' product of type **2**: this is clear from the mass spectra of the obtained dibromide, which contained a triplet of molecular peak with *m*/*z* 332, 334, and 336. Moreover, the obtained product has definitely the structure of **22**, confirmed by its NMR spectra. The NMR spectrum of **22** has only one set of signals expected for one isomer from two possible. Two signals of CH-groups at δ 48.6 and δ 51.0 ppm are observed and they confirm the presence of the 3,4-disubstituted cyclobutene fragment. The value of coupling constants $^1J_{CH}$ of CH-group of cyclobutene fragment is assigned to coupling constants for four-membered ring ($^1J_{CH}$ 141 and 139 Hz).[10] Two signals of CH_2-groups at δ 13.8 and δ 17.4 ppm have large C–H coupling constants ($^1J_{CH}$ 165 and 164 Hz), which are typical for cyclopropanes and can be assigned to a 1,1-disubstituted cyclopropane fragment.[10] Thus, this rearrangement proceeds with the migration of the bond **a** of the substituted three-membered ring to give rearrangement dibromide **22**.

Then, we investigated the tetracyclic dibromide **24** in the reaction with MeLi. The dibromide **24** contains unsubstituted cyclopropane rings incorporated in the spirosystem of type **1**, which was shown to be inert in the case of dibromide **24**. This reaction gave a complex mixture of products; the major of them was identified as the ether **25**, which was formed due to capture of solvent molecule by some carbene (carbenoid) intermediate. Compound **25** is rather unstable and can be characterized only by spectroscopy.



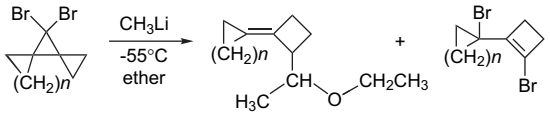
24 **25**

In order to clarify the formation of ether **25**, we note that carbenes, including the cyclopropylidene, can form the products of intermolecular insertion into α-C–H-bond of the solvent, i.e., ether.[11–13] Thus, isolation of the ether **25** might indicate the transient formation of the corresponding methylenecyclobutylidene in the process (vide infra), which is able to react with the diethyl ether solvent.

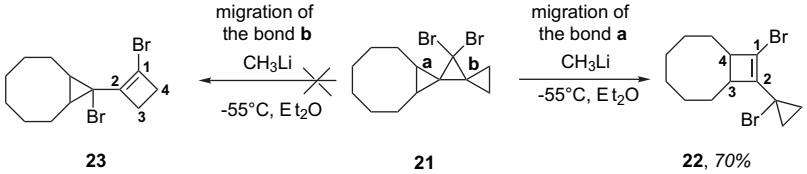
The behavior of tricyclic dibromide **26a** was also investigated in this rearrangement. By analogy with dibromide **24** one may expect either the formation of the ether **27a** or rearranged dibromide type **2**. However, the previous literature data have shown that the treatment of dibromide **26a** by MeLi or *t*-BuLi (−45 °C) provided a mixture of eight different products with the formation of ether **27a** as the major one (up to 40%).[13] In this reaction bromo containing compounds were obtained; however, they are not the product of dibromospiropentane rearrangement or related compounds, which could have been obtained by its dehydrobromination.

Thus, we decided to reinvestigate the reaction of dibromide **26a** with MeLi at −55 °C. The product **27a** was obtained in a high yield (89%) under these conditions. To our surprise, we did not observe other products in this reaction.

We should mention here also that this result is in contrast with behavior of dibromide **26b**, which did indeed give the rearranged dibromide **27b** in high yield (77%).[1,14]



26a *n*=4
26b *n*=1 **27a** *n*=4 **27b** *n*=1

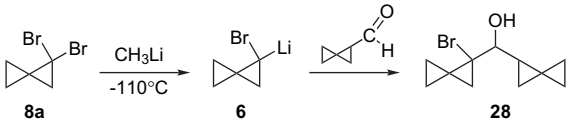


23 **21** **22**, *70%*

3. Discussion

The first conclusion, which can be derived from the results obtained, that this rearrangement under investigation, which was observed by us previously only for a few cases,[1,2] has in fact the general character, and it was established now for a variety of *gem*-dihalogenated spirocyclopropanes of type **1**. This is remarkable because there are many publications concerning the reactions of dibromides of type **1** with Alk-Li, where the rearranged products were not observed.[1b,15] This may be explained either by inconvenient conditions for the rearrangement or by loss of the minor rearranged product/s during purification.

The mechanistic rationalization of the whole process can be presented as follows (Scheme 2). For clarity we will use pure carbocationic stepwise presentation, thus, ignoring the problem of concerted mechanistic steps. First step of the reaction of dibromides of type **1** with MeLi seems to lead to the formation of lithium carbenoid of type **6** via halogenophilic attack. While this step is well documented in literature,[11,12,16] we decided to prove unambiguously the intermediate of Li-carbenoid **6** as well as its anionic character. We have found that the addition of cyclopropyl aldehyde to the intermediate **6** (R=R^1=H) leads to its trapping with the formation of alcohol **28**.[17]



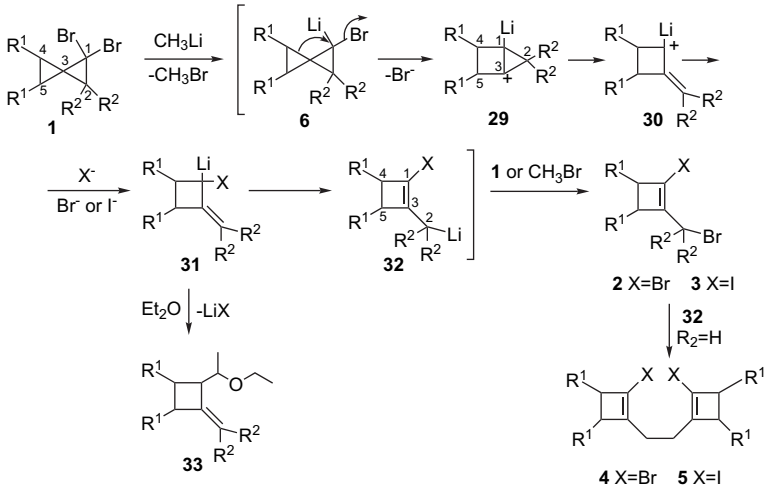
Next logical step is the manifestation of electrophilic nature of intermediate carbenoid **6**, which means principle acceptance of nucleophilic attack on carbenoid center.[5]

It means that we assume the second step of the whole process as **6**→**29**, in which the C–C bond of second three-membered ring (being enough nucleophilic as compared with normal C–C bonds) acts as a nucleophile leading to an S_N2-like displacement of the leaving group –Br from the electrophilic carbenoid carbon atom to give cation **29**. The driving forces for this step could be release of cyclopropane strain.

Geometry of this cation is not very favorable and it subsequently rearranges into cation **30**. It is to be noted that analogous pure carbocationic rearrangement during the deamination of aminospiro[2.2]pentane with intermediacy of unsubstituted cations of types **29** and **30** was observed.[18] However, the introduction of structure **30** is feasible but still risky.[18,19] Lithium being extremely electron donor substituent should stabilize the carbocationic center, but we do not know any theoretical calculations or experimental evidences for such type of Li-substituted cations. On the other hand, the acceptance of intermediate cation **30** permits to explain competitive participation of such external nucleophile as I^- in the next step of the reaction, **30**→**31**. Indeed, this step leads to the formation of a new carbenoid **31**, where X=Br or I.

Depending on the structure, this carbenoid **31** plays role as an intermediate for two ramificated pathways (Scheme 2). One of them is the insertion reaction into C–H bond of the ether used as solvent, which gives the compounds of type **33** (above mentioned **25**, **27a**). This insertion probably proceeds via formation of the corresponding carbene, namely substituted methylenecyclobutylidene. As it was mentioned above, it is well known that carbenes demonstrate the ability to insertion reactions into C–H and C–C bonds.[20] Also, it is known that cyclopropylidene, which is obtained from dibromocyclopropanes under treatment with alkyllithium reagents under temperatures above −80 °C, exhibits also the insertion into C–H and C–C bonds.[11,12]

Second route of the transformation of carbenoid **31** leads to 'dimeric' products of types **4** and **5**. One feasible explanation can be the following. This route includes several steps, the first being the following. The carbenoid **31**, which is lithium allylic derivative, may be prone to [1,3]-sigmatropic migration of C–Li bond to give the lithium derivative **32**. The sigmatropic tautomerism of allyllithium derivatives (or even acceptance of pure ionic structure) is well documented in literature (see for example Ref. 21). In turn, Li-derivative **32** can compete with starting MeLi in halogenophilic attack on dibromide **1** to give the carbenoid **6** and bromide **2** (or **3**; Scheme 2).

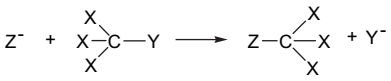


Scheme 2.

Further transformations of bromide **2** or **3** (Scheme 2) crucially depend on the nature of substituents R^2. In the case of $R^2 \neq H$ the *tert*-C–Br fragment is non-reactive may be due to steric reasons and the bromide/s can be isolated. In contrast, if R^2=H, the primary C–Br reacts with Li-derivative **32** to give coupling 'dimeric' products **4** or **5**. An example of this route in X-philic reactions has been illustrated in literature.[22,23]

Thus, the suggested mechanistic rationalization of the discovered rearrangement permits to explain the whole course of this process. The principle point of suggested scheme is the acceptance of carbocationic pathways in Li-carbenoid intermediates.

Some points discussed above may have more general value and can be generalized to be a guideline for future studies. Why the concept of ability of carbenoids of type Li–C–Br (*anions*: Li–C–Br↔Li^+ $^-$C–Br) to undergo *nucleophilic* attack, having been clearly documented by experiments, still has not acquired general understanding? Because these facts were not treated by a general theory of nucleophilic substitution. Indeed, while there exist endless numbers of publications concerning the detailed theoretical description of the process of nucleophilic substitution (Eq. 1), one point (to the best of our knowledge) is not clear enough: namely, the *influence of X-substituents on this process for the whole range of possibilities*.



(1)

Two opposite limiting cases can be roughly outlined. The increasing of X-electronegativities should lead to a decrease in the rate of S_N2-substitution and, in extreme case, has to change the reactivity mode to SET or X-philic pathway.[22–24]

In turn, the decrease in X-electrophilicities should lead to the increase in the rate of S_N2-substitution. Strong stabilization may lead also to a change of mode reactivity (e.g., from S_N2 to S_N1 pathway). The questions may be posed: which mode will be the case with X=Li or MgX, or another related extreme donor? Which kind of new reactivity pathway can be detected?

In this connection the structure of Li-carbenoid **6**, may be considered as an example of such limiting case. In summary the theoretical investigation of the process of Eq. 1 for the whole range of X-electronegativities including limiting cases may be useful in understanding where and why one can expect existence of unusual pathways, leading to unusual reactions.

4. Experimental

4.1. General

NMR spectra were recorded on a 'Bruker DPX-400' spectrometer (400.13 and 100.62 MHz, for ^1H and ^{13}C, respectively) at room temperature; the chemical shifts δ were measured in parts per million with respect to solvent (^1H: $CDCl_3$, δ=7.24 ppm; ^{13}C: $CDCl_3$, δ=77.13 ppm). Mass spectra were taken on Finnigan MAT 95 XL (70 eV) using electron impact ionization (EI) and GC–MS coupling. Microanalyses were performed on a Karlo Erba 1106 instrument. Analytical thin-layer chromatography (TLC) was carried out with Silufol silica gel plates (supported on aluminum); the revelation was done by UV lamp (254 and 365 nm) and chemical staining (iodine vapor). Melting points (mp) were determined on a Electrothermal 9100 capillary apparatus and are uncorrected. Column chromatography was performed using silica gel 60 (230–400 mesh, Merck). Petroleum ether (PE) used refers to the 40–60 °C boiling point fraction. All reagents, except commercial products of satisfactory quality were purified with literature procedures prior to use. Starting compounds: methylenecyclopropane,[25] 1-methylenespiro[2.3]hexane,[26] 1-methylenespiro[2.5]octane,[27] 9-methylenedispiro[3.0.3.1]nonane,[28] 7-methylenebicyclo[4.1.0]heptane,[29] 9-methylenebicyclo-[6.1.0]nonane,[29] 9-cyclopropylidenebicyclo[6.1.0]nonane,[30] cyclopropylidenecyclohexane,[31] 1,1′,1″-methanediylylidenetricyclopropane,[32] and 1,1-dibromospiro[2.2]pentane (**8a**)[33] were synthesized by known procedures.

4.1.1. 1,1-Diiodospiro[2.2]pentane (8b).[34] To a stirred solution of *t*-BuOK (3.2 g, 28 mmol) and olefin (5.4 g, 7.5 mL, 100 mmol) in *t*-BuOH (30 mL) at −30 °C under argon, iodoform (10.0 g, 25 mmol) was added. After 1 h the resulting mixture was allowed to −(5–10) °C and then, after 6 h, quenched with cold water (50 mL). The aqueous layer was extracted with petroleum ether (3×20 mL) and combined organic layers were dried over anhydrous $MgSO_4$ and concentrated in vacuo. The crude product were purified by column chromatography (silica gel, petroleum ether). Yield: 4.42 g (55%), yellow oil, R_f=0.75 (petroleum ether). ^1H NMR: δ 1.01–1.07 (m, 2H), 1.42–1.49 (m, 2H), 2.22 (s, 2H). ^{13}C NMR: δ −55.8 (CI_2), 16.3 (J=169, 2×CH_2), 22.0 (C), 32.2 (J=167, CH_2). MS (EI, 70 eV) *m*/*z* (rel. int., %): 320 (9) $[M]^+$, 193 (53) $[M-I]^+$, 165 (14), 127 (7), 66 (100).

4.2. General procedure 1. Preparation of the substituted *gem*-dibromospiropentanes 11–15, 21, 24, 26a, 26b

To a stirred mixture of *t*-BuOK (4.3 g, 38 mmol) and olefin (19 mmol) in petroleum ether (15 mL) at 0 °C under argon, a solution of bromoform (5.82 g, 2 mL, 23 mmol) in petroleum ether (5 mL) was added dropwise. After 20 min the resulting mixture was slowly allowed to warm to room temperature and then, after 4–48 h, quenched with cold water (40 mL). The aqueous layer was extracted with Et_2O (3×20 mL) and combined organic layers were dried over anhydrous $MgSO_4$ and concentrated in vacuo. The crude dibromides were purified by distillation.

4.2.1. 2′,2′-Dibromospiro[bicyclo[4.1.0]heptane-7,1′-cyclopropane] (11). Reaction mixture was stirred for 4 h. Yield: 2.71 g (51%), white solid, mp 49 °C, bp 62–65 °C/2 mmHg. ^1H NMR: δ 1.10–1.38 (m, 4H), 1.39–1.52 (m, 2H), 1.58–1.63 (m, 2H), 1.81 (s, 2H, cy-Pr), 1.81–1.95 (m, 2H). ^{13}C NMR: δ 21.0 (2×CH, cy-Pr), 21.3 (2×CH_2, cy-Hex), 21.7 (2×CH_2, cy-Hex), 26.2 (CH_2, cy-Pr), 32.3

(C), 35.6 (CBr_2). Anal. Calcd for $C_9H_{12}Br_2$: C, 38.61; H, 4.32%. Found: C, 38.56; H, 4.00%.

4.2.2. 2′,2′-Dibromospiro[bicyclo[6.1.0]nonane-9,1′-cyclopropane] (12). Reaction mixture was stirred for 6 h. Yield: 3.75 g (64%), white solid, mp 61.5 °C, bp 95–98 °C/2 mmHg. ^{1}H NMR: δ 1.01–1.19 (m, 2H, 2×CH, cy-Pr), 1.39–1.56 (m, 6H), 1.58–1.69 (m, 2H), 1.70–1.78 (m, 2H), 1.75 (s, 2H, cy-Pr), 1.79–1.89 (m, 2H). ^{13}C NMR: δ 24.7 (2×CH_2, cy-Oct), 25.2 (2×CH, cy-Pr), 26.5 (2×CH_2, cy-Oct), 26.7 (2×CH_2, cy-Oct), 28.7 (CH_2, cy-Pr), 30.6 (C), 35.8 (CBr_2). Anal. Calcd for $C_{11}H_{16}Br_2$: C, 42.89; H, 5.24%. Found: C, 42.66; H, 5.50%.

4.2.3. 1,1-Dibromotrispiro[2.0.3^4.0.3^8.0^3]undecane (13). Reaction mixture was stirred for 6 h. Yield: 3.66 g (63%), colorless liquid, bp 85 °C/2 mmHg. ^{1}H NMR: δ 1.79 (s, 2H, cy-Pr), 1.80–1.89 (m, 2H), 1.92–2.16 (m, 8H), 2.50–2.62 (m, 2H). ^{13}C NMR: δ 16.2 (J=138, 2×CH_2, cy-Bu), 21.9 (J=141, 2×CH_2, cy-Bu), 25.3 (J=141, 2×CH_2, cy-Bu), 27.2 (J=166, CH_2, cy-Pr), 31.9 (2×C), 36.0 (C), 36.4 (C). Anal. Calcd for $C_{11}H_{14}Br_2$: C, 43.17; H, 4.61%. Found: C, 43.07; H, 4.68%.

4.2.4. 1,1-Dibromodispiro[2.0.3.1]octane (14). Reaction mixture was stirred for 4 h. Yield: 4.19 g (83%), colorless liquid, bp 55–56 °C/2 mmHg. ^{1}H NMR: δ 1.05 (d, ^2J=5.2, 1H, cy-Pr), 1.15 (d, ^2J=5.2, 1H, cy-Pr), 1.83 (d, ^2J=6.2, 1H, cy-Pr), 1.98 (d, ^2J=6.2, 1H, cy-Pr), 1.88–2.24 (m, 5H), 2.71–2.82 (m, 1H). ^{13}C NMR: δ 17.0 (J=136, CH_2, cy-Bu), 21.7 (J=162, CH_2, cy-Pr), 25.8 (J=136, CH_2, cy-Bu), 28.3 (J=166, CH_2, cy-Pr), 29.3 (J=137, CH_2, cy-Bu), 31.3 (C), 33.1 (C), 33.4 (C). Anal. Calcd for $C_8H_{10}Br_2$: C, 36.13; H, 3.79%. Found: C, 36.46; H, 3.90%.

4.2.5. 1,1-Dibromodispiro[2.0.5.1]decane (15). Reaction mixture was stirred for 6 h. Yield: 2.90 g (52%), colorless liquid, bp 92–93 °C/2 mmHg. ^{1}H NMR: δ 0.84 (d, ^2J=4.5, 1H, cy-Pr), 0.95 (d, ^2J=4.5, 1H, cy-Pr), 1.15–1.41 (m, 2H), 1.42–1.66 (m, 6H), 1.74–1.88 (m, 2H), 1.79 (d, ^2J=6.2, 1H, cy-Pr), 1.92 (dd, ^2J=6.2, ^4J=0.8, 1H, cy-Pr). ^{13}C NMR: δ 22.8 (J=164, CH_2, cy-Pr), 25.3 (CH_2), 25.7 (CH_2), 26.0 (CH_2), 27.7 (J=165, CH_2, cy-Pr), 30.4 (C), 32.2 (C), 32.3 (CH_2), 34.0 (CH_2), 36.2 (CBr_2). Anal. Calcd for $C_{10}H_{14}Br_2$: C, 40.85; H, 4.80%. Found: C, 40.87; H, 4.75%.

4.2.6. 3′,3′-Dibromodispiro[bicyclo[6.1.0]nonane-9,1′-cyclopropane-2′,1″-cyclopropane] (21).[34] Reaction mixture was stirred for 16 h. Yield: 0.44 g (44%), colorless oil, R_f=0.6 (petroleum ether). ^{1}H NMR: δ 0.77–0.91 (m, 4H), 1.05–1.79 (m, 12H), 1.80–1.89 (m, 2H). ^{13}C NMR: δ 9.0 (2×CH_2), 23.8 (2×CH_2), 26.4 (2×CH_2), 27.7 (2×CH), 28.8 (2×CH_2), 29.7 (C), 31.9 (C), 41.5 (C). MS (EI, 70 eV) m/z (rel. int., %): 336 (0.2), 334 (0.6), 332 (0.2) [M]$^+$, 240 (30), 238 (60), 236 (32), 173 (40), 171 (44), 159 (35), 157 (33), 131 (58), 117 (58), 91 (100).

4.2.7. 1,1-Dibromo-2,2-dicyclopropylspiro[2.2]pentane (24).[35] Reaction mixture was stirred for 24 h. Yield: 2.67 g (46%), colorless liquid, bp 92–95 °C/2 mmHg. ^{1}H NMR: δ 0.18–0.26 (m, 2H), 0.41–0.49 (m, 2H), 0.52–0.68 (m, 4H), 0.91–0.99 (m, 2H), 1.01 (br s, 4H). ^{13}C NMR: δ 2.9 (J=161, 2×CH_2), 3.6 (J=161, 2×CH_2), 10.0 (J=165, 2×CH_2), 15.8 (J=160, 2×CH), 31.7 (C), 35.8 (C), 49.1 (CBr_2). Anal. Calcd for $C_{11}H_{14}Br_2$: C, 43.17; H, 4.61%. Found: C, 43.21; H, 4.57%.

4.2.8. 10,10-Dibromodispiro[2.0.5.1]decane (26a).[13] Reaction mixture was stirred for 48 h. Yield: 4.24 g (76%), white solid, mp 50 °C, R_f=0.8 (petroleum ether). ^{1}H NMR: δ 0.98–1.05 (m, 2H), 1.11–1.17 (m, 2H), 1.33–1.62 (m, 6H), 1.67–1.81 (m, 4H). ^{13}C NMR: δ 9.9 (2×CH_2), 25.0 (2×CH_2), 25.6 (CH_2), 33.7 (2×CH_2), 33.7 (C), 35.3 (C), 49.6 (CBr_2). Anal. Calcd for $C_{10}H_{14}Br_2$: C, 40.85; H, 4.80%. Found: C, 40.59; H, 5.01%.

4.2.9. 7,7-Dibromodispiro[2.0.2.1]heptane (26b).[33] Reaction mixture was stirred for 5 h. Yield: 2.92 g (61%), white solid, mp 71 °C, bp 75–76 °C/8 mmHg. ^{1}H NMR: δ 1.07–1.13 (m, 4H), 1.23–1.29 (m, 4H). ^{13}C NMR: δ 11.2 (4×CH_2), 31.9 (C), 40.7 (CBr_2).

4.3. General procedure 2. Reaction of the substituted *gem*-dihalogenospiropentanes 8a, 8b, 11–15, 21, 24, 26a, 26b with alkyllithium

To a stirred solution of *gem*-dihalogenospiropentanes (3.3 mmol) in Et_2O (10 mL) at −(55–60) °C under argon, methyllithium (solution in Et_2O, 2.75 mL, 1.6 M, 4.4 mmol) was added dropwise for 45 min. After 1 h the resulting mixture was slowly allowed to 0 °C and then quenched with cold water (20 mL). The aqueous layer was extracted with Et_2O (3×10 mL) and combined organic layers were dried over anhydrous $MgSO_4$ and concentrated in vacuo. The crude products were purified by column chromatography (silica gel, petroleum ether).

The reaction of 1,1-dibromospiro[2.2]pentane (**8a**) (0.75 g, 3.3 mmol) with butyllithium (solution in hexane, 2.75 mL, 1.6 M, 4.4 mmol) or *tert*-butyllithium (solution in pentane, 2.93 mL, 1.5 M, 4.4 mmol) proceeds according to the method described above.

4.3.1. 1,1′-Ethane-1,2-diylbis(2-bromocyclobutene) (10a).[1a] Yield in reaction **8a** with methyllithium: 0.29 g (60%); yield in reaction **8a** with butyllithium: 0.28 g (58%); yield in reaction **8a** with *tert*-butyllithium: 0.16 g (34%); colorless oil, R_f=0.8 (petroleum ether). ^{1}H and ^{13}C NMR data for the **10a** are the same as those reported in literature.[1a]

4.3.2. 1,1′-Ethane-1,2-diylbis(2-iodocyclobutene) (10b).[34] Yield: 0.29 g (45%), colorless oil, R_f=0.6 (petroleum ether). ^{1}H NMR: δ 2.09 (s, 4H), 2.70 (s, 8H). ^{13}C NMR: δ 27.5 (J=129, 2×CH_2), 34.4 (J=142, 2×CH_2), 36.4 (J=143, 2×CH_2), 83.5 (CI_2), 156.1 (C). MS (EI, 70 eV) m/z (rel. int., %): 386 (21) [M]$^+$, 259 (45) [M−I]$^+$, 231 (8), 245 (81), 132 (94), 131 (100), 117 (68), 91 (55), 77 (20), 65 (9), 51 (8), 39 (7).

4.3.3. 7,7′-Ethane-1,2-diylbis(8-bromobicyclo[4.2.0]oct-7-ene) (16). Yield: 0.29 g (44%), colorless oil, R_f=0.6 (petroleum ether). ^{1}H NMR: δ 1.31–1.75 (m, 16H), 2.05–2.39 (m, 4H), 2.83–3.05 (m, 4H). ^{13}C NMR: δ 17.7 (2×CH_2), 18.1 (CH_2), 18.2 (CH_2), 22.5 (2×CH_2), 23.2 (CH_2),

23.2 (CH_2), 24.1 (CH_2), 24.3 (CH_2), 40.8 (CH), 41.1 (CH), 45.5 (2×CH), 113.5 (C), 113.6 (C), 149.7 (C), 149.9 (C). Anal. Calcd for $C_{18}H_{24}Br_2$: C, 54.02; H, 6.04%. Found: C, 54.05; H, 6.04%.

4.3.4. 9,9′-Ethane-1,2-diylbis(10-bromobicyclo[6.2.0]-dec-9-ene) (17). Yield: 0.34 g (45%), colorless oil, R_f=0.6 (petroleum ether). ^{1}H NMR: δ 1.22–1.48 (m, 16H), 1.53–1.79 (m, 8H), 2.03–2.25 (m, 4H), 2.65–2.73 (m, 2H), 2.75–2.82 (m, 2H). ^{13}C NMR: δ 23.1 (CH_2), 23.4 (CH_2), 25.2 (4×CH_2), 25.9 (2×CH_2), 26.3 (2×CH_2), 29.6 (2×CH_2), 29.9 (2×CH_2), 47.4 (CH), 47.8 (CH), 52.2 (2×CH), 114.3 (C), 114.5 (C), 148.2 (C), 148.4 (C). Anal. Calcd for $C_{22}H_{32}Br_2$: C, 57.91; H, 7.07%. Found: C, 57.79; H, 7.21%.

4.3.5. 9,9′-Ethane-1,2-diylbis(10-bromodispiro[3.0.3.2]-dec-9-ene) (18). Yield: 0.57 g (76%), colorless oil, R_f=0.4 (petroleum ether). ^{1}H NMR: δ 1.63–1.91 (m, 8H), 1.98–2.17 (m, 16H), 2.37 (s, 4H). ^{13}C NMR: δ 15.0 (J=135, 2×CH_2), 15.8 (J=135, 2×CH_2), 23.4 (J=130, 2×CH_2), 27.1 (J=134, 4×CH_2), 28.4 (J=135, 4×CH_2), 55.9 (C), 59.8 (C), 118.3 (C), 149.1 (C). Anal. Calcd for $C_{22}H_{28}Br_2$: C, 58.42; H, 6.24%. Found: C, 58.72; H, 6.44%.

4.3.6. 1,1′-Ethane-1,2-diylbis(2-bromospiro[3.3]hept-1-ene) (19a), 2-bromo-1-[2-(1-bromospiro[3.3]hept-1-en-2-yl)ethyl]spiro[3.3]hept-1-ene (19b), and 2,2′-ethane-1,2-diylbis(1-bromospiro[3.3]hept-1-ene) (19c). Yield for the mixture of three isomers (A:B:C=4:2:1): 0.31 g (50%), colorless oil, R_f=0.7 (petroleum ether). ^{1}H NMR (for mixture of three isomers): δ 1.71–1.96 (m, 4H+4H+4H), 2.01–2.34 (m, 8H+8H+8H), 2.37 (br s, 4H, A), 2.49 (br s, 4H, C), 2.54 (br s, 4H, B), 2.73 (br s, 4H, B), 2.76 (br s, 4H, A), 2.82 (br s, 4H, C). ^{13}C NMR (for mixture of three isomers): δ 15.7 (2×CH_2), 16.27 (2×CH_2), 16.30 (2×CH_2), 23.5 (2×CH_2+2×CH_2), 25.1 (2×CH_2), 29.3 (2×CH_2+2×CH_2), 29.7 (2×CH_2), 30.75 (2×CH_2+2×CH_2), 30.80 (2×CH_2), 44.0 (C), 44.3 (C), 45.0 (C), 50.5 (2×CH_2+2×CH_2), 51.5 (2×CH_2), 109.3 (C), 109.5 (C), 117.2 (C), 143.7, 151.3 (C), 151.5 (C). MS (EI, 70 eV) m/z (rel. int., %): 374 (0.2), 372 (0.6), 370 (0.2) $[M]^+$, 293 (5), 291 (5) $[M-Br]^+$, 211 (24), 183 (27), 155 (38), 141 (38), 128 (40), 115 (38), 105 (56), 91 (100), 77 (58), 65 (27), 53 (22), 39 (27). Anal. Calcd for $C_{16}H_{20}Br_2$: C, 51.64; H, 5.42%. Found: C, 51.47; H, 5.69%.

4.3.7. 1,1′-Ethane-1,2-diylbis(2-bromospiro[3.5]non-1-ene) (20). Yield: 0.53 g (75%), colorless oil, R_f=0.6 (petroleum ether). ^{1}H NMR: δ 1.09–1.24 (m, 2H), 1.25–1.41 (m, 4H), 1.48–1.74 (m, 14H), 2.23 (s, 4H), 2.47 (s, 4H). ^{13}C NMR 23.4 (J=130, 2×CH_2), 24.4 (J=123, 4×CH_2), 25.5 (J=119, 2×CH_2), 34.4 (J=124, 4×CH_2), 48.0 (J=141, 2×CH_2), 50.4 (2C), 109.4 (C), 155.3 (C). Anal. Calcd for $C_{20}H_{28}Br_2$: C, 56.09; H, 6.59%. Found: C, 56.00; H, 6.27%.

4.3.8. 9-Bromo-10-(1-bromocyclopropyl)bicyclo[6.2.0]-dec-9-ene (22).[34] Yield: 0.31 g (70%), colorless oil, R_f=0.6 (petroleum ether). ^{1}H NMR: δ 0.82–0.91 (m, 2H), 1.05–1.16 (m, 2H), 1.18–1.81 (m, 11H), 2.03–2.11 (m, 1H), 2.72–2.78 (m, 1H), 2.86–2.92 (m, 1H). ^{13}C NMR: δ 13.9 (J=165, CH_2, cy-Pr), 17.4 (J=164, CH_2, cy-Pr), 24.9 (CH_2), 25.3 (CH_2), 25.9 (CH_2), 26.3 (CH_2), 29.5 (CH_2), 29.7 (CBr), 29.8 (CH_2), 48.6 (J=141, CH, cy-Bu), 51.1 (J=139, CH, cy-Bu), 114.5 (C), 147.3 (C). MS (EI, 70 eV) m/z (rel. int., %): 336 (1), 334 (2), 332 (1) $[M]^+$, 255 (10), 253 (10) $[M-Br]^+$, 174 (25), 173 (66), 159 (25), 145 (32), 131 (57), 117 (49), 105 (62), 91 (92), 84 (81), 67 (75), 55 (76), 51 (69), 49 (100), 43 (65), 39 (95).

4.3.9. 1-[2-(Dicyclopropylmethylene)cyclobutyl]ethyl ethyl ether (25).[34] Yield: 0.42 g (58%), colorless oil. ^{1}H NMR (for fragment cy-Bu-CH(CH_3)OEt): δ 1.12 (t, ^3J=7.1, 3H, CH_3), 1.12 (d, ^3J=6.3, 3H, CH_3), 2.79–2.86 (m, 1H, CH, cy-Bu), 3.44 (q, ^3J=7.1, 2H, CH_2O), 3.70 (dq, ^3J=6.3, ^3J=6.3, 1H, CHO). ^{13}C NMR: δ 3.2 (CH_2), 3.5 (CH_2), 4.5 (CH_2), 5.0 (CH_2), 10.5 (CH), 12.3 (CH), 15.6 (2×CH_3), 17.9 (CH_2), 28.9 (CH_2), 47.0 (CH), 63.9 (CH_2), 76.0 (CH), 125.4 (C), 131.0 (C). MS (EI, 70 eV) m/z (rel. int., %): 220 (15) $[M]^+$, 147 (58) $[M-(CH_3)CHOEt)]^+$, 121 (23), 105 (23), 91 (26), 73 (100), 67 (11), 45 (58).

4.3.10. [2-(1-Ethoxyethyl)cyclobutylidene]cyclohexane (27a).[13] Yield: 0.61 g (89%), colorless oil, R_f=0.7 (petroleum ether). ^{1}H NMR: δ 1.15 (dd, ^3J=6.8, ^3J=6.3, 3H, CH_3), 1.19 (d, ^3J=5.5, 3H, CH_3), 1.39–1.53 (m, 6H), 1.61–1.76 (m, 2H), 1.88–1.94 (m, 2H), 1.99–2.07 (m, 2H), 2.40–2.56 (m, 2H), 3.14–3.22 (m, 1H), 3.39–3.53 (m, 2H, CH_2O), 3.53–3.61 (m, 1H, CHO). ^{13}C NMR: δ 15.7 (J=126, CH_3), 15.9 (J=126, CH_3), 17.8 (J=136, CH_2), 26.6 (CH_2), 26.8 (CH_2), 27.6 (CH_2), 27.7 (CH_2), 29.2 (CH_2), 29.7 (CH_2), 46.3 (J=134, CH), 63.9 (J=140, CH_2O), 76.6 (J=139, CH), 129.4 (C), 131.9 (C). MS (EI, 70 eV) m/z (rel. int., %): 209 (1) $[M+1]^+$, 208 (1) $[M]^+$, 207 (1) $[M-1]^+$, 179 (2), 149 (65), 134 (13), 133 (22), 121 (44), 107 (68), 93 (70), 81 (78), 73 (100), 67 (73), 55 (80), 45 (96).

4.3.11. 1-Bromo-2-(1-bromocyclopropyl)cyclobutene (27b).[1a] Yield: 0.66 g (77%), colorless liquid, R_f=0.35 (petroleum ether). ^{1}H and ^{13}C NMR data for the **27b** are the same as those reported in literature.[1a]

4.3.12. (1-Bromospiro[2.2]pent-1-yl)(spiro[2.2]pent-1-yl)methanol (28). To a stirred solution of *gem*-dibromospiropentane **8a** (0.96 g, 3.3 mmol) in THF (30 mL) and pentane (10 mL) at −100 °C under argon, butyllithium (2.2 mL, 1.5 N, 3.3 mmol) was added dropwise for 15 min. The mixture was stirring at the same temperature for 1 h and then at −(110–115) °C spiro[2.2]pentane-1-carbaldehyde (0.32 g, 3.3 mmol) was added for 10 min. After 3 h the resulting mixture was quenched with cold 0.1 N H_2SO_4 (50 mL). The aqueous layer was extracted with Et_2O (3×10 mL) and combined organic layers were dried over anhydrous $MgSO_4$ and concentrated in vacuo. The crude products were purified by column chromatography (silica gel, petroleum ether). Yield: 0.38 g (47%), colorless oil, n_D^{20}=1.5140. ^{1}H NMR (mixture of four isomers): δ 0.65–1.70 (m, 4×13H), 2.82 (d, ^3J=7.8, 1H, C*H*OH), 2.94 (d, ^3J=7.4, 1H, C*H*OH), 3.13 (d, ^3J=7.1, 1H, C*H*OH), 3.44 (d, ^3J=3.4, 1H, C*H*OH). Anal. Calcd for $C_{11}H_{15}BrO$: C, 54.34; H, 6.22%. Found: C, 54.67; H, 6.58%.

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Nitroso group transfer from *N*-nitrososulfonamides to thiolate ions. Intrinsic reactivity

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Abstract—The nitroso group transfer from *N*-nitrososulfonamides to thiolate ions was studied. Based on the results, the reaction rate is strongly dependent on the nature of the leaving group $\left(\alpha_{lg} \approx -1.30\right)$, but virtually independent of the basicity of the thiol $(\beta_{nuc} \approx 0.10)$. This dependence is ascribed to the presence of a nucleophile desolvation equilibrium (β_d) that is followed by the attack of the thiolate ion on the nitroso group $\left(\beta'_{nuc}\right)$ via a concerted mechanism. The equilibrium constants for the loss of a nitroso group from a nitrosothiol and an *N*-nitrososulfonamide were used to obtain the equilibrium constants for the different reactions involved. By using rate–equilibrium correlations, the parameters α_{lg}^{norm}, β_d, and $\beta_{nuc}^{\prime norm}$ were obtained.

1. Introduction

The physiological properties of NO,[1,2] particularly those of vasodilation[3] and of the inhibition of platelet aggregation,[4] can explain the enormous interest aroused by the chemistry of *S*-nitrosothiols (RSNO) in the last few years. They have also been identified as bodily fluids, notably as *S*-nitrosoglutathione[5] and *S*-nitrosoalbumins.[6] Indeed, the current belief[7] is that NO is transported around the body as RSNO (mostly as the nitrosoalbumins), from which NO can be released under certain conditions.

S-Nitrosothiols are very readily generated in solution by conventional nitrosation of thiols, and examples have been known for about a 100 years.[8] Nitrosation of thiols has been examined mechanistically and follows the pattern of amine nitrosation, in which both acid- and halide ion-catalysis occur.[9] In contrast with the corresponding nitrosation of alcohols by sodium nitrite in mildly acidic solution, the equilibrium position corresponding to thiol nitrosation lies well over the right, with equilibrium constant[10] of 10^5–$10^6\ M^{-1}$. *S*-Nitrosothiols can also be obtained in a neutral or alkaline medium via transnitrosation, the nitroso group being transferred from an NO group donor to a suitable acceptor. One well-known example is the nitroso group transfer from *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide to cysteine.[11]

Recently, our group[12] calculated the equilibrium constants for the loss of an NO^+ group from a protonated *N*-nitrosamine $(pK_{NO}^{R_2NH^+NO})$ and an *N*-methyl-*N*-nitrosobenzenesulfonamide (pK_{NO}^{X-NO}). The relation $\Delta pK_{NO} = pK_{NO}^{R_2NH^+NO} - pK_{NO}^{X-NO}$ allows one to calculate the equilibrium constant for the transfer of a nitroso group from an *N*-nitrososulfonamide to an amine. In fact, this allowed us to derive a single rate–equilibrium correlation spanning a range of 10 pK_{NO} units and including both thermodynamically favorable and unfavorable reactions. In the light of the Marcus theory, the calculated intrinsic barriers for nitroso group transfer reactions reveal that the presence of electron-withdrawing groups on the aromatic ring of *N*-methyl-*N*-nitrosobenzenesulfonamide does not alter such barriers.

The purpose of this work was to expand a previous treatment of the nitroso group transfer from an N–N=O donor to an acceptor sulfur atom. To this end, we studied the nitroso group transfer from *N*-nitrososulfonamides to thiols (Scheme 1). The *N*-nitrososulfonamides used borne substituents of variable electron-releasing ability on their aromatic rings. The thiols used [viz. methyl thioglycolate (MTG), methylmercaptopropionate (MMP), mercaptoethanol (ME), and ethanothiol (EtSH)] were chosen on the grounds of their basicity.

Keywords: Kinetics; Mechanism; Nitrosation; Thiol; Sulfonamide.
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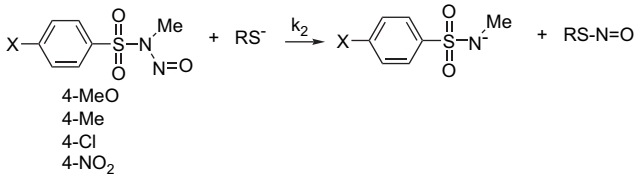


Scheme 1.

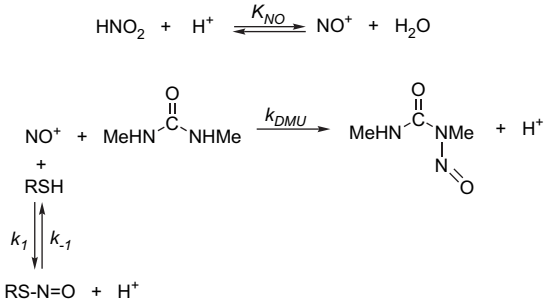
2. Results

Previous studies revealed that the formation of nitrosothiols by nitroso group transfer occurs via the basic form of the thiol.[11] Figure 1 illustrates the good linear relationship between the observed rate constant and the thiolate concentration (reaction showed in Scheme 1) in the nitrosation of mercaptoethanol:

$$k_{obs} = k_2[RS^-] \quad (1)$$

The slopes of the plots of Figure 1 were used to calculate the bimolecular rate constants shown in Table 1. From such rate constants and the pK_a values for the corresponding thiols and sulfonamides, we calculated parameters β_{nuc} and α_{lg}, which are also shown in Table 1. As can be seen, the bimolecular rate constants increased very slightly with increase in nucleophile basicity ($\beta_{nuc} < 0.1$); on the other hand, the nature of the leaving group was much more influential than the corresponding deprotonation equilibria ($|\alpha_{lg}| > 1$). Accurately interpreting these values in quantitative terms requires their normalization with respect to the corresponding equilibrium process.

As noted earlier, the equilibrium constants for the formation of nitrosothiols from the parent thiols and NO^+ were very high (10^5–10^6 M^{-1}) and difficult to quantify owing the instability of the products. In this work, we used an alternative approach based on the nitrosation of 1,3-dimethyl urea in an acid medium containing variable concentrations of the thiol (see Scheme 2).



Scheme 2.

Kinetic experiments were performed at a constant sodium nitrite concentration (1.00×10^{-4} M), [DMU]=5.00×10^{-3} M, [$HClO_4$]=0.50 M and an ionic strength ($NaClO_4$) of 1.00 M. As can be seen in Figure 2, the observed rate constant increased with increasing thiol concentration. The linear increase in k_{obs} was the result of two simultaneous, competitive processes, namely, the nitrosation of DMU[13] and that of the thiol.[14] Based on the nitrosation mechanisms for DMU and thiols in an acid medium, the observed rate constant can be expressed as

$$k_{obs} = k_{DMU}K_{NO}[H^+][DMU] + k_1K_{NO}[RSH][H^+] + k_{-1}[H^+] \quad (2)$$

where k_{DMU} is the bimolecular rate constant for the nitrosation of dimethyl urea, and k_1 and k_{-1} are the formation and

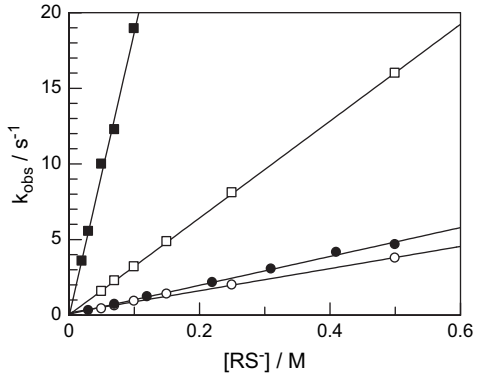


Figure 1. Influence of the thiolate concentration on k_{obs} for the nitrosation of mercaptoethanol (reaction showed in Scheme 1) by (○) 4-MeO, (●) 4-Me, (□) 4-Cl, and (■) 4-NO_2. T=25.0 °C.

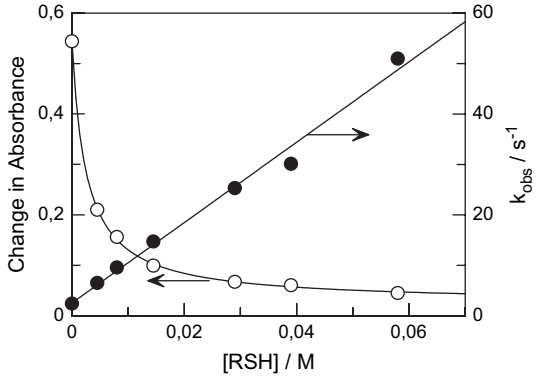


Figure 2. Influence of the mercaptoethanol concentration on k_{obs} and on the variation of the absorbance at 250 nm during the nitrosation of DMU. [DMU]=5.00×10^{-3} M; [$HClO_4$]=0.50 M; [$NaNO_2$]=1.00×10^{-4} M; ionic strength=1.00 M ($NaClO_4$). T=25.0 °C.

Table 1. Bimolecular rate constants, k_2 (M^{-1} s^{-1}), for the nitroso group transfer from *N*-nitrosobenzene-sulfonamides to thiolate ions

pK_a^{lg}	pK_a^{nuc}	7.81	9.45	9.72	10.6	β_{nuc}
		MTG	MMP	MercEt	EtSH	
11.7	4-MeO	9.76±0.05	11.7±0.1	13.5±0.2	14.6±0.5	0.06±0.01
11.6	4-Me	12.6±0.7	15.8±0.2	17.6±0.6	19.2±0.5	0.07±0.01
11.1	4-Cl	34.6±0.3	46.3±0.5	57.9±0.3	64.1±0.7	0.10±0.02
10.7	4-NO_2	194±2	227±8	351±9	375±8	0.10±0.03
	α_{lg}	−1.2±0.1	−1.2±0.1	−1.3±0.1	−1.4±0.1	

decomposition rate constants, respectively, of the nitrosothiol and K_{NO} is the equilibrium constant for the formation of NO^+ from HNO_2 and H^+. Eq. 2 allowed us to calculate the nitrosation rate constant for the thiol, k_1. Because the formation equilibrium constants for nitrosothiols are usually very large, k_{-1} must be very small relative to k_1 and k_{DMU}; this precludes the kinetic determination of k_{-1}.

As can be seen from Figure 2, the addition of RSH decreased the absorbance change at 250 nm due to the nitrosation of DMU. Such a decrease was a result of the formation of the nitrosothiol, which absorbs at 330 nm. Therefore, the absorbance change can be related to the amounts of nitrosodimethyl urea (MNU-NO) and nitrosothiol (RSNO) formed. The variation in absorbance in the absence of added thiol, $(\Delta A)_0$, and in its presence, ΔA, can be related with the nitrosation equilibrium constant of the thiol through the following equation:

$$\frac{(\Delta A)_0-\Delta A}{(\Delta A)_0}=\frac{K_{NO}K_1[RSH]}{1+K_{NO}K_1[RSH]} \quad (3)$$

where K_{NO} is the equilibrium constant for the formation of NO^+ from HNO_2 and H^+, and was taken[15] to be $3.5\times10^{-7}\ M^{-1}$; and K_1 is the equilibrium constant for the formation of the nitrosothiol, $K_1 = k_1/k_{-1}$.

Figure 3 illustrates the good fitting of the absorbance changes of Figure 2, and those obtained using different dimethyl urea concentrations to Eq. 3. The fit allows the product K_1K_{NO} to be established, $K_1K_{NO} = (270 \pm 17)\ M^{-1}$. By using the value of K_{NO}, we can get the nitrosation equilibrium constant for mercaptoethanol, $K_1 = 7.71 \times 10^8$. From similar plots to that of Figure 3 we have obtained the formation equilibrium constants for the nitrosothiols used in this work (see Table 2).

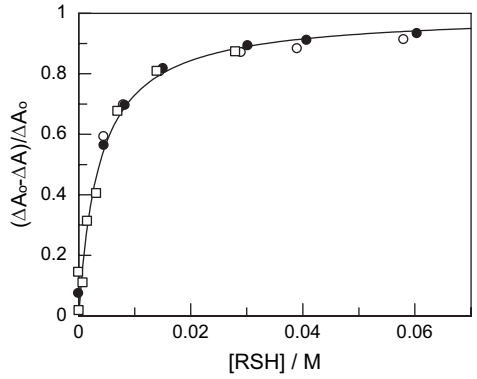


Figure 3. Plot of $(\Delta A)_0-\Delta A/(\Delta A)_0$ versus the thiol concentration, Eq. 3, for the nitrosation of DMU in the presence of variable concentrations of mercaptoethanol. $[HClO_4]$=0.50 M; $[NaNO_2]=1.00\times10^{-4}$ M; ionic strength=1.00 M ($NaClO_4$). T=25.0 °C. (○) $[DMU]=5.00\times10^{-3}$ M; (●) $[DMU]=3.00\times10^{-3}$ M; (□) $[DMU]=2.00\times10^{-3}$ M.

Table 2. Formation equilibrium constants for the studied nitrosothiols at an ionic strength of 1.00 M ($NaClO_4$) at 25.0 °C

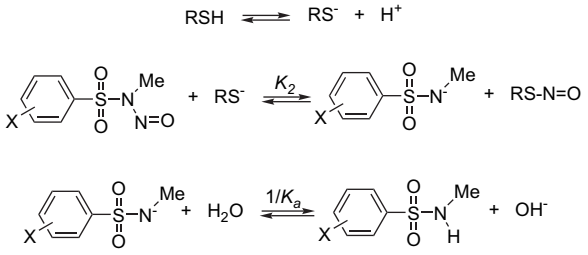
Thiol	MTG	MMP	MercEt	EtSH
pK_a	7.81	9.45	9.72	10.6
K_1	1.8×10^8	6.1×10^8	7.7×10^8	9.0×10^8

3. Discussion

The bimolecular rate constants k_2 for the nitroso group transfer from *N*-nitrosobenzene-sulfonamides to thiolate ions (Table 1) were much greater than those obtained with secondary amines as the nucleophiles. In fact, the rate constant for the nitroso group transfer from *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide to mercaptoethanol ($pK_a = 9.72$) is roughly 300 times greater than that for another nucleophile such as piperazine ($pK_a = 9.82$).[12] This higher strength of the sulfur nucleophiles is well documented in the literature as evidenced by the different values in Ritchie's N_+ scale.[16]

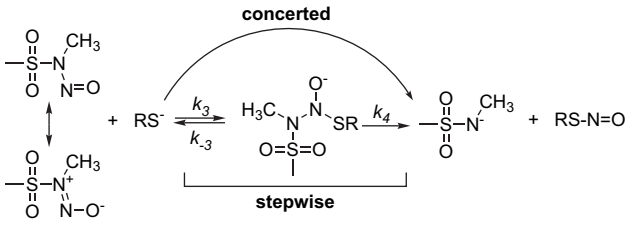
3.1. Reaction mechanism

The mechanism by which a nitroso group is transferred from an *N*-nitrosobenzenesulfonamide to a thiol involves the nucleophilic attack of the thiolate ion to the nitroso group and the release of the anion from the sulfonamide, which constitutes the rate-determining step of the process. Subsequently, the sulfonamide ion is protonated to an extent proportional to the acidity of the medium (see Scheme 3).



Scheme 3.

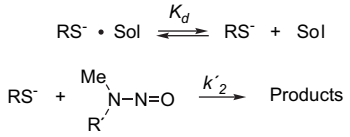
There are at least two possible mechanisms for the nitroso group transfer. One is of the addition–elimination type and the other involves the direct (concerted) displacement of the N═O group as in the alkaline hydrolysis of alkyl nitrites.[17] Because the Bronsted slopes, α_{lg}, of Table 1 are smaller than −1, the formation of the transition state is more strongly influenced by the development of negative charge than the acidity equilibrium of the sulfonamide. *N*-nitroso compounds stabilize by resonance between two different structures:[18] R–N–N═O↔R–N^+═N–O^-. A possible explanation is that the transnitrosation reaction involves a two unit change in charge at the sulfonamide nitrogen, from positive in the nitroso compound (Ar–SO_2–$N(CH_3)^+$═N–O^-) to negative in the product (Ar–SO_2–$N(CH_3)^-$) (Scheme 4). This change or charge of more than one unit, $\alpha_{lg} \approx -1.3$, in the reaction versus only one in the reference reaction, pK_a^{lg} (the acid dissociation of the sulfonamide), is only compatible with a concerted mechanism for nitroso group transfer, ruling out the stepwise mechanism shown in Scheme 4.



Scheme 4.

The variation of the nucleophilic reactivity with the basicity of the nucleophile shows behavior clearly different from that of the nitrogen or carbon nucleophiles. The nitroso group transfer rate constant increases slightly by 55% by increasing the basicity of the nucleophile approximately 615 times. The same increase in basicity brings a rise of more than 10^4% in the rate constants of amine[19] or carbanion nitrosation[20] by *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide. Such a difference of behavior is made clear when establishing a Bronsted correlation giving values of $\beta_{nuc} \approx 0.8$ for the nitrosation of primary and secondary amines by *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide,[12] whereas the values obtained for the nitrosation of the sulfur nucleophiles studied is close to $\beta_{nuc} \approx 0.08$.

This change in the sensitivity of the reaction to the basic strength of the nucleophile is unusual although not without precedent and it has traditionally been regarded as a consequence of the effect of desolvation on the reaction rate or on reactivity–structure correlations. In fact, there are many cases where the rate of certain nucleophilic attacks has been found to decrease as the basicity of the nucleophile is increased, leading to negative Bronsted exponents. This behavior has been observed for some phosphoryl transfer reactions to amines,[21] and for reactions of highly reactive carbocations with amines[22] and for reactions of thiolate ions with Fischer carbene complexes.[23] In the same way, values of the Bronsted exponent close to zero have been found for reactions of diphenylketene with amines.[24] Studies carried out by Jencks[21] indicate that these anomalous Bronsted exponents result form a requirement for partial desolvation of the nucleophile prior to reaction. The desolvation is usually considered to be a pre-equilibrium that occurs in a separate step, in such a way that a two-step model like that illustrated in Scheme 5 can be adopted for a nucleophilic attack.



Scheme 5.

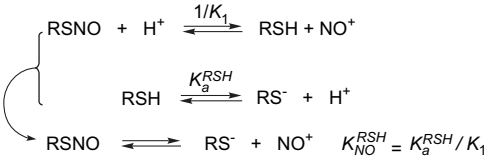
As Scheme 5 shows, the experimental value of the rate constant for the process of nucleophilic attack corresponds to the product $K_d k'_2$, where K_d is the equilibrium constant for the partial desolvation of the nucleophile. Taking into account this approach, we can assume that β_{nuc} is given by Eq. 4:

$$\beta_{nuc} = \frac{d \log k_2}{d\, pK_a^{RSH}} = \frac{d \log K_d k'_2}{d\, pK_a^{RSH}} = \frac{d \log K_d}{d\, pK_a^{RSH}} + \frac{d \log k'_2}{d\, pK_a^{RSH}} = \beta_d + \beta'_{nuc} \quad (4)$$

In view of the fact that the higher the basicity of RS^-, the more difficult the desolvation is, $\beta_d < 0$ can be expected. Thus, if β_{nuc} is low, β_{nuc} may be dominated by β_d and be close to zero or even negative. The values reported in the literature where β_{nuc} values are quite low for thiolate ion addition to a variety of electrophiles are very common.[25]

3.2. Determination of equilibrium constants

Accurately interpreting Bronsted exponents with a view to characterizing the structure of the transition state entails normalizing β_{nuc} and α_{lg} through β_{eq}. In the nitroso group transfer reactions studied here, the equilibrium is displaced to the formation of the nitrosothiol to such an extent that it precludes its experimental determination. Moreover, nitrosothiols are unstable compounds and decompose under a wide variety of conditions. The equilibrium constant for a process involving the loss of an NO^+ group from a nitrosothiol can be calculated from the equilibrium constant for the formation of the nitrosothiol from its parent thiol and NO^+, K_1, and the acidity constant for the thiol, K_a^{RSH} (see Scheme 6).



Scheme 6.

Table 3 lists the K_{NO}^{RSH} values obtained for various thiols in the form of pK_{NO}^{RSH}, as well as the previously reported values for the corresponding nitrososulfonamides.[12] Based on the results, the most basic sulfonamides or thiols produce the most stable *N*-nitroso compounds, which is consistent with the above-described behavior of *N*-nitrosamines. The dependence of the stability of *N*-nitrosamines on the basicity of the parent amines was previously demonstrated in the nitroso group transfer from an *N*-nitrosamine to another amine in an acid medium.[26,27] The structure and stereochemistry of *N*-nitrosamines should reflect delocalization

Table 3. Equilibrium constants for the loss of an NO^+ group from a nitrosothiol or *N*-nitrososulfonamide and for the nitroso group transfer from a nitrososulfonamide to a thiol, K_2

pK_{NO}		16.1	18.23	18.61	19.55	β_{nuc}^{norm}
		MTG	MMP	MercEt	EtSH	
		$\log K_2 = \Delta pK_{NO} = pK_{NO}^{acceptor} - pK_{NO}^{donor}$				
20.12	4-MeO	−4.02	−1.89	−1.51	−0.57	0.05±0.01
19.83	4-Me	−3.73	−1.6	−1.22	−0.28	0.05±0.01
18.83	4-Cl	−2.73	−0.6	−0.22	0.72	0.08±0.02
17.55	4-NO_2	−1.45	0.68	1.06	2.00	0.08±0.03
	α_{lg}^{norm}	0.51±0.03	0.50±0.01	0.54±0.02	0.55±0.03	

of the lone-pair electrons of the amino nitrogen into the π-system of the N=O group. Electron diffraction studies[28] have shown that N–N and N–O bond orders are ca. 1.5, which is consistent with a structure in between the valence structures of Scheme 7.

Scheme 7.

Independent evidence for considerable charge development in the ground state comes from dipole moments for aliphatic *N*-nitrosamines.[29] As the basicity of the amine increases, there will also be an increase in the stability of the resonance form with a positive charge on the nitrogen atom and consequently the stability of the *N*-nitrosamine will increase.

The equilibrium constant for the rate-determining step of the nitroso group transfer reaction, K_2, can be calculated from pK_{NO} for the *N*-nitrososulfonamide (the nitroso group donor) and the nitrosothiol (the acceptor), using the relation $\log K_2 = \Delta pK_{NO} = pK_{NO}^{acceptor} - pK_{NO}^{donor}$. Table 3 lists the $\log K_2$ values thus obtained. As can be seen, based on the equilibrium constant for the nitroso group transfer, the process will be thermodynamically unfavorable and lead to a mixed equilibrium between the *N*-nitrososulfonamide and the nitrosothiol in most cases. However, the sulfonamide will be protonated in a diffusion-controlled step at a later stage of the process. As a result, the backward reaction of the nitroso group transfer from a nitrosothiol to the neutral form of the sulfonamide will simply not occur. Also, nitrosothiols decompose rapidly,[30] so the nitroso group transfer will be quantitative.

3.3. Characterization of the transition state

The equilibrium constant for the rate-determining step in the nitroso group transfer, K_2, allows one to derive rate–equilibrium relationships. In fact, the resulting plots can be used to calculate α_{lg}^{norm} and β_{nuc}^{norm} (see Fig. 4), which are measures of charge development on the nitrogen atom of the *N*-nitrososulfonamide and the sulfur atom of the thiolate ion, respectively. The α_{lg}^{norm} and β_{nuc}^{norm} values thus obtained are listed in Table 3. As can be seen, the α_{lg}^{norm} values are close to 0.50 and

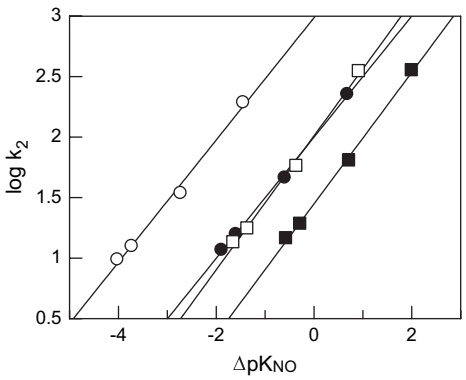


Figure 4. Plot of log k_2 versus ΔpK_{NO} for the nitroso group transfer from *N*-nitrososulfonamides to (○) MTG, (●) MMP, (□) MercEt, and (■) EtSH. The slopes of the lines provide an α_{lg}^{norm} value of 0.50.

independent of the particular substituent of the *N*-nitrososulfonamide, consistent with the previous results for the nitroso group transfer from *N*-nitrososulfonamides to amines.[12]

The β_{nuc}^{norm} values obtained for the nitroso group transfer from *N*-nitrososulfonamides to amines are also close to 0.50, which is consistent with a symmetric transition state for the reaction. The small values obtained in this work ($\beta_{nuc}^{norm} = 0.05$) suggest the presence of a thiolate desolvation equilibrium encompassed by the rate constant. Based on Scheme 5, $\beta_{nuc}^{norm} = \beta_d + \beta_{nuc}^{\prime norm}$, where β_d is the Bronsted slope for the desolvation of the thiolate ion and $\beta_{nuc}^{\prime norm}$ that for the nitroso group transfer.

Previous studies on the nitroso group transfer from *N*-nitrososulfonamides[12] revealed that stabilization by the SO_2 group of the negative charge that develops on the nitrogen atom of the sulfonamide during the reaction must be the result of polarization effects rather than of conjugative dπ–pπ bonding or negative hyperconjugation. This is consistent with the results for the mechanism by which carbanions with a sulfur atom[31] or sulfonyl group[32] in α are stabilized.[32] The absence of resonance effects from the stabilization of negative charge in α to sulfur atoms is supported by the fact that $\alpha_{lg}^{norm} + \beta_{nuc}^{norm} = 1$. One can therefore assume that the Bronsted exponent for the nitroso group transfer from *N*-nitrososulfonamides to thiolate ions (Scheme 5), $\beta_{nuc}^{\prime norm}$, should be 0.50. Accordingly, β_d should be −0.45, which is consistent with the expectations ($\beta_d < 0$) as the difficulty of desolvating the thiolate ion should increase with increasing basicity of RS^-.

3.4. Intrinsic rate constants

The Marcus theory[33] for *outer sphere electron transfer reactions* relates kinetic and thermodynamic barriers in a chemical reaction. This theory has allowed the experimental results for a large number of processes including proton,[34] hydride ion,[35] and methyl group[36,37] transfers to be explained. Such a wide scope suggests that the Marcus theory should also be applicable to nitroso group transfers.

The Marcus theory[33] predicts non-linearity in free energy relations by introducing a quadratic term:

$$\Delta G^{\neq} = \Delta G_0^{\neq} + \frac{\Delta G^0}{2} + \frac{\Delta G^{0^2}}{16\Delta G_0^{\neq}} \quad (5)$$

where $\Delta G^{\neq}$ is the free energy of activation for the reaction concerned, ΔG^0 the free energy change for the process and $\Delta G_0^{\neq}$ the *intrinsic barrier* corresponding to the free energy of activation at $\Delta G^0 = 0$. The associated rate constant and intrinsic rate constant are k and k_0, respectively. In proton transfer reactions, ΔG^0 is determined by the pK_a difference between the proton donor and acceptor. An identical formalism can be used with nitroso group transfers. In our case, ΔG^0 will be determined by the pK_{NO} difference between the nitroso group donor (i.e., the *N*-nitrososulfonamide) and acceptor (thiolate ion).

We used the results of Figure 4 to calculate the intrinsic rate constant, k_0, for the nitroso group transfer from *N*-nitrososulfonamides to thiols as the rate constants at $\Delta pK_{NO} = 0$.

The intrinsic constants thus obtained increased with decreasing basicity of the thiol; thus, $\log k_0$ was 3.0 for MTG ($pK_a = 7.81$), 2.0 for MMP ($pK_a = 9.45$), 2.0 for MercEt ($pK_a = 9.72$), and 1.75 for EtSH ($pK_a = 10.6$). These results are consistent with the need to desolvate thiolate ions, the desolvation increasing in difficulty with increase in the basicity of the thiol. In order to suppress the effect of desolvation on $\log k_0$, we used a β_d value of -0.45 to establish the following equation

$$\log k_2 + 0.45pK_a^{RSH} = \text{intercept} + \beta'_{nuc}pK_a^{RSH} \quad (6)$$

Figure 5 illustrates the good correlation between $\log k_2 + 0.45pK_a^{RSH}$ and ΔpK_{NO} for the nitroso group transfer from *N*-nitrososulfonamides to various thiolate ions. The figure is similar to the traditional log plots of proton transfer rate constants versus acid ionization constants. As can be seen, the rate constant for the nitroso group transfer and the equilibrium constants are linearly related. This is consistent with the previous results for the nitroso group transfer from *N*-nitrososulfonamides to secondary amines.[12] The linearity of the rate–equilibrium constant empirical correlation apparent from Figure 5 includes both thermodynamically favorable and unfavorable reactions. It should be noted that this linear relationship contradicts reported Bronsted exponent changes for a wide range of proton transfers from carbon acids.[38] However, a number of absolutely linear rate–equilibrium plots for proton abstraction by OH^- ions exist[39,40] that span 19 pK_a units and include favorable and unfavorable proton transfer processes.

The results of Figure 5 can be used to calculate the intrinsic rate constants for the nitroso group transfer from *N*-nitrososulfonamides to secondary amines ($\log k_0 = -0.5$) and thiols ($\log k_0 = 6.3$). The large difference between the reactivity of amines and thiolate ions is a result of the latter being highly effective nucleophiles and superior to oxyanions or amines of comparable pK_a in many nucleophilic addition reactions.[41] One of the reasons for such a high reactivity is their high carbon basicity (i.e., their high equilibrium constants for nucleophilic addition).[42] Within the framework of hard–soft acid–base interactions,[43] this can be understood as the soft (polarizable) electrophile having stronger affinity for the soft sulfur bases than for the hard nitrogen or oxygen bases.

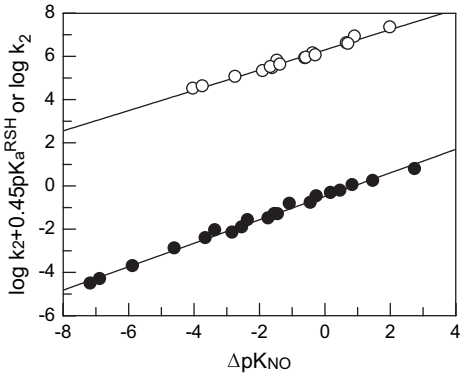


Figure 5. Plot of (○) $\log k_2 + 0.45pK_a^{RSH}$ versus ΔpK_{NO} for nitroso group transfer from *N*-nitrososulfonamides to thiolate ions and (●) $\log k_2$ versus ΔpK_{NO} for nitroso group transfer from *N*-nitrososulfonamides to secondary amines.

Albery and Kreevoy[36] examined methyl transfer reactions in the light of the Marcus theory and determined the intrinsic barriers $\Delta G_0^{\neq}$ for various identity reactions where the nucleophile and leaving group were the same. In this way, they obtained the $\Delta G_0^{\neq}$ values 147, 213, 133, 111, 99, and 97 kJ mol^{-1} (corresponding to the $\log k_0$ values -12.98, -24.45, -10.53, -6.67, -4.57, and -4.22) for H_2O, CN^-, F^-, Cl^-, Br^-, and I^-, respectively. These results for methyl group transfers show that the intrinsic rate constant is very strongly dependent on the nature of the nucleophile, in fact, it varies by a factor of 10^6 from F^- to I^-.

The above-described high thermodynamic affinity was recently shown not to fully account for the high nucleophilicity of thiolate ions.[25f] In fact, an important contribution comes from an enhanced intrinsic rate constant. This was demonstrated for the reaction of thiolate ions with α-nitrostilbene in 1:1 Me_2SO/water, for which $\log k_0 = 3.43$, is much greater than for the reaction of piperidine/morpholine with the same substrate ($\log k_0 = 1.43$).[44] An attractive explanation for the enhanced k_0 value is that the soft acid–soft base interaction, which is responsible for the high thermodynamic stability of the thiolate ion adduct develops ahead of C–S bond formation. This is interesting because it contrasts with most other product-stabilizing factors development of which typically lags behind bond formation at the transition state, thereby lowering k_0.[45] One common feature of product-stabilizing factors such as resonance, solvation or intramolecular hydrogen bonding is that they are 'created' by the reaction (i.e., they would not exist in the absence of bond formation). At best, these factors could conceivably develop synchronously with bond formation, but not possibly ahead of it. By contrast, soft–soft interactions are rooted in the polarizability of the interacting molecules and may not require a substantially developed bond to make themselves felt.

4. Conclusions

On the basis of this study, the following results are particularly worthy of note.

(1) The nitroso group transfer from *N*-nitrososulfonamides to thiolate ions takes place directly via concerted mechanism. The rate of the reaction is strongly dependent on the nature of the leaving group, but virtually independent of the basicity of the thiolate ion. This is a result of the presence of a prior equilibrium for the desolvation of thiolate ions.

(2) The calculated equilibrium constants for the nitroso group transfers allowed charge development on the nucleophile and leaving group in the transition state to be quantified. Because the sulfonyl group is not involved in the establishment by resonance of the negative charge on the nitrogen atom in α, the nucleophilic sensitivity of the reaction can be resolved into a desolvation term and a bond formation term.

(3) The establishment of a rate–equilibrium relation for the nitroso group transfer allowed us to derive a quantitative explanation for the observed reactivity similarly to proton transfer reactions. The absolute linearity of the rate–equilibrium correlation was interpreted in the light of the

Marcus theory of electron transfer as applied to the nitroso group transfer process. The accelerating effect of substituents withdrawing charge from the aromatic ring of the *N*-nitrososulfonamide is mainly due to the greater thermodynamic driving force of the reaction. Hence, the intrinsic reactivities of nitrososulfonamides remain virtually constant.

5. Experimental

N-methylbenzenesulfonamides were synthesized by reacting the corresponding benzenesulfonyl chlorides with excess methylamine in water. The resulting products were extracted with dichloromethane and washed with a solution of sodium hydrogen carbonate and water. *N*-methyl-*p*-toluenesulfonamide and its nitroso derivative were supplied by Ega-Chemie and Merck, respectively. *N*-Methyl-*N*-nitrososulfonamides were prepared from a biphasic water/dichloromethane mixture. The aqueous phase, containing sodium nitrite, and the organic phase, containing the parent sulfonamide, were mixed together and slowly supplied with concentrated (5 M) perchloric acid. Following stirring for 1 h, the organic phase was separated and washed with water, *N*-methyl-*N*-nitrososulfonamides being finally recrystallized in a 80% yield from a dichloromethane/petroleum ether mixture. This method has the advantage that it avoids the hydrolysis of the nitroso derivatives by sequestering them in the organic phase as they form. All other reagents were obtained in the highest available purity from Aldrich and used as received.

The pK_a values of thiols in water at 25.0 °C, ionic strength = 1.00 M ($NaClO_4$) were determined potentiometrically. The obtained values are compatible with those existing in the bibliography.[46] The pK_a values of the sulfonamides were known from the previous studies.[46c]

All kinetic experiments were performed with a large excess of nucleophile relative to *N*-methyl-*N*-nitrososulfonamide, the concentration of the latter ranged from $(1–2)\times10^{-4}$ M. The pH was controlled by using the nucleophile itself as buffering agent in solutions with NaOH. Because of their low solubility in water, the *N*-methyl-*N*-nitrososulfonamides were dissolved in a small amount of organic solvent (dioxane) prior to preparing the aqueous solutions. The final concentration of organic solvent in the reaction medium was smaller than 3.3% (v/v) in all instances. The reaction kinetics was studied by monitoring the formation of the nitrosothiol via the absorbance at 330 nm, which was measured with an Applied Photophysics stopped-flow spectrophotometer. The reaction was found to be first order up to about 90% conversion. Each kinetic run was repeated at least five times in order to obtain an average value for the *pseudo* first-order rate constant, k_{obs}. The standard error for k_{obs} was always less than 3%. All experiments were performed at 25.0 °C.

The equilibrium constant of nitrosothiol formation was determined from the nitrosation of 1,3-dimethyl urea (DMU) in the presence of variable concentrations of a thiol. Experiments were conducted with [$NaNO_2$] much smaller than those of DMU and RSH. The reaction was monitored at 250 nm, where nitrosothiols absorb negligibly.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2006.06.060.

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Preparation of optically pure fused polycyclic scaffolds by Ugi reaction followed by olefin and enyne metathesis

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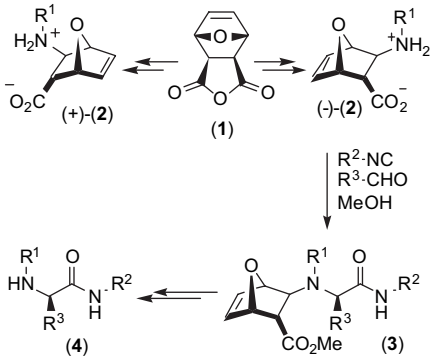
Abstract—Optically pure fused polycyclic scaffolds containing up to eight stereocentres have been synthesised by olefin metathesis and tandem enyne metathesis/Diels–Alder addition of Ugi multicomponent reaction adducts generated from 7-oxa-[2.2.1]-bicyclic amino acid derivatives.

1. Introduction

Multicomponent reactions (MCRs) are a very important class of reactions, which have recently attracted the attention of several academic and industrial researchers, due to their ability to generate quickly and efficiently large collections of compounds amenable for HTS.[1] In this area, the Ugi four-component reaction occupies a leading position since it represents a valuable method to access alpha-acylamino-amides in a straightforward manner.[2]

The power of this reaction has been dramatically increased during the last decades by modifications of the classic procedure. Such modifications include the use of non-classical building blocks, the employment of bifunctional components or the exploitation of post-condensation modifications. All these aspects have been extensively reviewed recently by Doemling.[3] Within our research in the field of intramolecular MCRs, we have recently reported the completely stereoselective Ugi 5-Centre-4-Component Reaction (U-5C-4CR) of an *N*-alkylated bicyclic β-amino acid derivative **2** with a wide variety of aldehydes and isocyanides;[4] and we have demonstrated that the stereochemistry of the newly generated carbon centre is governed by the configuration of the bicyclic unit, which was efficiently prepared in both enantiomerically pure forms from common substrate **1**.[5] We have shown that the bicyclic moiety can be used as a chiral auxiliary and that it can be easily removed at the end of the reaction, yielding optically pure amino acid derivatives **4**. In Scheme 1, the reaction sequence is summarised starting from the symmetric anhydride **1** and using (−)-**2** as chiral auxiliary.



Scheme 1. Optically pure bicyclic amino acid derivatives as chiral auxiliaries in stereoselective Ugi reactions.

[2.2.1]-Bicyclic alkenes have been extensively used in intramolecular ROM/RCM reactions to construct polycyclic ring systems, mainly due to the driving force constituted by the strain release in the ROM step; the conservation of the starting chiral information throughout the process is another advantage of these transformations.[6] However, the final compounds obtained via this route generally do not display many additional handles for decorating the framework with diverse building blocks, a useful feature to render these scaffolds better suited for structure optimisation studies and biological screenings. Moreover, the substances used in these studies are generally available as racemic mixtures, although an example of ROM/RCM on an optically pure 7-oxa-[2.2.1]-bicyclic amino acid derivative has been recently reported.[7]

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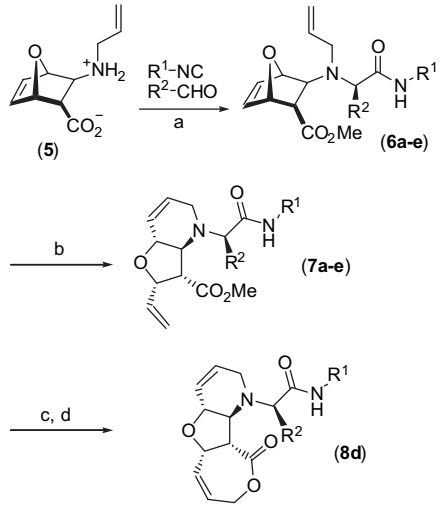
On the other hand, the quest for polyfunctional, structurally complex compounds, acting as smart tools both in understanding the role and functions of emerging biological targets and in validating their biological responses, is becoming more important in the drug discovery process.[8]

In this paper, we report our studies on the coupling of the above-mentioned stereoselective Ugi reaction with two complexity-generating reactions,[9] i.e, ring-opening/ring-closing metathesis (ROM/RCM) and Diels–Alder addition to prepare optically pure polycyclic scaffolds.

2. Results and discussion

Optically pure (−)-*N*-allyl-3-amino-7-oxa-[2.2.1]-bicyclohept-5-ene-2-carboxylic acid was prepared in good yield using a procedure similar to that used to prepare *N*-methyl and *N*-benzyl derivatives,[5] and reacted with different combinations of aldehydes and isocyanides in methanol at room temperature for 2–4 days (Scheme 2). The resulting Ugi adducts were isolated in good yields (Table 1) and as a single diastereoisomer, as previously reported for similar compounds. Optical integrity was previously demonstrated by converting the amino acids, derived from a *retro* Diels–Alder process followed by an enamine deprotection, into the corresponding Mosher's amides.[5]



Scheme 2. Ugi reaction followed by ROM/RCM. Reagents and conditions: (a) MeOH, room temperature; (b) Grubb's II generation catalyst, CH_2Cl_2, room temperature; (c) allyl alcohol, NaH, room temperature; (d) Grubb's I generation catalyst, CH_2Cl_2, reflux.

The subsequent ROM/RCM step was investigated under various conditions; in particular, two different catalysts were tested, and also the presence of an ethylene atmosphere replacing the inert gas was analysed. Optimal conditions were found with 10 mol % of the Grubb's II generation catalyst (benzylidene-1,3-bis-(2,4,6-trimethylphenyl)-2-(imidazolidinylidene)dichloro(tricyclohexylphosphine) ruthenium) under an ethylene atmosphere at a 5 mM concentration of substrate in dichloromethane (Table 1). The reactions with and without ethylene did not display a dramatic difference in reactivity, while the use of Grubb's I generation catalyst (benzylidene-bis(tricyclohexylphosphine)dichlororuthenium) resulted in very poor yield of the desired product. The compounds obtained in this way were fully characterised and their structure unequivocally determined by two-dimensional NMR experiments. Bicyclic systems have been recently used as scaffolds to prepare conformationally constrained peptides and peptidomimetics.[10] We are currently investigating the properties of these molecules as reverse-turn inducers in RGD-based peptidomimetic inhibitors of integrins.[11]

Table 1. Ugi reaction followed by ROM/RCM

Entry	R^1	R^2	Yield (**6**)	Catalyst	Conditions	Yield (**7**)
a	*t*-Bu	*i*-Bu	61	II	Ar	61
b	*t*-Bu	4-NO_2–Ph	70	II	Ar	71
c	Bn	*i*-Bu	71	II	CH_2=CH_2	88
d	Cyclohex	*i*-Pr	69	II	CH_2=CH_2	87
e	*t*-Bu	4-Cl–Ph	46	II	CH_2=CH_2	95
f	*t*-Bu	*i*-Bu	—[a]	I	Ar	10

[a] See, entry a.

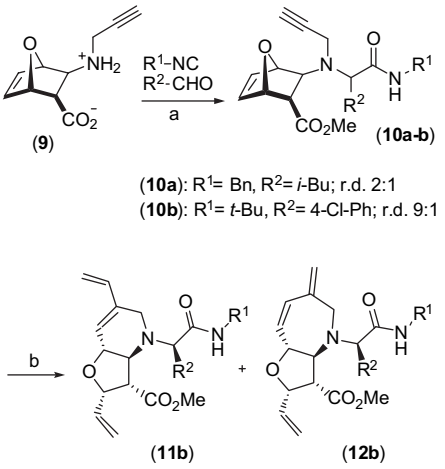
Compound **7d** was further elaborated: after conversion of the methyl ester into the corresponding allyl ester, conditions for the ring-closing metathesis between the two terminal double bonds were investigated. Surprisingly, no product was isolated when the reaction was performed at room temperature, either with generation (I) or (II) catalyst, and with or without an ethylene atmosphere. However, while with the generation (I) catalyst the starting material was recovered unreacted, with the generation (II) catalyst complete consumption of compound **7d** gave rise to polymerisation products.

The desired tricyclic product **8d** was eventually obtained in a satisfactory 78% yield when the reaction was performed with the less reactive catalyst (10%) at reflux in a 5 mM dichloromethane solution under an argon atmosphere. It is worth noting that a similar reaction was reported by Winkler and co-workers, but in that case a larger amount of catalyst (30%) and higher dilution (0.5 mM) were found necessary to isolate the final product in 57% yield.[7]

In order to increase the diversity of these polycyclic molecules, we also explored the possibility of substituting the allyl group on the nitrogen atom with a propargyl group, and to perform the Ugi and the enyne matathesis reactions on the resulting derivative. Bicyclic amino acid **9** was therefore reacted with different combinations of aldehydes and isocyanides and to our surprise stereoselection in this case was not complete (Scheme 3). At this stage, we cannot give an explanation of these unexpected results since, if it can be postulated that a propargyl group, being less bulky than an allyl group, could in principle have a lower effect on the stereoselection, it is difficult to use the same rationale when complete stereoselection is observed with an even less bulky methyl group.[4]

In the case of compound **10b**, however, stereoselection was satisfactory and the major diastereoisomer could be separated from the minor one by chromatographic purification.[12] This propargyl derivative was then subjected to ROM/RCM under different conditions (Table 2).

The reaction did not proceed at all without an ethylene atmosphere, and the starting material was recovered unreacted. There are many reports in which the presence of ethylene



Scheme 3. Ugi reaction with propargylic amino acid followed by ROM/RCM. Reagents and conditions: (a) MeOH, room temperature; (b) See text.

Table 2. ROM/RCM on propargylic derivative **10b**

Entry	Solvent	Catalyst	Conditions	Yield (**11b**)	Yield (**12b**)
a	CH_2Cl_2	II	Ar	—	—
b	CH_2Cl_2	I	Ar	—	—
c	CH_2Cl_2	II	CH_2=CH_2	64	26
d	CH_2Cl_2	I	CH_2=CH_2	79	—
e	Toluene	II	CH_2=CH_2	40[a]	15
f	THF	II	CH_2=CH_2	29[b]	8

[a] Twenty percent of open adduct was also isolated.
[b] Seventeen percent of open adduct was also isolated.

gas was found beneficial during enyne metathesis and indeed also in our case, the reaction proceeded smoothly with the aid of this gas.[13] Interestingly, when the Grubb's II generation catalyst was used, two products were isolated: the 6-*exo* compound **11b** and the less common 7-*endo* compound **12b**;[14] however, when the Grubb's I generation catalyst was used instead, only the 6-*exo* derivative **11b** could be isolated in 78% yield. Other solvents such as toluene and THF were also investigated, but none of them gave results better than DCM.

The diene resulting from this reaction could be further transformed via a Diels–Alder reaction in the presence of a dienophile, and representative compounds **13** and **14** were obtained. Since complete stereocontrol was observed also during the Diels–Alder reaction, highly complex enantiomerically pure polycyclic derivatives could be assembled with this three-step process. Detailed NOE experiments have been conducted on compound **13**, whose structure has been univocally identified as the one shown in Scheme 4. The Diels–Alder reaction could be also performed in situ without the isolation of the diene, by addition of the corresponding alkene; however, due to the high dilution required for the ROM/RCM process, the Diels–Alder reaction was found to be extremely slow under these conditions.

3. Conclusions

In conclusion, in the present paper it has been shown how, starting from simple, easily available precursors, the synthesis of enantiomerically pure fused polycyclic scaffolds containing up to eight stereocentres can be achieved in a straightforward manner, taking advantage of a complexity–diversity generating strategy based on a Ugi multicomponent reaction, a ring-opening/ring-closing metathesis and a Diels–Alder cycloaddition.

Moreover, it is noteworthy that the use of different aldehydes, isocyanides and unsaturated moieties on the secondary amine in the Ugi reaction, together with the presence of differently manipulable functionalities on the final adducts, offers the possibility to diversely decorate these scaffolds to generate new DOS libraries, useful for application in chemical genetics.[15]

With this aim, we are now continuing to work on this project and the results of this research will be reported in due course.

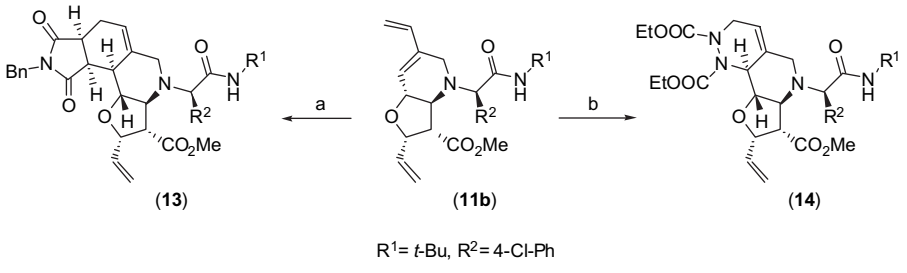
4. Experimental

4.1. General remarks

TLC analyses have been performed on silica TLC plates MERCK 60 F254 (0.25 mm thick). Spots have been observed under the UV light (λ=254 nm) or stained with iodine vapours or with a solution of $(NH_4)MoO_4 \cdot 4H_2O$ and $Ce(SO_4)_2$ in diluted H_2SO_4. Flash chromatographies have been performed on ICN Biomedicals 60A silica (230–400 mesh). Optical rotations were determined with a Jasco DP-181 polarimeter, using a Jasco cylindrical cell 10× 100 mm. Microanalyses were performed on a Heraeus CHN-O-Rapid instrument. Anhydrous solvents and all reagents have been bought from FLUKA or ALDRICH.

4.2. NMR spectra

^{1}H NMRs have been recorded on a VARIAN 'MERCURY 300' at 300 MHz. Chemical shifts are reported in parts per million using TMS (0.00 ppm) as internal standard.



Scheme 4. Diels–Alder reactions on bicyclic diene **11b**. Reagents and conditions: (a) *N*-benzylmaleimide, CH_2Cl_2, room temperature, 78%; (b) Diethylazodicarboxylate, DCM, room temperature, 59%.

J constants are reported in Hertz. ^{13}C NMRs have been recorded on a VARIAN 'MERCURY 300' at 75 MHz. When not indicated, spectra have been recorded at room temperature in $CDCl_3$ as the solvent. Peak attribution is made with the aid of DEPT, 2D COSY, HSQC and TOCSY experiments.

4.3. GC–MS analysis

GC–MS analyses have been performed on an HP-5890 series II with an HP-1 column (530 μm, length 12 m, internal diameter 0.2 mm). Ultrapure helium has been used as carrier gas. Mass spectra (electron impact) were recorded on an HP-5971A spectrometer, coupled to the above chromatograph. Conditions for the GC–MS analyses are as follows: flow: 0.9 mL/min; initial temperature: 100 °C; initial time: 2 min.; rate: 20 °C/min; final temperature: 280 °C; final time: 4 min; injector temperature: 250 °C.

4.4. General procedure for the Ugi reaction

The amino acid (0.29 mmol) was suspended in dry methanol (1 mL), and aldehyde (0.32 mmol) and isocyanide (0.32 mmol) were added in sequence. The reaction was stirred at room temperature under nitrogen for 2–5 days, then concentrated in vacuo and analysed by ^{1}H NMR to determine the diastereomeric ratio. The crude material was then purified by flash chromatography.

4.5. General procedure for the ROM/RCM reaction

The bicyclic derivative (0.18 mmol) was dissolved in dry dichloromethane (30 mL) under argon or ethylene atmosphere. The Grubb's catalyst (0.018 mmol), dissolved in dry dichloromethane (6 mL), was added via syringe and the reaction left stirring overnight. The solvent was then evaporated under reduced pressure and the crude material purified by flash chromatography. For compound **8d**, the reaction was performed at reflux.

4.6. General procedure for the Diels–Alder reaction

The diene (0.18 mmol) and the dienophile (0.27 mmol) are dissolved in dry dichloromethane (2 mL) and stirred for 24 h. The solvent is then evaporated and the crude material purified by flash chromatography.

4.7. Detailed compound characterisation

4.7.1. (1*S*,2*S*,3*S*,4*R*)-Methyl 3-(*N*-((*R*)-1-(*tert*-butylcarbamoyl)-3-methylbutyl)-*N*-allylamino)-7-oxa-bicyclo[2.2.1]hept-5-ene-2-carboxylate (6a). M.W. 378.51; R_f 0.67 (eluent: EtOAc/PE 3:7); $[\alpha]_D^{20}$ −55.4 (*c* 0.66, $CHCl_3$).

^{1}H NMR (300 MHz): δ 0.90 [3H, d, *J* 7]; 0.94 [3H, d, *J* 7]; 1.33 [9H, s]; 1.40 [1H, m]; 1.65–1.85 [2H, m]; 2.93 [1H, t, *J* 4]; 3.31 [1H, d, *J* 4]; 3.32–3.40 [2H, m]; 3.45 [1H, dd, *J* 15, 7]; 3.66 [3H, s]; 4.90 [1H, br s]; 5.07 [1H, d, *J* 4]; 5.11 [1H, dd, *J* 10, 1]; 5.30 [1H, dd, *J* 16, 1]; 5.70–5.82 [1H, m]; 6.34 [1H, dd, *J* 6, 2]; 6.46 [1H, dd, *J* 6, 2]; 6.70 [1H, s].

^{13}C NMR (75 MHz): δ 22.5 (CH_3); 23.0 (CH_3); 25.7 (CH); 28.6 (CH_3); 38.2 (CH_2); 47.2 (CH); 50.4 (C); 50.6 (CH_2); 51.9 (CH_3); 59.8 (CH); 64.3 (CH); 78.5 (CH); 82.8 (CH); 116.8 (CH_2); 135.5 (CH); 136.0 (CH); 137.1 (CH); 172.2 (C); 172.9 (C).

Elem. Anal. Calcd for: C, 66.64; H, 9.05; N, 7.40. Found C, 66.81; H, 9.04; N, 7.39.

4.7.2. (1*S*,2*S*,3*S*,4*R*)-Methyl 3-(*N*-((*R*)-(*tert*-butylcarbamoyl)(4-nitrophenyl)methyl)-*N*-allylamino)-7-oxa-bicyclo[2.2.1]hept-5-ene-2-carboxylate (6b). M.W. 443.49; R_f 0.34 (eluent: EtOAc/PE 3:7); $[\alpha]_D^{20}$ −85.8 (*c* 1.26, $CHCl_3$).

^{1}H NMR (300 MHz): δ 1.40 [9H, s]; 2.98 [1H, t, *J* 4]; 3.09 [1H, dd, *J* 15, 8]; 3.42 [1H, d, *J* 4]; 3.60 [1H, m]; 3.71 [3H, s]; 3.91 [1H, br s]; 4.80 [1H, s]; 5.05 [1H, d, *J* 4]; 5.26 [1H, d, *J* 10]; 5.32 [1H, d, *J* 13]; 5.66–5.80 [1H, m]; 6.18 [1H, dd, *J* 6, 1]; 6.29 [1H, dd, *J* 6, 2]; 7.25 [1H, s]; 7.49 [2H, dd, *J* 7, 2]; 8.24 [2H, dd, *J* 7, 2].

^{13}C NMR (75 MHz): δ 28.4 (CH_3); 47.4 (CH); 50.7 (C); 51.2 (CH_2); 52.1 (CH_3); 63.8 (CH); 65.8 (CH); 78.1 (CH); 80.7 (CH); 118.7 (CH_2); 123.3 (CH); 131.2 (CH); 135.4 (CH); 135.5 (CH); 136.1 (CH); 144.5 (C); 147.3 (C); 169.4 (C); 171.7 (C).

Elem. Anal. Calcd for: C, 62.29; H, 6.59; N, 9.47. Found C, 62.41; H, 6.60; N, 9.45.

4.7.3. (1*S*,2*S*,3*S*,4*R*)-Methyl 3-(*N*-((*R*)-1-(benzylcarbamoyl)-3-methylbutyl)-*N*-allylamino)-7-oxa-bicyclo[2.2.1]hept-5-ene-2-carboxylate (6c). M.W. 412.52; R_f 0.47 (eluent: EtOAc/PE 3:7); $[\alpha]_D^{20}$ −55.5 (*c* 0.65, $CHCl_3$).

^{1}H NMR (300 MHz): δ 0.91 [3H, d, *J* 7]; 0.96 [3H, d, *J* 7]; 1.40–1.50 [1H, m]; 1.70–2.00 [2H, m]; 2.80 [1H, t, *J* 4]; 3.26 [1H, d, *J* 4]; 3.40–3.55 [3H, m]; 3.56 [3H, s]; 4.35 [1H, dd, *J* 15, 6]; 4.47 [1H, dd, *J* 15, 6]; 4.86 [2H, br s]; 5.09 [1H, dd, *J* 10, 1]; 5.17 [1H, dd, *J* 16, 1]; 5.65–5.80 [1H, m]; 6.32 [1H, dd, *J* 6, 1]; 6.42 [1H, dd, *J* 6, 1]; 7.20 [1H, br s]; 7.25–7.40 [5H, m].

^{13}C NMR (75 MHz): δ 22.5 (CH_3); 23.1 (CH_3); 25.7 (C); 38.2 (CH_2); 43.3 (CH_2); 47.4 (CH); 50.2 (CH_2); 52.0 (CH_3); 59.6 (CH); 64.3 (CH); 78.2 (CH); 82.6 (CH); 117.1 (CH_2); 127.3 (CH); 127.8 (CH); 128.6 (CH); 135.8 (CH); 135.9 (CH); 136.8 (CH); 138.6 (C); 172.3 (C); 173.6 (C).

Elem. Anal. Calcd for: C, 69.88; H, 7.82; N, 6.79. Found C, 69.81; H, 7.80; N, 6.80.

4.7.4. (1*S*,2*S*,3*S*,4*R*)-Methyl 3-(*N*-((*R*)-1-(cyclohexylcarbamoyl)-2-methylpropyl)-*N*-allylamino)-7-oxa-bicyclo[2.2.1]hept-5-ene-2-carboxylate (6d). M.W. 390.52; R_f 0.56 (eluent: EtOAc/PE 3:7); $[\alpha]_D^{20}$ −37.1 (*c* 0.52, $CHCl_3$).

^{1}H NMR (300 MHz): δ 0.81 [3H, d, *J* 7]; 0.96 [3H, d, *J* 7]; 1.10–2.50 [11H, m]; 2.66 [1H, d, *J* 10]; 2.91 [1H, dd, *J* 4, 3]; 2.99 [1H, dd, *J* 15, 8]; 3.42 [1H, d, *J* 3]; 3.60 [3H, s]; 3.70–3.90 [2H, m]; 4.85 [1H, br s]; 5.00–5.15 [3H, m]; 5.65–5.80 [1H, m]; 6.16 [1H, d, *J* 8]; 6.32 [1H, dd, *J* 6, 2]; 6.43 [1H, dd, *J* 6, 2].

^{13}C NMR (75 MHz): δ 19.6 (CH_3); 20.1 (CH_3); 24.8 (CH_2); 24.9 (CH_2); 25.5 (CH_2); 27.8 (CH); 33.0 (CH_2); 33.3 (CH_2);

46.3 (CH); 47.4 (CH); 50.3 (CH_2); 51.9 (CH_3); 64.0 (CH); 67.9 (CH); 78.6 (CH); 83.6 (CH); 116.0 (CH_2); 135.8 (CH); 136.1 (CH); 137.5 (CH); 171.5 (C); 172.6 (C).

Elem. Anal. Calcd for: C, 67.66; H, 8.78; N, 7.17. Found C, 67.79; H, 8.76; N, 7.16.

4.7.5. (1*S*,2*S*,3*S*,4*R*)-Methyl 3-(*N*-((*R*)-(*tert*-butylcarbamoyl)(4-chlorophenyl)methyl)-*N*-allylamino)-7-oxa-bicyclo[2.2.1]hept-5-ene-2-carboxylate (6e). M.W. 432.94; R_f 0.58 (eluent: EtOAc/PE 3:7); $[\alpha]_D^{20}$ −64.8 (*c* 0.80, $CHCl_3$).

^{1}H NMR (300 MHz): δ 1.38 [9H, s]; 2.95 [1H, t, *J* 4]; 3.07 [1H, dd, *J* 15, 8]; 3.36 [1H, d, *J* 4]; 3.55–3.65 [1H, m]; 3.70 [3H, s]; 3.87 [1H, br s]; 4.66 [1H, s]; 5.02 [1H, d, *J* 4]; 5.24 [1H, d, *J* 10]; 5.30 [1H, d, *J* 16]; 5.66–5.80 [1H, m]; 6.16 [1H, dd, *J* 6, 2]; 6.24 [1H, dd, *J* 6, 1]; 7.21 [2H, d, *J* 7]; 7.28 [1H, s]; 7.35 [2H, d, *J* 7].

^{13}C NMR (75 MHz): δ 28.5 (CH_3); 47.4 (CH); 50.5 (C); 51.0 (CH_2); 52.1 (CH_3); 63.9 (CH); 66.3 (CH); 78.2 (CH); 80.8 (CH); 118.5 (CH_2); 128.6 (CH); 131.7 (CH); 133.8 (C); 135.1 (C); 135.2 (CH); 135.9 (CH); 136.6 (CH); 170.3 (C); 171.9 (C).

Elem. Anal. Calcd for: C, 63.81; H, 6.75; Cl, 8.19; N, 6.47. Found C, 63.97; H, 6.76; Cl, 8.19; N, 6.46.

4.7.6. (2*S*,3*S*,3a*S*,7a*R*)-Methyl 4-((*R*)-1-(*tert*-butylcarbamoyl)-3-methylbutyl)-2,3,3a,4,5,7a-hexahydro-2-vinylfuro[3,2-*b*]pyridine-3-carboxylate (7a). M.W. 378.51; R_f 0.57 (eluent: EtOAc/PE 2:8); $[\alpha]_D^{20}$ +86.8 (*c* 1.26, $CHCl_3$).

^{1}H NMR (300 MHz): δ 0.84 [3H, d, *J* 7]; 0.88 [3H, d, *J* 7]; 1.38 [9H, s]; 1.40–1.80 [3H, m]; 2.83 [1H, dd, *J* 8, 6]; 3.20–3.40 [4H, m]; 3.72 [3H, s]; 4.05–4.15 [1H, m]; 4.85 [1H, t, *J* 9]; 5.22 [1H, ddd, *J* 10, 1, 1]; 5.33 [1H, ddd, *J* 17, 1, 1]; 5.60–5.80 [2H, m]; 5.99 [1H, ddd, *J* 10, 3, 2]; 6.93 [1H, s].

^{13}C NMR (75 MHz): δ 22.0 (CH_3); 23.1 (CH_3); 24.4 (CH); 28.9 (C); 37.8 (CH_2); 47.6 (CH_2); 51.3 (CH and C); 52.5 (CH_3); 62.4 (CH); 64.0 (CH); 79.9 (CH); 80.9 (CH); 119.4 (CH_2); 124.7 (CH); 127.8 (CH); 134.6 (CH); 169.6 (C); 173.6 (C).

GC–MS 9.0 min (100%) [378 (M), 278 (100%, M−*t*-BuNHCO)].

Elem. Anal. Calcd for: C, 66.64; H, 9.05; N, 7.40. Found C, 66.62; H, 9.05; N, 7.41.

4.7.7. (2*S*,3*S*,3a*S*,7a*R*)-Methyl 4-((*R*)-(*tert*-butylcarbamoyl)(4-nitrophenyl)methyl)-2,3,3a,4,5,7a-hexahydro-2-vinylfuro[3,2-*b*]pyridine-3-carboxylate (7b). M.W. 443.49; R_f 0.31 (eluent: EtOAc/PE 3:7); $[\alpha]_D^{20}$ −48.8 (*c* 1.56, $CHCl_3$)

^{1}H NMR (300 MHz): δ 1.35 [9H, s]; 3.00 [1H, dd, *J* 11, 10]; 3.25 [1H, dd, *J* 11, 8]; 3.33 [1H, dq, J_d 17, J_q 3]; 3.40–3.55 [1H, m]; 3.52 [3H, s]; 4.20 [1H, s]; 4.31 [1H, m]; 4.74 [1H, dd, *J* 10, 8]; 5.19 [1H, dt, J_d 10, J_t 1]; 5.26 [1H, d, *J* 17]; 5.65 [1H, ddd, *J* 17, 10, 8]; 5.70–5.80 [1H, m]; 6.07 [1H, br d, *J* 10]; 6.66 [1H, s]; 7.60 [2H, d, *J* 9]; 8.17 [2H, d, *J* 9].

^{13}C NMR (75 MHz): δ 28.5 (CH_3); 51.2 (CH); 51.3 (C); 51.8 (CH_3); 52.1 (CH_2); 64.9 (CH); 70.6 (CH); 78.4 (CH); 80.7 (CH); 119.4 (CH_2); 123.1 (CH); 125.5 (CH); 127.4 (CH); 129.2 (CH); 134.2 (CH); 146.1 (C); 147.3 (C); 167.7 (C); 172.2 (C).

GC–MS 12.1 min (100%) [443 (M), 343 (100%, M−*t*-BuNHCO), 208 (30%, M−*t*-BuNHCOC$H_6H_4NO_2$), 96 (60%)].

Elem. Anal. Calcd for: C, 62.29; H, 6.59; N, 9.47. Found C, 62.37; H, 6.58; N, 9.47.

4.7.8. (2*S*,3*S*,3a*S*,7a*R*)-Methyl 4-((*R*)-1-(benzylcarbamoyl)-3-methylbutyl)-2,3,3a,4,5,7a-hexahydro-2-vinylfuro[3,2-*b*]pyridine-3-carboxylate (7c). M.W. 412.52; R_f 0.66 (eluent: EtOAc/PE 3:7); $[\alpha]_D^{20}$ +88.8 (*c* 0.60, $CHCl_3$).

^{1}H NMR (300 MHz): δ 0.85 [3H, d, *J* 7]; 0.89 [3H, d, *J* 7]; 1.40–1.80 [3H, m]; 3.01 [1H, dd, *J* 8, 6]; 3.20–3.40 [4H, m]; 3.63 [3H, s]; 4.05–4.15 [1H, m]; 4.43 [1H, dd, *J* 15, 6]; 4.52 [1H, dd, *J* 15, 6]; 4.70–4.80 [1H, m]; 5.20 [1H, dd, *J* 10, 1]; 5.29 [1H, dt, J_d 16, J_t 1]; 5.60–5.80 [2H, m]; 5.99 [1H, ddd, *J* 11, 4, 2]; 7.20–7.40 [5H, m]; 7.50 [1H, br s].

^{13}C NMR (75 MHz): δ 22.0 (CH_3); 23.0 (CH_3); 24.5 (CH); 37.7 (CH_2); 43.2 (CH_2); 47.9 (CH_2); 52.3 (CH); 52.5 (CH_3); 62.0 (CH); 63.9 (CH); 79.8 (CH); 80.9 (CH); 119.3 (CH_2); 124.8 (CH); 127.1 (CH); 127.5 (CH); 127.8 (CH); 128.5 (CH); 134.5 (CH); 138.8 (C); 170.6 (C); 173.7 (C).

GC–MS 11.1 min (100%) [412 (M), 278 (100%, M−BnNHCO), 91 (30%)].

Elem. Anal. Calcd for: C, 69.88; H, 7.82; N, 6.79. Found C, 70.09; H, 7.84; N, 6.80.

4.7.9. (2*S*,3*S*,3a*S*,7a*R*)-Methyl 4-((*R*)-1-(cyclohexylcarbamoyl)-2-methylpropyl)-2,3,3a,4,5,7a-hexahydro-2-vinylfuro[3,2-*b*]pyridine-3-carboxylate (7d). M.W. 390.52; R_f 0.65 (eluent: EtOAc/PE 3:7); $[\alpha]_D^{20}$ +123.0 (*c* 1.00, $CHCl_3$).

^{1}H NMR (300 MHz): δ 0.81 [3H, d, *J* 6]; 0.92 [3H, d, *J* 6]; 1.15–2.20 [12H, m]; 3.10–3.30 [4H, m]; 3.63 [3H, s]; 3.70–3.80 [1H, m]; 4.08 [1H, br s]; 4.86 [1H, t, *J* 9]; 5.23 [1H, dd, *J* 10, 1]; 5.33 [1H, d, *J* 16]; 5.60–5.80 [2H, m]; 5.97 [1H, ddd, *J* 10, 4, 2]; 6.97 [1H, d, *J* 7].

^{13}C NMR (75 MHz): δ 19.9 (CH_3); 20.2 (CH_3); 24.8 (CH_2); 24.8 (CH_2); 25.5 (CH_2); 26.0 (CH); 32.9 (CH_2); 33.8 (CH_2); 47.5 (CH_2); 48.0 (CH); 52.6 (CH_3); 52.9 (CH); 63.6 (CH); 71.5 (CH); 80.0 (CH); 80.8 (CH); 119.4 (CH_2); 124.5 (CH); 127.7 (CH); 134.7 (CH); 167.7 (C); 174.0 (C).

GC–MS 10.4 min (100%) [390 (M), 264 (100%, M−cyclohexNHCO)].

Elem. Anal. Calcd for: C, 67.66; H, 8.78; N, 7.17. Found C, 67.74; H, 8.76; N, 7.18.

4.7.10. (2*S*,3*S*,3a*S*,7a*R*)-Methyl 4-((*R*)-(*tert*-butylcarbamoyl)(4-chlorophenyl)methyl)-2,3,3a,4,5,7a-hexahydro-2-vinylfuro[3,2-*b*]pyridine-3-carboxylate (7e). M.W. 432.94; R_f 0.16 (eluent: EtOAc/PE 2:8); $[\alpha]_D^{20}$ −69.6 (*c* 0.72, $CHCl_3$).

^{1}H NMR (300 MHz): δ 1.30 [9H, s]; 3.00 [1H, dd, *J* 12, 10]; 3.21 [1H, dd, *J* 12, 8]; 3.32 [1H, dq, J_d 17, J_q 3]; 3.40–3.50 [1H, m]; 3.48 [3H, s]; 4.04 [1H, s]; 4.20–4.30 [1H, m]; 4.68 [1H, dd, *J* 10, 8]; 5.15 [1H, d, *J* 10]; 5.24 [1H, d, *J* 17]; 5.63 [1H, ddd, *J* 17, 10, 8]; 5.65–5.75 [1H, m]; 6.05 [1H, br d, *J* 10]; 6.32 [1H, s]; 7.30 [4H, m].

^{13}C NMR (75 MHz): δ 28.5 (CH_3); 51.0 (CH); 51.4 (C); 51.8 (CH_3); 52.1 (CH_2); 65.4 (CH); 71.5 (CH); 77.6 (CH); 80.8 (CH); 119.1 (CH_2); 125.7 (CH); 127.8 (CH); 128.3 (CH); 129.9 (CH); 133.7 (C); 134.5 (CH); 136.9 (C); 169.2 (C); 171.7 (C).

GC–MS 11.1 min (100%) [332 (40%, M−*t*-BuNHCO), 208 (50%, M−*t*-BuNHCOCHC$_6$H$_4$Cl), 96 (100%)].

Elem. Anal. Calcd for: C, 63.81; H, 6.75; Cl, 8.19; N, 6.47. Found C, 63.71; H, 6.74; Cl, 8.21; N, 6.47.

4.7.11. (5a*S*,6a*R*,10a*S*,10b*S*)-10-((*R*)-1-(*tert*-Butylcarbamoyl)-3-methylbutyl)-5a,6a,9,10,10a,10b-hexahydropyrido[2,3-*b*]furo[3,2-*c*]oxepin-1-(3*H*)-one (8d). M.W. 388.50; R_f 0.16 (eluent: EtOAc/PE 2:8); $[\alpha]_D^{20}$ +129.0 (*c* 0.70, $CHCl_3$).

^{1}H NMR (300 MHz): δ 0.83 [3H, d, *J* 7]; 0.95 [3H, d, *J* 7]; 1.15–2.00 [10H, m]; 2.00–2.20 [1H, m]; 2.40 [1H, d, *J* 10]; 3.20–3.40 [4H, m]; 3.75–3.85 [1H, m]; 4.10 [1H, br s]; 4.54 [1H, ddd, *J* 13, 7, 1]; 4.64 [1H, dd, *J* 13, 7]; 5.11 [1H, br d, *J* 10]; 5.74 [1H, ddd, *J* 10, 6, 3]; 6.03 [1H, ddd, *J* 10, 4, 2]; 6.10–6.20 [1H, m]; 6.26 [1H, br d, *J* 10]; 7.46 [1H, d, *J* 7].

^{13}C NMR (75 MHz): δ 20.0 (CH_3); 20.3 (CH_3); 24.7 (CH_2); 25.6 (2×CH_2); 26.7 (CH); 32.8 (CH_2); 33.5 (CH_2); 47.8 (CH_2); 48.0 (CH); 54.2 (CH); 62.4 (CH_2); 65.7 (CH); 70.6 (CH); 76.4 (CH); 79.7 (CH); 124.3 (CH); 126.2 (CH); 128.6 (CH); 138.1 (CH); 168.3 (C); 174.2 (C).

GC–MS 11.3 min (100%) [388 (M), 262 (100%, M−cyclohexNHCO), 124 (40%)].

Elem. Anal. Calcd for: C, 68.01; H, 8.30; N, 7.21. Found C, 67.84; H, 8.28; N, 7.22.

4.7.12. (1*S*,2*S*,3*S*,4*R*)-Methyl 3-(*N*-((*RS*)-1-(benzylcarbamoyl)-3-methylbutyl)-*N*-propargylamino)-7-oxa-bicyclo[2.2.1]hept-5-ene-2-carboxylate (10a). Mixture of diastereoisomers; M.W. 410.51; R_f 0.34 (eluent: EtOAc/PE 3:7).

^{1}H NMR (300 MHz): δ 0.90–1.00 [9H, m]; 1.60–1.90 [3H, m]; 2.15 [0.67H, d, *J* 2]; 2.18 [0.33H, d, *J* 2]; 2.84 [0.33H, t, *J* 4]; 2.94 [0.67H, t, *J* 4]; 3.40 [0.67H, d, *J* 4]; 3.48 [0.33H, d, *J* 4]; 3.50–3.75 [3H, m]; 3.58 [2H, s]; 3.62 [1H, s]; 4.30–4.50 [2H, m]; 4.94 [0.67H, d, *J* 5]; 4.96 [1H, s]; 5.06 [0.33H, d, *J* 5]; 6.25–6.45 [2H, m]; 6.83 [0.33H, t, *J* 6]; 7.18 [0.67H, t, *J* 6]; 7.20–7.35 [5H, m].

^{13}C NMR (75 MHz): δ 22.4 (CH_3); 22.7 and 22.9 (CH_3); 25.1 and 25.3 (CH); 36.7 and 37.0 (CH_2); 38.4 and 38.5 (CH_2); 43.2 (CH_2); 47.6 and 48.1 (CH); 51.8 and 51.9 (CH_3); 60.6 and 61.6 (CH); 63.4 and 64.5 (CH); 71.9 and 72.0 (CH); 78.2 and 81.3 (CH); 78.3 (CH); 81.5 and 82.4 (CH); 127.2 and 127.3 (CH); 127.7 and 127.8 (CH); 128.5 (CH); 135.2 and 135.7 (CH); 136.0 and 136.4 (CH); 138.3 and 138.4 (C); 171.9 and 172.3 (C); 172.5 and 173.1 (C).

Elem. anal. (mixture) calcd for: C, 70.22; H, 7.37; N, 6.82. Found C, 70.14; H, 7.35; N, 6.83.

4.7.13. (1*S*,2*S*,3*S*,4*R*)-Methyl 3-(*N*-((*R*)-(*tert*-butylcarbamoyl)(4-chlorophenyl)methyl)-*N*-propargylamino)-7-oxa-bicyclo[2.2.1]hept-5-ene-2-carboxylate (10b). Major diastereoisomer; M.W. 430.92; R_f 0.32 (eluent: EtOAc/PE 3:7); $[\alpha]_D^{20}$ −72.2 (*c* 0.50, $CHCl_3$).

^{1}H NMR (300 MHz): δ 1.37 [9H, s]; 2.26 [1H, t, *J* 2]; 3.10 [1H, t, *J* 4]; 3.36 [1H, dd, *J* 18, 2]; 3.40 [1H, d, *J* 4]; 3.66 [1H, dd, *J* 18, 2]; 3.69 [3H, s]; 4.29 [1H, br s]; 4.77 [1H, s]; 5.06 [1H, d, *J* 4]; 6.22 [1H, dd, *J* 6, 2]; 6.29 [1H, dd, *J* 6, 2]; 7.02 [1H, s]; 7.26–7.36 [4H, m].

^{13}C NMR (75 MHz): δ 28.4 (CH_3); 37.4 (CH_2); 47.1 (CH); 51.0 (C); 52.0 (CH_3); 64.2 (CH); 68.0 (CH); 72.8 (CH); 78.3 (CH); 80.7 (C); 81.0 (CH); 128.6 (CH); 131.3 (CH); 133.9 (C); 134.6 (C); 135.6 (CH); 136.0 (CH); 169.6 (C); 171.7 (C).

Elem. Anal. Calcd for: C, 64.11; H, 6.32; Cl, 8.23; N, 6.50. Found C, 64.23; H, 6.31; Cl, 8.21; N, 6.48.

4.7.14. (2*S*,3*S*,3a*S*,7a*R*)-Methyl 4-((*R*)-(*tert*-butylcarbamoyl)(4-chlorophenyl)methyl)-2,3,3a,4,5,7a-hexahydro-2,6-divinylfuro[3,2-*b*]pyridine-3-carboxylate (11b). M.W. 458.98; R_f 0.77 (eluent: EtOAc/PE 4:6); $[\alpha]_D^{20}$ −25.5 (*c* 0.58, $CHCl_3$).

^{1}H NMR (300 MHz): δ 1.31 [9H, s]; 2.98 [1H, dd, *J* 12, 10]; 3.19 [1H, dd, *J* 12, 9]; 3.32 [1H, br d, *J* 16]; 3.47 [3H, s]; 3.72 [1H, br d, *J* 16]; 4.07 [1H, s]; 4.35 [1H, br d, *J* 9]; 4.69 [1H, dd, *J* 10, 7]; 5.02 [1H, d, *J* 18]; 5.04 [1H, d, *J* 11]; 5.14 [1H, ddd, *J* 10, 2, 1]; 5.22 [1H, ddd, *J* 17, 2, 1]; 5.62 [1H, ddd, *J* 17, 10, 8]; 6.04 [1H, br s]; 6.26 [1H, dd, *J* 18, 11]; 6.28 [1H, s]; 7.20–7.40 [4H, m].

^{13}C NMR (75 MHz): δ 28.6 (CH_3); 51.1 (CH); 51.5 (C); 51.8 (CH_2); 51.8 (CH_3); 65.8 (CH); 72.0 (CH); 78.4 (CH); 81.1 (CH); 113.7 (CH_2); 119.1 (CH_2); 126.0 (CH); 128.4 (CH); 129.9 (CH); 133.7 (C); 134.4 (CH); 135.9 (CH); 136.6 (C); 136.9 (C); 169.2 (C); 171.6 (C).

GC–MS 12.3 min (100%) [458 (M); 358 (100%, M−*t*-BuNHCO), 234 (70%, M−*t*-BuNHCOCHC$_6$H$_4$Cl), 122 (90%)].

Elem. Anal. Calcd for: C, 65.42; H, 6.81; Cl, 7.72; N, 6.10. Found C, 65.48; H, 6.80; Cl, 7.70; N, 6.12.

4.7.15. (*Z*,2*S*,3*S*,3a*S*,8a*R*)-Methyl 4-((*R*)-(*tert*-butylcarbamoyl)(4-chlorophenyl)methyl)-3,3a,4,5,6,8a-hexahydro-6-methylene-2-vinyl-2*H*-furo[3,2-*b*]azepine-

3-carboxylate (12b). M.W. 458.98; R_f 0.64 (eluent: EtOAc/PE 4:6); $[\alpha]_D^{20}$ −96.4 (*c* 0.50, $CHCl_3$).

^{1}H NMR (300 MHz): δ 1.36 [9H, s]; 2.94 [1H, dd, *J* 9, 6]; 3.37 [3H, s]; 3.43 [1H, br d, *J* 15]; 3.60 [1H, dd, *J* 9, 6]; 3.94 [1H, br d, *J* 15]; 4.07 [1H, s]; 4.54 [1H, dd, *J* 9, 8]; 4.93 [1H, br d, *J* 9]; 4.95 [1H, s]; 5.16 [1H, ddd, *J* 10, 1, 1]; 5.17 [1H, s]; 5.30 [1H, ddd, *J* 17, 2, 1]; 5.57 [1H, ddd, *J* 17, 10, 7]; 5.99 [1H, br d, *J* 11]; 6.20 [1H, dd, *J* 11, 2]; 6.56 [1H, s]; 7.20–7.40 [4H, m].

^{13}C NMR (75 MHz): δ 28.7 (CH_3); 51.3 (C); 51.4 (CH_3); 55.6 (CH_2); 56.4 (CH); 70.8 (CH); 71.2 (CH); 79.3 (CH); 80.5 (CH); 118.6 (CH_2); 121.3 (CH_2); 128.7 (CH); 131.3 (CH); 131.3 (CH); 132.2 (CH); 133.2 (CH); 134.1 (C); 134.9 (C); 140.5 (C); 169.9 (C); 171.5 (C).

GC–MS 12.5 min (100%) [458 (M); 358 (100%, M−*t*-BuNHCO), 234 (50%, M−*t*-BuNHCOCHC$_6$H$_4$Cl), 125 (100%)].

Elem. Anal. Calcd for: C, 65.42; H, 6.81; Cl, 7.72; N, 6.10. Found C, 65.28; H, 6.82; Cl, 7.72; N, 6.11.

4.7.16. (2*S*,3*S*,3a*S*,8*S*,9*R*,9a*S*,9b*R*)-*N*-Benzyl-4-((*R*)-(*tert*-butylcarbamoyl)(4-chlorophenyl)methyl)-3-(methoxycarbonyl)-2,3,3a,4,5,7,8,9,9a,9b-decahydro-2-vinylfuro[3,2-*c*]isoquinoline-8,9-dicarboximide (13). M.W. 646.18; R_f 0.22 (eluent: EtOAc/PE 3:7); $[\alpha]_D^{20}$ +60.2 (*c* 0.60, $CHCl_3$).

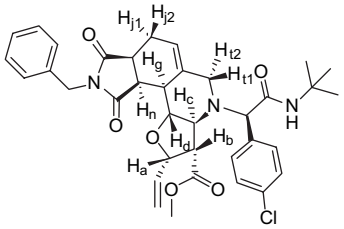
^{1}H NMR (300 MHz): δ 1.36 [9H, s]; 2.05–2.15 [1H, m]; 2.41 [1H, ddd, *J* 11, 6, 4]; 2.69 [1H, ddd, *J* 15, 7, 1]; 3.05 [1H, br d, *J* 16]; 3.10 [1H, ddd, *J* 8, 8, 1]; 3.23 [1H, t, *J* 10]; 3.32 [1H, d, *J* 16]; 3.38 [1H, t, *J* 10]; 3.40 [1H, dd, *J* 8, 6]; 3.58 [3H, s]; 3.94 [1H, s]; 4.51 [1H, d, *J* 14]; 4.62 [1H, d, *J* 14]; 4.67 [1H, t, *J* 10]; 4.89 [1H, dd, *J* 10, 8]; 5.22 [1H, d, *J* 10]; 5.33 [1H, d, *J* 17]; 5.57 [1H, br s]; 5.67 [1H, ddd, *J* 17, 10, 8]; 6.72 [1H, s]; 7.20–7.40 [9H, m].

^{13}C NMR (75 MHz): δ 25.1 (CH_2); 28.6 (CH_3); 39.9 (CH); 40.5 (CH); 42.3 (CH_2); 42.7 (CH); 49.0 (CH_2); 51.5 (C); 52.2 (CH_3); 54.8 (CH); 65.5 (CH); 67.8 (CH); 76.9 (CH); 81.2 (CH); 119.3 (CH_2); 122.1 (CH); 127.8 (CH); 128.2 (CH); 128.2 (CH); 128.5 (CH); 129.7 (CH); 133.1 (C); 134.5 (CH); 135.7 (C); 136.2 (C); 136.3 (C); 168.1 (C); 173.5 (C); 176.9 (C); 179.0 (C).

Elem. Anal. Calcd for: C, 66.91; H, 6.24; Cl, 5.49; N, 6.50. Found C, 67.09; H, 6.23; Cl, 5.50; N, 6.51.

NOE analysis. Given the configurations of C_c and C_d (respectively *S* and *R*), the structure is confirmed by a NOESY signal between H_g and H_c (the two protons are cis, therefore the configuration of C_g is *S*) and by the absence of a signal between H_d and H_n (the two protons are trans, therefore the configuration of C_n is *R*).

Detailed NOE absorbances H_d–H_{t1}: 7%; H_d–H_b: 7%; H_d–H_a: 6%; H_g–H_c: strong, overlapped with H_g–H_n; H_g–H_{j1}: 8%; H_g–H_{t2}: 4%. The signals of H_c and H_n are overlapped in the ^{1}H NMR; it is not possible to determine an NOE absorbance between these two protons.



4.7.17. (2*S*,3*S*,3a*S*,9a*S*,9b*R*)-Diethyl 4-((*R*)-(*tert*-butylcarbamoyl)(4-chlorophenyl)methyl)-2,3,3a,4,5,9,10b-hexahydro-3-methoxycarbonyl-2-vinylfuro[3,2-*h*]pyrido[4,3-*c*]pyridazine-8,9(7*H*,10a*H*)-dicarboxylate (14). M.W. 633.13; R_f 0.49 (eluent: EtOAc/PE 1:1); $[\alpha]_D^{20}$ −104.9 (*c* 0.35, $CHCl_3$).

^{1}H NMR (CD_3OD, 300 MHz): δ 1.20–1.30 [6H, m]; 1.28 [9H, s]; 3.10 [1H, t, *J* 10]; 3.20–3.60 [5H, m]; 3.47 [3H, s]; 4.12 [1H, s]; 4.15–4.30 [4H, m]; 4.43 [1H, dd, *J* 17, 6]; 4.60 [1H, t, *J* 8]; 5.15 [1H, d, *J* 10]; 5.27 [1H, d, *J* 17]; 5.55–5.70 [1H, m]; 5.68 [1H, br s]; 7.20–7.40 [4H, m]; 7.81 [1H, s].

^{13}C NMR (CD_3OD, 75 MHz): δ 14.9 (CH_3); 28.7 (CH_3); 43.4 (CH_2); 52.4 (CH); 52.8 (CH_3); 57.4 (CH_2); 60.1 (C); 63.6 and 64.0 (CH_2); 69.5 (CH); 71.0 (CH); 80.8 (CH); 81.6 (CH); 119.0 (CH_2); 123.6 (CH); 129.3 (CH); 131.9 (CH); 134.9 (C); 136.2 (CH); 138.2 (C); 156.7 and 157.2 (C); 172.2 (C); 172.7 (C).

GC–MS 17.4 min (100%) [532 (200%, M−*t*-BuNHCO), 408 (30%, M−*t*-BuNHCOCHC$_6$H$_4$Cl), 207 (75%), 125 (95%), 95 (100%)].

Elem. Anal. Calcd for: C, 58.81; H, 6.53; Cl, 5.60; N, 8.85. Found C, 58.63; H, 6.53; Cl, 5.59; N, 8.87.

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Supplementary data

Complete product characterisation data of all new compounds and detailed NOE experiments for compound **13**. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2006.06.061.

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Netamines A–G: seven new tricyclic guanidine alkaloids from the marine sponge *Biemna laboutei*

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Abstract—In our continuing program to identify bioactive compounds from marine invertebrates, the MeOH/EtOAc (1:1) extract of three collections of the Madagascar sponge, *Biemna laboutei*, was found to be cytotoxic to a series of human tumor cells. From the two sponges, seven new guanidine alkaloids, designated netamines A–G (**1**–**7**), have been isolated and their structures elucidated. Compounds **3** and **4** were found to be cytotoxic against three tumor cells with GI_{50} values in the micromolar range.

1. Introduction

The first report of a complex sponge-derived polycyclic guanidine alkaloid was on the pentacyclic ptilomycalin A, which we isolated from both a Caribbean sample of *Ptilocaulis spiculifer* and a Red Sea collection of *Hemimycale* sp.[1,2] Shortly thereafter, the structure of hydroxylated ptilomycalins, designated crambescidins 800, 816, 830, and 844, isolated from the Mediterranean sponge *Crambe crambe* and other sponges have been reported.[3–8] Pentacyclic guanidine alkaloids have also been reported from Brazilian specimens of *Monanchora unguiculata*[4] and Caribbean collection of *Batzella* spp.[5] Tricyclic guanidine alkaloids bearing the (5,6,8b)-triazaperhydroacenaphthylene skeleton (ptilocaulin derivatives and mirabilins) were reported from *Batzella* spp.,[5,9] *P. spiculifer*,[10] and curiously, from two New Caledonian starfish[11] (probably due to sequestration of these alkaloids from prey-sponges). Due to similar morphological characters and secondary metabolites, it is suggested that the above mentioned sponges should eventually be united in one sponge genus which, for priority reasons, has to be *Crambe*.[4]

Many of the cyclic guanidine derivatives show noteworthy biological activities, e.g., HIV gp120-human CD4-binding inhibition, $p56^{lck}$-CD4 dissociation induction, Ca^{2+} channel blocker activities, cytotoxicity, and antifungal and antimicrobial activities.[2,5,9,11]

As part of a continuing program to discover bioactive compounds from marine invertebrates,[12–14] we found that the extracts of the Poecilosclerida sponge *Biemna laboutei* (Hooper, 1996) to be cytotoxic. The sponge was collected twice near the Sainte-Marie Island on the east coast of Madagascar, in May 2004 and once at Itampule, Madagascar, in January 2005.

2. Results and discussion

The $CHCl_3$/MeOH (1:1) extracts of the frozen *B. laboutei* samples were subjected to solvent-partitioned, i.e., aq MeOH against hexane and CH_2Cl_2 and the CH_2Cl_2 fraction was chromatographed on Sephadex LH-20, eluted with hexane/MeOH/$CHCl_3$ (2:1:1), to afford a complex cytotoxic mixture of nitrogen-atom containing compounds. From the later mixture we isolated upon repeated Sephadex LH-20 and silica gel chromatography, and in several cases also RP-18 HPLC, seven compounds designated netamines A–G (compounds **1**–**7**) (in 9, 0.5, 2.5, 3, 12, 0.6, 2×10^{-3} wt %) thus far (Fig. 1). Additional hydroxylated netamines, which exist in minute quantities, are under investigation.

The molecular formula of netamine A (**1**) was established by HRFABMS to be $C_{19}H_{35}N_3$ *m/z* 306.2903 $[M+H]^+$ (calcd

Keywords: Marine sponges; Guanidine alkaloids; Heterocyclic.
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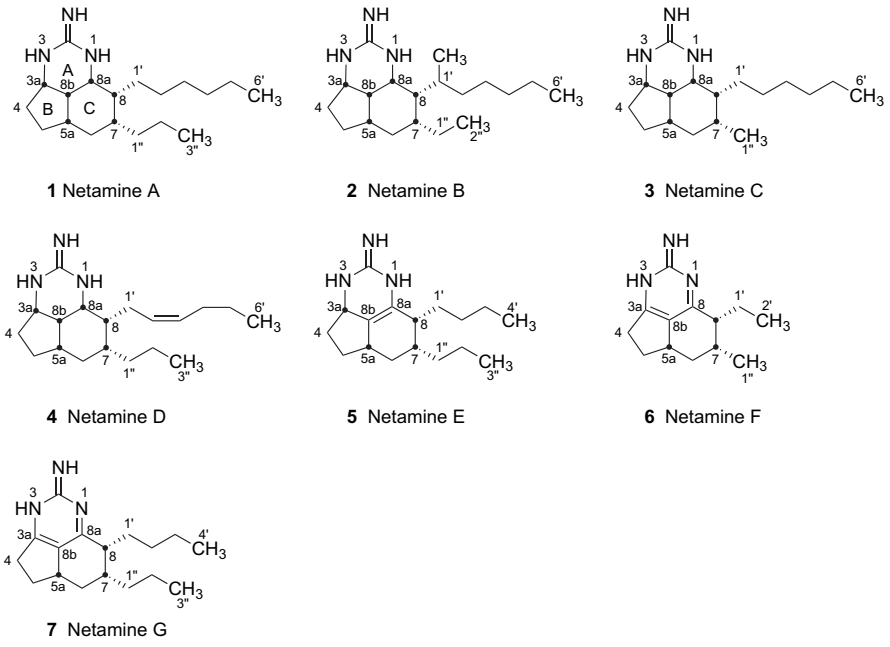


Figure 1. Netamines A–G (**1**–**7**).

306.2901) indicating four degrees of unsaturation. Analysis of the 1D and 2D ^{1}H and ^{13}C NMR data for **1** (Table 1) exhibited a single sp^2 carbon atom (δ 156.0), which together with three NH groups (δ_H 7.80, 7.85, and 6.90), suggested a guanidine moiety. In the absence of a carbon–carbon double bond, besides the guanidine imine, netamine A (**1**) had to be tricyclic to account for the four degrees of unsaturation. Two methines next to nitrogens (C-3a and C-8a, δ_C 53.6, δ_H 3.80 dd and δ_C 49.4, δ_H 3.57 dd, respectively) were a good starting point for the structure elucidation of the ring system. The latter two methines were connected to each other via another methine (C-8b) resonating at δ_C 35.2 and δ_H 2.30. The COSY experiment (Table 1) further connected the latter methine to another CH-group (C-5a, δ_C 35.3, δ_H 2.05 m). The high degree of overlapping of methylene protons made interpretation of the COSY map difficult. Assistance from selective 1D TOCSY experiments, applying different mixing times (10–100 ms), provided several proton sequences (3a, 4, 5, 5a and 5a, 6, 7). CH correlations deduced from an HMBC experiment (Table 1; Fig. 2) gave the crucial information for constructing a (5,6,8b)-triazaperhydroacenaphthylene ring system. The ^{15}NH HMBC agreed well with the suggested heterocyclic ring system namely, $^{3}J_{NH}$ correlations observed between H-8b, H-4 and a nitrogen atom resonating at 84.1 ppm ($^{1}J_{NH}$=93 Hz) established this nitrogen to be N-3. $^{3}J_{NH}$ correlations from H-8b to a second nitrogen atom resonating at 87.5 ppm determined it to be N-1 ($^{1}J_{NH}$=93 Hz). The proton on N-2 (δ_N 65.3 ppm) gave $^{3}J_{NH}$ correlations to both N-1 and N-3 ($^{1}J_{NH}$=93 Hz) (Fig. 3). Essentially, the latter unsaturated ring system is known in ptilocaulin,[15] isoptilocaulin,[3] and several mirabilins,[16] however, the saturated system is new. Indeed, a saturated ring system with unknown stereochemistry of the five chiral centers has been reported

Table 1. 1D and 2D NMR data for netamine A (**1**) in $CDCl_3$

Position	δ_C, ppm[a,b]	δ_H, ppm (mult, *J* Hz)	COSY (^{1}H–^{1}H)[c]	HMBC (H–C)
1	—	7.80 (br s)		—
2	156.0s	6.90 (br s)		3a, 8a, NH-1, NH-2, NH-3
3	—	7.85 (br s)		—
3a	53.6d	3.80 (dd, 6.2, 3.9)	4, 8b	4β, 5a, 5β, 8a, 8b, NH-2
4	33.5t	1.63 (m) 1.93 (m)	3a, 5	3a, 8b
5	30.5t	1.36 (m) 1.95 (m)	4, 5a	3a, 5a
5a	35.3d	2.05 (m)	5, 6, 8b	3a, 6β, 8a, 8b,
6	32.5t	0.98 (q, 12.0) 1.73 (dt, 12.7, 4.8)	5a, 7	8b
7	39.3d	1.13 (m)	6, 8, 1″	6β, 6α, 7, 8, 8a
8	43.4d	1.51 (m)	7, 8a, 1′	1″, 6β, 6α, 7, 8a, NH-1
8a	49.4d	3.57 (dd, 5.0, 1.5)	8, 8b	1′, 5a, 5β, 8, 8b, NH-1
8b	35.2d	2.30 (dt, 11.1, 5.9)	3a, 8a	3a, 4β, 5a, 8a, NH-1
1′	31.7t	1.27 (m)	8, 2″	7
2′	27.7t	1.28 (m)	1′	8
3′	29.6t	1.32 (m)		8
4′	35.1t	1.27 (m)		6′
5′	22.6t	1.28 (m)	6′	6′
6′	14.2q	0.89 (t, 7.0)	5′	
1″	40.2t	1.40 (m) 1.48 (m)	7, 2″	3″, 6α
2″	20.4t	1.28 (m) 1.39 (m)		3″
3″	14.0q	0.90 (t, 7.0)	2″	

[a] $CDCl_3$, using a Bruker ARX-500 instrument.
[b] Multiplicities were determined by DEPT and HSQC experiments.
[c] The CH correlations were assigned by an HSQC experiment.

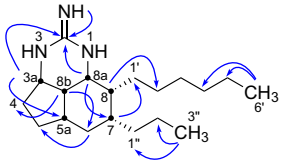


Figure 2. Key HMBC correlations in netamin A (**1**).

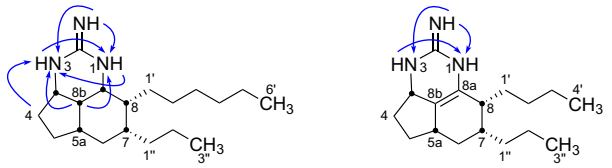


Figure 3. ^{15}N HMBC correlations in netamine A (**1**) and netamine E (**5**).

synthetically.[17] Additional HMBC correlations for **1** clearly indicated that the heterocyclic ring system is substituted at positions C-7 and C-8 by two alkyl groups ($\delta_{CH(7)}$ 39.3 d and $\delta_{CH(8)}$ 43.4 d)—including a combined nine carbon atoms. A fragmentation of 86 m.u. in the MS measurement (*m/z* 220 [M+H$^+$−C_6H_{13}]), demonstrated that one chain is a hexyl group and, therefore, the second chain has to be a propyl substituent. The latter propyl group was confirmed by CH correlations from CH_3 (3″) to CH_2 (1″) and CH_2 (2″). The propyl group was determined to be attached to C-7 on the basis of HMBC correlations of H-6α to C-1″, and H-7 to C-1″, C-2″, and C-1′, and therefore, the hexyl group substitutes the C-8 atom. The stereochemistry of netamine A (**1**) was unequivocally determined by NOE correlations shown in Figure 4 (because of overlapping protons, other NOE's

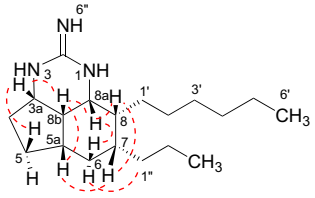


Figure 4. Key NOEs for netamine A–D (**1**–**4**).

are less clear). Namely, an NOE between the center H-8b (δ 2.30) and protons H-3a, H-5a, and H-8a (δ 3.80, 2.05, and 3.57) determined all four to be directed to the same β-side of the tricyclic ring system, thereby forming a twisted 'cap' shape system. Next, the stereochemistry of C-7 was determined from the multiplicity of H-6α, i.e., the latter proton, appearing as a quartet with a coupling constant of ca. 12 Hz, has to be axial and thus H-5a and H-7 also have to be axial. Hence, the propyl chain at C-7 has to be α-equatorial. The latter three axial protons require the cyclohexane ring (C) to be in a slightly twisted chair conformation. Additional NOE's between H-5a and H-7 and between H-6β (equatorial) and H-7 further supported the suggested configuration of C-7. An NOE between H-6α and H-1″ unequivocally confirmed the stereochemistry of C-7. Next, the stereochemistry at C-8 was determined from NOEs from both H-8a(β) and H-7(β) to H-8, namely H-8 also has to be in the β-configuration, and therefore, the two chains are cis to each other.

The second isolated compound, netamine B (**2**) was only obtained in minute amounts (2 mg, 0.5×10^{-3}% dry weight). Its mass spectrum suggested the same formula, $C_{19}H_{35}N_3$, as that of **1**, implying, together with the NMR spectroscopic data, an isomeric structure. The major difference in the ^{1}H NMR spectrum of **2** was an additional methyl group, δ_H 1.01 d (*J*=6.5 Hz). The CH correlations of this methyl to C-1′ and C-8 determined its location on C-1′. As in **1**, the two side chains include nine carbon atoms that differ, however, from the chains of **1**. Namely, C-7 carries an ethyl group (C-1″, -2″) (Table 2) (*m/z* 279.2 [M+H$^+$−C_2H_5]), confirmed by CH correlations from CH_3-2″ to CH_2-1″ and CH-7, and C-8 the rest of the chain-carbons. The above mentioned CH_3 (d), together with the correlations of a second triplet methyl group, CH_3-6′ (δ_H 0.83, δ_C 13.9) to C-5′ and C-4′, established the *iso*-heptyl structure of the C-8 side chain. Similar NOEs to the one measured for **1** suggested the same stereochemistry for **2** (Fig. 4).

From a second Saint-Marie collection of *B. laboutei* were isolated netamines C (**3**) and D (**4**). Their structure determination was done similar to that of compounds **1** and **2**. Netamine C, with two carbon atoms less than **1**, was found to be its C-7-CH_3 lower homolog, and netamine D (**4**), with two protons less, the 2′(3′)-dehydro analog of **1**. In the structure

Table 2. ^{13}C NMR spectral data of netamines A–H (**1**–**8**)[a,b]

C	1	2	3	4	5	6	7	8
2	156.0s	156.0s	154.9s	154.8s	155.0q	163.0s	162.8s	156.0s
3a	53.6d	53.7d	53.7d	53.6d	54.3d	175.0s	174.8s	53.7d
4	33.5t	34.3t	33.4t	33.3t	34.4t	33.6t	34.0t	33.5t
5	30.5t	30.2t	30.4t	30.4t	30.8t	33.1t	32.9t	30.6t
5a	35.3d	34.4d	35.8d	35.4d	38.0d	37.8d	37.3d	35.1d
6	32.5t	33.2t	35.1t	35.1t	36.9t	39.6t	35.9t	32.5t
7	39.3d	35.6d	34.5d	38.9d	38.7d	33.2d	38.5d	39.3d
8	43.4d	45.0d	45.0d	43.6d	42.5d	47.7d	44.0d	43.6d
8a	49.4d	49.8d	49.8d	48.7d	129.2s	166.0s	166.1s	49.4d
8b	35.2d	35.2d	34.8d	34.9d	119.8s	127.0s	126.4s	35.3d
1′	31.7t	31.5d	34.7t	32.2t	29.1t	22.8t	30.5t	34.8t
2′	27.7t	27.4t	27.5t	127.3d	27.8t	9.5q	27.5t	29.9t
3′	29.6t	29.3t	29.4t	132.0d	24.7t	—	23.1t	22.9t
4′	35.1t	34.5t	31.7t	29.5t	14.9q	—	14.1q	14.2q
5′	22.6t	23.1t	22.6t	22.7t	—	—	—	—
6′	14.2q	22.4t	14.0q	13.8q	—	—	—	—
7′	—	13.8q	—	—	—	—	—	—
1″	40.2t	34.8t	23.1q	40.0t	37.7t	20.8q	36.8t	40.2t
2″	20.4t	14.0q	—	20.3q	21.7t	—	20.1t	20.2t
3″	14.0q	—	—	14.1q	15.2q	—	14.3q	14.0q

[a] $CDCl_3$, using a Bruker ARX-500 instrument (except **5** which was in CD_3OD).
[b] Multiplicities were determined by DEPT and HSQC experiments.

elucidation of **4**, due to the double bond label, the identification of the side chains and their locations was more facile. Namely, it was found that a hex-2-enyl group is attached to C-8 and a propyl group to C-7. The *Z*-configuration of the double bond was determined on the basis of the 11.0 Hz coupling constant between the two olefinic protons as well as an NOE between them.

Netamine E (**5**) was isolated from the third, Itampule sponge specimen, and assigned the molecular composition of $C_{17}H_{29}N_3$ (CIMS *m/z* 276.2 $[M+H]^+$). According to the spectral data of compound **5** (Tables 2 and 4), it was determined to possess a 8a(8b)-isomeric ptilocaulin type structure. CH correlations, derived from an HMBC experiment [correlations from H-3a, H-7, and H-8 to C-8a (129.2), and from H-3a, H-8, and H-5a to C-8b (119.8)], confirmed the unsaturated tricyclic ring system. The δ_N values of the three NH groups (δ_N 70.4, 85.3, and 96.0), which agreed well with the nitrogen chemical shifts of **1**, fully supported the suggested structure. The 8a(8b)-double bond assisted with the differentiation of the side chains, as H-8 is in an allylic position resonating at δ_H 1.98 ppm. Again, as with most other netamines, the 'lower' chain was a higher homolog of the C-7 methyl found in ptilocaulin and the mirabilins. The C-7 chain of **5** was determined to be a propyl group, as in **1** and **4**, and the C-8 chain to be a shorter butyl substituent (*m/z* 219.2 $[M+H^+-C_4H_9]$). The stereochemistry elucidation of **5** started from the multiplicity of H-6α, namely, as explained above for **1**–**4**, a quartet with a 12 Hz coupling constant pointed to two vicinal (H-5a and 7) axial proton atoms. The key NOEs, depicted in Figure 5, established the all cis-geometry of the heterocyclic methine protons (H-3a, 5a, 7, and 8).

Hydrogenation of compound **5** over Pd mainly afforded compound **8** (Scheme 1) which, according to the carbon chemical shifts, which is an excellent probe for the ring's stereochemistry (Table 2), has the same stereochemistry as netamines A–D.

Two additional compounds, netamines F and G, were isolated from the latter sponge and differed from netamines A–D by possessing an unsaturated ring system, as in mirabilins A–C,[16] however, with a different stereochemistry. The imine, rather than the tautomeric aminopyrimidine, structure of the latter was recently determined by Hamann via X-ray diffraction analysis of a mixture of two ptilocaulins.[18] Netamine F (**6**) is the *cis*-7-methyl-8-ethyl analog and **7** the 1(8a),3a(8b)-dehydro analog of **5** (Fig. 1). Comparison of the NMR data (Tables 2 and 4) of netamines F and G (**6** and **7**) with compounds **1**–**5** (Tables 2 and 3) and mirabilins A–C[16] clearly pointed to the 7,8-disubstituent-1(8a),3a(8b)-unsaturated tricyclic ring system. Netamine F (**6**), the *cis*-7-methyl-8-ethyl substituted compound (Fig. 6), is the smallest homolog among the netamines while netamine G (**7**) possesses the same skeleton as **5**. Indeed,

Table 3. ^{1}H NMR spectral data of netamines A–D (**1**–**4**)[a]

H	1	2	3	4
1-NH	7.80 (br s)	7.75 (br s)	6.93 (br s)	6.94 (br s)
2-NH	6.90 (br s)	6.90 (br s)	7.53 (br s)	7.55 (br s)
3-NH	7.85 (br s)	7.80 (br s)	7.67 (br s)	7.64 (br s)
3a	3.80 (dd, 6.2, 3.9)	3.80 (m)	3.86 (m)	3.83 (m)
4	1.63 (m)	1.62 (m)	1.63 (m)	1.64 (m)
	1.93 (dd, 13.0, 5.0)	1.85 (m)	1.84 (dd)	1.84 (dd)
5	1.36 (m)	1.28 (m)	1.30 (m)	1.34 (m)
	1.95 (m)	1.95 (m)	1.94 (m)	1.98 (m)
5a	2.05 (m)	2.05 (m)	2.08 (m)	2.07 (m)
6	0.98 (q, 12.0)	0.92 (m)	1.06 (m)	0.94 (m)
	1.73 (dt, 12.7, 4.8)	1.73 (m)	1.63 (m)	1.73 (ddd)
7	1.13 (m)	2.03 (m)	1.21 (m)	1.16 (m)
8	1.51 (m)	1.30 (m)	1.36 (m)	1.55 (m)
8a	3.57 (dd, 5.0, 1.5)	3.48 (br d)	3.51 (br d)	3.55 (br d)
8b	2.30 (dt, 11.1, 5.9)	2.30 (dt)	2.32 (m)	2.32 (m)
1′	1.27 (m)	1.25 (m)	1.28 (m)	2.00 (m)
				2.04 (m)
2′	1.28 (m)	1.30 (m)	1.31 (m)	5.34 (m)
3′	1.32 (m)	1.28 (m)	1.28 (m)	5.47 (dt)
4′	1.27 (m)	1.28 (m)	1.28 (m)	2.00 (m)
5′	1.28 (m)	1.25 (m)	1.27 (m)	1.38 (m)
		1.35 (m)		
6′	0.89 (t, 7.0)	0.82 (t)	0.89 (t)	0.91 (t)
7′	—	1.01 (d)	—	—
1″	1.40 (m)	1.25 (m)	1.02 (d)	1.28 (m)
	1.48 (m)	1.35 (m)		1.41 (m)
2″	1.28 (m)	0.85 (t)	—	1.28 (m)
	1.39 (m)			1.37 (m)
3″	0.90 (t, 7.0)	—	—	0.88 (t)

[a] $CDCl_3$, using a Bruker ARX-500 instrument.

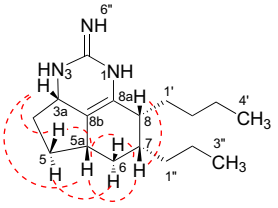


Figure 5. Key NOEs for netamine E (**5**).

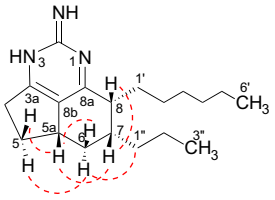
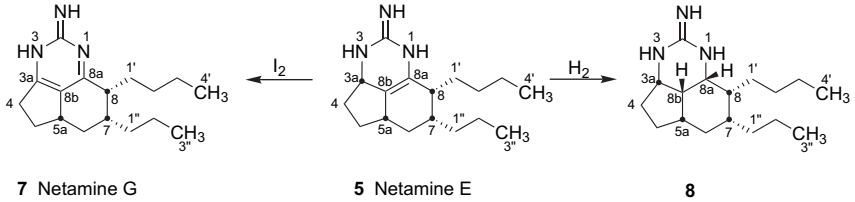


Figure 6. Key NOEs for netamine F–G (**6**–**7**).



Scheme 1. Reduction and oxidation of netamine E (**5**).

Table 4. ^{1}H NMR spectral data of netamines E–H (**5–7**)

H	**5**[a]	**6**[b]	**7**[b]
1-NH	9.82 (br s)	—	—
2-NH	8.65 (br s)	—	—
3-NH	7.43 (br s)	—	—
3a	4.29 (br t, 8.0)	—	—
4	1.70 (m)	2.58 (dd, 17.0, 8.0)	2.65 (dd)
	2.22 (m)	2.90 (m)	2.95 (m)
5	1.30 (m)	1.52 (m)	1.55 (m)
	2.05 (m)	2.35 (dt, 12.0, 7.0)	2.40 (m)
5a	2.45 (m)	2.90 (m)	2.90 (m)
6	0.80 (m)	0.92 (q, 12.0)	0.88 (m)
	2.15 (m)	2.02 (ddd, 12.3, 4.8, 3.2)	2.22 (dt)
7	1.60 (m)	1.86 (m)	1.75 (m)
8	1.98 (m)	2.30 (m)	2.35 (m)
1′	1.70 (m)	1.79 (m)	1.76 (m)
		2.13 (m)	2.04 (m)
2′	1.25 (m)	0.81 (t, 7.3)	1.12 (m)
			1.31 (m)
3′	1.38 (m)	—	1.31 (m)
4′	0.95 (m)	—	0.89 (t)
1″	1.25 (m)	1.08 (d, 6.6)	1.27 (m)
	1.55 (m)		1.58 (m)
2″	1.35 (m)	—	1.31 (m)
	1.50 (m)		1.50 (m)
3″	0.95 (m)	—	0.96 (t)

[a] CD_3OD, using a Bruker ARX-500 instrument (NH values were taken in DMSO-d_6).
[b] $CDCl_3$, using a Bruker Avance-400 instrument.

dehydrogenation of **5** with iodine afforded compound **7** (Scheme 1).

Netamines A–G are presumably derived via intramolecular cyclization of guanidine-substituted polyketides (C_{12} for **6** through C_{16} for **3**, **5**, and **7** to C_{18} for **1**, **2**, and **4**) as earlier suggested by Capon.[16] Outstanding is netamine B (**2**) with the *iso*-heptyl side chain. The netamines add new structures to the group of the tricyclic guanidine alkaloids. Four of the netamines (A–D) possess the unprecedented saturated all cis ring system with six chiral centers. ^{15}N chemical shifts, measured from ^{15}NH HMBC, have been shown to be an additional important tool for the structure elucidation of these guanidines.

3. Biological activity

The in vitro activity of netamines C and D was evaluated against three human tumor cell lines: NSCL (A549), colon (HT29), and breast (MDA-MB-231) (Table 5).[19] All other compounds were less active.

Considering the bioactivities of the guanidine alkaloids all new compounds have to be further examined in a variety of tests.

Table 5. Cytotoxicity data (GI_{50}, μM) for netamines C (**3**) and D (**4**)

Compound	Cell lines/GI_{50} (μM)		
	A549	HT29	MDA-MB-231
3	4.3	2.4	2.6
4	6.6	5.3	6.3

4. Experimental

4.1. General

Optical rotations were obtained with a Jasco P-1010 polarimeter. IR spectra were obtained with a Bruker FTIR Vector 22 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on Bruker ARX-500 and Avance-400 spectrometers. ^{1}H, ^{13}C, COSY, HSQC, and HMBC were recorded using standard Bruker pulse sequences. EIMS, CIMS, and HRMS measurements were recorded on a Fisons, Autospec Q instrument.

4.2. Biological material

B. laboutei (Hooper, 1996) was collected twice from the east coast of Madagascar at the Saint-Marie Island, in May 2004 at a depth of 30 m and once from Itampule, 150 km west-south of Tuléar, Madagascar, in January 2005 at a depth of 20 m. The sponge causes dermaties, order Poecilosclerida, family Desmacellidae.

4.3. Extraction and isolation

The frozen sponge *B. labouti* (344 g) was homogenized and extracted with $CHCl_3$/MeOH (2:1). The organic extract was concentrated to yield a crude extract (6.2 g). The crude extract was subjected to partitioning by the Kupchan method.[20] The dichloromethane fraction (640 mg) was repeatedly chromatographed on a Sephadex LH-20 column, eluting with hexane/MeOH/$CHCl_3$ (2:1:1) to obtain compounds **1** (30 mg, 0.009 wt %), **2** (2 mg, 0.0006 wt %), **5** (40 mg, 0.0012 wt %), and **7** (8 mg, 0.002 wt %). A fraction of the Sephadex LH-20 column (containing compound **6**) was subjected to VLC over silica gel, using hexane with increasing proportions of ethyl acetate as eluent. Compound **6** (2 mg, 0.0006 wt %) was afforded by elution with 50% ethyl acetate in hexane.

A frozen second wet sample of *B. labouti* (529 g) was exhaustively extracted with water (3×1 L) and then with CH_2Cl_2/MeOH (1:1) (3×1 L). The organic extract was concentrated to yield a gummy material (5.2 g). This material was subjected to VLC on Lichroprep RP-18 with a stepped gradient from H_2O/MeOH (3:1) to MeOH. Next, one fraction (535 mg), eluted with H_2O/MeOH (1:3) (875 mg), was subjected to preparative HPLC (Symmetry Prep C-18, 19×150 mm, gradient H_2O+0.1% TFA/CH_3CN+0.1% TFA, from 35 to 45% CH_3CN in 20 min, UV detection, 15 ml min^{-1}) to yield **3** (14 mg, 0.0025 wt %) and impure **4** (17 mg, 0.003 wt %) as their TFA salts. The final purification of **4** was achieved by semi preparative HPLC (Symmetry Prep C-18, 7.8×150 mm, isocratic H_2O+0.1% TFA/CH_3CN+0.1% TFA 54:46, UV detection, 2.5 ml min^{-1}), obtaining 8.8 mg of pure compound **4**.

4.3.1. Netamine A (1). Pale yellow oil; $[\alpha]_D^{20}$ +2.2 (*c* 0.65, CH_2Cl_2); IR (CH_2Cl_2) ν_{max} 3244, 3030, 2930, 1667 cm^{-1}; ^{1}H and ^{13}C NMR see Tables 2 and 3; HRFABMS *m/z* $[M+H]^+$ 306.2903 (calcd for $C_{19}H_{35}N_3$, 306.2901), 220.1 (10) ($[M+H]^+-C_6H_{13}$).

4.3.2. Netamine B (2). Pale yellow oil; $[\alpha]_D^{20}$ +5.3 (*c* 0.05, CH_2Cl_2); IR (CH_2Cl_2) ν_{max} 3250, 3930, 2850, 1667 cm^{-1};

^{13}C and ^{1}H NMR see Tables 2 and 3; HRFABMS *m/z* $[M+H]^+$ 306.2893 (calcd for $C_{19}H_{35}N_3$, 306.2901), 279.2 (100) ($[M+H]^+-C_2H_5$).

4.3.3. Netamine C (3). Pale yellow oil; $[\alpha]_D^{25}$ +3.7 (*c* 0.09, MeOH); IR (KBr) ν_{max} 3260, 2931, 2860, 1669 cm^{-1}; ^{13}C and ^{1}H NMR see Tables 2 and 3; (+)-HRESIMS *m/z* $[M+H]^+$ 278.2602 (calcd for $C_{17}H_{32}N_3$, 278.2590).

4.3.4. Netamine D (4). Pale yellow oil; $[\alpha]_D^{25}$ −5.8 (*c* 0.03, MeOH); IR (KBr) ν_{max} 3428, 2932, 2868, 1662 cm^{-1}; ^{13}C and ^{1}H NMR see Tables 2 and 3; (+)-HRESIMS *m/z* $[M+H]^+$ 304.2760 (calcd for $C_{19}H_{34}N_3$, 304.2747).

4.3.5. Netamine E (5). Pale yellow oil; $[\alpha]_D^{21}$ +35.0 (*c* 0.80, CH_2Cl_2); IR (CH_2Cl_2) ν_{max} 3272, 2959, 2872, 1670 cm^{-1}; ^{13}C and ^{1}H NMR see Tables 2 and 4; CIMS *m/z* 276.3 $[M+H]^+$, 233.2 (10) ($[M+H]^+-C_3H_7$), 219.2 (100) ($[M+H]^+-C_4H_9$), 176.1 (70) ($[M+H]^+-C_7H_{16}$); HRCIMS *m/z* 276.2442 (calcd for $C_{17}H_{29}N_3$, 276.2448).

4.3.6. Netamine F (6). Pale yellow oil; $[\alpha]_D^{20}$ +108.0 (*c* 0.05, CH_2Cl_2); IR (CH_2Cl_2) ν_{max} 3852, 3749, 3648, 1698, 1541 cm^{-1}; ^{13}C and ^{1}H NMR see Tables 2 and 4; EIMS *m/z* 217.2 $[M]^+$, 188.2 (100) ($M^+-C_2H_5$), 173.2 (95) ($M^+-C_3H_8$); HREIMS *m/z* 217.1591 (calcd for $C_{13}H_{19}N_3$, 217.1587).

4.3.7. Netamine G (7). Pale yellow oil; $[\alpha]_D^{21}$ +27.0 (*c* 0.20, CH_2Cl_2); IR (CH_2Cl_2) ν_{max} 2959, 2872, 1601 cm^{-1}; ^{13}C and ^{1}H NMR see Tables 2 and 4; EIMS *m/z* 273.2 $[M^+]$, 230.2 (20) ($[M]^+-C_3H_7$), 216.2 (80) ($[M]^+-C_4H_9$), 173.1 (100) ($M^+-C_7H_{16}$); HREIMS *m/z* 273.2219 (calcd for $C_{17}H_{27}N_3$, 273.2213).

4.3.8. Hydrogenation of netamine E (5). Netamine E (8.5 mg) in methanol (10 ml) was hydrogenated over 10% Pd/C (10 mg), for 4 h at 3 atm pressure. The solution was filtered through Celite, then evaporated and chromatographed on Sephadex LH-20 to afford compound **8** (4 mg).

4.3.9. Oxidation of netamine E (5). Netamine E (2 mg) in CH_2Cl_2 (5 ml) was stirred at room temperature with I_2 (10 mg) for half an hour, then washed with a saturated solution of sodium sulphite, dried and evaporated to afford netamine G (**7**) (2 mg).

4.4. Biological activity

A colorimetric type assay, using the sulforhodamine B reaction, was adopted for quantitative measurement of the cell growth and viability, according to the technique described in the literature.[19] The in vitro activity of the compounds was evaluated against three human tumor cell lines: NSCL (A549), colon (HT29), and breast (MDA-MB-231).

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Ring opening of 2-acylaziridines by acid chlorides

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Abstract—Good nucleophilicity of the ring nitrogen in chiral (2*R*,1′*R*)-2-acyl-(1′-phenylethyl)aziridines initiated the reaction with various acid chlorides to form the corresponding acylaziridinium ion intermediates whose rings were opened by the chloride anion to yield the β-amino-α-chlorocarbonyl compounds. The subsequent displacement of the chloride with the internal oxygen nucleophile originated from methylchloroformate, acetyl chloride, and methyl chlorooxoacetate yielded oxazolidin-2-ones, β-amino-α-acetyloxypropionates, and morpholin-2,3-diones, respectively.

1. Introduction

Aziridine, nitrogen containing three-membered ring, is a cousin of oxirane with similar ring strain energy.[1] However, its property including proton affinity is quite different from oxirane when the ring nitrogen has a substituent other than simple hydrogen.[2] Basicity of the ring nitrogen of aziridine is dependent to the substituent whether it is electron donating or electron withdrawing.[3] Its difference would be observed by the comparison of electrostatic potentials of *N*-methyl and *N*-acetyl aziridines shown in Figure 1.[4] Electrostatic potentials of those two examples are very different not only in the nitrogen but carbons of the aziridine ring.

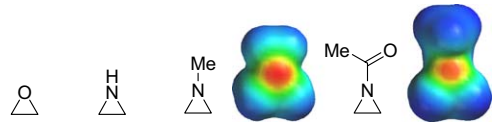
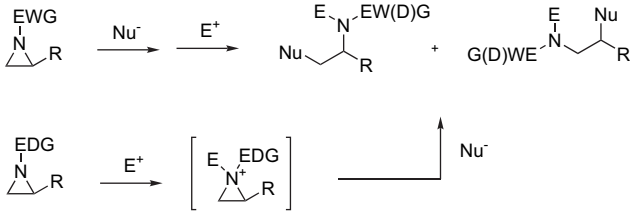


Figure 1. The chemical structures of oxirane, aziridine, *N*-methylaziridine, and *N*-acetylaziridine and two electrostatic potential maps of *N*-methylaziridine and *N*-acetylaziridine.

Naturally, the reactivity of aziridine is quite different whether the substituent on the ring nitrogen is electron donating or electron withdrawing.[1] When there is an electron-withdrawing substituent including *N*-sulfonyl, *N*-phosphonyl or *N*-carbonyl, the ring is activated with release of the electron density of the ring nitrogen as shown in *N*-acetyl aziridine in Figure 1. This aziridine reacts with the nucleophile to yield ring-opened products (Scheme 1).[5] However, aziridine with electron-donating substituent such as alkyl is quite inert toward nucleophiles. Ring opening of this aziridine requires the prior activation with a proper electrophile including protic or Lewis acid to form the corresponding azaridinium ion shown in the bracket of Scheme 1 that reacts with coming nucleophiles.[6,7] Regiochemical pathway of the ring-opening reaction is dependent to the substituent of R in Scheme 1. Most of the reactions proceeded in the less hindered position of C-3 to yield α-amino product. When R is allyl, benzyl or acyl substituents, the breakage of the bond between C-2 and the ring nitrogen occurs to give the β-amino product (Scheme 1).[8]



(EWG = Electron-withdrawing group, EDG = Electron-donating group)

Scheme 1.

Many examples of aziridine ring openings were disclosed only with electron-withdrawing substituents on the ring

Keywords: 2-Acylaziridine; Acid chloride; Ring opening; Morpholin-2,3-dione; Isoserine.
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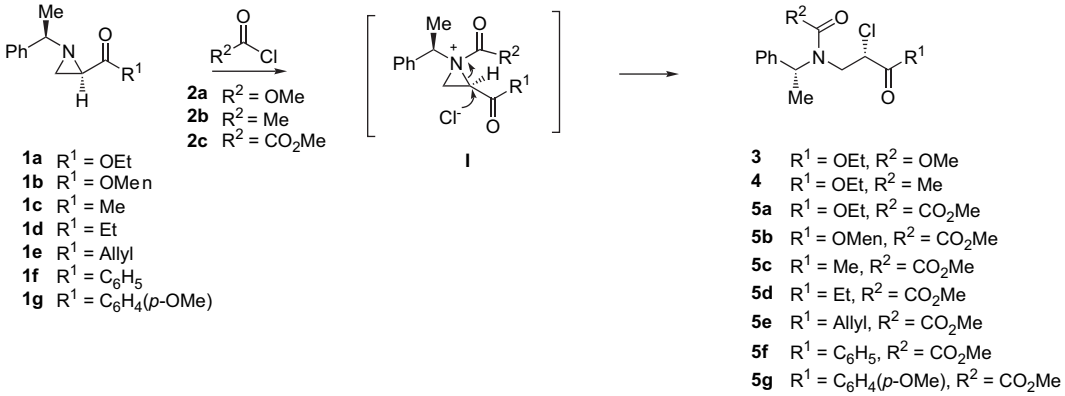
nitrogen such as *N*-sulfonyl, *N*-phosphonyl, and *N*-carbonyl.[1,5] However, they have certain limitations due to the paucity of reliable methodology to accomplish free amine with removal of *N*-substituents under mild condition. A few years ago, we have succeeded multi-kilo scale production of the enantiomerically pure aziridine bearing phenylethyl group at the ring nitrogen.[6] Their synthetic utility have been studied for many years taking advantage of their regio- and stereoselectivities of most reactions due to the rigidity of the aziridine ring.[6,9] Those aziridines represented by (1′*R*)-(1′-phenylethyl)aziridine-2-carboxylates are now commercially available in optically pure forms and are able to provide us many enantiomerically pure α- or β-amino esters and their derivatives.[6] In this report, the reaction of the (1′*R*)-(1′-phenylethyl)aziridine-2-carboxylate with acid chloride for the synthesis of valuable cyclic and acyclic hydroxy amines with important mechanistic implication of the aziridine ring opening reactions is described.

2. Results and discussion

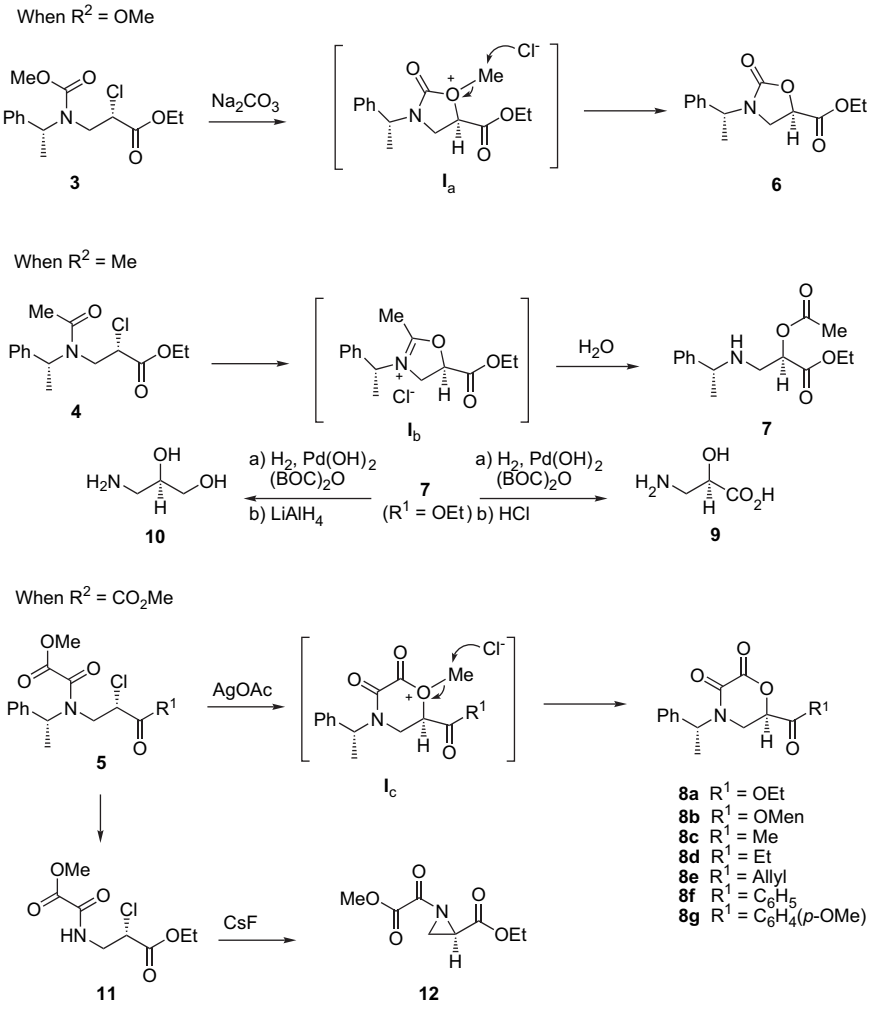
Ring-opening reactions of 2-acylaziridines with phenylethyl group at the ring nitrogen proceed to yield aziridinium ion for initial activation with the assistance of protic acid or Lewis acid as an electrophile followed by nucleophilic attack of the ring. The molecule bearing both characters as an electrophile and a nucleophile is acid chloride that is able to activate the aziridine ring and to provide the nucleophile to lead the ring-opening reactions. At first, the ring nitrogen of (1′*R*)-(1′-phenylethyl)-2-acylaziridines (**1**) is basic enough to react with acid chlorides (**2**) to yield the aziridinium ion (**I**) with the chloride free (Scheme 2). This aziridinium ion shown in the bracket of Scheme 2 is highly activated and ready to react with the coming nucleophile. The nucleophile available in the reaction mixture is the chloride ion liberated originally from the acid chloride during the formation of the aziridinium ion. The acyclic β-amino-α-chlorocarbonyl products (**3**–**5**) were resulted by nucleophilic ring opening of the aziridinium ion intermediate by the chloride. Various acid chlorides were applicable including alkoxychloroformate, acetyl chloride, and methyl chlorooxoacetate. All ring-opening reactions were highly specific without detectable amount of regio- or stereoisomers. This implies that the bond between C-2 and the ring nitrogen was labile as we expected and the reaction proceeded with complete inversion of the configuration during the attack of the coming nucleophile. The reaction was successful at room temperature under mild condition with wide scope of substrates such as aziridine-2-carboxylates (**1a**, **1b**) and 2-acylaziridines (**1c**–**g**) and various acid chlorides including methylchloroformate (**2a**), acetyl chloride (**2b**), and methyl chlorooxoacetate (**2c**) to afford β-amino-α-chloro carbonyl compounds in 53–98% yield (Scheme 2).

Acyclic β-amino-α-chlorocarbonyl compound was further reacted in many ways depending on the characteristics of R^2 (Scheme 3). When R^2 is OMe originated from methylchloroformate (**2a**), internal nucleophilic reaction by oxygen leads the formation of the oxazolidine-2-one ring (**6**) with removal of the chloride as in $\mathbf{I}_a$. The reaction pathway was disclosed in our early study by the isolation of the acyclic β-amino-α-chlorocarbonyl compound and by the stereochemical outcome of the reaction product.[10] However, the initial product of ethyl (2*R*,1′*R*)-[1-(1′-phenylethyl)]aziridine-2-carboxylate with acetyl chloride (**4**) is readily reacted by the internal oxygen nucleophile with the replacement of the chloride to make the possible dihydrooxazole ring compound as shown in $\mathbf{I}_b$. In the presence of small amount of moisture in the air or in silica gel, the intermediate $\mathbf{I}_b$ was hydrolyzed to yield (2*R*,1′*R*)-1-acetyloxy-2-[*N*-(1′-phenylethyl)]aminopropionate (**7**) in 75% yield. The removal of the phenylethyl group by hydrogenation in the presence of $(BOC)_2O$ and the subsequent hydrolysis provided (+)-(*R*)-isoserine (**9**).[11] Reduction by LAH after debenzylation of α-acyloxy-β-aminopropionate (**7**) afforded (2*R*)-3-amino-1,2-propandiol (**10**). In both of those two cases, the configurations of the stereogenic centers were identified to be '*R*' originated from the C-2 of the aziridine ring with retention of the configuration. This stereochemical outcome supports the double displacements with the inversion of the configuration during the ring opening and the removal of the chloride as we expected from the early observation.[10]

When R^2 was methoxycarbonyl originated from methyl chlorooxoacetate all of the acyclic reaction products **5** were stable enough to be isolated bearing the chloride at α-position and to be kept at room temperature under air. Preparation of acyclic β-amino-α-chloro compounds (**5**) was achieved from either aziridine-2-carboxylates or 2-acylaziridines with



Scheme 2.



Scheme 3.

various substituents of R^1 including OEt (**5a**), OMen (**5b**), Me (**5c**), Et (**5d**), allyl (**5e**), phenyl (**5f**), and *p*-methoxyphenyl (**5g**) in high yields. Removal of the phenylethyl from **5a** was successful to yield **11** with methanesulfonic acid and anisole[10] while catalytic hydrogenation did not work. Compound **11** was attempted to be cyclized with the removal of chlorine using several different bases including NaH, Na_2CO_3, Et_3N, and CsF. Among them, CsF was the best to afford a new (2*R*)-aziridine-2-carboxylate (**12**) as a single isomer judged by chiral GC and HPLC in 62% yield. Its configuration was possibly speculated as '*R*' derived from double inversions as observed in earlier cases for the preparation of **6**, **9**, and **10**. This was further confirmed by the formation of morpholin-2,3-diones in the next series of reactions.

As shown in the cases of oxazolidin-2-one (**6**) and α-acetyloxy-β-aminopropionate (**7**) from the acyclic chloropropanoates (**3** and **4**), cyclization of **5** afforded morpholin-2,3-dione (**8**) via nucleophilic displacement of chlorine by oxygen in the presence of AgOAc. This cyclization proceeded well in 52–86% yield from diverse acyclic compounds **5** originated from the early reactions of 2-acylaziridines (**1**) bearing various substituents. The stereochemistry was identified by the X-ray crystalline structure of morpholin-2,3-dione (**8a**) obtained from ethyl (2*R*)-aziridine-2-carboxylate (**1a**) (Fig. 2).[12]

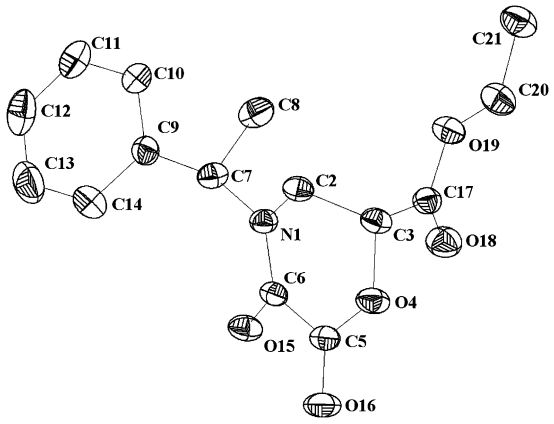


Figure 2. The X-ray structure of (6*R*,1′*R*)-6-ethoxycarbonyl-4-(1′-phenylethyl)morpholin-2,3-dione (**8a**).

The configuration '*R*' at C-2 of the starting aziridine was completely retained at C-6 of the final product **8a** whose position was denoted by C3 in Figure 2. This tells us that the reaction proceeds in double inversions during the aziridine ring opening as in Scheme 2 and the internal cyclization to form the morpholin-2,3-dione ring by the oxygen nucleophile as in $\mathbf{I}_c$ in Scheme 3.

In conclusion, good nucleophilicity of the ring nitrogen in the chiral (2*R*,1′*R*)-2-acyl-[1-(1′-phenylethyl)]aziridine initiated the reaction with various acid chlorides to form the acylaziridinium ion intermediates whose rings were opened by the chloride anion released from acid chloride to yield β-amino-α-chlorocarbonyl compounds. The subsequent displacement of the chloride with the internal oxygen nucleophile originated from methylchloroformate, acetyl chloride, and methyl chlorooxoacetate yielded oxazolidin-2-ones, β-amino-α-acetyloxypropionates, and morpholin-2,3-diones, respectively. *This is a new type of aziridine ring-opening reaction based on dual role of acid chlorides as an activator of the aziridine ring and a provider of the nucleophile for the aziridine ring opening*.

3. Experimental

3.1. General methods

^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian 200 (200 MHz for ^{1}H and 50.3 MHz for ^{13}C). Chemical shifts were given in parts per million using TMS as an internal standard. Mass spectra were obtained using a Hewlett Packard Model 5985B spectrometer or a Kratos Concept 1-S double focusing mass spectrometer. Elemental analysis was taken on a Perkin–Elmer 240 DS elemental analyzer. Melting point was measured by Mel-II capillary melting point apparatus. Optical rotations were measured on Rudolph Research Autopole 3 polarimeter. The silica gel used for column chromatography was Merck 230–400 mesh. Thin layer chromatography was carried out with Merck 60F-254 plates with 0.25 mm thickness.

3.2. Reactions of ethyl (2*R*,1′*R*)-(1′-phenylethyl)aziridine-2-carboxylate with acetyl chloride

Into the solution of ethyl (2*R*,1′*R*)-(1′-phenylethyl)aziridine-2-carboxylate (**1a**, 154 mg, 0.70 mmol) in anhydrous 20 mL CH_3CN, acetyl chloride (66 mg, 0.84 mmol) was added drop wise at room temperature. This solution was stirred for 0.5 h at room temperature until all the starting material was consumed. The reaction mixture was filtered and the filtrate was evaporated under reduced pressure to yield yellowish hydroscopic solid, which is unstable at room temperature under air. Mp 86 °C (decomposed). ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.34–7.65 (m, 5H), 5.59 (d, J=10.2 Hz, 1H), 4.17–4.29 (m, 1H), 4.13 (q, J=7.2 Hz, 2H), 3.18–3.22 (m, 2H), 2.30 (s, 3H), 1.93 (d, J=6.8 Hz, 3H), 1.17 (t, J=7.2 Hz, 3H). ^{13}C NMR (50.3 MHz, $CDCl_3$) δ 170.5, 166.7, 135.4, 129.7, 127.9, 127.1, 67.0, 62.5, 59.0, 44.8, 21.3, 20.6, 14.0. The above solid was dissolved in CH_2Cl_2 and was neutralized by Na_2CO_2 solution. The resultant organic layer was washed twice with brine and concentrated under reduced pressure. Purification by silica gel chromatography (EtOAc/*n*-hexane, 1:3) provided 147 mg of analytically pure product (**7**) in 75% yield. Liquid. $[\alpha]_D$ 19.1 (*c* 0.5, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.26–7.34 (m, 5H), 4.06–4.32 (m, 4H), 3.83 (q, J=7.2 Hz, 1H), 3.23–3.28 (m, 1H), 2.05 (s, 3H), 1.39 (d, J=7.0 Hz, 3H), 1.27 (t, J=7.2 Hz, 3H). ^{13}C NMR (50.3 MHz, $CDCl_3$) δ 173.0, 170.7, 144.7, 128.6, 127.3, 127.0, 66.0, 61.2, 57.8, 56.8, 25.4, 20.9, 14.3. HRMS (EI) calcd for $C_{15}H_{21}NO_4$: 279.1471, found 279.1477. Anal. Calcd for $C_{15}H_{21}NO_4$: C, 64.5; H, 7.58; N, 5.01. Found: C, 64.3; H, 7.66; N, 5.25.

3.3. General procedure for reactions of either (2*R*,1′*R*)-(1′-phenylethyl)aziridine-2-carboxylate or (2*R*,1′*R*)-2-acyl-(1′-phenylethyl)aziridine with methyl chlorooxoacetate

To the solution of either (2*R*,1′*R*)-(1′-phenylethyl)aziridine-2-carboxylate or (2*R*,1′*R*)-2-acyl-(1′-phenylethyl)aziridine (1.25 mmol) in CH_3CN under nitrogen at room temperature was added methyl chlorooxoacetate (184 mg, 1.50 mmol). The mixture was stirred for 1 h at room temperature and concentrated in vacuo. The mixture was extracted with CH_2Cl_2 and water. The combined organic extracts were dried, filtered, and concentrated under reduced pressure. Purification by silica gel chromatography (EtOAc/*n*-hexane, 1:3) provided analytically pure product.

3.3.1. Ethyl (2*S*,1′*R*)-2-chloro-3-[2-methoxy-2-oxo-*N*-(1′-phenylethyl)acetamido]propionate (5a). Yield 89%. Liquid. $[\alpha]_D$ 55.2 (*c*, 1.0 MeOH); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.33–7.58 (m, 5H), 4.96 (q, J=8.4 Hz, 1H), 4.57–4.66 (m, 1H), 4.12–4.27 (m, 6H), 3.08–3.20 (m, 1H), 1.70 (d, J=6.8 Hz, 3H), 1.15–1.24 (m, 3H). ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 168.1, 163.2, 163.1, 138.0, 129.0, 128.5, 127.3, 62.5, 56.7, 53.1, 53.0, 46.1, 18.0, 13.9. HRMS (EI) calcd for $C_{16}H_{20}ClNO_5$: 341.1031, found 341.1028.

3.3.2. (−)-Menthyl (2*S*,1′*R*)-2-chloro-3-[2-methoxy-2-oxo-*N*-(1′-phenylethyl)acetamido]-propionate (5b). Yield 98%. Liquid. $[\alpha]_D$ 18.1 (*c* 1.0, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.19–2.37 (m, 5H), 4.83 (q, J=6.8 Hz, 1H), 4.65 (t, J=4.0 Hz, 1H), 4.25–4.41 (m, 1H), 3.31–3.48 (m, 2H), 1.90–2.11 (m, 3H), 1.82–0.17 (m, 21H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 168.4, 163.2, 163.0, 137.7, 129.0, 128.5, 127.6, 57.0, 52.8, 52.5, 47.0, 46.1, 40.5, 34.1, 31.4, 26.0, 23.3, 22.1, 20.7, 17.4, 16.1. HRMS (EI) calcd for $C_{24}H_{34}ClNO_5$: 451.2126, found 451.2122.

3.3.3. *N*-{(2*S*)-Chloro-3-oxobutyl}-*N*-{(1*R*)-phenylethyl}-oxalamic acid methyl ester (5c). Yield 76%. Liquid. $[\alpha]_D$ 43.4 (*c* 2.5, MeOH); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.28–7.54 (m, 5H), 5.01 (q, J=6.8 Hz, 1H), 4.67 (t, J=6.6 Hz, 1H), 3.89 (s, 3H), 3.21–3.35 (m, 2H), 2.27 (s, 3H), 1.49–1.65 (m, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 201.6, 163.2, 162.9, 137.5, 129.1, 129.0, 127.6, 58.3, 57.0, 53.0, 44.8, 26.7, 17.3. HRMS (EI) calcd for $C_{15}H_{18}ClNO_4$: 311.0924, found 311.0933.

3.3.4. *N*-{(2*S*)-Chloro-3-oxopentyl}-*N*-{(1*R*)-phenylethyl}-oxalamic acid menthol ester (5d). Yield 88%. Liquid. $[\alpha]_D$ 40.1 (*c* 1.0, MeOH); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.19–7.42 (m, 5H), 5.02 (q, J=7.2 Hz, 1H), 4.90 (q, J=7.0 Hz, 1H), 3.69 (s, 3H), 3.38–3.54 (m, 2H), 2.35–2.47 (m, 2H), 1.52 (d, J=7.0 Hz, 3H), 1.01 (t, J=6.5 Hz, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 204.3, 163.2, 163.1, 138.0, 129.0, 129.0, 127.3, 57.7, 56.8, 53.0, 45.4, 33.0, 18.0, 7.7. HRMS (EI) calcd for $C_{16}H_{20}ClNO_4$: exact mass: 325.1081, found 325.1085.

3.3.5. *N*-{(2*S*)-Chloro-3-oxohex-5-enyl}-*N*-{(1*R*)-phenylethyl}oxalamic acid methyl ester (5e). Yield 84%. Liquid.

$[\alpha]_D$ 44.9 (*c* 1.0, MeOH); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.18–7.43 (m, 5H), 6.86 (q, *J*=7.0 Hz, 1H), 6.24 (d, *J*=15.8 Hz, 2H), 4.84 (q, *J*=8.2 Hz, 1H), 3.85–4.09 (m, 1H), 3.79 (s, 3H), 3.61 (d, *J*=6.6 Hz, 2H), 3.38 (d, *J*=6.2 Hz, 2H), 1.83 (m, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 192.4, 163.3, 163.2, 146.6, 137.7, 129.1, 128.9, 127.9, 127.6, 57.2, 54.9, 53.0, 45.5, 29.0, 18.7. HRMS (EI) calcd for $C_{17}H_{20}ClNO_4$: 337.1081, found 337.0177.

3.3.6. *N*-{(2*S*)-Chloro-3-oxo-3-phenylpropyl}-*N*-{(1*R*)-phenylethyl}oxalamic acid methyl ester (5f). Yield 67%. Liquid. $[\alpha]_D$ 59.6 (*c* 0.9, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.89–8.04 (m, 10H), 5.75 (q, *J*=7.4 Hz, 1H), 4.95 (t, *J*=7.2 Hz, 1H), 3.86 (s, 3H), 3.61 (d, *J*=7.6 Hz, 2H), 1.65–1.73 (m, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 193.2, 163.5, 163.2, 137.7, 134.2, 129.1, 128.9, 127.9, 127.5, 57.4, 53.0, 51.4, 46.0, 17.4. HRMS (EI) calcd for $C_{20}H_{20}ClNO_4$: 373.1081, found 373.1088.

3.3.7. *N*-{(2*S*)-Chloro-3-oxo-3-*p*-methoxylphenylpropyl}-*N*-{(1*R*)-phenylethyl}oxalamic acid methyl ester (5g). Yield 81%. Liquid. $[\alpha]_D$ 52.2 (*c* 1.0, MeOH); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.26–7.49 (m, 9H), 5.70 (q, *J*=6.2 Hz, 1H), 4.92 (t, *J*=7.0 Hz, 1H), 3.95 (s, 3H), 3.86 (s, 3H), 3.60 (d, *J*=6.6 Hz, 2H), 1.59–1.69 (m, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 191.7, 164.4, 163.5, 163.2, 137.6, 131.6, 131.3, 128.8, 128.6, 127.9, 114.2, 57.4, 55.6, 53.0, 51.3, 46.3, 17.4. HRMS (EI) calcd for $C_{21}H_{22}ClNO_5$: 403.1187, found 403.1179.

3.4. (2*R*)-Isoserine (9)

To the solution of ethyl (2*R*,1′*R*)-2-acetyloxy-3-(1′-phenylethyl)aminopropionate (**7**, 450 mg, 1.61 mmol) in MeOH were added $(Boc)_2O$ (530 mg, 2.43 mmol) and 340 mg of Pd/C. The reaction mixture was stirred at room temperature with atmospheric pressure of H_2 for 12 h, and then the catalyst was filtered. The solution was concentrated in vacuo. This crude product was dissolved in MeOH containing 2 M HCl. The reaction mixture was stirred for 1 h under reflux. The mixture was cooled to room temperature and concentrated under vacuum. The residue was purified by ion-exchange chromatography on Amberlite® IR-120H (H^+ form), eluting first with water and then with 5% NH_4OH to yield 128 mg isoserine in 76% yield. White solid. Mp= 197–198 °C. $[\alpha]_D$ −32.1 (*c* 0.5, H_2O); Lit.[11b] $[\alpha]_D$ −32.1 (*c* 0.5, H_2O).

3.5. (2*R*)-3-Amino-1,2-propandiol (10)

To the solution of **7** (279 mg, 1.0 mmol) in MeOH were added $(BOC)_2O$ (327 mg, 1.50 mmol) and 213 mg of $Pd(OH)_2$. The reaction mixture was stirred at room temperature with atmospheric pressure of H_2 for 12 h. Then the catalyst was filtered and the filtrate was concentrated in vacuo. Purification by silica gel chromatography (EtOAc/*n*-hexane, 1:3) provided oil whose solution in THF was added $LiAlH_4$ (95 mg, 2.5 mmol) at 0 °C. The mixture was filtered and concentrated in vacuo. This was dissolved 2 M HCl solution in MeOH and the resultant mixture was stirred for 1 h under reflux. The mixture was cooled to room temperature and concentrated under reduced pressure to afford **10** (29 mg) in 32% yield. Viscous oil. $[\alpha]_D$ 28.5 (*c* 0.5, MeOH); Lit.[13] $[\alpha]_D$ 28 (*c* 4, 5 M HCl). ^{1}H NMR (D_2O, 200 MHz) δ 3.45 (br s, 2H), 3.19–3.42 (m, 1H), 2.88 (d, *J*=4.4 Hz, 2H). ^{13}C NMR (D_2O, 50.3 MHz) δ 70.2, 62.9, 42.2.

3.6. Ethyl (2*S*)-2-chloro-3-(2-methoxy-2-oxoacetamido)-propionate (11)

To the solution of **5a** (839 mg, 2.45 mmol) in $CHCl_3$ under nitrogen were added methanesulfonic acid (1.179 g, 12.27 mmol) and anisole (662 mg, 6.13 mmol). The solution was refluxed for 4 h and then cooled to room temperature. The solution was quenched by saturated $NaHCO_3$ solution. The aqueous layer was extracted with CH_2Cl_2, and the combined organic extracts were dried, filtered, and concentrated under reduced pressure. Purification by silica gel column chromatography (EtOAc/*n*-hexane, 1:2) provided debenzylated product **11** as oil (308 mg, 53% yield). Liquid. $[\alpha]_D$ −22.0 (*c* 1.5, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.39 (t, *J*=6.4 Hz, 1H), 4.14 (q, *J*=7.0 Hz, 2H), 3.85 (s, 3H), 3.73–3.89 (m, 2H), 1.26 (t, *J*=7.2 Hz, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 168.0, 160.6, 156.7, 62.8, 54.0, 53.9, 42.8, 14.0. HRMS (EI) calcd for $C_8H_{12}ClNO_5$: 237.0404, found 237.0397.

3.7. Ethyl (2*R*)-1-methoxyoxalylaziridine-2-carboxylate (12)

To the solution of **11** (89 mg, 0.37 mmol) in CH_3CN was added cesium fluoride (70 mg, 0.44 mmol). The solution was refluxed for 3 h and then cooled to room temperature. The mixture was extracted with CH_2Cl_2 and water. The combined organic extracts were dried, filtered, and concentrated under reduced pressure. Purification by silica gel chromatography (EtOAc/*n*-hexane, 1:2) provided 31 mg **12** in 42% yield. Liquid. $[\alpha]_D$ 34.5 (*c* 0.5, EtOAc); ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.31 (q, *J*=7.0 Hz, 2H), 4.23–4.36 (m, 3H), 4.00 (s, 3H), 1.44 (t, *J*=7.0 Hz, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 169.3, 157.1, 156.6, 62.2, 59.3, 53.7, 42.7, 14.0. HRMS (EI) calcd for $C_8H_{11}NO_5$: 201.0637, found 201.0643.

3.8. General procedure for the preparation of morpholin-2,3-diones (8) from either (2*S*,1′*R*)-2-chloro-3-{*N*-methoxyoxalyl-(1′-phenylethyl)amino}propionate (5a, 5b) or *N*-{(2*S*)-chloro-3-oxoalkyl}-*N*-{(1*R*)-phenylethyl}oxalamic acid methyl ester (5c–g)

To solution of **5** (0.91 mmol) in CH_3CN was added silver acetate (1.10 mmol). The solution was refluxed for 3 h and then cooled to room temperature. The mixture was extracted with CH_2Cl_2 and water. The combined organic extracts were dried, filtered, and concentrated under reduced pressure. Purification by silica gel chromatography (EtOAc/*n*-hexane, 1:3) provided **8** as oil.

3.8.1. (6*R*,1′*R*)-6-Ethoxycarbonyl-4-(1′-phenylethyl)morpholine-2,3-dione (8a). Yield 84%. Liquid. $[\alpha]_D$ 87.4 (*c* 2.4, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.32–7.58 (m, 5H), 6.01 (q, *J*=7.4 Hz, 1H), 4.91–4.94 (m, 1H), 3.82–4.12 (m, 2H), 3.48–3.72 (m, 2H), 1.47 (d, *J*=7.0 Hz, 3H), 0.97 (t, *J*=6.8 Hz, 3H). ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 167.4, 156.1, 152.7, 138.1, 128.9, 128.5, 127.7, 72.8, 63.1, 51.3, 41.1, 14.8, 13.7. HRMS (EI) calcd for $C_{15}H_{17}NO_5$: 291.1107,

found 291.1115. Anal. Calcd for $C_{15}H_{17}NO_5$: C, 61.9; H, 5.88; N, 4.81. Found: C, 61.6; H, 5.69; N, 4.87.

3.8.2. (6*R*,1′*R*)-6-(−)-Menthylcarbonyl-4-(1′-phenylethyl)morpholine-2,3-dione (8b). Yield 86%. Liquid. $[\alpha]_D$ 7.3 (*c* 2.0, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 7.24–7.58 (m, 5H), 4.96 (q, *J*=7.2 Hz, 1H), 4.64–4.72 (m, 1H), 3.64–3.82 (m, 1H), 3.32–3.47 (m, 2H), 1.82–0.17 (m, 21H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 169.8, 167.9, 166.9, 138.7, 128.9, 128.8, 127.5, 78.0, 71.6, 52.7, 47.0, 40.5, 34.2, 31.4, 26.4, 23.6, 22.0, 20.7, 20.5, 17.0. HRMS (EI) calcd for $C_{23}H_{31}NO_5$: 401.2202, found 401.2211.

3.8.3. (6*R*,1′*R*)-6-Acetyl-4-(1′-phenylethyl)morpholine-2,3-dione (8c). Yield 82%. Liquid. $[\alpha]_D$ 64.2 (*c* 1.5, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 7.19–7.43 (m, 5H), 4.94 (q, *J*=7.0 Hz, 1H), 4.61–4.74 (m, 2H), 3.48–3.61 (m, 1H), 2.28–2.35 (m, 3H), 1.55 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 202.1, 178.2, 152.8, 137.5, 129.1, 128.7, 127.4, 79.1, 52.0, 40.3, 26.8, 15.1. HRMS (EI) calcd for $C_{14}H_{15}NO_4$: 261.2732, found 261.2736.

3.8.4. (6*R*,1′*R*)-6-Propionyl-4-(1′-phenylethyl)morpholine-2,3-dione (8d). Yield 60%. Liquid. $[\alpha]_D$ 85.1 (*c* 1.0, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 7.16–7.44 (m, 5H), 5.04 (q, *J*=7.4 Hz, 1H), 4.85 (q, *J*=7.0 Hz, 1H), 3.66–3.81 (m, 2H), 2.53 (q, *J*=7.0 Hz, 2H), 1.81 (d, *J*=7.0 Hz, 3H), 0.93 (t, *J*=7.1 Hz, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 206.5, 170.1, 163.4, 138.1, 129.0, 128.8, 127.5, 75.5, 52.9, 42.1, 33.0, 20.5, 7.1. HRMS (EI) calcd for $C_{15}H_{17}NO_4$: 275.2998, found 275.3004.

3.8.5. (6*R*,1′*R*)-6-But-3-enoyl-4-(1′-phenylethyl)morpholine-2,3-dione (8e). Yield 52%. Liquid. $[\alpha]_D$ 88.3 (*c* 1.0, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 7.11–7.32 (m 5H), 5.24 (q, *J*=7.0 Hz, 1H), 571–5.84 (m, 3H), 5.03 (q, *J*=8.2 Hz, 1H), 3.91 (d, *J*=6.2 Hz, 2H), 3.14–3.29 (m, 2H), 1.69–1.86 (m, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 192.4, 160.3, 158.9, 137.1, 132.9, 128.6, 128.3, 127.7, 115.3, 91.8, 50.1, 46.5, 38.0, 21.7. HRMS (EI) calcd for $C_{16}H_{17}NO_4$: 287.1158, found 287.1151.

3.8.6. (6*R*,1′*R*)-6-Benzoyl-4-(1′-phenylethyl)morpholine-2,3-dione (8f). Yield 72%. Liquid. $[\alpha]_D$ 65.9 (*c* 1.5, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 7.93–8.21 (m, 10H), 6.15 (q, *J*=7.0 Hz, 1H), 4.85 (q, *J*=7.0 Hz, 1H), 3.80 (d, *J*=4.0 Hz, 2H), 1.67 (d, *J*=7.2 Hz, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 195.2, 170.0, 163.4, 138.2, 134.0, 129.0, 128.9, 128.8, 128.4, 128.4, 127.9, 127.6, 71.6, 52.9, 43.4, 20.4. HRMS (EI) calcd for $C_{19}H_{17}NO_4$: 323.1158, found 323.1150.

3.8.7. (6*R*,1′*R*)-6-*p*-Methoxybenzoyl-4-(1′-phenylethyl)-morpholine-2,3-dione (8g). Yield 81%. Liquid. $[\alpha]_D$ 81.2 (*c* 1.5, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ ^1H NMR (200 MHz, $CDCl_3$) δ 7.19–7.37 (m, 9H), 5.16 (q, *J*= 7.2 Hz, 1H), 4.91 (q, *J*=7.0 Hz, 1H), 3.79 (s, 3H), 3.34–3.46 (m, 2H), 1.69 (d, *J*=6.8 Hz, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 193.6, 170.1, 164.3, 163.5, 138.3, 131.5, 130.9, 128.8, 128.4, 127.7, 127.5, 71.4, 57.0, 55.6, 53.0, 20.5. HRMS (EI) calcd for $C_{20}H_{19}NO_5$: 353.1263, found 353.1265.

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12. Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 295629. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44(0)-1223–336033 or e-mail: deposit@ccdc.cam.ac.uk].
13. Aldrich® catalog for (R)-3-aminopropandiol, No. 09267.

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Bioactive prenylated xanthones and anthraquinones from *Cratoxylum formosum* ssp. *pruniflorum*

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Abstract—Ten new compounds (**1**–**10**), pruniflorone A–J, together with 21 known compounds (**11**–**31**) were isolated from the roots and barks of *Cratoxylum formosum* ssp. *pruniflorum*. Their structures were determined by spectroscopic methods. Compounds **1** and **11** were also confirmed by X-ray diffraction data. In addition, antibacterial and cytotoxic activities of the isolates were also evaluated.

1. Introduction

Cratoxylum belongs to the family Guttiferae, which is distributed in several Southeast Asian countries. Six species are found in Thailand;[1] *Cratoxylum arboresens*, *Cratoxylum cochinchinense*, *Cratoxylum maingayi*, *Cratoxylum sumatranum* ssp. *neriifolium*, *Cratoxylum formosum* ssp. *formosum* (Jack) Dyer and *Cratoxylum formosum* (Jack) Dyer ssp. *pruniflorum* (Kurz) Gogel. The last two species, which are subspecies of *C. formosum*, can be differentiated through the young twigs, leaves, pedicels and sepals. Those of *C. formosum* ssp. *formosum* are glabrous, whereas *C. formosum* ssp. *pruniflorum* are densely villous.[2] Some species of this genus have been used for the treatment of diuretic, stomachic and tonic effects,[3] as well as for diarrhoea and flatulence,[4] and for food poisoning and internal bleeding.[5] They produce various types of secondary metabolites, including xanthones,[6a–c] triterpenoids[6b,7] and flavonoids.[3] We have previously isolated a number of xanthones from the roots of *C. formosum* ssp. *formosum*.[8] As a continuation of our study on this genus, we report herein nine new xanthones (**1**–**9**): pruniflorones A–I, and nine known xanthones (**11**–**19**) from the roots, a new anthraquinone (**10**): pruniflorone J, six known anthraquinones (**20**–**25**) and six known xanthones (**26**–**31**) from the barks of *C. formosum* ssp. *pruniflorum*. In addition, the antibacterial and cytotoxic activities of selected compounds are also reported.

2. Results and discussion

The CH_2Cl_2 extracts of the roots and barks of *C. formosum* ssp. *pruniflorum* were subjected to column chromatography to give nine new xanthones (**1**–**9**) and a new anthraquinone (**10**). All isolated new xanthones gave characteristic signals in the UV spectrum, showing absorption bands in the range of 236–261 and 312–380 nm. The IR spectra also exhibited characteristic conjugated carbonyl and hydroxyl functionalities in the range of 1632–1646 and 3170–3414 cm^{-1}, respectively. Moreover, the ^1H and ^{13}C NMR spectral data suggested that the isolated new xanthones **1**–**6** had the 1,3,6,7-oxygenated xanthone skeleton, whereas xanthones **7**–**8** and **9** were 1,3,5,6- and 1,3,6-oxygenated xanthones, respectively.

Pruniflorone A (**1**) was isolated as a pale yellow powder, which was further recrystallized from $CHCl_3$–MeOH (4:1, v/v) to yield pale yellow single crystals. The X-ray structure (Fig. 1) confirmed a molecular structure with a prenylated xanthone skeleton and a molecular formula $C_{25}H_{30}O_7$. Its structure was supported by ^1H and ^{13}C NMR spectral data (Tables 1 and 2). The ^1H NMR spectral data of **1** (Table 1) showed two aromatic protons at δ 6.29 (s, H-4) and 6.72 (s, H-5), and two methoxyl groups at δ 3.90 (s, 3-OMe) and 3.84 (s, 7-OMe). In addition, the ^1H NMR spectral data also exhibited a dimethylchromane ring[9] at δ 2.73 (2H, br t,

Keywords: *Cratoxylum formosum* ssp. *pruniflorum*; Pruniflorone; Xanthone; Anthraquinone; Antibacterial activity; Cytotoxic activity.
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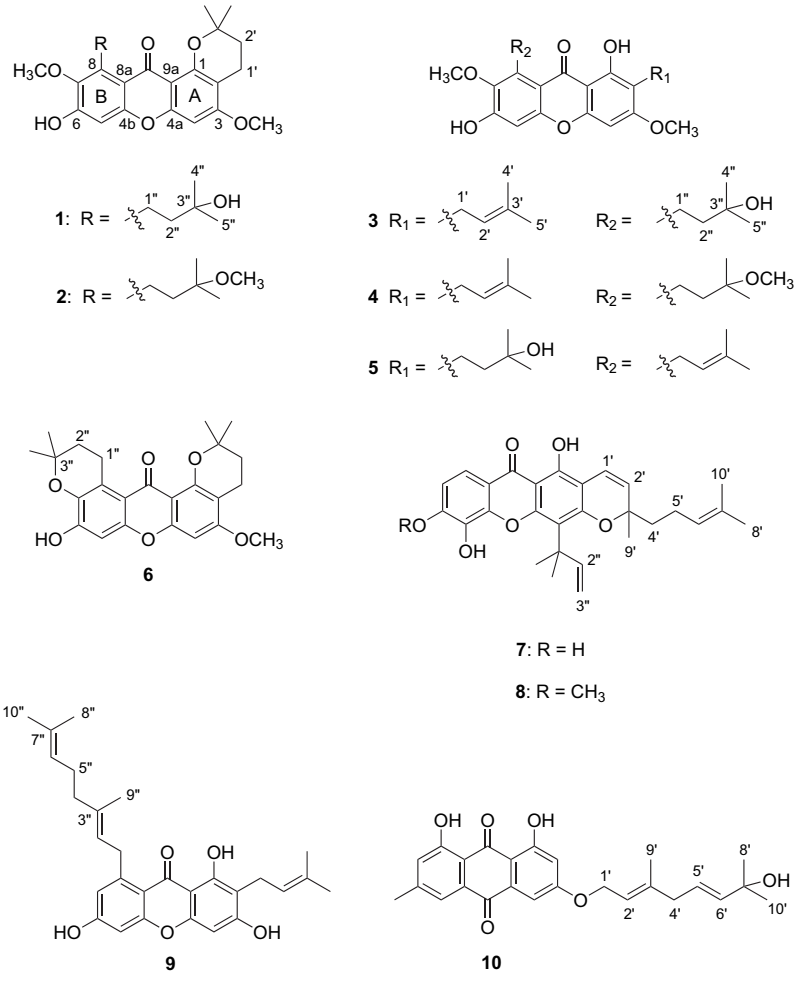


Structures of pruniflorone A-J

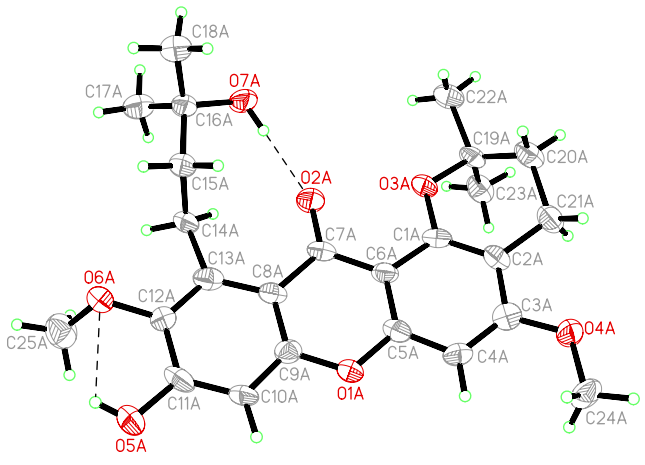


Figure 1. The ORTEP plot of **1**. There are two molecules in asymmetric unit of **1**, only one molecule was shown for clarity.

J=7.5 Hz, H-1′), 1.72 (2H, br t, J=7.5 Hz, H-2′) and 1.34 (6H, s, H-4′ and H-5′). A 3-hydroxyl-3-methylbutyl group[9] was evident from signals at δ 3.38 (2H, m, H-1″), 1.78 (2H, m, H-2″) and 1.30 (6H, s, H-4″ and H-5″). From these data, the structure of pruniflorone A was deduced to be **1**.

Pruniflorone B (**2**) was obtained as a yellow powder with a molecular ion peak at m/z 456.2116 $[M]^+$ in the HREIMS, corresponding to a molecular formula of $C_{26}H_{32}O_7$. The UV, ^1H and ^{13}C NMR spectral data of **2** (Tables 1 and 2) were similar to those of **1**, indicating the presence of a xanthone skeleton. However, an additional methoxyl singlet signal was apparent in both the ^1H+^{13}C spectra (δ_H 3.35, δ_C 49.2). This methoxyl group was located at C-3″ from the HMBC correlation with C-3″ (75.1). The completed HMBC correlations were summarized in Table 3. Thus, the structure of pruniflorone B was assigned as **2**.

Pruniflorone C (**3**) showed a molecular formula of $C_{25}H_{30}O_7$ by HREIMS. The ^1H and ^{13}C NMR spectra of **3** (Tables 1 and 2) were similar to those of **1**, except for the presence of signals for a prenyl group at δ_H 3.33 (2H, d, J=7.2 Hz, H-1′), δ_C 21.1; δ_H 5.21 (1H, br t, J=7.2, H-2′), δ_C 122.1; δ_H 1.79 (3H, s, H-4′), δ_C 17.5; δ_H 1.68 (3H, s, H-5′), δ_C 25.6 and δ_C 131.6 (C-3′) instead of the dimethylchromane ring present in **1**. HMBC data confirmed the position of the prenyl group in **3** (Table 3) at C-2 by the ^2J correlation of H-1′ with C-2, and the ^3J correlations of H-1′ with C-1 and C-3. The structure of pruniflorone C was therefore assigned as **3**.

Pruniflorone D (**4**) showed a molecular formula of $C_{26}H_{32}O_7$ by HREIMS. The ^1H NMR spectral data (Table 1) of **4** were similar to those of **3**, except for an additional methoxyl singlet signal at δ_H 3.32, δ_C 49.2. This group replaced the hydroxyl group at C-3″ in **3**. HMBC correlations between

Table 1. ^{1}H NMR spectral data of **1–6** (δ in ppm, multiplicities, J in Hz)

Position	**1**[a]	**2**[b]	**3**[c]	**4**[b]	**5**[c]	**6**[a]
4	6.29 s	6.33 s	6.32 s	6.34 s	6.38 s	6.35 s
5	6.72 s	6.76 s	6.75 s	6.82 s	6.78 s	6.74 s
1′	2.73 br t (7.5)	2.63 br t (7.0)	3.33 d (7.2)	3.36 d (7.5)	2.72 m	2.64 t (6.9)
2′	1.72 br t (7.5)	1.83 br t (7.0)	5.21 br t (7.2)	5.23 br t (7.5)	1.71 m	1.82 t (6.9)
4′	1.34 s	1.41 s	1.79 s	1.81 s	1.29 s	1.46 s
5′	1.34 s	1.41 s	1.68 s	1.69 s	1.29 s	1.46 s
1″	3.38 m	3.37 m	3.39 m	3.38 m	4.12 d (6.9)	3.58 t (6.9)
2″	1.78 m	1.79 m	1.77 m	1.77 m	5.26 m	1.84 t (6.9)
4″	1.30 s	1.31 s	1.32 s	1.30 s	1.85 s	1.36 s
5″	1.30 s	1.31 s	1.32 s	1.30 s	1.69 s	1.36 s
1-OH			[d]	13.60 s	[d]	
3-OMe	3.90 s	3.89 s	3.90 s	3.91 s	3.92 s	3.90 s
7-OMe	3.84 s	3.83 s	3.84 s	3.86 s	3.80 s	
3″-OMe		3.35 s		3.32 s		

[a] Recorded at 300 MHz in $CDCl_3$.
[b] Recorded at 500 MHz in $CDCl_3$.
[c] Recorded at 300 MHz in $CD_3OD/CDCl_3$.
[d] Exchangeable with CD_3OD.

3″-OMe and δ_C 74.9 (C-3″), and H-2″ with δ_C 22.2 (C-1″) and 74.9 (C-3″) (Table 3) proved this assignment. Thus, the structure of pruniflorone D was assigned as **4**.

Pruniflorone E (**5**) was isolated as a yellow gum, which showed a molecular ion peak at m/z 442.2000 $[M]^+$ in the HREIMS, corresponding to a molecular formula of $C_{25}H_{30}O_7$. The UV and IR spectra of **5** exhibited the same patterns as those of **3** and **4**. Extensive 1D and 2D NMR analysis of **5** showed that this xanthone has the same substituents as **3**; a 3-hydroxyl-3-methylbutyl, a prenyl and two methoxyl groups (Tables 1 and 2). However, the ^{1}H NMR spectral data of **5** (Table 1) and **3** exhibited different chemical shifts for the 3-hydroxyl-3-methylbutyl and prenyl moieties. From HMBC spectral data (Table 3), the methylene protons of H-1′ of a 3-hydroxyl-3-methylbutyl group showed correlations with δ_C 163.3 (C-3), 159.8 (C-1) and 103.6 (C-2), and the methoxyl group at δ_H 3.92 showed a cross peak with δ_H 6.38 (H-4) in the NOESY spectrum (Table 4). It was therefore apparent there that the 3-hydroxyl-3-methylbutyl and methoxyl substituent groups were located at C-2 and C-3 in ring A, respectively. In addition, the signal for H-1″ of the prenyl group (δ 4.12, 2H, d, J=6.9 Hz) in **5** appears further downfield than the expected values for this functionality (ca. δ 3.5–3.3).[10] This can be explained by the fact that H-1″ is in a deshielding region of the carbonyl functionality. The HMBC experiment was also used to confirm the position of attachment of the prenyl group in **5** (Table 3) at C-8 by the ^2J correlation of H-1″ with C-8. Thus, the structure of pruniflorone E was assigned as **5**, a constitutional isomer of **3**.

Table 2. ^{13}C NMR (75 MHz) spectral data of **1–6** in $CD_3OD/CDCl_3$

Position	**1**	**2**[a]	**3**[a]	**4**[a,b]	**5**[a]	**6**[b]
1	155.2	155.3	159.1	159.8	159.8	155.6
2	105.6	105.4	111.3[c]	111.5	103.6	105.2
3	162.0	161.4	163.3	163.5	163.3	161.3
4	89.6	89.6	88.7	88.8	88.9	89.7
5	101.3	100.8	101.7	101.4	101.7	99.7
6	154.6	154.5	156.0	154.5	155.1	150.1
7	143.3	142.3	143.1	142.6	143.0	137.7
8	138.4	138.5	138.4	138.8	137.2	122.0
9	177.5	176.4	181.8	182.0	181.9	177.4
4a	157.1	156.9	155.1	155.2	155.4	157.2
4b	155.2	152.9	155.6	155.8	155.6	151.7
8a	113.8	115.5	111.2[c]	112.5	112.0	114.1
9a	107.2	107.9	103.5	103.8	111.9	107.8
1′	16.9	17.1	21.1	21.4	16.9	17.1
2′	31.3	31.4	122.1	122.3	42.1	31.5
3′	75.7	75.2[c]	131.6	131.7	71.1	75.2[c]
4′	26.1	26.5	17.5	17.8	28.9	26.6
5′	26.1	26.5	25.6	25.8	28.9	26.6
1″	21.8	21.8	21.8	22.2	26.4	22.6
2″	43.9	39.7	44.0	39.9	123.2	33.1
3″	70.7	75.1[c]	70.8	74.9	131.9	75.3[c]
4″	28.7	25.3	28.7	25.2	18.1	26.5
5″	28.7	25.3	28.7	25.2	25.8	26.5
3-OMe	55.6	55.7	55.3	55.8	55.8	55.7
7-OMe	60.9	62.0	61.2	62.2	61.4	
3″-OMe		49.2		49.2		

[a] Recorded at 125 MHz.
[b] Recorded in $CDCl_3$.
[c] May be interchangeable.

Pruniflorone F (**6**), a pale yellow powder, was deduced as $C_{24}H_{26}O_6$ from an exact mass measurement. The ^{1}H and ^{13}C NMR spectral data of **6** (Tables 1 and 2) were closely related to those of **1**. The major difference was the replacement of the ^{1}H NMR signals for the methoxyl and 3-hydroxyl-3-methylbutyl groups at C-7 and C-8, respectively, of **1** with a dimethylchromane ring at δ 3.58 (2H, t, J=6.9 Hz, H-1″), 1.84 (2H, t, J=6.9 Hz, H-2″) and 1.36 (6H, s, H-4″ and H-5″) in **6**. The observed HMBC correlations (Table 3) confirmed the assignment of this structure.

Pruniflorone G (**7**), a brown powder, was deduced as $C_{28}H_{30}O_6$ from an exact mass measurement. The ^{1}H NMR spectral data of **7** (Table 5) were similar to those of gerontoxanthone I (29),[11] except for the appearance of the signal of a chromene ring bearing a methyl group and six-carbon side chain (Table 5) instead of the prenyl moiety present in gerontoxanthone I. The proposed structure was further supported by the appearance of an abundant fragment ion m/z 379 ($[M]^+ -83$), resulting from loss of the 4-methylpent-3-enyl moiety. The location of a chromene ring was confirmed by HMBC (Table 6), in which the methine proton H-1′ at δ_H 6.83 was correlated with δ_C 81.1 (C-3′), 105.2 (C-2), 156.8 (C-1) and 159.2 (C-3), while the methine proton H-2′ at δ_H 5.58 was correlated with δ_C 26.9 (C-9′), 41.8

Table 3. HMBC (300 MHz) spectral data of **1**–**6** in $CDCl_3$

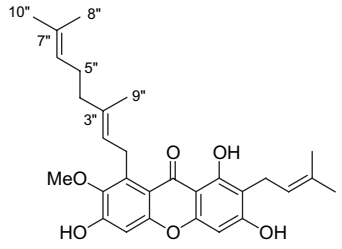
Position	**1**[a]	**2**[b]	**3**[a]	**4**[b]	**5**[a]	**6**
4	C-2, C-3, C-9, C-4a, C-9a	C-2, C-3, C-9, C-4a, C-9a	C-2, C-3, C-9, C-4a, C-9a	C-2, C-3, C-9, C-4a, C-9a	C-2, C-3, C-9, C-4a, C-9a	C-3, C-9, C-4a, C-9a
5	C-7, C-8, C-9, C-4b, C-8a	C-6, C-7, C-9, C-4b, C-8a	C-6, C-7, C-8, C-4b, C-8a, C-9	C-6, C-9, C-4b, C-8a	C-6, C-7, C-9, C-4b, C-8a	C-6, C-7, C-9, C-4b, C-8a
1′	C-1, C-2, C-3, C-2′, C-3′	C-1, C-2, C-3, C-2′, C-3′	C-1, C-2, C-3, C-2′, C-3′	C-1, C-2, C-3, C-2′, C-3′	C-1, C-2, C-3, C-2′	C-1, C-2, C-3, C-2′, C-3′
2′	C-2, C-1′, C-3′, C-4′, C-5′	C-2, C-1′, C-3′, C-4′, C-5′		C-2, C-4′, C-5′	C-3′, C-4′, C-5′	C-2, C-1′, C-3′, C-4′, C-5′
4′	C-1, C-1′, C-2′	C-2′, C-3′	C-2′, C-3′, C-5′	C-2′, C-3′	C-2′, C-3′	C-2′, C-3′
5′	C-1, C-1′, C-2′	C-2′, C-3′	C-2′, C-3′, C-4′	C-2′, C-3′	C-2′, C-3′	C-2′, C-3′
1″	C-7, C-8, C-8a, C-3″	C-7, C-8, C-8a, C-2″, C-3″	C-7, C-8, C-8a, C-3″	C-7, C-8, C-8a, C-2″	C-8	C-7, C-8, C-8a, C-2″, C-3″
2″	C-8, C-3″, C-4″, C-5″	C-8, C-1″, C-3″, C-4″, C-5″	C-8, C-3″	C-1″, C-3″, C-4″, C-5″		C-8, C-1″, C-3″, C-4″, C-5″
4″	C-1″, C-2′, C-3″	C-2″, C-3″	C-1″, C-2″, C-3″	C-2″, C-3″	C-2″, C-3″, C-5″	C-2″, C-3″
5″	C-1″, C-2″, C-3″	C-2″, C-3″	C-1″, C-2″, C-3″	C-2″, C-3″	C-2″, C-3″, C-4″	C-2″, C-3″
1-OH				C-1, C-2, C-9a		
3-OMe	C-3	C-3	C-3	C-3	C-3	C-3
7-OMe	C-7	C-7	C-7	C-7	C-7	
3″-OMe		C-3″		C-3″		

[a] Recorded in $CD_3OD/CDCl_3$.
[b] Recorded at 500 MHz.

(C-4′), 81.1 (C-3′), 105.2 (C-2) and 159.2 (C-3), respectively. From these data, the structure of pruniflorone G was assigned as **7**.

Pruniflorone H (**8**) was obtained as a yellow powder, which showed a molecular ion peak at *m*/*z* 476.2215 $[M]^+$ in the HREIMS corresponding to a molecular formula of $C_{29}H_{32}O_6$. The ^1H and ^{13}C NMR spectral data of **8** (Table 5) showed characteristics similar to those of **7**, except that an additional signal of a methoxyl group was observed at δ_H 3.32, δ_C 56.6 in **8**, this methoxyl group was located at C-6 due to the correlation with δ_C 149.0 (C-6) from HMBC experiment (Table 6). Thus, the structure of pruniflorone H was assigned as **8**.

Pruniflorone I (**9**) showed a molecular formula of $C_{28}H_{32}O_5$ by HREIMS. The ^1H and ^{13}C NMR spectral data of **9** (Table 7) showed characteristics similar to those of cowanin,[12] except for the appearance of an aromatic proton at δ_H 7.18 (s, H-7), δ_C 123.7 instead of the methoxyl group at δ_H 3.80 (s, 7-OMe) present in cowanin. Therefore, the structure of pruniflorone I was deduced as **9**.



cowanin

Pruniflorone J (**10**) was isolated as orange viscous oil, which was assigned as $C_{25}H_{26}O_6$ from an exact mass measurement. The UV spectrum of **10** exhibited absorption maxima at 269, 283, 366 and 440 nm, suggesting an anthraquinone as a basic structure.[13] IR absorption bands at 1673 and 1625 $cm^{-1}$and ^{13}C NMR chemical shifts at 190.8 and 182.0 also indicated the presence of carbonyl and chelated carbonyl groups, respectively. Chelated hydroxyl protons were shown at δ_H 12.30 (1H, s) and 12.13 (1H, s). The ^1H and ^{13}C NMR spectral data of **10** (Table 8) showed characteristics similar to those of 3-geranyloxy-6-methyl-1,8-dihydroxyanthraquinone,[14] except for the appearance of *trans*-olefinic protons at δ_H 5.62 (1H, dd, J=6.5, 15.5 Hz, H-5′) and 5.69 (1H, d, J=15.5 Hz, H-6′) in **10** instead of methylene protons at C-5′ and an olefin at C-6′. The chemical shift of the methylene protons at C-4′ was shifted downfield (δ_H 2.79 (2H, d, J=6.5 Hz, H-4′)) compared to δ_H 2.11,[14] due to the double allylic status of these protons. The location of H-4′ at C-4′ was supported by HMBC correlations. The chemical shift of C-7′ (δ 70.8) suggested an oxy-quarternary carbon, whose position was confirmed by HMBC correlations with H-5′ and H-6′. Thus, the structure of pruniflorone J was assigned as **10**.

The following known compounds were also isolated from the roots and barks of *C. formosum* ssp. *pruniflorum*: dulxisxanthone F (**11**),[15] β-mangostin (**12**),[16] α-mangostin (**13**),[16] formoxanthone A (**14**),[8] 3-isomangostin (**15**),[16] 3,4-dihydro-5,9-dihydroxy-8-methoxy-7-(3-methoxy-3-methylbutyl)-2,2-dimethyl-2*H*,6*H*-pyrano-[3,2-*b*]xanthen-6-one (**16**),[17]

Table 4. NOESY (300 MHz) spectral data of **1–6** in $CDCl_3$

Position	**1**[a]	**2**[b]	**3**[a]	**4**[b]	**5**[a]	**6**
4	3-OMe	3-OMe	3-OMe	3-OMe	3-OMe	3-OMe
5						
1′	H-2′	H-2′	H-2′, H-4′	H-2′		H-2′
2′	H-1′, H–4′, H-5′	H-1′, H-4′, H-5′	H-1′, H-5′	H-1′, H-5′	H-4′, H-5′	H-1′, H-4′, H-5′
4′	H-2′	H-2′	H-1′		H-2′	H-2′
5′	H-2′	H-2′	H-2′	H-2′	H-2′	H-2′
1″	7-OMe, H-2″	H-2″	H-2″	H-2″		H-2″
2″	H-1″, H-4″, H-5″	H-4″, H-5″, 3″-OMe	H-1″, H-4″, H-5″	H-1″, H-4″, H-5″, 3″-OMe	H-5″	H-1″, H-4″, H-5″
4″	H-2″	H-2″	H-2″	H-3″		H-2″
5″	H-2″	H-2″	H-2″	H-3″	H-2″	H-2″
1-OH						
3-OMe	H-4	H-4	H-4	H-4		H-4
7-OMe						
3″-OMe				H-4″, H-5″, 3″-OMe		

[a] Recorded in $CD_3OD/CDCl_3$.
[b] Recorded at 500 MHz.

3,4-dihydro-5,9-dihydroxy-7-(3-hydroxy-3-methylbutyl)-8-methoxy-2,2-dimethyl-2*H*,6*H*-pyrano[3,2-*b*]xanthen-6-one (**17**),[17] isocudraniaxanthone B (**18**),[18] 10-*O*-methylmacluraxanthone (**19**),[19] 3-geranyloxy-6-methyl-1,8-dihydroxyanthraquinone (**20**),[14] 11-hydroxy-5-methoxy-2,2,9-trimethyl-2*H*-anthra-[1,2-*b*]pyran-7,12-dione (**21**),[20] vismiaquinone A (**22**),[21] madagascin (**23**),[22] physcion (**24**),[23] emodin (**25**),[24] formoxanthone B (**26**),[8] macluraxanthone (**27**),[25] xanthone V_1 (**28**),[26] gerontoxanthone I (**29**),[11] 6-deoxyjacareubin (**30**)[27] and 3,4-dihydrojacareubin (**31**).[28] These compounds were identified by comparison of their spectroscopic data with those reported in the literature. In addition, the X-ray structure of **11** was reported here for the first time (Fig. 2).

Table 5. ^1H and ^{13}C NMR spectral data of **7** and **8** in $CDCl_3$

Position	**7**		**8**	
	^1H (J in Hz)[a]	^{13}C (δ)[b]	^1H (J in Hz)[a]	^{13}C (δ)[b]
1		156.8		156.6
2		105.2		104.9
3		159.2		159.3
4		112.7		113.0
5		131.0		133.4
6		149.0		151.4
7	6.96 d (9.0)	112.8	6.97 d (9.0)	108.2
8	7.69 d (9.0)	117.5	7.75 d (9.0)	116.8
9		180.7		181.0
4a		154.1		154.8
4b		144.5		144.3
8a		113.7		114.2
9a		102.9		103.0
1′	6.83 d (9.9)	116.7	6.81 d (9.9)	116.7
2′	5.58 d (9.9)	125.6	5.56 d (9.9)	125.6
3′		81.1		81.1
4′	1.91 m[c]	41.8	1.89 m[c]	41.7
	1.72 m[c]		1.70 m[c]	
5′	2.14 m	23.2	2.10 m	23.3
6′	5.13 br t (7.2)	123.7	5.12 br t (7.5)	123.7
7′		132.1		132.0
8′	1.60 s	17.6	1.59 s	17.6
9′	1.47 s	26.9	1.45 s	26.9
10′	1.69 s	25.7	1.68 s	25.6
1″		41.4		41.3
2″	6.75 dd (10.8, 17.7)	156.7	6.66 dd (10.5, 17.4)	154.9
3″	5.05 dd (1.2, 10.8)	103.3	5.04 dd (1.2, 10.5)	104.5
	5.23 dd (1.2, 17.7)		5.18 dd (1.2, 17.4)	
4″	1.66 s	28.0	1.66 s	28.4
5″	1.66 s	28.4	1.66 s	28.4
1-OH	13.50 s		13.50 s	
6-OMe			3.32 s	56.6

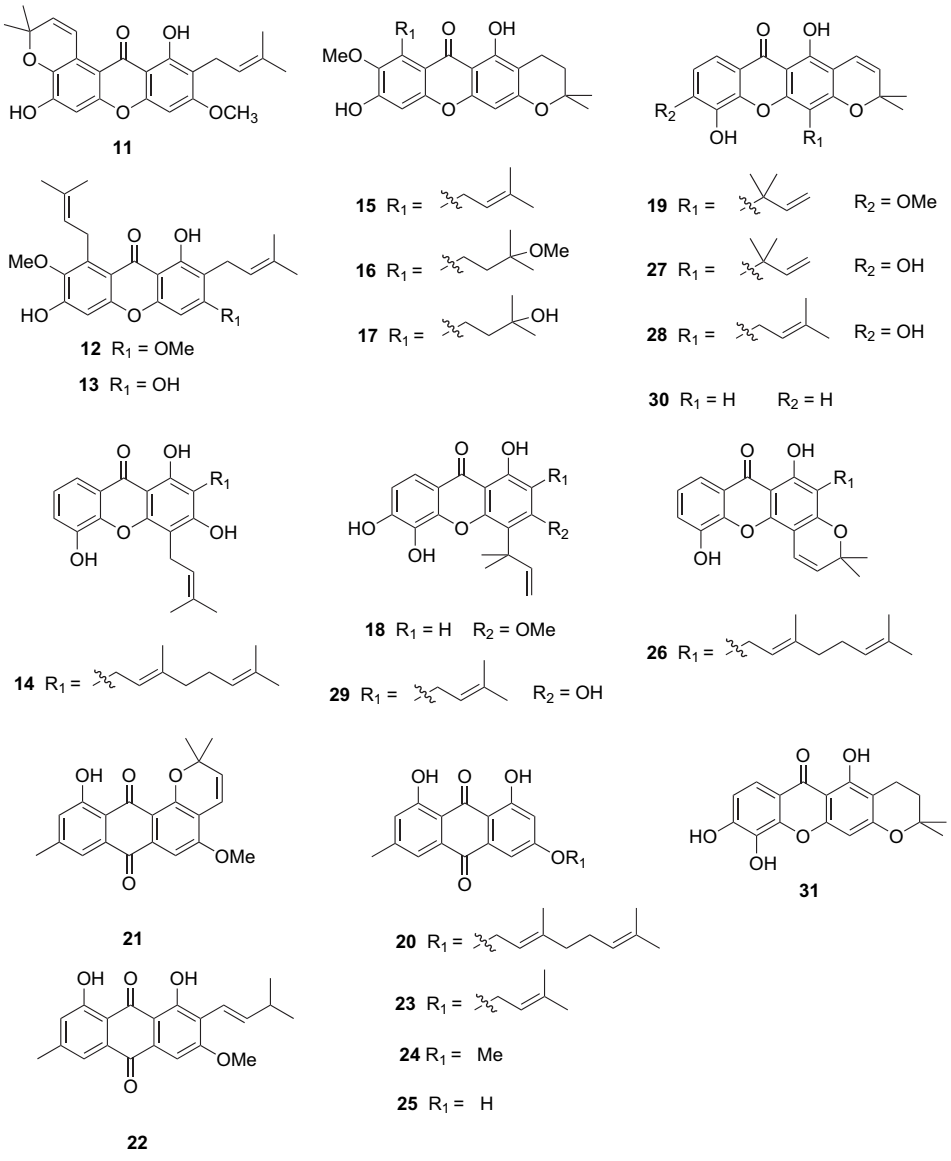
[a] Recorded at 300 MHz.
[b] Recorded at 75 MHz.
[c] Reduced from HMQC experiment.

Only stable compounds of sufficient quantity were evaluated for their antibacterial activity against both Gram-positive (*Bacillus substilis* and *Staphylococcus aureus*) and Gram-negative (*Streptococcus faecalis*, *Salmonella typhi*, *Shigella sonei* and *Pseudomonas aeruginosa*) bacteria. Cytotoxicity against MCF-7 (breast adenocarcinoma), HeLa (Human cervical cancer), HT-29 (colon cancer) and KB (human oral cancer) cell lines was also evaluated. The results of antibacterial activity of the tested compounds are given in Table 9. Pruniflorone E (**5**) and **13** showed potent antibacterial activity against *B. substilis*, *S. aureus* and *S. faecalis*, whereas pruniflorone C (**3**) and **31** exhibited strong activity against *B. substilis* and *S. aureus*. Compounds **28** and **29** showed strong and broad spectrum of antibacterial activity compared to vancomycin. For this investigation, only **29** showed inhibition against *S. sonei* and *P. aeruginosa*. It is interesting to note that, compounds **12**, **17** and **30** were highly active specifically against *S. aureus* therefore it might be worthwhile to further investigate the structure–activity relationships (SAR) of these compounds against *S. aureus*. Compounds **20**–**25** exhibited no antibacterial activity. According to the MIC values shown in Table 9, it seems that the isoprenyl or 3-hydroxyl-3-methylbutyl moiety at C-2 and C-8, and the catechol unit are both important for antibacterial activity, whereas isoprenyl unit at C-8, which was cyclized to 3,3-dimethylchromene or 3,3-dimethylchromane rings might decrease the antibacterial activity as shown in compounds **6** and **11**. In addition to antibacterial activity, compound **29** strongly inhibited all cancer cell lines used in this investigation compared to camptothecin, whereas compounds **12**, **13**, **28** and **31** showed less inhibitory activity. Compounds **1**, **5**, **6**, **11** and **20**–**25** were found to be inactive for cytotoxic activity (Table 10).

3. Experimental

3.1. General experimental procedures

Melting points were determined on the Fisher-John melting point apparatus. Optical rotations were measured on



a JASCO P-1020 digital polarimeter. UV and IR spectra were recorded on SPECORD S 100 (Analytikjena) and Perkin–Elmer FTS FTIR spectrophotometer, respectively. The ^{1}H and ^{13}C NMR spectra were recorded on a 500 MHz Varian UNITY INOVA and/or 300 MHz Bruker FTNMR Ultra Shield™ spectrometers in $CDCl_3$ or CD_3OD with TMS as

Table 6. HMBC and NOESY (300 MHz) spectral data of **7** and **8** in $CDCl_3$

Position	**7**		**8**	
	HMBC	NOESY	HMBC	NOESY
7		H-8	C-5, C-6, C-8a	H-8, 6-OMe
8	C-6, C-9	H-7	C-6, C-9	H-7
1′	C-1, C-2, C-3, C-3′	H-2′	C-1, C-2, C-3, C-3′	H-2′
2′	C-2, C-3, C-3′ C-4′, C-9′	H-1′, H-4′	C-2, C-3′	H-1′
4′	C-3′, C-5′	H-2′, H-6′	C-3′	
5′	C-3′, C-4′, C-6′, C-7′	H-6′	C-4′	
6′	C-5′, C-8′, C-10′	H-4′, H-5′	C-4′	
8′	C-6′, C-7′		C-6′, C-7′	
9′	C-2′, C-3′, C-4′		C-3′, C-4′	
10′	C-6′, C-7′		C-6′, C-7′	
2″	C-4, C-1″	H-3″	C-4, C-1″	H-3″
3″	C-1″, C-2″	H-2″	C-1″, C-2″, C-4″, C-5″	H-2″, H-4″, H-5″
4″	C-4, C-1″		C-4, C-1″, C-2″	H-3″
5″	C-4, C-1″		C-4, C-1″, C-2″	H-3″
1-OH	C-1, C-2, C-9a		C-1, C-2, C-9a	
6-OMe			C-6	H-7

Table 7. NMR (300 MHz) spectral data of **9** in $CDCl_3$

Position	**9**			
	^{1}H (J in Hz)	^{13}C (δ)	HMBC	NOESY
1		160.7		
2		108.5		
3		162.1		
4	6.19 s	93.2	C-2, C-3, C-9, C-4a, C-9a	
5	7.17 s	116.7	C-6, C-9, C-4b, C-8a	
6		152.0		
7	7.18 s	123.7	C-6, C-8	
8		127.1		
9		183.4		
4a		155.3		
4b		151.3		
8a		118.4		
9a		104.1		
1′	3.35 d (6.9)	21.5	C-1, C-2, C-3, C-2′, C-3′	H-2′, H-4′
2′	5.19 br t (6.9)	121.4	C-4′	H-1′, H-5′
3′		135.5		
4′	1.66 s	25.8	C-2′, C-3′	H-1′
5′	1.74 s	17.9	C-2′, C-3′	H-2′
1″	4.20 d (6.6)	25.6	C-7, C-8, C-4a, C-8a, C-2′, C-3′	H-2″, H-9″
2″	5.16 br t (6.6)	121.5	C-8, C-4′, C-9′	H-1″, H-4″
3″		138.6		
4″	1.98 m	39.7	C-3′, C-9′	H-2″
5″	1.98 m	26.4	C-4′, C-6′, C-7′	H-6″
6″	4.94 m	123.8	C-4′, C-5′, C-8′	H-5″, H-8″
7″		132.0		
8″	1.55 s	25.8	C-6′, C-7′	H-6″
9″	1.77 s	16.4	C-2′, C-3′	H-1″
10″	1.48 s	17.7	C-6′, C-7′	
1-OH	13.54 s		C-1, C-2, C-9a	

Table 8. NMR (500 MHz) spectral data of **10** in $CDCl_3$

Position	**10**			
	^{1}H (J in Hz)	^{13}C (δ)	HMBC	NOESY
1-OH	12.30 s	165.1	C-1, C-2, C-9a	
2	6.68 d (2.5)	107.6	C-1, C-4	H-1′
3		165.8		
4	7.37 d (2.5)	108.7	C-3, C-10, C-9a	H-1′
5	7.62 br s	121.3	C-7, C-10, C-8a, C-6(Me)	6-Me
6		148.4		
7	7.08 br s	124.5	C-5, C-8, C-8a, C-6(Me)	6-Me
8-OH	12.13 s	163.0	C-7, C-8, C-8a	
9		190.8		
10		182.0		
4a		135.2		
4b		133.2		
8a		113.7		
9a		110.1		
1′	4.68 d (6.5)	65.8	C-3, C-2′, C-3′	
2′	5.50 br t (6.5)	119.0	C-4′	
3′		141.5		
4′	2.79 d (6.5)	42.1	C-2′, C-3′, C-5′, C-6′, C-9′	
5′	5.62 dd (6.5, 15.5)	123.9	C-4′, C-7′	
6′	5.69 d (15.5)	140.5	C-4′, C-7′, C-8′, C-10′	
7′		70.8		
8′	1.33 s	29.8	C-6′, C-7′	
9′	1.77 s	16.8	C-2′, C-3′, C-4′	
10′	1.33 s	29.8	C-6′, C-7′	
6-Me	2.45 s	22.2	C-5, C-6, C-7	H-5, H-7

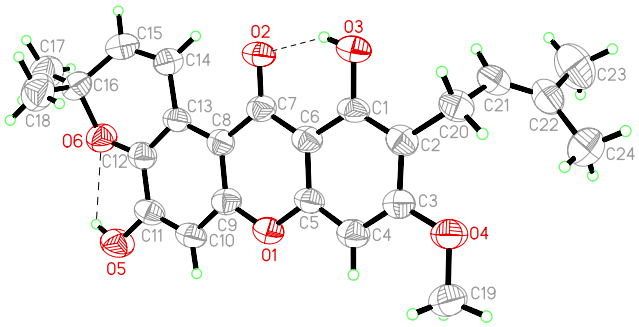


Figure 2. The ORTEP plot of **11**.

the internal standard. Chemical shifts are reported in δ (ppm) and coupling constants (J) are expressed in Hertz. EI and HREIMS were measured on a Kratos MS 25 RFA spectrometer. Quick column chromatography (QCC) and column chromatography (CC) were carried out on silica gel 60 F_{254} (Merck) and silica gel 100 (Merck), respectively.

3.2. Plant material

Barks and roots of *C. formosum* ssp. *pruniflorum* were collected in May 2004 from Nong Khai Province, northeastern part of Thailand. Identification was made by Professor Puangpen Sirirugsa, Department of Biology, Faculty of Science, Prince of Songkla University and a specimen (No. 0012677) was deposited at Prince of Songkla University Herbarium.

3.3. Isolation and extraction

Air-dried roots (5.30 kg) were extracted with CH_2Cl_2 (2×20 L, for 5 days) at room temperature. The crude CH_2Cl_2 extracts were evaporated under reduced pressure to afford a brownish crude (30.04 g) extract. The crude extract was subjected to QCC on silica gel using hexane as the first eluent and then increasing polarity with EtOAc and acetone, respectively, to give eight fractions (FR1–FR8). Fraction FR2 was separated by CC eluting with CH_2Cl_2–hexane (4:1, v/v) to afford four subfractions (FR2A–FR2D). Subfraction FR2A was further purified by CC with EtOAc–hexane (3:7, v/v) to give **12** (45.0 mg). Subfraction FR2B was further purified by CC eluting with acetone–hexane (1:9, v/v) to give **13** (15.0 mg). Subfraction FR2C was further purified by CC on reversed-phase silica gel C-18 with MeOH to give **7** (8.0 mg) and **18** (2.5 mg). Fraction FR3 (2.56 g) was separated by CC with acetone–hexane (3:17, v/v) to give **14** (15.0 mg), **6** (5.8 mg) and **11** (10.2 mg), which was further recrystallized in $CHCl_3$–MeOH (4:1, v/v) to yield yellow needle single crystals. Fraction FR4 was subjected to CC with acetone–hexane (1:4, v/v) to afford five subfractions (FR4A–FR4E). Subfraction FR4B was separated by CC with acetone–hexane to give three fractions (FR4BA–FR4BC). Subfraction FR4BC was further purified by CC on reversed-phase silica gel C-18 with MeOH to give **9** (15.0 mg). Subfraction FR4D was further purified by CC on reversed-phase silica gel C-18 with MeOH to give **8** (3.0 mg) and **19** (3.0 mg). Fraction FR6 was purified by CC with acetone–hexane (1:4, v/v) to give **2** (3.3 mg), **15** (5.0 mg) and **16** (5.0 mg). Fraction FR7 was further purified by CC with EtOAc–hexane (2:3, v/v) to give **3** (8.2 mg),

Table 9. Antibacterial activity of compounds isolated from *C. formosum* ssp. *pruniflorum*

Compound	Minimum inhibitive concentration (μg/mL)					
	B. substilis	*S. aureus*	*S. faecalis*	*S. typhi*	*S. sonei*	*P. aeruginosa*
1	300	18.7	—	—	300	—
3	<1.1	<1.1	150	—	300	—
5	<1.1	<1.1	<1.1	—	300	18.7
6	300	9.3	4.6	—	300	37.5
10	300	75	150	—	300	—
11	75	18.7	—	—	300	—
12	18.7	<1.1	75	—	—	—
13	<1.1	<1.1	<1.1	—	18.7	18.7
14	18.7	37.5	—	—	—	—
17	9.3	<1.1	—	—	—	—
27	4.6	4.6	2.3	9.6	—	—
28	<1.1	<1.1	<1.1	<1.1	—	9.3
29	<1.1	<1.1	4.6	37.5	<1.1	<1.1
30	4.6	<1.1	75	—	150	150
31	<1.1	<1.1	37.5	—	—	37.5

— = Inactive at >50 μg/mL.

Table 10. In vitro cytotoxic activity of compounds isolated from *C. formosum* ssp. *pruniflorum*

Compound	Cell line			
	MCF-7	HeLa	HT-29	KB
	IC_{50} (μg/mL)			
12	3.6	4.9	4.8	4.6
13	3.7	3.2	4.5	3.2
28	>25.0	4.7	6.0	2.7
29	0.6	0.7	0.7	0.6
31	>5.0	3.4	>5.0	>5.0

4 (1.5 mg) and **5** (2.0 mg). Fraction FR8 was separated by CC with a gradient of acetone–hexane to give four fractions (FR8A–FR8D). Subfraction FR8C was further purified by CC with a gradient of acetone–hexane to give **17** (2.1 mg) and **1** (32.2 mg), which was further recrystallized from $CHCl_3$–MeOH (4:1, v/v) to yield pale yellow single crystals.

Ground-dried barks (4.00 kg) were extracted with CH_2Cl_2 and acetone (each 2×20 L, for 5 days) at room temperature, successively. The crude extracts were evaporated under reduced pressure to afford brownish crude CH_2Cl_2 (76.28 g) and acetone (21.74 g) extracts. The crude CH_2Cl_2 extract was subjected to QCC eluting with increasing polarities of EtOAc and acetone in hexane to afford 10 fractions (F1–F10). Fraction F1 (2.01 g) was separated by CC with acetone–hexane (1:19, v/v) to afford three subfractions (F1A–F1C). Subfraction F1B was further purified by CC with EtOAc–hexane (1:9, v/v) to give **22** (3.3 mg) and **23** (5.6 mg). Fraction F2 (58.06 g) was further separated by CC using a gradient of hexane with EtOAc to afford eight subfractions (F2A–F2H) and **27** (150.0 mg). Subfraction F2C (120.02 g) was further purified by CC with EtOAc–hexane (1:4, v/v) to give **10** (5.2 mg) and **20** (68.2 mg). Subfraction F2D was purified by CC with CH_2Cl_2–hexane (3:2, v/v) to give three fractions (F2DA–F2DC). Subfraction F2DB was further purified by prep TLC with CH_2Cl_2–hexane (3:7, v/v) to give **26** (1.5 mg). Subfraction F2G was subjected to CC with acetone–hexane (1:9, v/v) to give **24** (5.0 mg). Fraction F3 was separated by CC with acetone–hexane (1:9, v/v) to afford five fractions (F3A–F3E). Subfraction F3D was purified by CC with acetone–hexane (3:17, v/v) to give **29** (25.0 mg). Fraction F6 was separated by CC with acetone–hexane (3:17, v/v) to afford seven subfractions (F6A–F6G). Subfraction F6B was further purified by CC with EtOAc–hexane (3:7, v/v) to give **28** (8.0 mg). The crude acetone was subjected to QCC eluting with a gradient of hexane–acetone to afford 12 fractions (FA1–FA12). Fraction FA2 (1.98 g) was further separated by CC with acetone–hexane (3:97, v/v) to give six subfractions (FA2A–FA2F). Subfraction FA2B (422.0 mg) was further purified by CC with acetone–hexane (1:19, v/v) to give **21** (3.0 mg). Fraction FA3 was further purified by CC with EtOAc–hexane (1:9, v/v) to give **30** (4.0 mg). Fraction FA7 was separated by CC with acetone–hexane (1:4, v/v) to give **25** (3.1 mg) and **31** (5.0 mg).

3.3.1. Pruniflorone A (1). Pale yellow needle crystals, mp 259-260 °C, $[\alpha]_D^{26}$ −5.1 (*c* 0.430, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 247 (4.29), 261 (4.34), 314 (4.17), 355 (3.55) nm; IR (KBr) ν_{max} 3414, 1642, 1614 cm^{-1}; HREIMS *m/z* $[M]^+$ 442.1994 (calcd for $C_{25}H_{30}O_7$, 442.1992); ^1H NMR ($CDCl_3$, 300 MHz), see Table 1; ^{13}C NMR (CD_3OD/$CDCl_3$, 75 MHz), see Table 2.

3.3.2. Pruniflorone B (2). Yellow powder, mp 215–217 °C, $[\alpha]_D^{26}$ −4.0 (*c* 0.165, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 246 (4.01), 299 (3.79), 334 (3.40) nm; IR (neat) ν_{max} 3177, 1639, 1611 cm^{-1}; HREIMS *m/z* $[M]^+$ 456.2116 (calcd for $C_{26}H_{32}O_7$, 456.2148); ^1H NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR (CD_3OD/$CDCl_3$, 125 MHz), see Table 2.

3.3.3. Pruniflorone C (3). Yellow solid, mp 134–136 °C, $[\alpha]_D^{27}$ −5.5 (*c* 0.145, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 245 (3.89), 259 (3.86), 313 (3.75), 353 (3.25) nm; IR (KBr) ν_{max} 3414, 1632, 1614 cm^{-1}; HREIMS *m/z* $[M]^+$ 442.1995 (calcd for $C_{25}H_{30}O_7$, 442.1992); ^1H NMR (CD_3OD/$CDCl_3$, 300 MHz), see Table 1; ^{13}C NMR ($CDCl_3$/CD_3OD, 125 MHz), see Table 2.

3.3.4. Pruniflorone D (4). Yellow viscous oil, $[\alpha]_D^{26}$ 17.5 (*c* 0.075, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 249 (5.00), 259 (4.98), 312 (4.92), 352 (4.35) nm; IR (neat) ν_{max} 3170, 1646, 1597 cm^{-1}; HREIMS *m/z* $[M]^+$ 456.2198 (calcd for

$C_{26}H_{32}O_7$, 456.2148); ^{1}H NMR ($CD_3OD/CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CD_3OD/CDCl_3$, 125 MHz), see Table 2.

3.3.5. Pruniflorone E (5). Yellow gum, $[\alpha]_D^{27}$ −4.4 (*c* 0.130, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 245 (3.91), 260 (3.88), 312 (3.78), 353 (3.25) nm; IR (KBr) ν_{max} 3414, 1635, 1614 cm^{-1}; HREIMS *m*/*z* [M]$^+$ 442.2000 (calcd for $C_{25}H_{30}O_7$, 442.1992); ^{1}H NMR ($CD_3OD/CDCl_3$, 300 MHz), see Table 1; ^{13}C NMR ($CD_3OD/CDCl_3$, 125 MHz), see Table 2.

3.3.6. Pruniflorone F (6). Pale yellow powder, mp 235–236 °C, $[\alpha]_D^{26}$ −9.2 (*c* 0.290, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 255 (3.94), 258 (3.99), 302 (3.77), 349 (3.40) nm; IR (KBr) ν_{max} 3177, 1614 cm^{-1}; HREIMS *m*/*z* [M]$^+$ 410.1728 (calcd for $C_{24}H_{26}O_6$, 410.1729); ^{1}H NMR ($CDCl_3$, 300 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 2.

3.3.7. Pruniflorone G (7). Brown powder, mp 143–145 °C, $[\alpha]_D^{27}$ −7.4 (*c* 0.425, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 243 (4.56), 288 (4.81), 335 (4.53) nm; IR (KBr) ν_{max} 3414, 1646, 1628, 1580 cm^{-1}; EIMS *m*/*z* 462 (11) [M]$^+$, 447 (5), 379 (100); HREIMS *m*/*z* [M]$^+$ 462.2063 (calcd for $C_{28}H_{30}O_6$, 462.2042); ^{1}H NMR ($CDCl_3$, 300 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 3.

3.3.8. Pruniflorone H (8). Yellow powder, mp 175–177 °C, $[\alpha]_D^{27}$ −56.5 (*c* 0.050, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 252 (4.06), 289 (4.22), 336 (4.01) nm; IR (KBr) ν_{max} 3400, 1632, 1597, 1573 cm^{-1}; EIMS *m*/*z* 476 (31) [M]$^+$, 461 (15), 393 (100), 279 (15), 167 (39), 149 (94), 97 (21), 85 (22), 83 (29); HREIMS *m*/*z* [M]$^+$ 476.2215 (calcd for $C_{29}H_{32}O_6$, 476.2199); ^{1}H NMR ($CDCl_3$, 300 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 3.

3.3.9. Pruniflorone I (9). Brown viscous oil, $[\alpha]_D^{27}$ −11.3 (*c* 1.150, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 264 (4.70), 310 (4.45), 380 (3.94) nm; IR (neat) ν_{max} 3400, 1642, 1608 cm^{-1}; HREIMS *m*/*z* [M]$^+$ 448.2277 (calcd for $C_{28}H_{32}O_5$, 448.2250); ^{1}H NMR ($CDCl_3$, 300 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 4.

3.3.10. Pruniflorone J (10). Orange viscous oil, $[\alpha]_D^{27}$ −98.4 (*c* 0.250, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 269 (4.33), 283 (4.32), 366 (3.37), 440 (3.86) nm; IR (neat) ν_{max} 3414, 1673, 1625 cm^{-1}; HREIMS *m*/*z* [M]$^+$ 422.1737 (calcd for $C_{25}H_{26}O_6$, 422.1729); ^{1}H NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 5.

3.4. X-ray crystallographic studies of 1 and 11

Crystallographic data were collected at 100.0(1) K with the Oxford Cyrosystem Cobra low-temperature attachment. The data were collected using a Bruker Apex2 CCD diffractometer with a graphite monochromated Mo Kα radiation at a detector distance of 5 cm and with APEX2 software.[29] The collected data were reduced using *SAINT* program,[29] and the empirical absorption corrections were performed using *SADABS* program.[29] The structures were solved by direct methods and refined by least-squares using the *SHELXTL* software package.[30] All non-hydrogen atoms were refined anisotropically, whereas all H atoms were placed in calculated positions with an O–H distance of 0.82 Å and C–H distances in the range 0.93–0.98 Å after checking their positions in the difference map. The U_{iso} values were constrained to be $1.5U_{eq}$ of the carrier atoms for methyl H atoms and $1.2U_{eq}$ for hydroxyl and the other H atoms. The final refinement converged well. Materials for publication were prepared using *SHELXTL*[30] and *PLATON*.[31]

Crystal data for **1**: $C_{25}H_{30}O_7$, M=442.49, 0.52×0.19×0.05 mm^3, monoclinic, $P2_1/n$, a=11.9303(4) Å, b=19.3361(7) Å, c=19.6631(7) Å, β=96.64(2), V=4505.1(3) Å^3, Z=8, D_x=1.305 Mg m^{-3}, μ(Mo Kα)=0.097 mm^{-1}, 79,107 reflection measured, 7928 unique reflections, R=0.0759, R_w=0.1699.

Crystal data for **11**: $C_{24}H_{24}O_6$, M=408.43, 0.54×0.22×0.08 mm^3, triclinic, P-1, a=8.1342(6) Å, b=8.9103(6) Å, c=14.2437(9) Å, α=82.229(4)°, β=80.494(4)°, γ=83.065(4)°, V=1003.70(12) Å^3, Z=2, D_x=1.351 Mg m^{-3}, μ(Mo Kα)=0.097 mm^{-1}, 25,932 reflection measured, 3926 unique reflections, R=0.1064, R_w=0.2883

The crystallographic-information files for **1** and **11** have been deposited in the Cambridge Crystallographic Data Center as CCDC293266 and CCDC293267, respectively. These data can be obtained free of charge via http://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, UK; fax: +44 1223 336033.

3.5. Bioassays

3.5.1. Antibacterial assay. The isolated compounds from roots and barks of *C. formosum* ssp. *pruniflorum* were tested against the microorganisms, *B. substilis* (obtained from Department of Industrial Biotechnology, PSU), *S. aureus* (TISTR517) (obtained from Microbial Resources Center (MIRCEN), Bangkok, Thailand), *S. faecalis*, *S. typhi*, *S. sonei* and *P. aeruginosa*. The last four microorganisms were obtained from Department of Pharmacognosy and Botany, PSU. The antibacterial assay employed was the same as described in Boonsri et al.[8] Vancomycin, which was used as a standard, showed antibacterial activity of 75 μg/mL.

3.5.2. Cytotoxic assay. The procedure for cytotoxic assay was performed by the sulphorhodamine B (SRB) assay as described by Skehan et al.[32] In this study, four cancer cell lines obtained from National Cancer Institute, Bangkok, Thailand, were used: MCF-7 (breast adenocarcinoma), KB (human oral cancer), HeLa (Human cervical cancer) and HT-29 (colon cancer). Camptothecin, which was used as a standard, showed cytotoxic activity in the range of 0.2–2.0 μg/mL.

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