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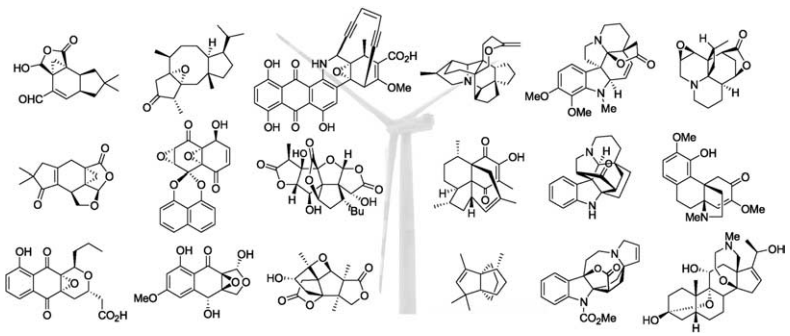
Tetrahedron

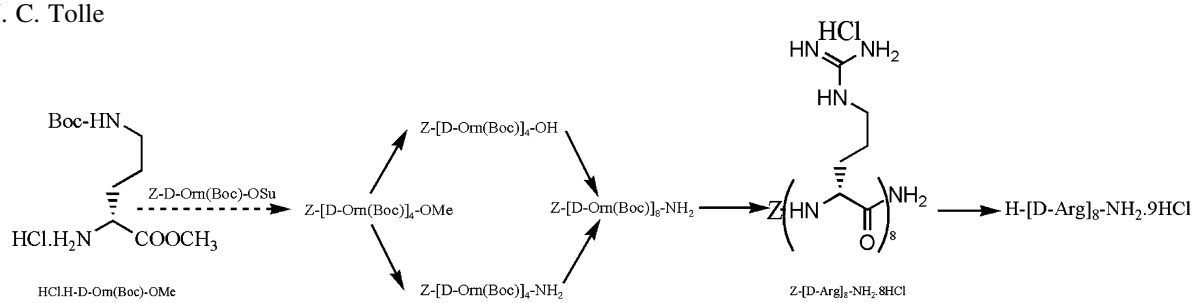
Tetrahedron Vol. 61, No. 37, 2005

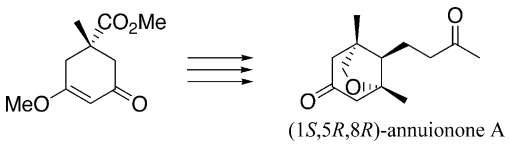
Contents

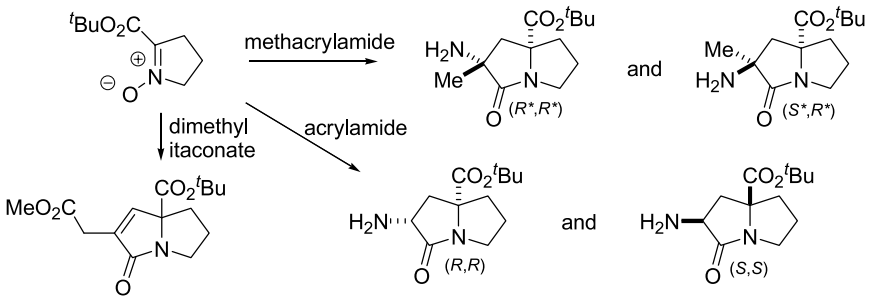
REPORT

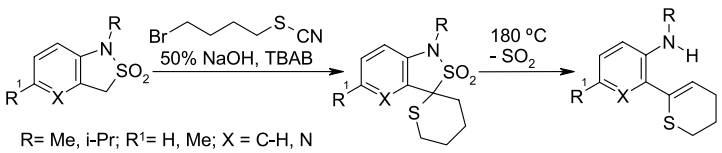


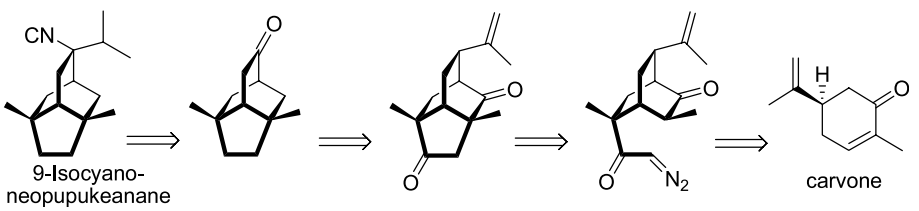
ARTICLES

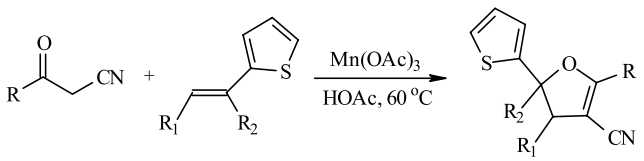


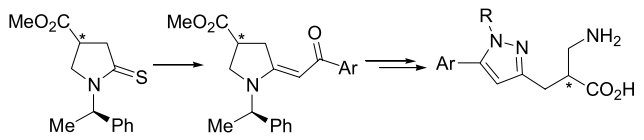


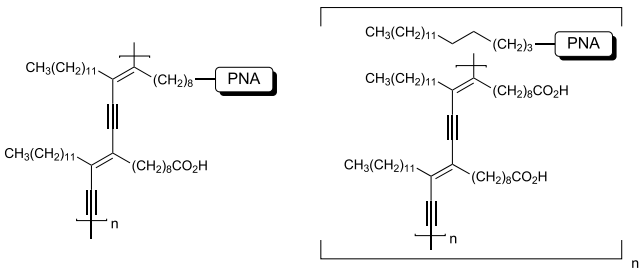




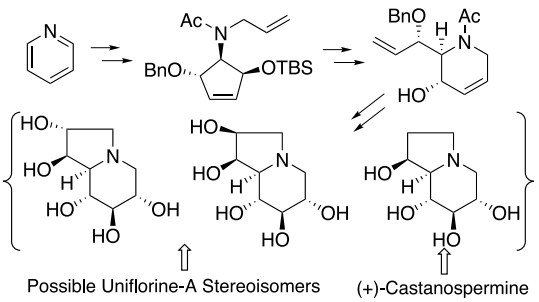


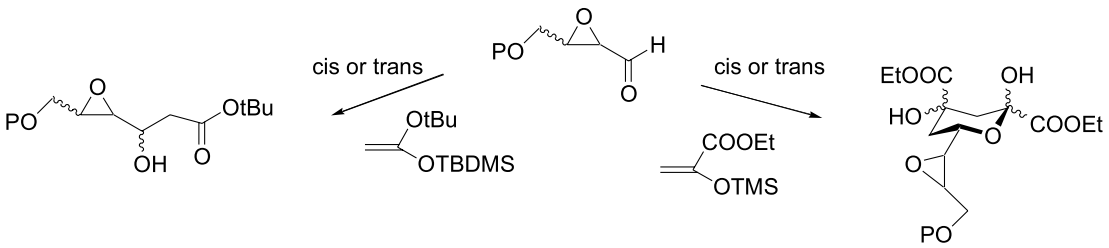




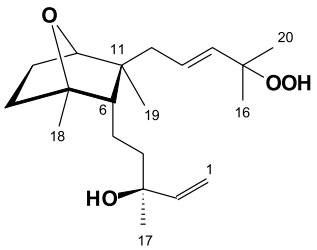


C- and *N*-terminal PNA monomers and oligomers have been prepared, from which polydiacetylene liposomes have been produced.

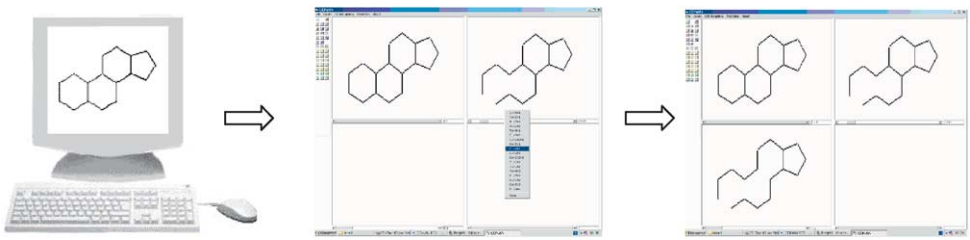


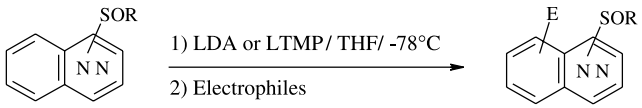


EWG = electron-withdrawing group
TMGA = *N,N,N′,N′*-tetramethylguanidinium azide
QN_3 = hexadecyltributylphosphonium azide

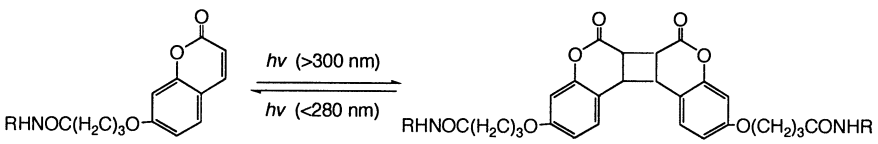


E-Dactylohydroperoxide A **4**

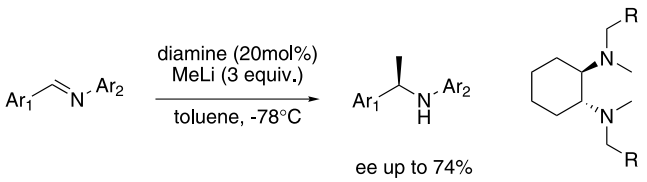




R = t-Bu, Ph, *p*-Tolyl

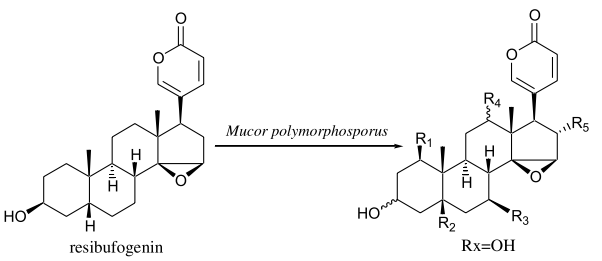


gel

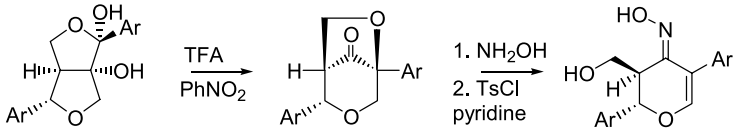


ee up to 74%

Biotransformation of the cytotoxic steroid resibufogenin by *Mucor polymorphosporus* afforded 22 products, and 15 of them are new compounds.



resibufogenin



(Ar = 3,4-methylenedioxyphenyl)

OTHER CONTENTS

*Corresponding author
ⓘ+ Supplementary data available via ScienceDirect

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ISSN 0040-4020

Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron
Tetrahedron 61 (2005) 8769–8807

Tetrahedron report number 732

Synthesis of propellane-containing natural products

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Received 31 May 2005

Available online 7 July 2005

Contents

Keywords: Total synthesis; Natural products; Propellanes.
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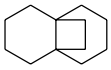
doi:10.1016/j.tet.2005.06.013

1. Propellanes and their synthesis

The first propellanes were synthesized in the 1930s during investigations into the Diels–Alder reaction.[1–6] However, the first 'propellane by design' was synthesized much later, in 1965.[7,8] Their nomenclature was introduced shortly thereafter. In 1966, Bloomfield and Irelan reported a synthesis of [4.4.2]propellane (**1**) and used the term propellerane in this context,[9] but the editors did not accept this nomenclature[10] and the compound was reported as 9,10-dihydro-9,10-ethanonaphthalene. In the same year, Ginsburg and co-workers introduced the name propellane in a paper that reported the syntheses of a variety of different propellanes, without editorial dissent.[11]



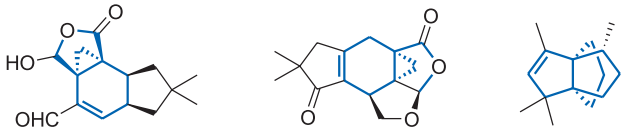
1

Webster's Unabridged Dictionary defines a propeller as 'a device having a revolving hub with radiating blades'[12] and, indeed, the structure of the molecules is in accordance with the name (see Fig. 1). The name propellane refers to a tricyclic system conjoined by a carbon–carbon single bond. The nomenclature, suggested by Ginsburg and co-workers, follows and simplifies that used for tricycloalkanes (Fig. 1).[11]

The first synthesis of a small-ring propellane, [3.2.1] propellane, was published in 1968.[13] Theoretically, the most interesting propellane is [1.1.1]propellane (**2**) (Fig. 1). The reports of the first modeling studies concluded that **2** should be more stable than the corresponding diradical that lacks the conjoining bond.[14,15] However, the researchers were uncertain that **2** could ever exist. A decade later, Wiberg and Walter reported the synthesis and isolation of a surprisingly stable molecule, [1.1.1]propellane.[16] The nature of the central bond of the molecule has been the subject of many studies. The theoretical and experimental results of many different research groups agree on the special nature of the bond.[17–19] Some groups have questioned the existence of the central bond,[14,15,17,20,21] but Wiberg, Bader, and Lau argue cogently that the bond definitely exists between the bridgehead carbons with a bond order of 0.73.[22,23] The controversy regarding the central bond revolves around differing explanations of similar experimental facts and theoretical results of the nature of the bonding interaction. In addition to Wiberg's results, other theories have also been suggested.[18,24–27]

Ginsburg[28–30] and Wiberg[19,31,32] have reviewed the syntheses and the theoretical studies of propellanes extensively, Wiberg concentrating on the small-ring propellanes and Ginsburg on the medium-sized rings. In his book entitled 'Propellanes', Ginsburg even describes the syntheses and structures of natural products possessing the propellane structure.[33]

Indeed, propellane structures are present in many different classes of natural products, for example, **3**–**5** (Fig. 2). Whereas theoretical studies have focused on small-ring propellanes containing more than one three- or four-membered ring, in the known natural products only one of the rings is small, the rest being five to eight-membered. The literature selected for this review describes the total syntheses of propellane-containing natural products. Particular emphasis is placed on propellanes having a three-membered ring in the ring system. The descriptions of the syntheses focus on the methods used for construction of the propellane ring systems of the molecules.



3: marasmic acid **4**: sterepolide **5**: modhephene

Figure 2. Natural products, which contain a propellane ring system.

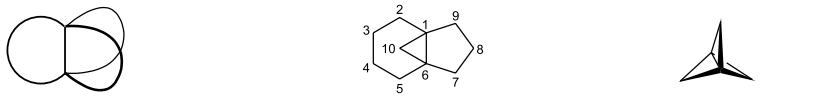
By far, one of the most popular avenues to synthesize any ring system has been the venerable Diels–Alder reaction.[34] This has also been the case with propellane ring structures. If the ~20 syntheses of modhephene (**5**), a [3.3.3]propellane, are disregarded because the molecule consists of five-membered rings (see Fig. 2), half of the syntheses analyzed here have utilized the Diels–Alder reaction as one of the key ring-forming transformations. The following sections describe the syntheses of natural products, which contain a propellane ring system. The literature until the beginning of 2005 is covered.

2. [*m.n*.1]Propellanes

There are quite a few natural products with the [*m.n*.1] propellane structure. However, only eight of them have been synthesized. Two of these contain a cyclopropane ring and, in six of them, the smallest ring is an epoxide. From a synthetic point of view this is a crucial difference, since constructing a cyclopropane ring is still considered a challenge, while many excellent methods exist for the formation of epoxides. The ring system of propellanes poses its own challenge for three-membered ring formation.

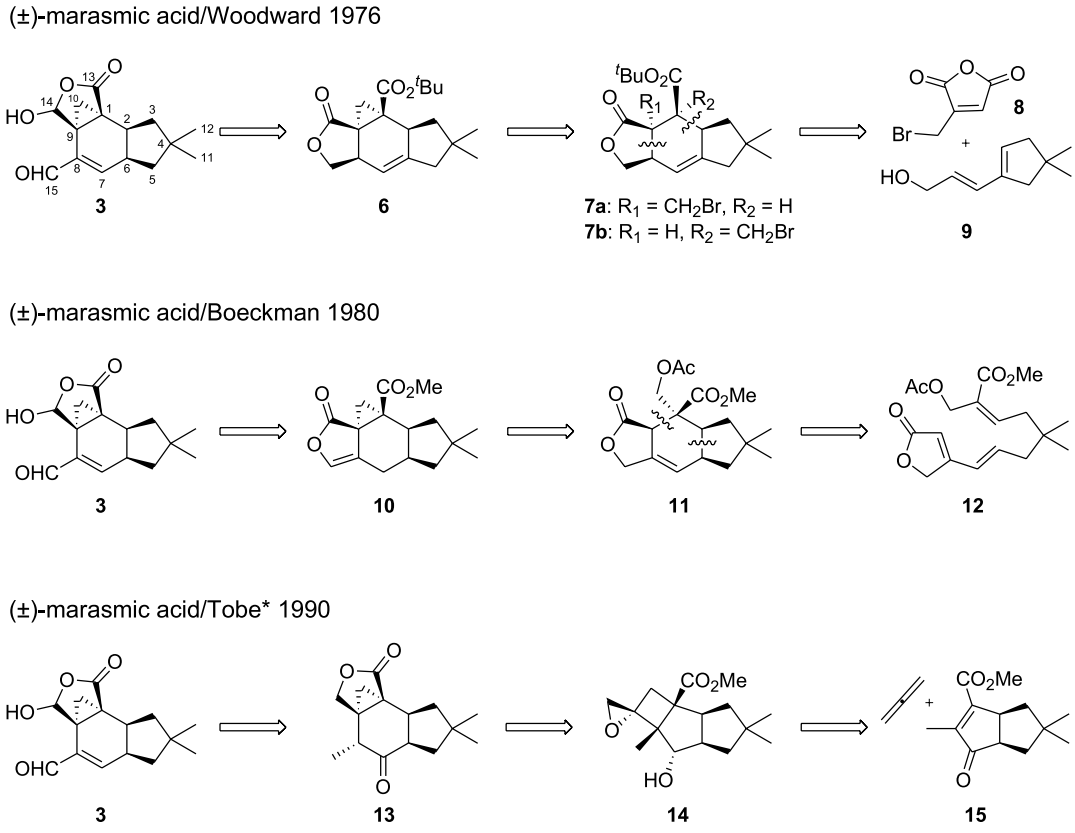
2.1. Cyclopropanes: marasmic acid and sterepolide

Marasmic acid (**3**) and sterepolide (**4**) can be described as [4.3.1]propellanes in which the smallest ring is a cyclopropane (see the highlighted parts in Fig. 2). The synthetic strategies that have been used to date for marasmic acid and



General structure of propellanes Numbering of [4.3.1]propellane [1.1.1]propellane (**2**)

Figure 1. Propellanes.

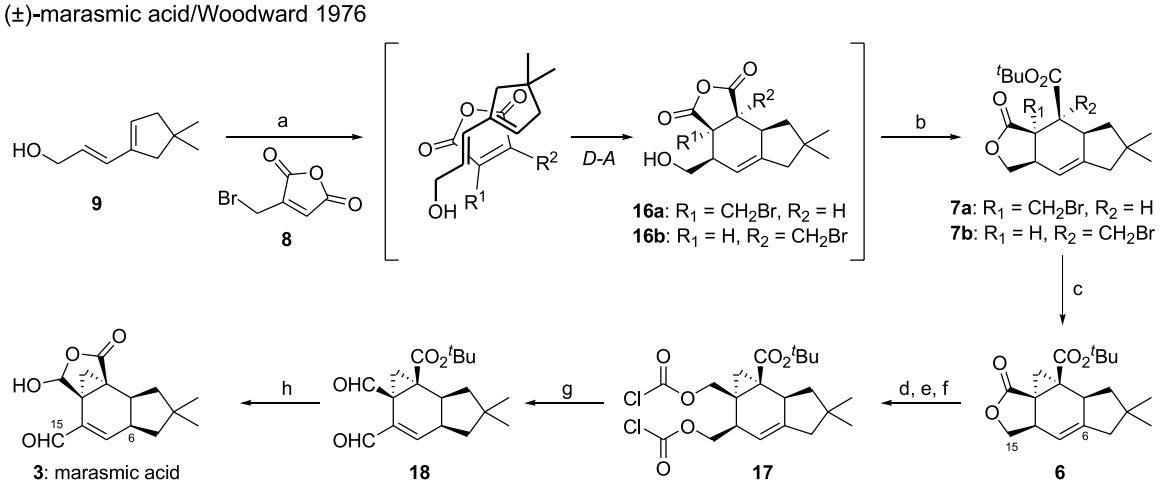


Scheme 1. Retrosynthetic analysis of marasmic acid.

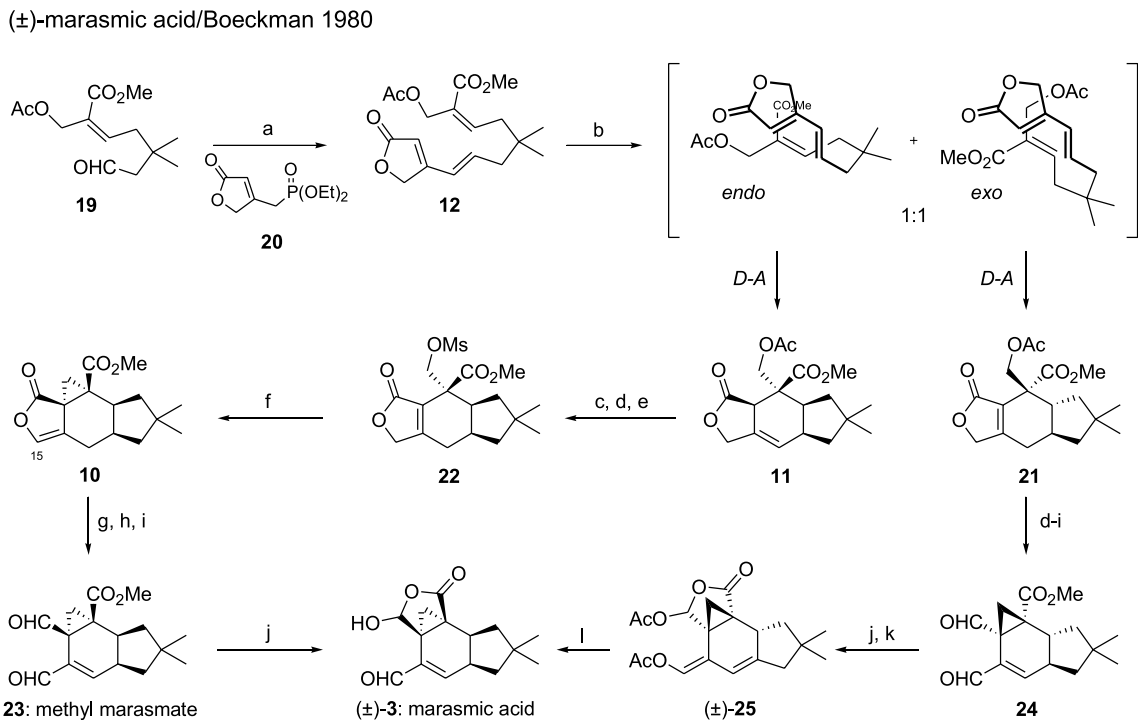
sterepolide do not utilize a cyclopropanation reaction to install the challenging cyclopropane ring; instead, most of them have adapted variants of the method developed by Woodward, which accesses the cyclopropane ring by a Diels–Alder-enolate alkylation sequence (vide infra).

2.1.1. Marasmic acid. Marasmic acid (**3**) is an antibacterial agent isolated for the first time by Kavanagh et al. from *Bacidiomycetes*.[35,36] To date, three different syntheses have been reported for this molecule. Retrosynthetic analysis of the syntheses (Scheme 1) reveals that two of the groups, those of Woodward[37–39] and Boeckmann,[40] have employed the Diels–Alder reaction as the key ring-forming step. While both of these used a similar enolate alkylation strategy to form the cyclopropane ring, the third synthesis by Tobe et al. used a 1-oxaspirohexane rearrangement to make the carbon framework of the molecule.[41]

Woodward group's synthesis of (±)-marasmic acid (Scheme 2)[37–39] commences with a completely *endo*-selective Diels–Alder reaction of diene **9** and 2-(bromomethyl)maleic anhydride (**8**), yielding a mixture



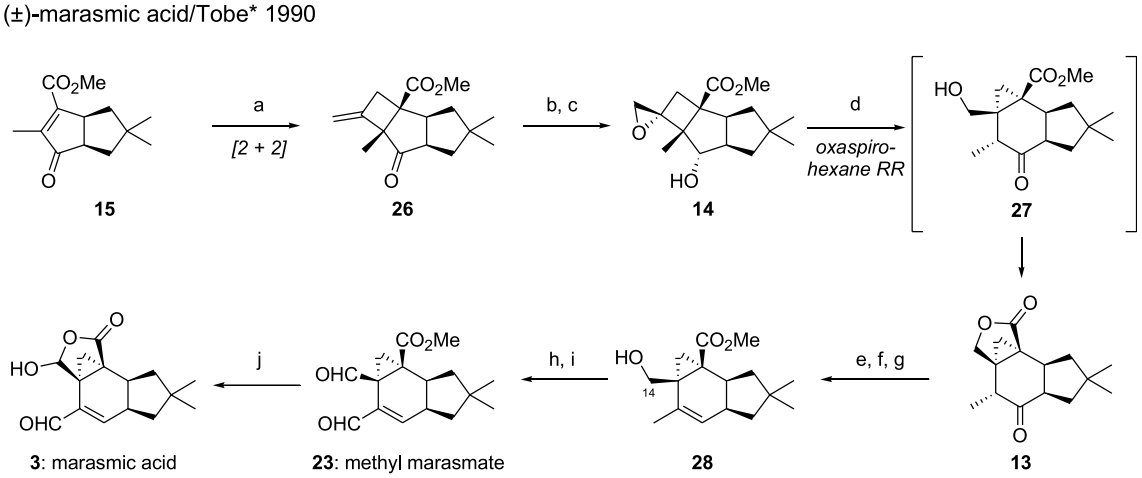
Scheme 2. (a) **8**, CH_2Cl_2, rt, 36 h, **16a**:**16b** 1:1; (b) isobutylene, *p*-TsOH, CH_2Cl_2, rt, 4 days; (c) *t*-BuOK, *t*-BuOH, PhH, rt, 10 min, 44% over three steps; (d) DIBAL-H, tol, −78 °C, 4 h; (e) $NaBH_4$, MeOH, rt, 15 min, 65% over two steps; (f) phosgene, quinoline, Et_2O, 0 °C→rt, 30 min, 91%; (g) DMSO, 15 °C, 25 min, then Et_3N, 15 °C, 20 min, 25%; (h) TFA, PhH, rt, 30 min, 50%.



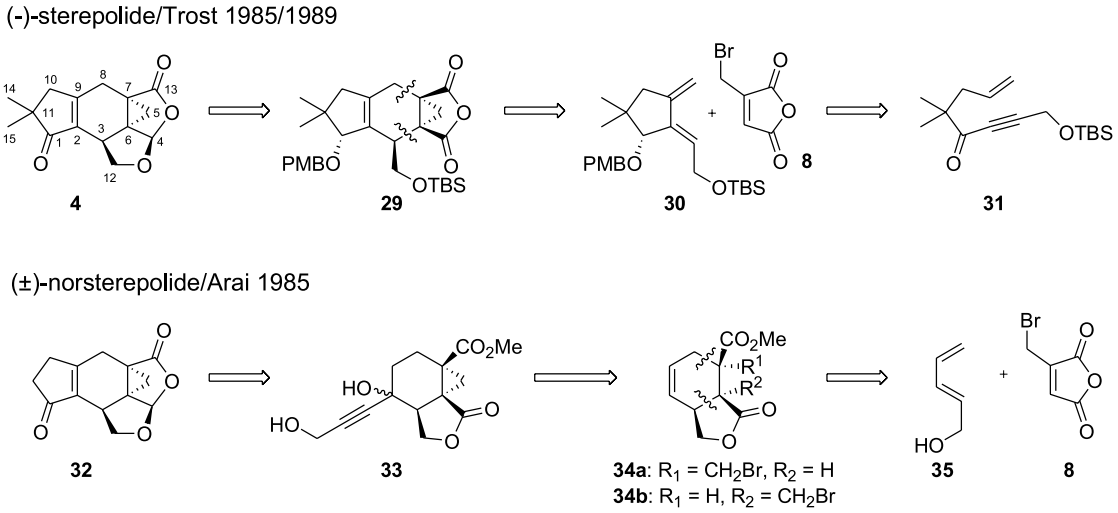
Scheme 3. (a) NaH, **20**, DMF, −5→0 °C, 30 min; (b) tol, 200 °C, 30 min, 92%, **11**:**21** 1:1; (c) *t*-BuOK, Et_2O, 0 °C, 30 min, quant.; (d) cat. *p*-TsOH, MeOH, 65 °C, 12 h, quant.; (e) MsCl, Et_3N, CH_2Cl_2, −5 °C, 30 min; (f) DBU, THF, 65 °C, 8 h, >90% over two steps; (g) PhSeBr, MeOH, rt, 2 h, 92%; (h) DIBAL-H, tol/THF 1:1, −78 °C, 12 h; (i) *m*-CPBA, CH_2Cl_2, −78 °C→rt, 4.5 h, 77% over two steps; (j) BBr_3, CH_2Cl_2, −20→0 °C, 4.5 h, 50%;[(k)[36] *p*-TsOH, isopropenyl acetate, reflux, 5 h; (l)[36] AcOH, HCl, H_2O, 'cold', 24 h, reactions not performed].

of *tert*-butyl esters **7a** and **7b** after treatment of the cycloaddition product with isobutylene under acidic conditions. Subjection of the esters to potassium *tert*-butoxide afforded a single cyclopropane **6** in 44% yield over the three steps. Having obtained the carbon framework of the molecule with the correct stereochemistry, the oxidation states of carbons C_6 and C_{15} had to be adjusted. To that end, lactone **6** was subjected to a sequence of reductions and chloroformate formation with quinoline and phosgene to give dichloroformate **17**. The dichloroformate **17** was then oxidized[42] to dialdehyde **18**, which gave (±)-marasmic acid (**3**) after de-esterification.

Boeckmann and co-workers chose to make the carbon skeleton of marasmic acid via an intramolecular Diels–Alder reaction (Scheme 3).[40] While Woodward's strategy provided essentially one diastereomer after the cyclopropane ring formation, Boeckmann's group was unable to avoid a 1:1 mixture of two diastereomers **11** and **21** resulting from *endo* and *exo* transition states, respectively. They were able to transform both diastereomers into marasmic acid, however, thus enhancing the productivity of the synthesis. After isomerization of the double bond of **11** to give an α,β-unsaturated lactone (see **22**), the acetate was transformed into a mesylate to set the stage for the



Scheme 4. (a) hν, allene, CH_2Cl_2, −78 °C, 3 h, 73%, 91% selectivity; (b) $NaBH_4$, MeOH, rt, 4 h, 98%; (c) *m*-CPBA, Na_2HPO_4, CH_2Cl_2, 0 °C, 89%; (d) H_2SO_4, CH_2Cl_2, 80%; (e) $NaBH_4$, THF/MeOH 1:2, rt, overnight, 9:1 selectivity; (f) MsCl, pyr, DMF, 80 °C, 1 h, 87% over two steps; (g) NaOH, H_2O, MeOH, reflux, overnight, 92%; (h) TBHP, SeO_2, CH_2Cl_2, rt, overnight; (i) $(COCl)_2$, DMSO, CH_2Cl_2, −60 °C, 10 min, then Et_3N, 15 min→rt, 19% over two steps; [(j)[40] BBr_3, CH_2Cl_2, −20→0 °C, 4.5 h, reaction not performed, formal total synthesis].

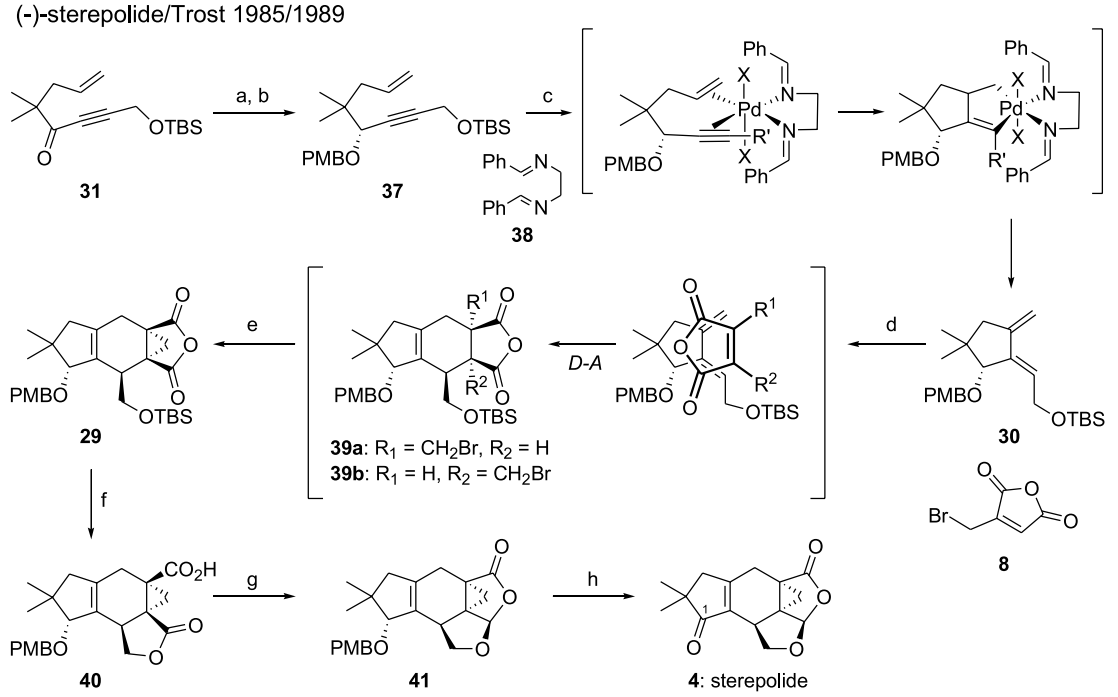


Scheme 5. Retrosynthetic analysis of sterepolide.

cyclopropane ring formation. Exposure of mesylate **22** to DBU in refluxing THF provided cyclopropane **10** in excellent yield. Next, a sequence of phenylselenide formation, DIBAL-H reduction and *m*-CPBA oxidation yielded methyl marasmate (**23**). Finally, **23** was treated with BBr_3 to give (±)-marasmic acid (**3**).

Tobe and co-workers have reported a 1-oxaspirohexane rearrangement as an entry to the norcarane skeleton present in the marasmane-type natural products (Scheme 4).[43] To utilize this rearrangement in the synthesis of marasmic acid,[41] an entry to cyclobutyl epoxide **14** was required. Thus, enone **15** was irradiated in the presence of allene to yield the head-to-head photoadduct **26** as the major product. Sodium borohydride reduction of the remaining ketone and *m*-CPBA epoxidation of the exocyclic double bond provided the rearrangement precursor **14** in a highly stereoselective manner. Exposure of **14** to concentrated sulfuric acid in methylene chloride for 1 h gave cyclopropanolactone **13** in 80% yield, and <5% of the β-Me isomer of **13** was observed. Next, ketone **13** was reduced and eliminated and the lactone ring was opened to enable the oxidation at C_{14}. Allylic oxidation[44] of **28** followed by Swern oxidation gave methyl marasmate (**23**), completing a formal total synthesis (denoted with an asterisk in Scheme 4) of (±)-marasmic acid (**3**).

2.1.2. Sterepolide. Two different syntheses have been reported for the pentacyclic framework of sterepolide (**4**), a metabolite of the fungus *Stereum purpureum* (Scheme 5).[45]



Scheme 6. (a) $LiAlH_4$/Darvon alcohol complex, Et_2O, −100 °C, quant., >98% ee; (b) NaH, PMBBr, cat. Bu_4NI, THF, rt, 82%; (c) 5% $Pd(OAc)_2$, 10% **38**, PhH, 70 °C, 81%; (d) **8**, PhH, 80 °C; (e) DBU, PhH, rt, 81% over two steps; (f) TBAF, THF, rt; (g) disiamylborane, Et_2O, 0 °C→rt, 75%; (h) DDQ, CH_2Cl_2, H_2O, then PDC, 3 Å MS, CH_2Cl_2, 83%.

Trost and co-workers have reported a racemic[46] and an asymmetric[47] synthesis relying on a palladium-catalyzed cyclization and a Diels–Alder–cyclopropane formation sequence, very similar to that, which Woodward and co-workers used in their synthesis of marasmic acid (vide supra). Arai et al. have synthesized norsterepolide, which lacks the *gem*-dimethyl groups of sterepolide, utilizing a Diels–Alder–cyclopropane formation sequence and a Nazarov cyclization to form the ring system.[48]

In 1985, Trost and Chung published a racemic synthesis of sterepolide.[46] In 1989, they published a refined, enantioselective route to the molecule and assigned its absolute stereochemistry (Scheme 6).[47] Thus, reduction of the acetylenic ketone **31** with $LiAlH_4$/Darvon alcohol complex gave the protected alcohol **37** in >98% ee after PMB protection. The stage was now set for the palladium-catalyzed cyclization. In the presence of palladium acetate and ligand **38**, the Diels–Alder precursor **30** formed smoothly in 81% yield. Heating diene **30** and 2-(bromomethyl)maleic anhydride (**8**) in benzene followed by base treatment yielded cyclopropanoanhydride **29** as a single diastereomer. Removal of the silyl protecting group then opened the anhydride and consequently closed the five-membered lactone ring. Reduction then closed the second lactone ring and deprotection followed by oxidation completed the total synthesis of (−)-sterepolide (**4**) and established its absolute stereochemistry as shown. On the basis of this stereochemical assignment, Trost has proposed that sterepolide and marasmic acid are biosynthetically derived from the same enantiomeric folding of farnesyl pyrophosphate (Fig. 3).[36,45] Interestingly, the final step of the synthesis also destroys the stereocenter at C_1 that has been employed as a stereochemical control element throughout the synthesis.

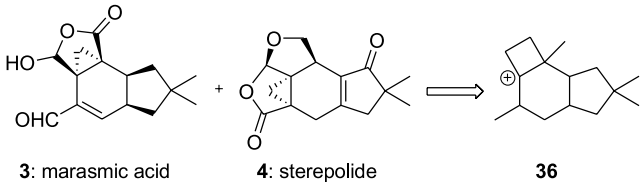
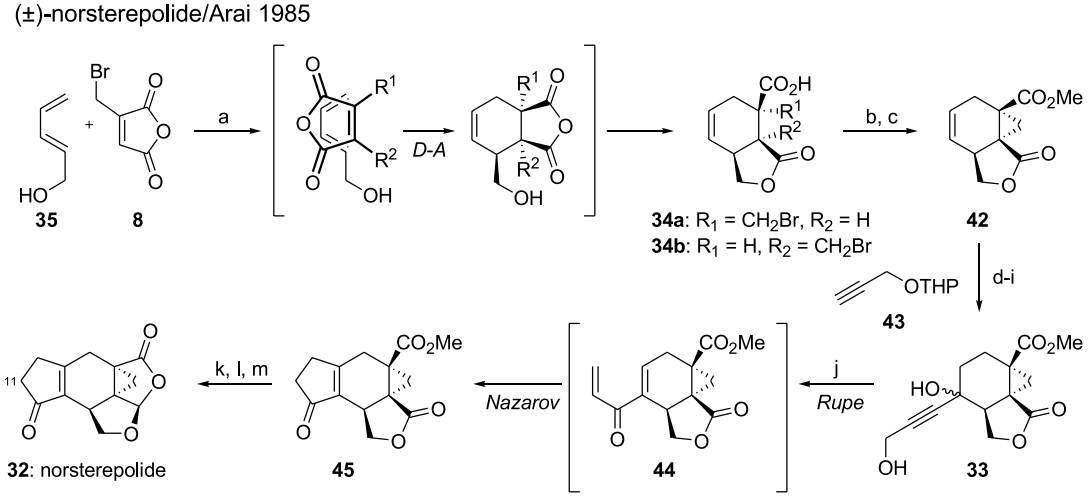


Figure 3. Biogenetic origin of marasmic acid and sterepolide.

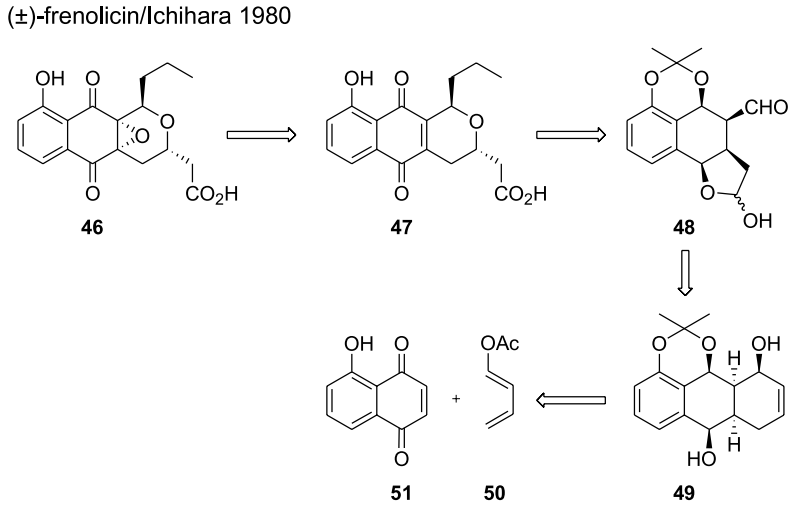
Arai and co-workers' synthesis[48] of norsterepolide (**32**) also commences with a Diels–Alder reaction of 2-(bromomethyl)maleic anhydride (**8**) and diene **35** (Scheme 7). The cycloaddition produced the *endo*-products **34a** and **34b** in a ratio of 4.5:1, which was inconsequential since, after esterification, the compound was treated with potassium *tert*-butoxide to close the cyclopropane ring in 90% yield. Next, the acetylenic side chain was introduced to prepare for the crucial Nazarov cyclization. Epoxidation, opening of the epoxide with hydrogen bromide, Jones oxidation and treatment with zinc to remove the bromide gave a ketone ready to be alkylated with **43**. Alkylation of the ketone with the lithium acetylide of **43** followed by deprotection of THP in acidic conditions produced diol **33**. Subjection of **33** to phosphorus pentoxide and methanesulfonic acid yielded the cyclopentenone **45** via a Rupe rearrangement followed by a Nazarov-type conrotatory electrocyclization.[49] The final lactone ring was closed by protecting the enone as a ketal, reducing the lactone and treating the resulting hemiacetal with acid to close the lactone and release the enone to give (±)-norsterepolide (**32**). Attempts were made to introduce the *gem*-dimethyl groups at C_{11}, but they proved unsuccessful.

2.2. Epoxides: frenolicin B, dynemicin A, fusicogigantone A, SF 2315B, diepoxin σ and arthrinone

This rather heterogeneous group of molecules is unified by the fact that they all have an epoxide ring fused to two other rings in their ring system. Most of them, namely frenolicin B, dynemicin A, SF 2315B, and diepoxin σ, are [4.4.1] propellanes, whereas fusicogigantone A is a [6.3.1] propellane and arthrinone a [4.3.1]propellane. The Diels–Alder reaction is a frequent feature of these syntheses; seven of the 10 routes described herein utilize the cycloaddition to



Scheme 7. (a) PhH, rt, 72 h, 72%, **34a**:**34b** 4.5:1; (b) CH_2N_2, MeOH, Et_2O, quant.; (c) *t*-BuOK, *t*-BuOH, PhH, rt, 1 h, 90%; (d) *m*-CPBA, cat. bis-(3-*t*-Bu-4-hydroxy-5-methylphenyl) sulfide, $NaHCO_3$, $CHCl_3$, reflux, 6 h, α:58%, β:16%; (e) 48% HBr, $CHCl_3$, 0 °C→rt, 1 h, α-OH and β-Br: 80%, α-Br and β-OH: 70%; (f) Jones reagent, acetone, rt, 6 h; (g) Zn, AcOH, rt, 15 h; (h) **43**, BuLi, −70→−30 °C, 1 h, then sm, −78 °C, 1 h; (i) *p*-TsOH, MeOH, rt, 48 h; 47% over two steps; (j) P_2O_5, MsOH, rt, 2.5 h, 34%; (k) ethylene glycol, PPTS, PhH, reflux, 5 h, 93%; (l) DIBAL-H, THF, −90 °C, 30 min, 84%; (m) *p*-TsOH, PhH, 55 °C, 7 h, 85%.

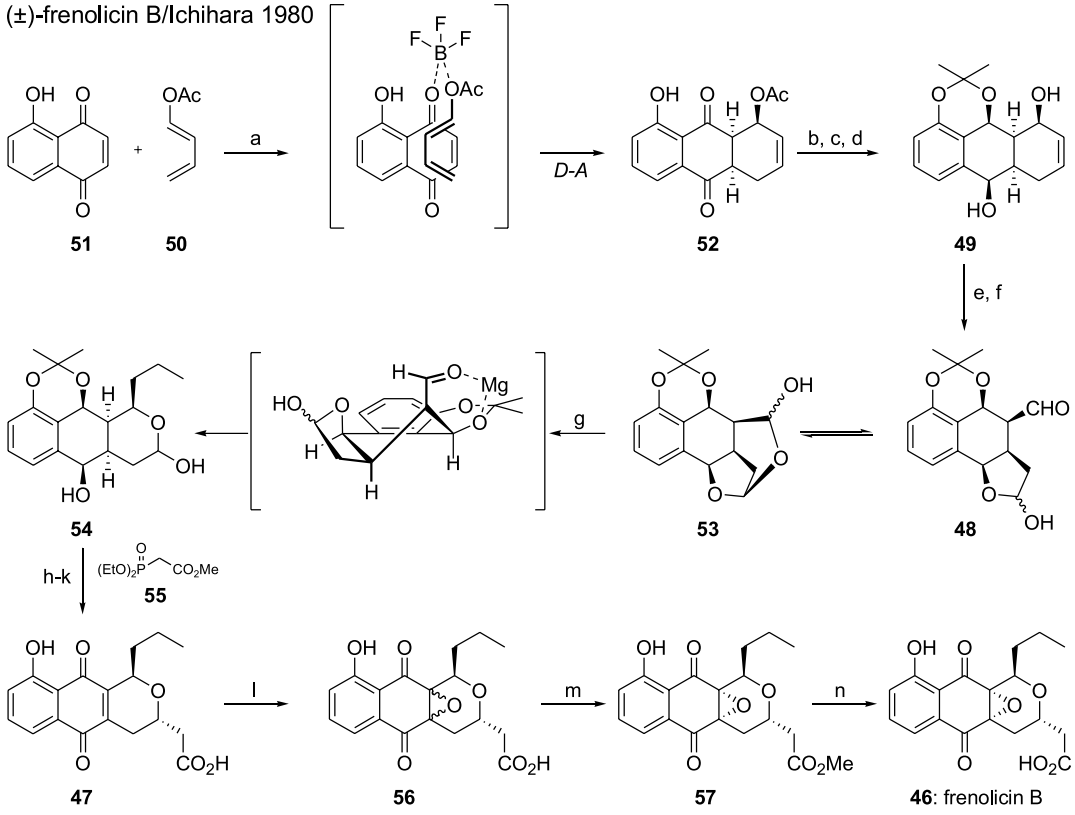


Scheme 8. Retrosynthetic analysis of frenolicin B.

form the carbon backbone of the molecule. Once the rings are in place, the epoxidations generally proceed stereoselectively and give good yields of the products.

2.2.1. Frenolicin B. Frenolicin B (**46**), a pyranonaphthoquinone antibiotic isolated from *Streptomyces fradiae* in 1960,[50,51] has been synthesized[52] only once, although six syntheses for deoxyfrenolicin (**47**) have been reported. Since those syntheses, by Naruta and co-workers,[53] Semmelhack et al.,[54–56] Uno,[57] Kraus et al.,[58] Moore and co-workers,[59,60] Brimble and Lynds,[61] as well as Xu et al.,[62] lack the formation of the epoxide, which makes the molecule a propellane, they will not be treated here. Scheme 8 shows Ichihara's retrosynthetic approach to frenolicin (**46**). The key steps include a Lewis acid-catalyzed Diels–Alder cyclization and an alkylation followed by cyclization of the pyran ring.

Ichihara's synthesis of racemic frenolicin starts with a highly selective boron trifluoride-catalyzed Diels–Alder



Scheme 9. (a) BF_3, PhH or $CHCl_3$, 55 °C, 97%; (b) $NaBH_4$, THF, 5 °C, quant.; (c) 2,2-dimethoxypropane, acetone, $BF_3{\cdot}OEt_2$, 70%; (d) $LiAlH_4$, Et_2O, 97%; (e) OsO_4, $NaIO_4$, *t*-BuOH, H_2O; (f) NaOAc, DABCO, 99% over two steps, (g) *n*-PrMgBr, Et_2O, 66%; (h) **55**, *n*-BuLi, DMSO, rt, 2 h, 61%; (i) PCC, CH_2Cl_2, rt, 98%; (j) DDQ, TsOH, MeOH, reflux, 9 h, then dioxane, reflux, 12 h; (k) KOH, MeOH, H_2O; (l) TBHP, triton B, dioxane, EtOH, rt, α:β 1:1; (m) CH_2N_2, then separation of diastereomers; (n) KOH, MeOH, H_2O, rt.

reaction of juglone (**51**) and acetoxybutadiene (**50**), which gives the tricyclic acetate **52** in excellent yield (Scheme 9).[63] Selective reduction, ketal formation and reduction of the remaining ketone and acetate provided the allylic alcohol **49**. Lemieux-Johnson oxidation of the double bond in **49** afforded an equilibrated mixture of aldehyde **48** and hemiacetal **53**. This mixture was then treated with *n*-propylmagnesium bromide to give hemiacetal **54** stereoselectively in a chelation-controlled addition. Next, a Horner–Wadsworth–Emmons reaction added the needed two-carbon side chain, and a sequence of PCC- and DDQ-oxidations followed by basic hydrolysis provided naphthoquinone **47**. Subjection of **47** to *tert*-butyl hydroperoxide in the presence of Triton B gave a 1:1 mixture of two unseparable epoxide diastereomers, which was methylated, separated and hydrolyzed to give (±)-frenolicin (**46**). The lack of selectivity in the epoxidation step can be explained by the fact that the two bulky substituents can reside either pseudo-equatorially/pseudo-axially or pseudo-axially/pseudo-equatorially (Fig. 4).

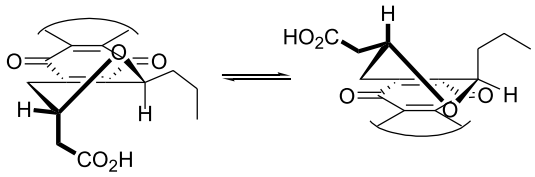
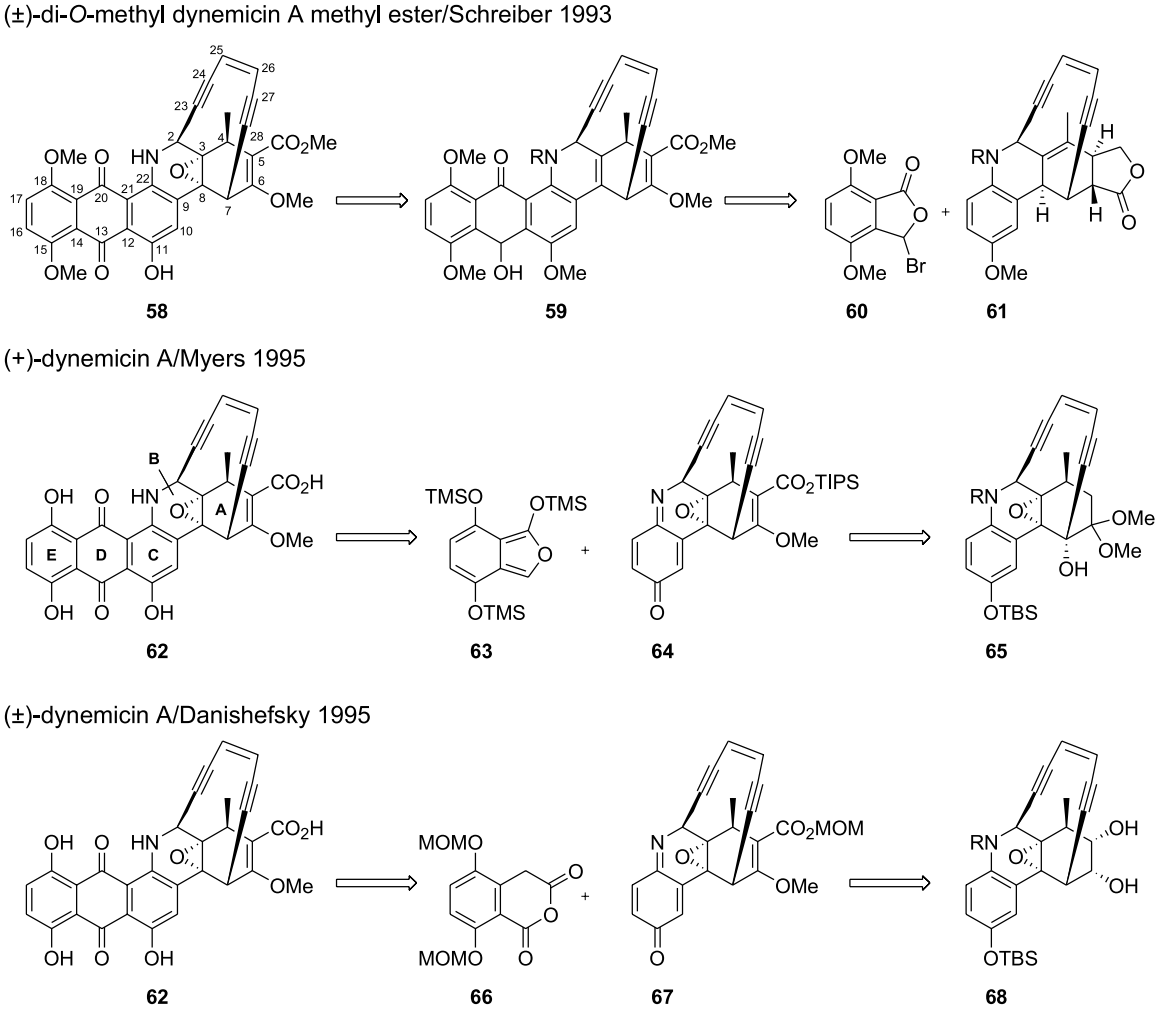
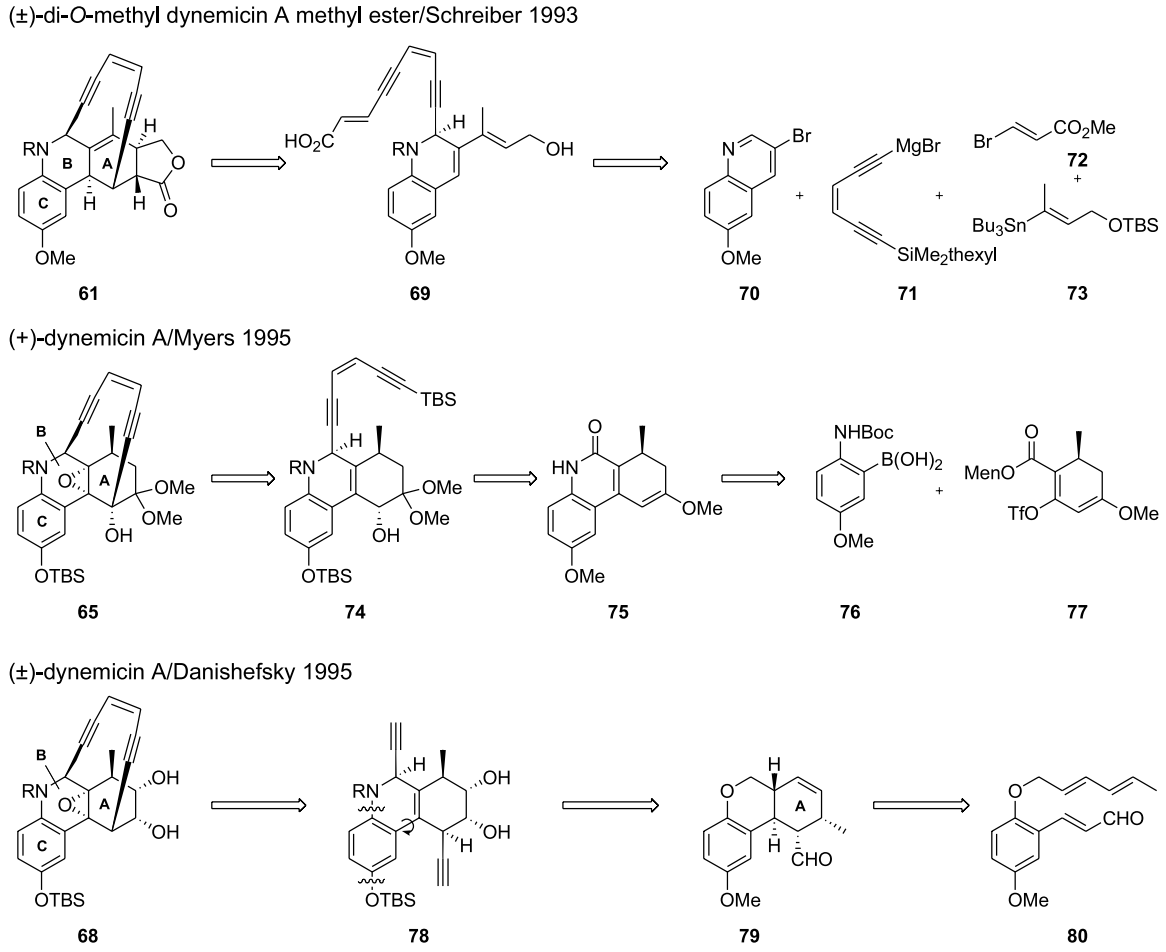


Figure 4. Conformations of alkene **47**.

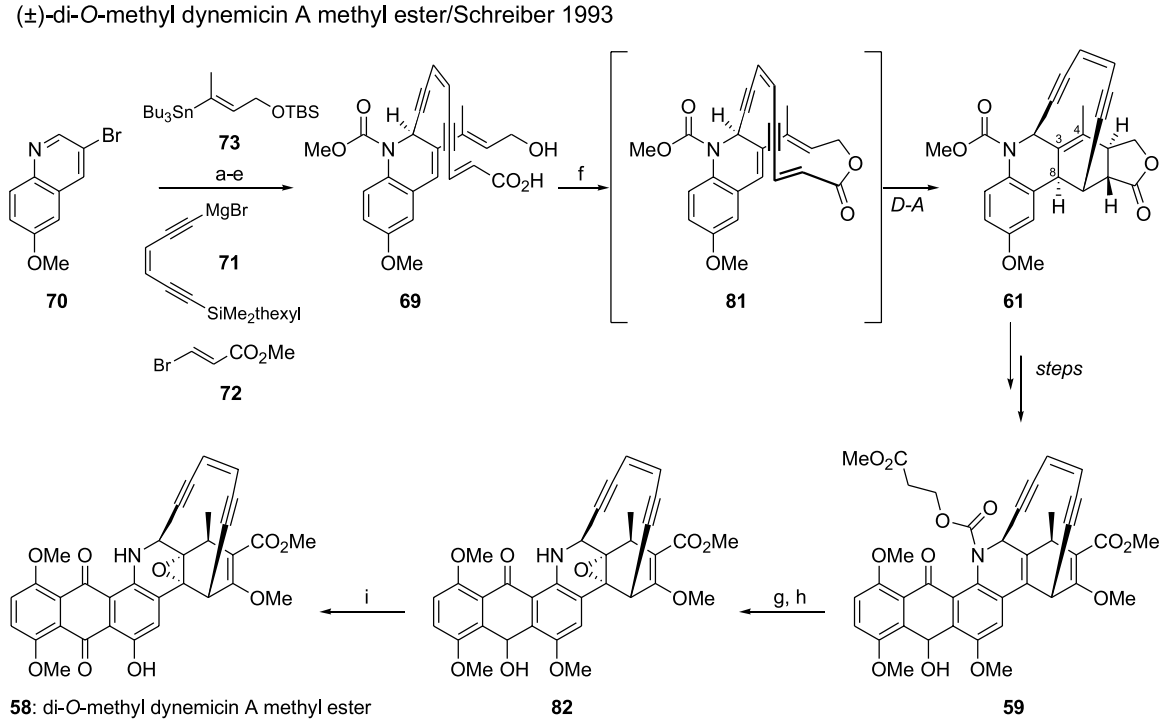
2.2.2. Dynemicin A. Dynemicin A (**62**), a potent antibacterial and anticancer agent isolated from *Micromonospora chersina*,[64,65] has attracted wide synthetic interest due to its biological activity and intriguing molecular structure. So far, two elegant total syntheses of dynemicin A and one of di-*O*-methyl dynemicin A methyl ester have appeared in the literature (Schemes 10 and 11). Five different synthetic approaches to model compounds of the dynemicin A ring framework have also been published. The total syntheses by the groups of Schreiber,[66–69] Myers[70,71] and Danishefsky[72–77] will be treated here with more detail. The groups of Nicolaou,[78–81] Isobe,[82–84] Magnus,[85] Maier[86] and Takahashi[87,88] have reported their efforts towards dynemicin A model compounds. Of these, the Nicolaou and Takahashi models include a [4.4.1]propellane ring system, so the retrosynthetic analysis of these syntheses are shown in Scheme 15. The syntheses of Isobe, Magnus and Maier did not result in the formation of the propellane skeleton and are outside the scope of this review. Maier has published a review that details the investigations into different dynemicin A analogs.[89]



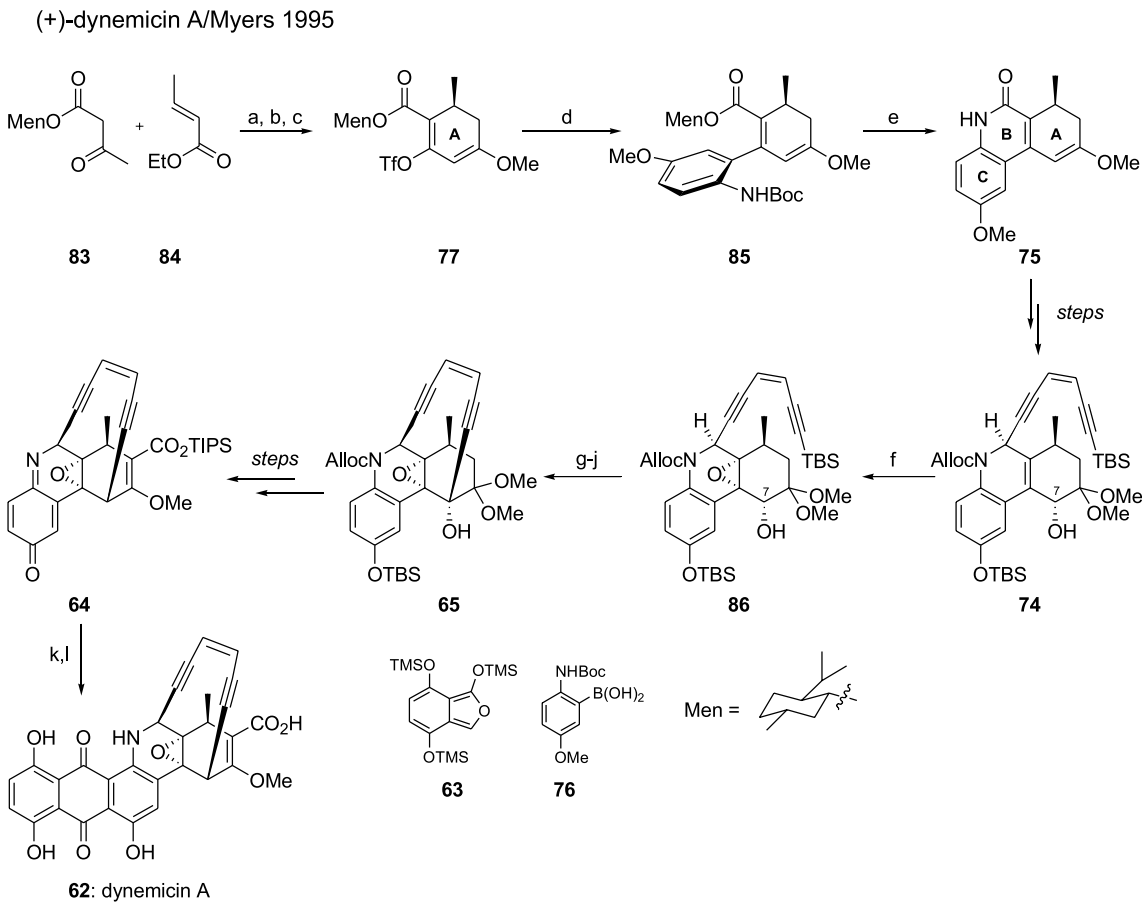
Scheme 10. Retrosynthetic analysis of dynemicin A.



Scheme 11. Retrosynthetic analysis of advanced intermediates of dynemicin A.



Scheme 12. (a) **73**, $Pd(PPh_3)_4$, 85%; (b) **71**, $ClCO_2Me$, THF; (c) TBAF, THF, 60% over two steps; (d) **72**, $Pd(PPh_3)_4$, CuI, 20%; (e) LiOH, H_2O, THF, 65%; (f) Bromo-tris-pyrrolidino-phosphonium hexafluoro-phosphate (PyBroP), Et_3N, CH_2Cl_2, rt, 13 h, 51%; *steps*; (g) *m*-CPBA, pH 7 buffer, CH_2Cl_2, rt, 2.5 h, 73%; (h) 0.11 M DBU, MeOH, rt, 1 h; (i) CAN, aq MeCN, 0 °C, 45 min.

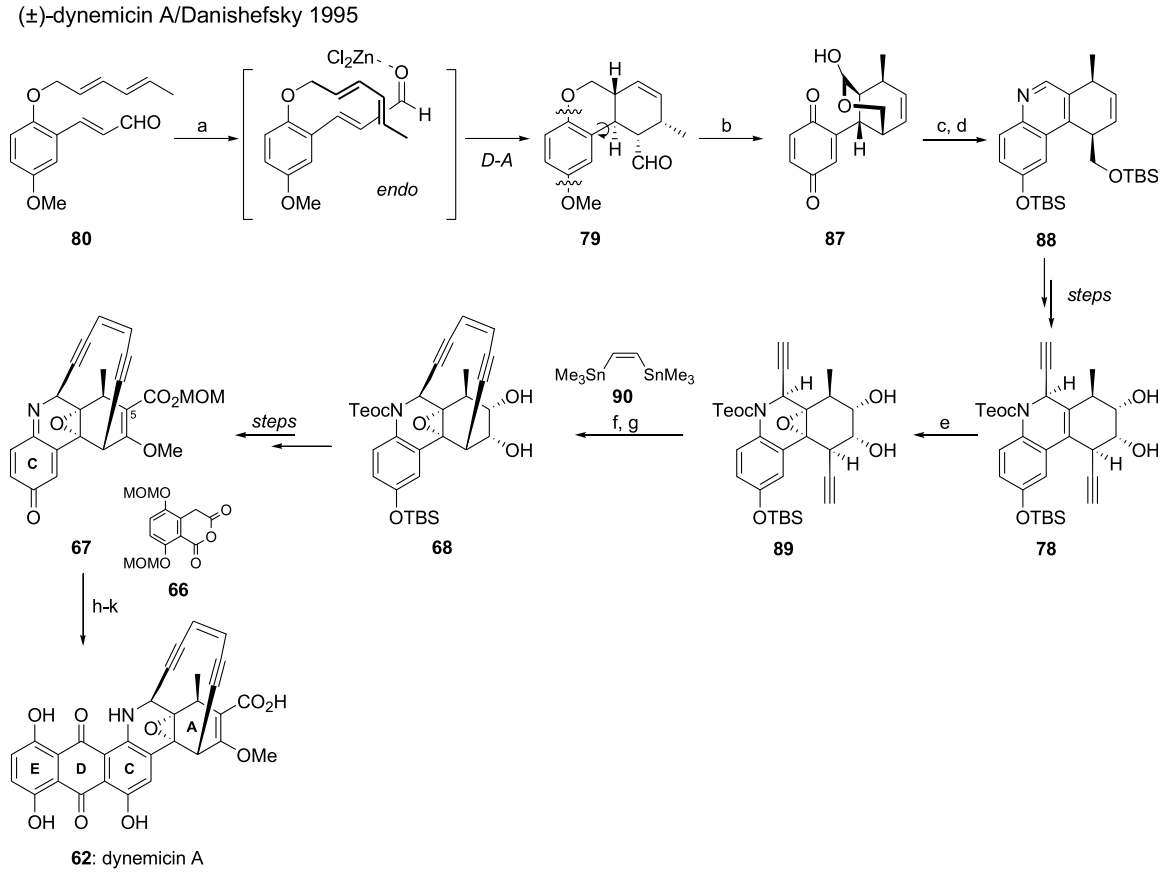


Scheme 13. (a) *t*-BuOK, *t*-BuOH, reflux, 3.5 h, 36% after recryst, 1:1 diastereoselectivity; (b) CSA, MeOH, rt, 12 h, 71%, 4:1 regioselectivity; (c) NaH, Et_2O, rt, 5 h, then Tf_2O, −78→0 °C, 30 min, 95%; (d) **76**, $Pd(PPh_3)_4$, $NaCO_3$, *p*-dioxane, reflux, 45 min, 90%; (e) 4-ClPhOH, 180 °C, 30 min, 84%; *steps*; (f) *m*-CPBA, pH 7 buffer, CH_2Cl_2, 0 °C, 19 h, 88%; (g) TBAF, THF, 0 °C, 15 min, quant.; (h) TBSCl, imid., DMF, rt, 1 h, 96%; (i) $(COCl)_2$, DMSO, CH_2Cl_2, −40 °C, 10 h, then Et_3N, −78→0 °C, 30 min, 92%; (j) KHMDS, $CeCl_3$, THF, −78 °C, 5 min, 94%; *steps*; (k) **63**, THF, −20→55 °C, 5 min; (l) MnO_2, $3HF{\cdot}Et_3N$, THF, rt, 9 min, 40% over two steps.

Retrosynthetic analyses of the dynemicin A syntheses are shown in Schemes 10 and 11. All of the groups have accessed the final compound via fairly similar ABC ring structures (**61**, **65**, and **68**), although the Schreiber group installed the epoxide in the late stages of the synthesis. The Schreiber group also decided to use an intramolecular Diels–Alder reaction (IMDA) to form the A ring and close the enediyne bridge of the molecule, whereas the Danishefsky group utilized IMDA only to form the A ring. The Myers group joined the A and C rings with a Suzuki cross-coupling reaction and subsequently closed the B ring by lactam formation.

The synthesis of di-*O*-methyl dynemicin A methyl ester by Schreiber and co-workers begins with the assembly of the intramolecular Diels–Alder substrate (Scheme 12).[66,68,69] Stille coupling of **70** with vinylstannane **73** followed by a 1,2-addition of acetylide **71** to the resulting pyridinium salt provided a diyne. Silyl deprotection gave an acetylide that was then coupled with vinyl bromide **72**. After ester hydrolysis, the IMDA substrate **69** was exposed to Yamaguchi macrolactonization conditions, effecting the macrocyclization, and, subsequently, the intramolecular Diels–Alder reaction to give lactone **61** in 50% yield. Next, the stereochemistry at C_4 and the oxidation state of carbons C_3 and C_8 were adjusted followed by functional group manipulation and construction of the anthraquinone fragment with a Diels–Alder reaction (see Scheme 10). Exposure of **59** to *m*-CPBA in pH 7 phosphate buffer gave epoxide **82** stereoselectively after deprotecton of the carbamate nitrogen with DBU. Finally, the secondary alcohol was oxidized with ceric ammonium nitrate to give (±)-di-*O*-methyl dynemicin A methyl ester (**58**).

The total synthesis of dynemicin A by Myers and co-workers starts with the construction of the A ring (Scheme 13).[70,71] Condensation of menthyl acetoacetate (**83**) and *trans*-ethyl crotonate (**84**) provided a 36% yield of the correct β-methyl diastereomer after recrystallization. Stirring the 1,3-diketone in methanolic camphorsulfonic acid led to the formation of an enol ether, which was then subjected to sodium hydride and triflic anhydride to yield the coupling precursor **77**. Suzuki cross-coupling of enol triflate **77** with arylboronic acid **76** in the presence of tetrakis(triphenylphosphine)palladium and sodium carbonate afforded the AC ring structure **85** in 90% yield. Next, heating the carbamate **85** in 4-chlorophenol cleaved the nitrogen-protecting group and closed the B ring of the molecule. After a series of functional group manipulations as well as incorporation of the enediyne side chain, allylic alcohol **74** was ready to be epoxidized. Initially, the Myers group tried to close the enediyne bridge with various



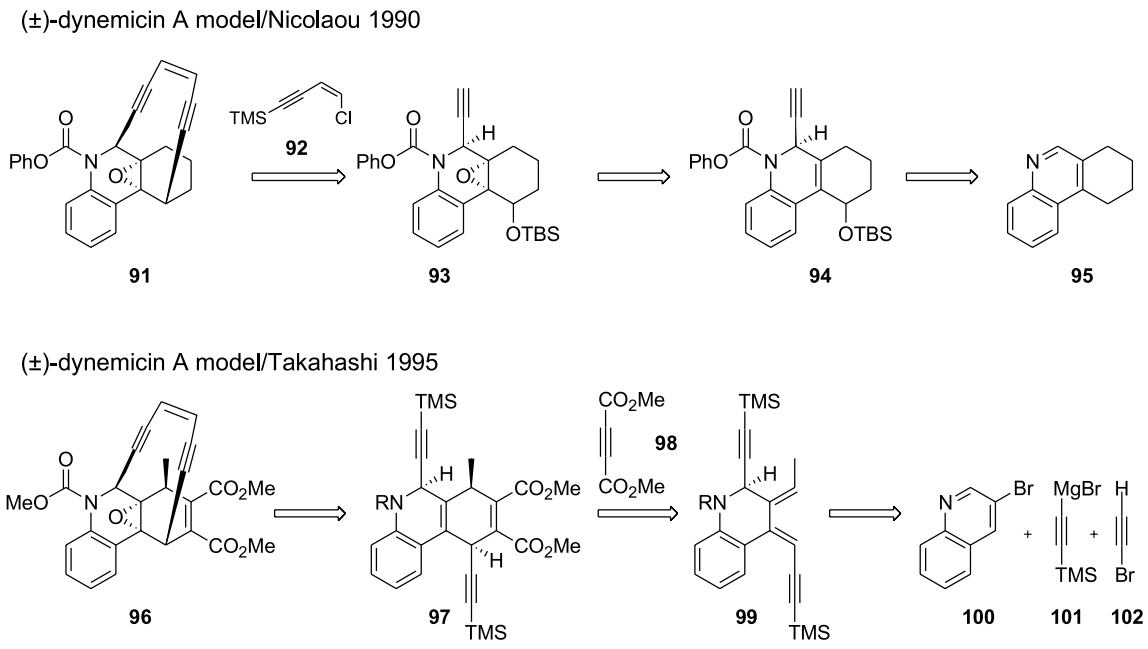
Scheme 14. (a) $ZnCl_2$, CH_2Cl_2, rt, 3 days, 60%, *endo*:*exo* 20:1; (b) CAN, MeCN, H_2O, 0 °C, 30 min, 90%; (c) NH_4OAc, AcOH, 100 °C, 1 h, 89%; (d) TBSCl, imid., CH_2Cl_2, 0 °C, 2 h, 98%; *steps*; (e) *m*-CPBA, CH_2Cl_2, rt, 8.5 h, 87%; (f) cat. $AgNO_3$, NIS, THF, rt, 3.5 h, 91%; (g) **90**, $Pd(PPh_3)_4$, DMF, 75 °C, 1.2 h, 81%; *steps*; (h) LiHMDS, **66**, THF, 0 °C, 35 min, then **67**, 0 °C, 35 min; (i) $PhI(OCOCF_3)_2$, THF, 0 °C, 5 min; (j) air, daylight, THF, high concentration, 20 h; (k) $MgBr_2$, Et_2O, 0 °C→rt, 12 h, 15% over four steps.

electrophiles at C_7 before the epoxidation without succeeding. In the event, buffered *m*-CPBA effected the epoxidation in 88% yield. Then, removal of the silyl groups, reprotection of the phenol and oxidation at C_7 provided the cyclizaton precursor. Indeed, deprotonation of the acetylene caused the enediyne bridge to attack the ketone at C_7 and gave alcohol **65** in excellent yield. After adjusting the oxidation states of various carbons, the stage was set for the final Diels–Alder reaction to complete the anthraquinone part of the molecule. The Diels–Alder reaction between the quinone imine **64** and isobenzofuran **63** proceeded in 5 min to give the di-TMS-ether, which was immediately oxidized with manganese dioxide to provide (+)-dynemicin A (**62**) in 40% yield over two steps.

Danishefsky and co-workers also decided to begin the construction of dynemicin A ring system with an intramolecular Diels–Alder reaction (Scheme 14).[72–77] A zinc chloride-catalyzed IMDA led selectively to the *endo* adduct **79** in 60% yield. Exposure of adduct **79** to ceric ammonium nitrate gave rise to quinone lactol **87** via oxidation of the aromatic ring and lactol formation between the unveiled alcohol and the aldehyde. Ammonium acetate followed by silylation then afforded **88** with both of its stereocenters arising from the Diels–Alder step. After installation of the acetylenic functions, olefin **78** was ready for the epoxidation step. Thus, epoxidation of olefin **78** with *m*-CPBA proceeded smoothly to give epoxide **89** in 87% yield. Next, the diacetylene **89** was transformed into a diiodide, which could then be treated with vinylbis(stannane) (**90**) and $Pd(PPh_3)_4$ to close the enediyne bridge, giving **68** in 81% yield. The enediyne bridge could not be closed without the presence of the epoxide (e.g., compound **78**), and a similar observation was also made by the Myers group (Scheme 13 and discussion). Then, after adjusting the oxidation state of the C ring and adding a carboxyl group at C_5, quinone imine **67** was ready to react with the anion of anhydride **66** to form the remaining rings of dynemicin A. The adduct was immediately oxidized with $PhI(OCOCF_3)_2$. Exposure of the product to air and daylight also oxidized ring D and the final step was to remove the MOM protecting groups. This was accomplished with magnesium bromide to provide (±)-dynemicin A (**62**) in 15% yield over the last four steps.

The synthesis of dynemicin A model compounds by Nicolaou and co-workers[78–81] is based on functionalizing quinoline derivative **91** (Scheme 15).[90,91] Takahashi and co-workers decided to form the A ring of their model compound **96** with a Diels–Alder reaction (Scheme 15).[87,88] The diene part **99** is derived from a [2,3]-Wittig rearrangement. The enediyne bridge was closed with a palladium-catalyzed coupling.

2.2.3. Fusicogigantone A. Fusicogigantone A (**103**) and fusicogigantepoxide (**112**) were isolated from *Pleurozia*



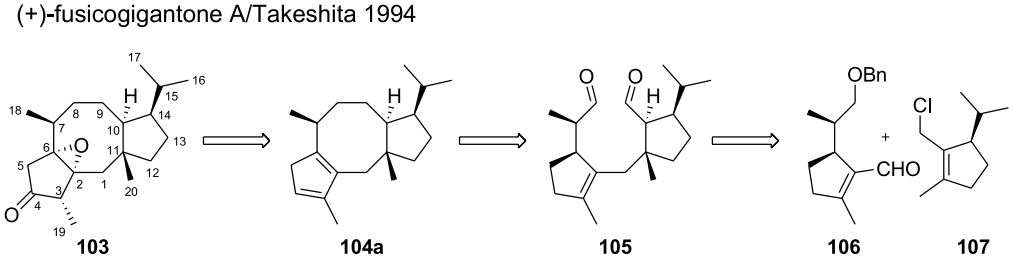
Scheme 15. Retrosynthetic analyses of dynemicin A model compounds.

gigantea collected from East Malaysia in 1990.[92] The only reported synthetic route to the fusicogigantones is from the group of Takeshita, the retrosynthetic analysis of, which is shown in Scheme 16. The key ring-forming steps are a singlet oxygen oxidation and a titanium-mediated McMurry ring closure of a dialdehyde.[93,94]

Optically active enal **106** and allyl chloride **107** were mixed with chromium dichloride to obtain alcohol **108** in good yield (Scheme 17). Next, the double bond in **108** was hydroborated, the benzyl group removed, the free secondary alcohol eliminated and the resulting double bond reduced with lithium and ethylamine. After a Swern oxidation of the diol, the resulting dialdehyde **105** was treated with titanium tetrachloride in the presence of zinc to obtain the cyclized α-*cis*-diol **109** in 38% yield together with the β-*cis*-diol in 7% yield. The diol was removed by orthoformate formation and subsequent reductive elimination.[95,96] The C_{8-9} double bond in **110** was hydrogenated to give a mixture of C_{2-6} and C_{2-3} double-bond-containing compounds. This mixture was treated with singlet oxygen generated by means of Rose Bengal photosensitization, followed by reduction with triphenylphosphine and dehydration with silica gel to obtain a 2:3 mixture of **104a** and **104b**. Oxidation of this mixture with singlet oxygen generated with tetraphenylporphyrin at −78 °C gave a mixture of **111a** and **111b** (observed by NMR), which were only stable below 0 °C. This mixture was left to stand at room temperature for 10 h, and **111a** gradually turned into (+)-fusicogigantone A (**103**) and (+)-fusicogigantepoxide (**112**). Heating the mixture to 60 °C for 1 h gave two more products, (+)-fusicogigantone B (**114**) and **113**, from the more stable endoperoxide **111b**. The products were separated by column chromatography. The authors report that the attack of $^1O_2$ had occurred exclusively from the α-side of the dienes in **104a** and **104b**.

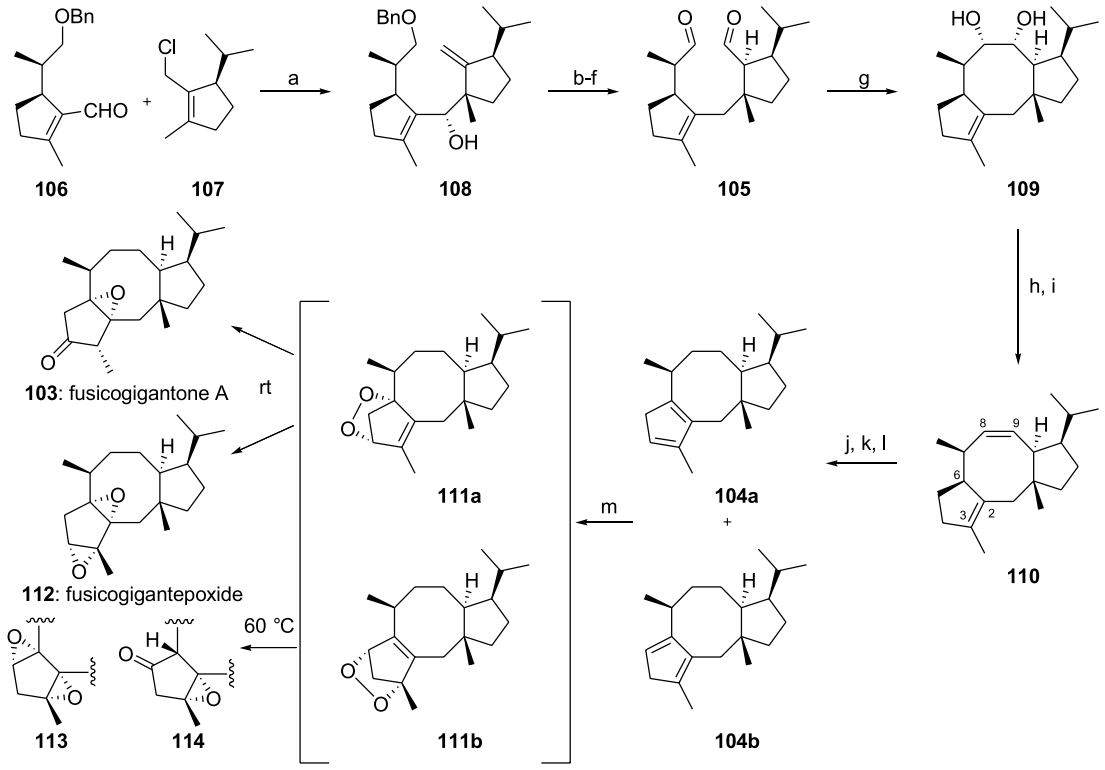
2.2.4. SF 2315B. Sulikowski and co-workers have reported a synthetic route[97,98] to the ring system of SF 2315B (**120**), an angucyclinone antibiotic isolated from a soil microorganism of the *Actinomycete* strain *Excellospora viridilutea*.[99,100] Their retrosynthetic analysis of the target is shown in Scheme 18. The key transformations in the synthesis are a highly selective Diels–Alder reaction to set up the carbon framework and an oxygenation to provide the correct oxidation pattern.

Sulikowski's synthesis of epoxyquinol **115** started with a highly regioselective Diels–Alder cycloaddition between 2-bromo-acetoxyjuglone (**119**) and diene **118** (Scheme 19). The reaction produced cycloadduct **121**, which was then dehydrobrominated with lithium hydroxide to give quinone **117**. Exposure of **117** to molecular oxygen and tetrabutylammonium fluoride provided epoxyalcohols **116** and **122**, which were separated by column chromatography.



Scheme 16. Retrosynthetic analysis of fusicogigantone A.

(+)-fusicogigantone A and (+)-fusicogigantepoxide/Takeshita 1994



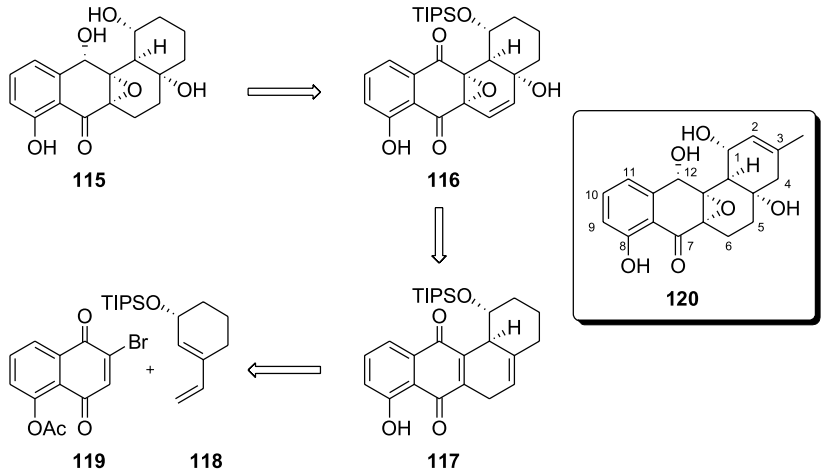
Scheme 17. (a) $CrCl_2$, $LiAlH_4$, DMF/THF 2:1, *i*-PrOH, 90%; (b) $Me_2CHCMe_2BH_2$, H_2O_2, ^-OH, 88%; (c) H_2, Pd/C, 82%; (d) HCl, THF, 99%, (e) Li, $EtNH_2$, 68%; (f) $(COCl)_2$, DMSO, Et_3N, 74%; (g) $TiCl_4$, Zn, PhH/THF 5:1, α-*cis* 38%, β-*cis* 7%; (h) $CH(OMe)_3$, PPTS, CH_2Cl_2, 93%; (i) Δ, Ac_2O, tol, 82%; (j) H_2, Pd/C, EtOH, EtOAc, 99%; (k) $^1O_2$; (l) PPh_3, silica, 90%, **104a**:**104b** 2:3; (m) $^1O_2$, tol, −78 °C→rt/60 °C, **103** 23%, **112** 12%, **113** 8%, **114** 8%.

Then, a sequence of hydrogenation, acetylation of the phenol, TIPS deprotection, C_1-hydroxyl-directed reduction of the C_{12}-ketone and removal of the acetyl group gave epoxyquinol **115**, which bears the complete array of stereogenic centers present in SF 2315B (**120**).

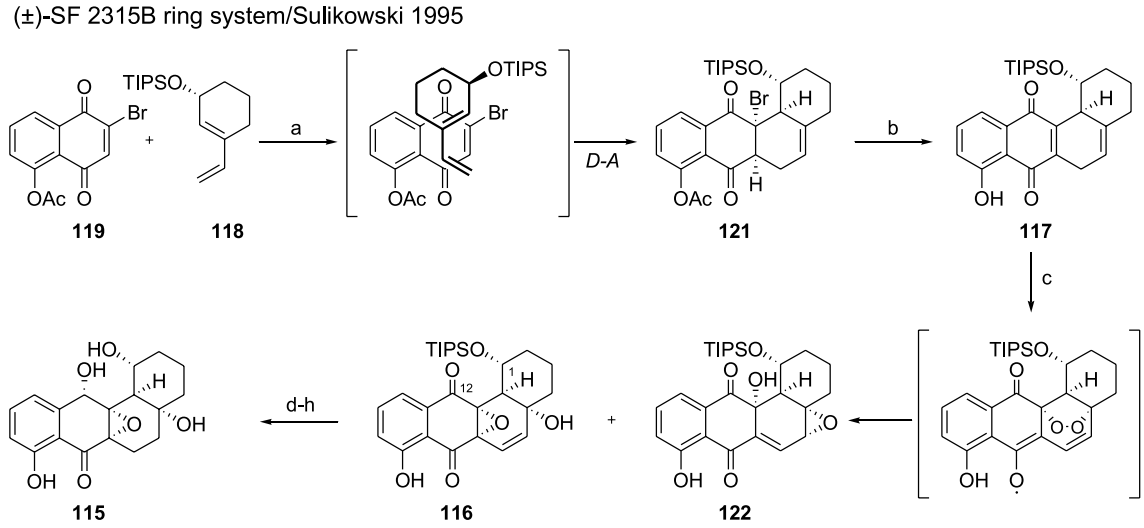
2.2.5. Diepoxin σ. Diepoxin σ (**123**) was isolated from fermentation broths of a nonsporulating fungus, LL-07F275, collected in Panama from a tree trunk in 1993.[101] To date, one total synthesis of the molecule has appeared in the literature by Wipf and Jung (Scheme 20).[102–104] The key steps in their synthesis include an Ullmann coupling followed by an oxidative spirocyclization to introduce the naphthalene ketal and a stereoselective epoxidation. Another notable feature is the use of a Diels–Alder-retro-Diels–Alder strategy to introduce chirality into the molecule as well as to protect the highly reactive naphthoquinone ring system.

The first task in the diepoxin σ synthesis by Wipf and Jung

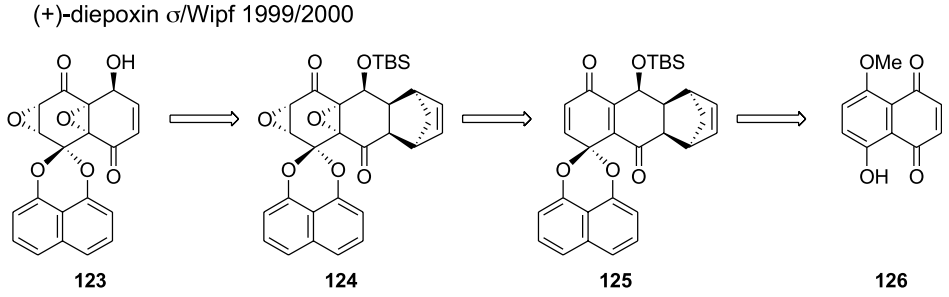
(±)-SF 2315B ring system/Sulikowski 1995



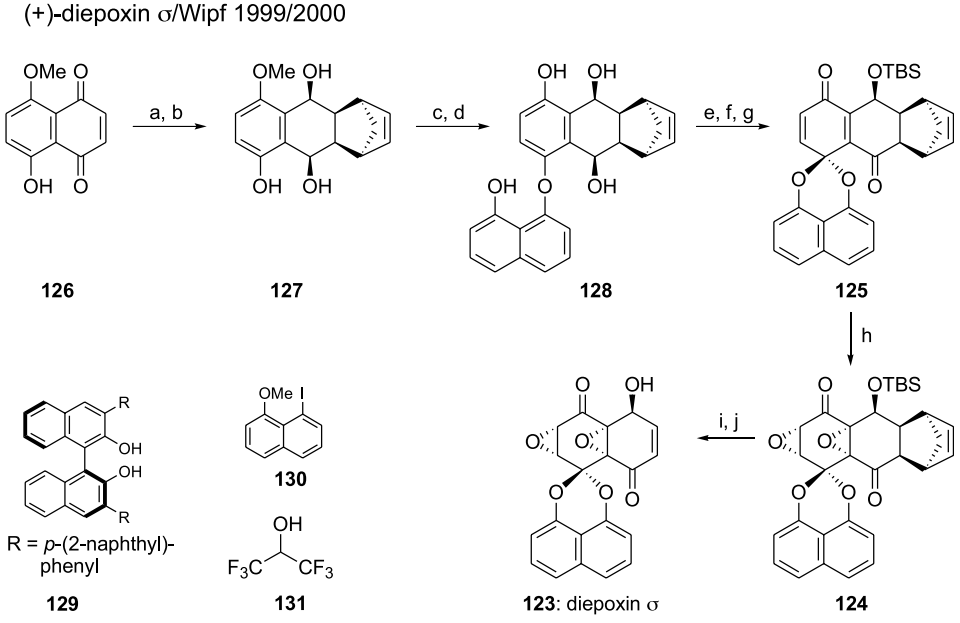
Scheme 18. Retrosynthetic analysis of SF 2315B ring system.



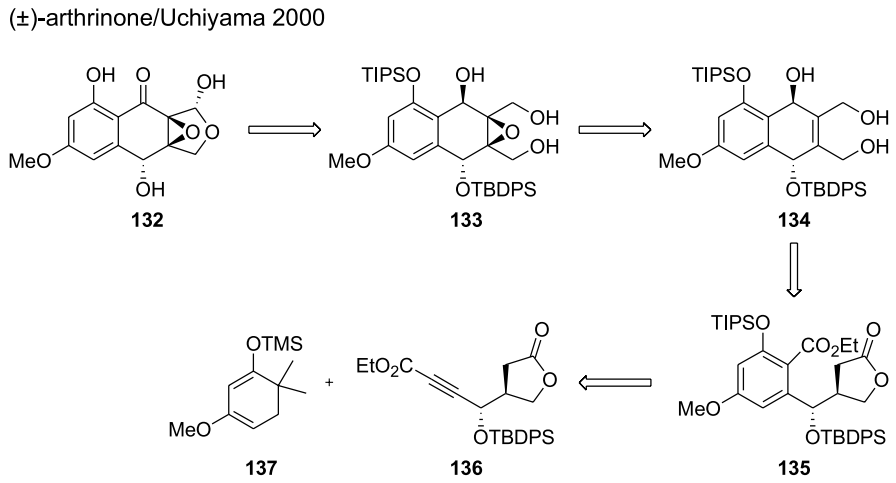
Scheme 19. (a) Tol, reflux, 71%; (b) LiOH, THF/MeOH 1:1, 0 °C, 30 min, 70%; (c) TBAF, O_2, THF, −78 °C→rt, **116** 33%, **122** 16%, **117** 20%; (d) H_2, PtO_2, EtOAc, 0 °C, 30 min, 76%; (e) Ac_2O, DMAP, pyr, CH_2Cl_2, rt, 30 min, 96%; (f) HF·pyr, MeCN, 0 °C→rt, 2 h, quant.; (g) $Me_4NB(OAc)_3$, AcOH, MeCN/THF 1:1, −10 °C, 53% (+38% of recovered sm); (h) Bu_4NOH, THF, 0 °C, 80%.



Scheme 20. Retrosynthetic analysis of diepoxin σ.



Scheme 21. (a) **129**, BH_3·THF, AcOH, THF, rt, 1 h, then cyclopentadiene, −78 °C, 2 h, 72%, 94% ee; (b) $NaBH_4$, THF, MeOH, −78→0 °C, 3 h, 88%; (c) **130**, Cu_2O, pyr, reflux, 20 h, 70%; (d) Ph_2PH, *n*-BuLi, THF, rt, 7 days, 95%; (e) $PhI(OAc)_2$, 4 Å MS, **131**, rt, 1 h, 61%; (f) TBSOTf, lutidine, CH_2Cl_2, −78 °C, 1 h, 91%; (g) PDC, DMF, rt, 24 h, 72%; (h) H_2O_2, K_2CO_3, THF, H_2O, 0 °C, 9 h, 88%; (i) PhOPh, reflux, 1 h; (j) HF, MeCN, H_2O, 0 °C→rt, 20 h, 73% over two steps.

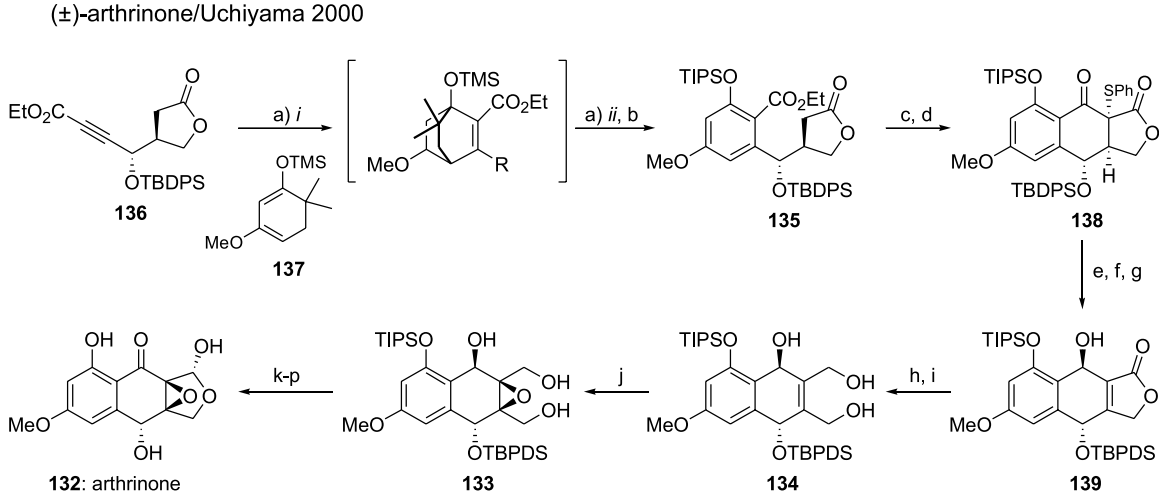


Scheme 22. Retrosynthetic analysis of arthrinone.

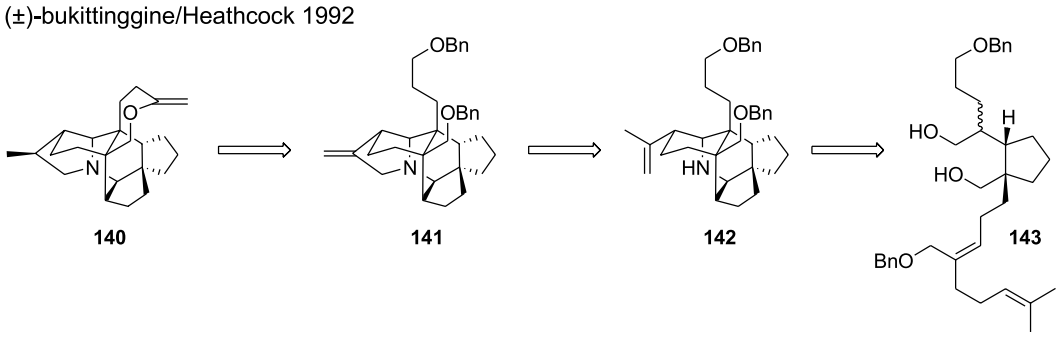
(Scheme 21) was the protection of the reactive double bond in **126** as a Diels–Alder adduct with cyclopentadiene.[102–104] In their preliminary work, Wipf and Jung developed a racemic synthesis of the molecule, after which they successfully performed the cycloaddition in an enantioselective manner. In the strictest sense, the synthesis is thus a formal total synthesis of (+)-diepoxin σ, but, because the same group performed the racemic synthesis, this is trivial. The Diels–Alder reaction of **126** and cyclopentadiene in the presence of borane and ligand **129** proceeded well to give 94% ee and 72% yield of the cycloadduct. Sodium borohydride reduction then gave the required precursor for the Ullmann ether coupling, which provided **128** after demethylation. The spiroketal ring was then closed with an oxidative cyclization in the presence of $PhI(OAc)_2$ in hexafluoro-2-propanol (**131**) followed by TBS protection of the less hindered secondary alcohol and PDC oxidation of the remaining alcohol to yield dienone **125**. Next, epoxidation of the two double bonds proceeded smoothly to give *syn*-diepoxide **124** in 88% yield as a single diastereomer. Now, the only remaining task was to cleave the protecting groups. Surprisingly, the enone double bond was unmasked with a retro-Diels–Alder reaction at 250 °C in boiling diphenyl ether without significant decomposition of the product. Then, treatment of the product with hydrogen fluoride gave (±)-diepoxin σ (**123**) in 73% yield over two steps.

2.2.6. Arthrinone. Arthrinone (**132**) is an antifungal metabolite, which was isolated from *Artrinium* sp. FA 1744 in 1994.[105] Its only total synthesis was reported in 2000 by Uchiyama and co-workers (Scheme 22).[106] The key ring-forming steps in the synthesis include a Diels–Alder–cycloreversion reaction of a diene and an acetylene to form a highly substituted aromatic ring, as well as a Dieckmann condensation.

The synthesis of arthrinone by Uchiyama et al. is illustrated in Scheme 23.[106] The Diels–Alder reaction between acetylene **136** and diene **137** proceeded smoothly and gave ester **135** in 81% yield after cycloreversion and TIPS protection. With all requisite carbon atoms in place,



Scheme 23. (a) i. **137**, 160 °C, ii. THF, 5% HCl, rt, 81%; (b) TIPSCl, imid., DMF, rt, quant.; (c) NaHMDS, THF, −78 °C; (d) NaH, THF, 0 °C, then PhSCl, 87% over two steps; (e) $Zn(BH_4)_2$, CH_2Cl_2, 0 °C, 83%; (f) *m*-CPBA, CH_2Cl_2, 0 °C, 90%, 9:1 selectivity; (g) $P(OMe)_3$, tol, reflux, 89% from major sulfide, 76% from minor; (h) DIBAL-H, CH_2Cl_2, −78 °C; (i) $NaBH_4$, MeOH, 0 °C, 77% over two steps; (j) *m*-CPBA, CH_2Cl_2, pH 7.9 phosphate buffer, 0 °C→rt, 72%; (k) TBSOTf, pyr, THF, −60 °C, 78%; (l) TBDPSCl, imid., DMF, rt, 91%; (m) 5% NaOH in EtOH, THF, rt, 61%; (n) MnO_2, acetone, rt, 91%; (o) cat. TPAP, NMO, 4 Å MS, CH_2Cl_2, rt; (p) TBAF, AcOH, THF, rt, 73% over two steps.



Scheme 24. Retrosynthetic analysis of bukittinggine.

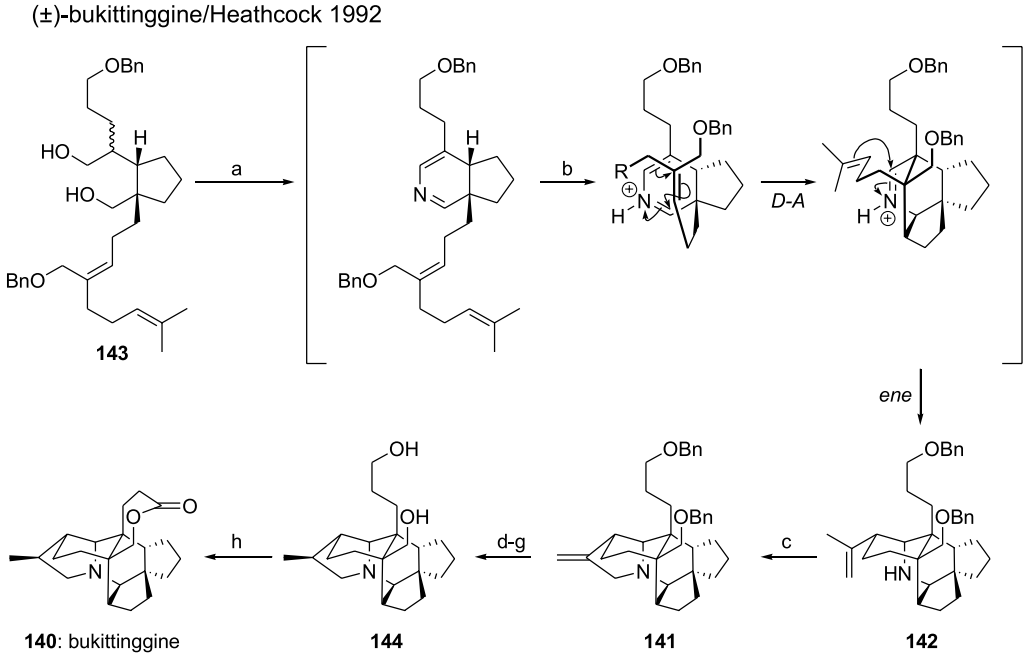
NaHMDS effected a Dieckmann condensation to close the required cyclohexane ring. Because the cyclization product was not stable, it was immediately converted into phenyl sulfide **138** to create unsaturation for the subsequent epoxidation. After reduction of the ketone in **138**, the sulfide was oxidized into two epimeric sulfoxides and eliminated to give the α,β-unsaturated lactone **139**. Attempts to insert the epoxide at this point in the synthesis, only resulted in aromatization of the cyclohexene ring. Thus, the lactone was reduced to diol **134** and the epoxidation took place to give epoxide **133** in 72% yield as a single diastereomer. The excellent selectivity obtained can be attributed to the directing effect of the allylic hydroxyl group. The tetrahydrofuran ring was then reconstructed using the following sequence: the less hindered primary alcohol was protected with a TBS group and the other with a TBDPS group, the TIPS and TBS groups were removed, the benzylic alcohol was oxidized with MnO_2 and the free primary alcohol with TPAP and, finally, the two TBDPS groups were removed to give (±)-arthrinone (**132**).

3. Other propellanes with all-carbon quaternary stereocenters

3.1. Bukittinggine

Bukittinggine (**140**) was isolated in 1990 from the leaves and branches of *Sapium baccatum* near the town of Bukittinggi in West Sumatra, Indonesia.[107] The Heathcock group synthesized this unique heptacyclic alkaloid in 1992.[108] The main transformation in their synthesis, a tetracyclization reaction developed in the group, suggests diol **143** as a starting material (Scheme 24).

The tetracyclization process is effected by a Swern oxidation of diol **143** followed by gaseous ammonia (Scheme 25). Exposure of the product to acetic acid then triggers an inverse-electron-demand Diels–Alder reaction followed by an ene reaction to give the pentacycle **142**. There has been some discussion as to whether the mechanism is stepwise or concerted,[109] but the Heathcock group has reported experimental evidence, which indicates



Scheme 25. (a) $(COCl)_2$, DMSO, CH_2Cl_2, −78 °C, 15 min, then Et_3N, −78→0 °C, 1 h, then NH_3(g), 0 °C→rt, 45 min; (b) NH_4OAc, AcOH, 30 min, rt, then 75 °C, 2 h, 76% from **143**; (c) $(CF_3CO_2)_2Pd$, PPh_3, *p*-benzoquinone, MeCN, 24 h, rt, 70%; (d) $BH_3\cdot THF$, THF, 0 °C→rt, 2 h, then $NaBO_3\cdot 4H_2O$, H_2O, 3 h, rt; (e) *p*-TsCl, DMAP, pyr, $CHCl_3$, 0 °C→rt, 46 h; (f) $LiEt_3BH$, THF, 0 °C→rt, 4 h, then $NaBO_3\cdot 4H_2O$, H_2O, 0 °C→rt, 2.5 h; (g) Na, NH_3, THF, −78 °C, 20 min; (h) Ag_2CO_3 on Celite®, PhH, reflux, 5 h; 52% over five steps.

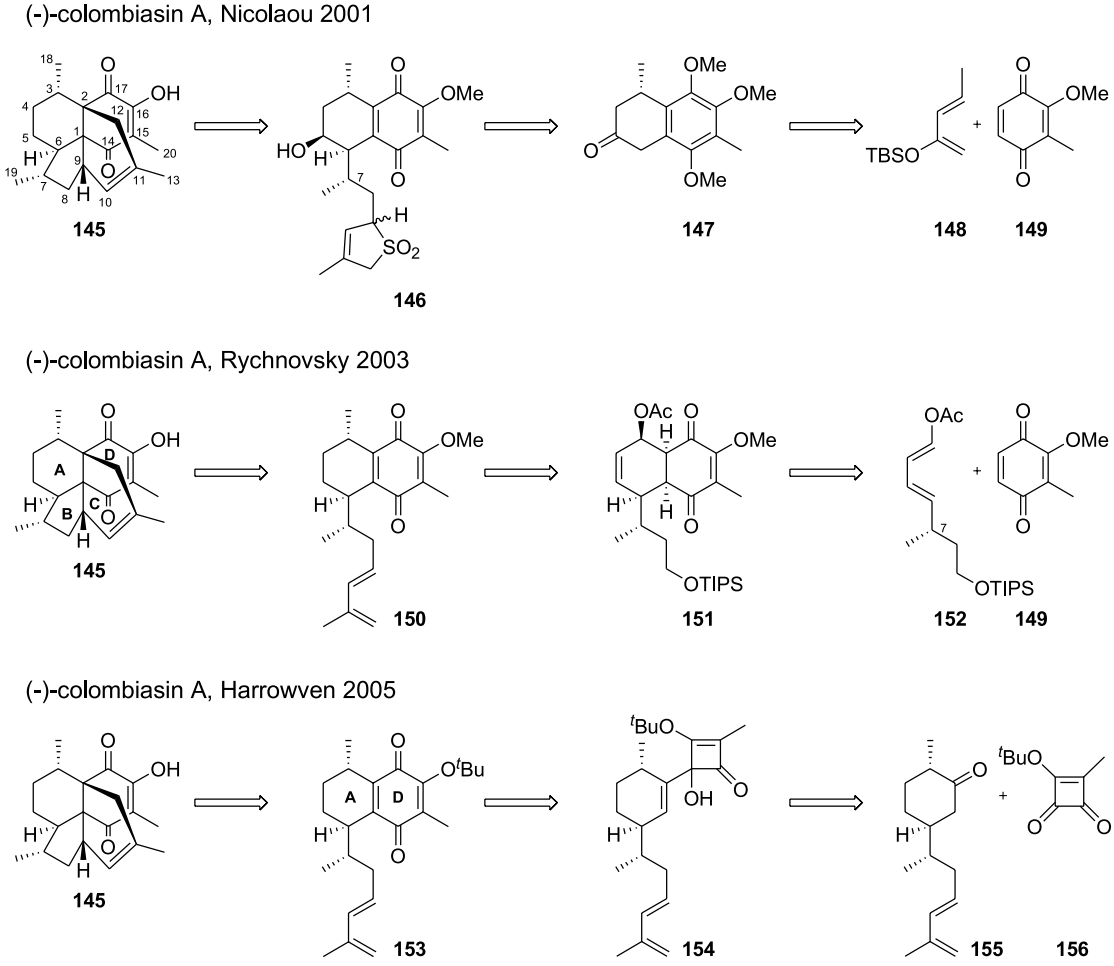
that the cyclization proceeds through a concerted, but asynchronous, Diels–Alder reaction.[110,111] Next, the pentacyclic amine **142** was treated with palladium triflate to cyclize the sixth ring of the molecule. The following sequence reduced the exocyclic double bond in **141** stereoselectively (>15:1): hydroboration and oxidation of the double bond and tosylation of the formed primary alcohol followed by reduction with lithium triethoxyborohydride. Removal of the benzyl protecting groups then gave diol **144**, which was ready for the final cyclization. Finally, oxidation of the diol with Fetizon's reagent provided (±)-bukittinggine (**140**) as the sole product.

3.2. Colombiasin A

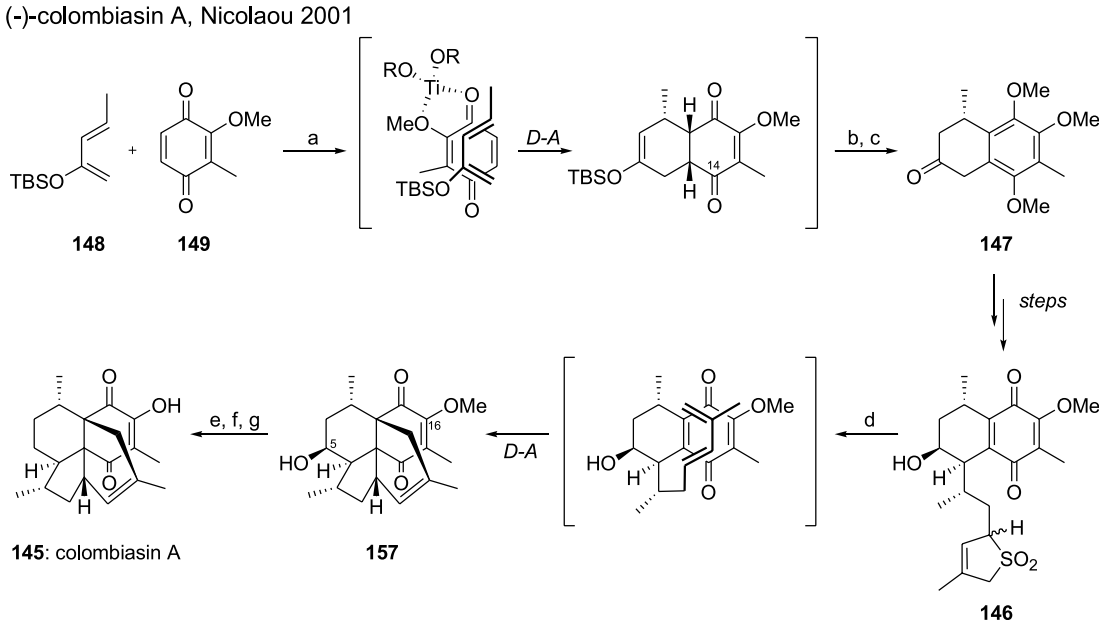
Colombiasin A (**145**), a diterpene isolated from the gorgonian octacoral *Pseudopterogorgia elisabethae*,[112] has attracted the interest of the synthetic community with its novel and challenging molecular framework. To date, three total syntheses, from the Nicolaou,[113,114] Rychnovsky,[115] and Harrowven[116,117] groups, have been published (Scheme 26). In addition, an approach to this complex ring system has appeared in the literature (Scheme 30).[118]

Unsurprisingly, all of the total syntheses rely heavily on the Diels–Alder reaction in the final stages of the synthesis (Scheme 26). The main difference in the first two synthetic routes, by Nicolaou et al. and Rychnovsky et al. is the point at which the side chain, which later forms the B and C rings, is installed. In the Nicolaou group strategy, it is attached after the initial Diels–Alder reaction, whereas the Rychnovsky group decided to include part of the side chain, including the crucial C_7 methyl group, in their first Diels–Alder substrate. The Harrowven group relied on a Moore rearrangement to construct the A and D rings of colombiasin A in their recent synthesis. In this route, the methyl group stems from a hydroboration, where a separable 5:2 mixture of diastereomers is obtained.

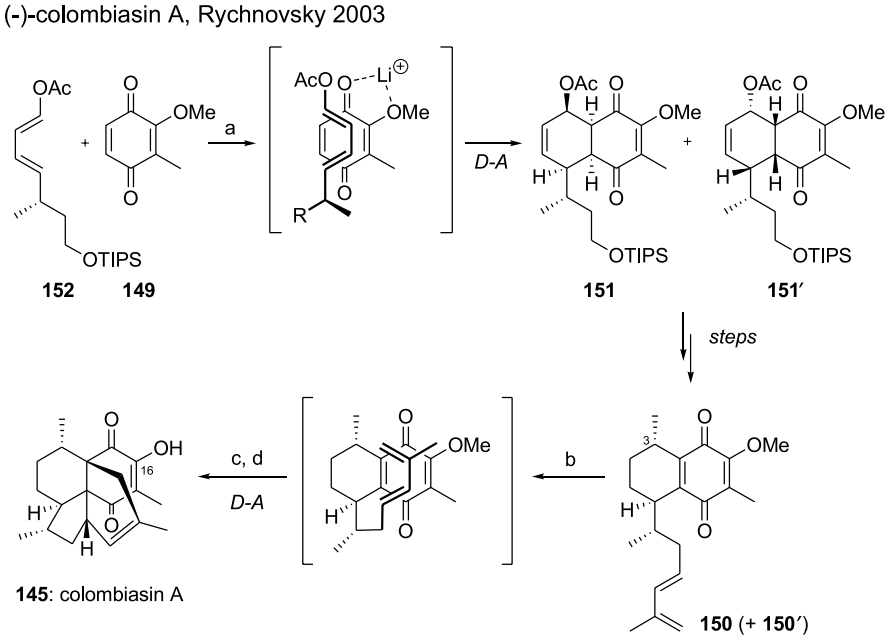
The Nicolaou group synthesis commences with a catalytic asymmetric Diels–Alder reaction (Scheme 27).[113,114] The reaction between diene **148** and quinone **149** in the presence of [(*S*)-BINOL-$TiCl_2$] provided the Diels–Alder adduct **147** after aromatization, methylation and desilylation in 94% ee and 85:15 regioselectivity. The main regioisomer was thought to arise from a bidentate coordination of titanium, as shown in Scheme 27. The minor regioisomer would then originate from monodentate coordination of titanium to the more electron-rich vinylogous ester carbonyl oxygen at C_{14} (colombiasin A



Scheme 26. Retrosynthetic analysis of colombiasin A.



Scheme 27. (a) [(*S*)-BINOL-$TiCl_2$], tol, −60→−10 °C, 7 h, 85:15 regioselectivity, 94% ee; (b) K_2CO_3, MeI, acetone, reflux, 48 h; (c) TFA, CH_2Cl_2, rt, 2 h, 70% over three steps, 94% ee; *steps*; (d) tol (sealed tube), 180 °C, 20 min, 89%, 100% selectivity; (e) NaH, THF/CS_2/MeI 4:1:1, 50 °C, 3 h, 95%; (f) AIBN, Bu_3SnH, tol, 110 °C, 30 min, 77%; (g) BBr_3, *cis*-cyclooctene, CH_2Cl_2, −78 °C, 43% based on 70% conversion.

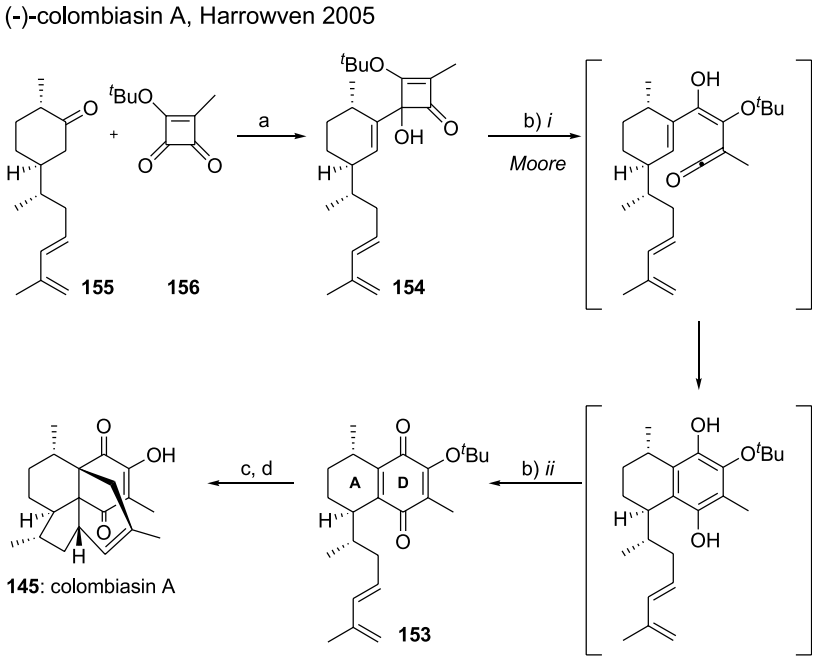


Scheme 28. (a) 5 M $LiClO_4$, Et_2O, rt, 24 h, 75%, **151**:**151′** 1.7:1; *steps*; (b) tol, 180 °C, 83%; (c) $AlCl_3$, $PhNMe_2$, CH_2Cl_2, 0 °C→rt, 73%; (d) separation of diastereomers.

numbering).[†] After further elaboration and attachment of the side chain, the sulfone-protected diene **146** was ready for the critical IMDA reaction. The reaction was performed at 180 °C in toluene in a sealed tube and provided tetracycle **157** in 89% yield as a single product. Deoxygenation at C_5 and demethylation at C_{16} then gave (−)-colombiasin A (**145**).

The Rychnovski group used a Lewis acid-catalyzed Diels–Alder reaction between diene **152** and quinone **149** to start the synthesis of the colombiasin A ring system (Scheme 28).[115] The reaction gave a 1.7:1 mixture of **151** and **151′**, that could only be separated in the last step of the synthesis, despite attempts to do so earlier. The use of chiral Lewis acids to catalyze the reaction was not successful. After

[†] Note that there is an error in the original paper[114] in Scheme 9; the transition states $\mathbf{TS_a}$ and $\mathbf{TS_b}$ provide the enantiomers of compounds **8** and **8′**. (Personal communication with Prof. G. Vassilikogiannakis.).



Scheme 29. (a) Trisylhydrazine, **155**, THF, rt, 2 h, then *n*-BuLi, −78 °C, then **156**, −20 °C, 36%; (b) i. MW, THF, 110 °C, then ii. air, rt, 80%; (c) tol, 150 °C, 61%; (d) $BF_3 \cdot OEt_2$, 0 °C, 78%.

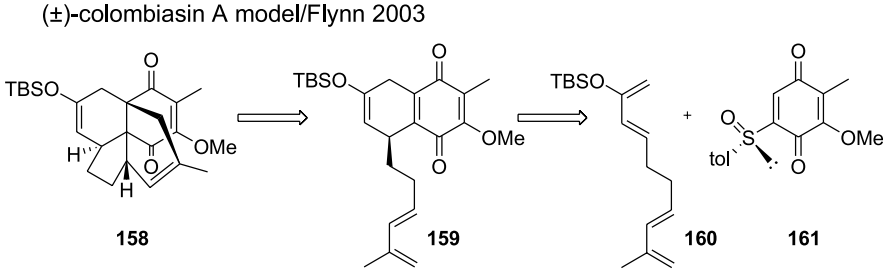
installing the C_3-methyl and the diene required for the IMDA, compound **150** was ready for cyclization. The final cyclization of diene **150** (and **150′**) was performed in a similar fashion to that used in the Nicolaou synthesis, followed by deprotection of the C_{16}-methoxy group and separation of the diastereomers, providing (−)-colombiasin A (**145**).

In the Harrowven group's synthesis of colombiasin A (Scheme 29),[116] the crucial intermediate **154** for the Moore reaction[119–121] was obtained from a Shapiro coupling between ketone **155** and diketone **156**. After some experimentation, it was found that an in situ formation of a trisylhydrazone from ketone **155**, which could then be treated with *n*-butyllithium and diketone **156**, was the best way to obtain the Moore substrate **154**. Heating **154** to 110 °C in a microwave effected the formation of the Diels–Alder substrate **153**. As the penultimate step, an intramolecular Diels–Alder reaction provided the tetracycle in a similar fashion to the Nicolaou (Scheme 27) and Rychnovski (Scheme 28) syntheses. Finally, trifluoroborane-etherate deprotected the alcohol to give (−)-colombiasin A (**145**).

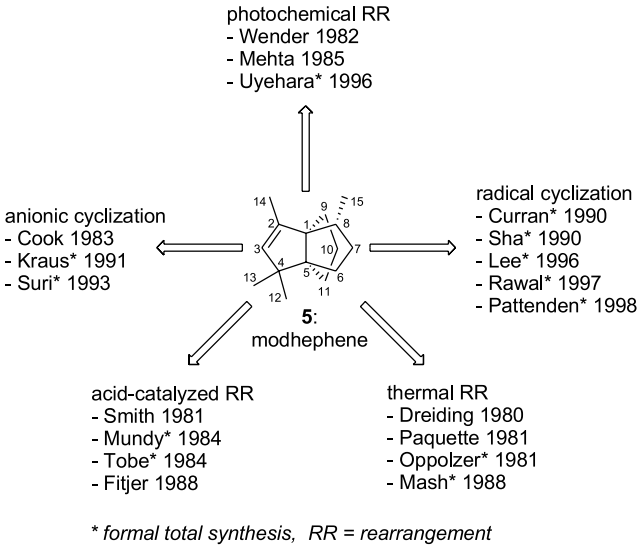
The research group of Flynn[118] has also published their efforts towards colombiasin A (Scheme 30). They used an enantioselective double Diels–Alder approach to construct the tetracyclic framework of colombiasin A. Their strategy is similar to Rychnovsky's (Scheme 28), but differs in execution of the actual route. As a source of chirality, they use sulfoxide **161**, which, after the first enantioselective Diels–Alder reaction, eliminates to give the substrate for the second intramolecular Diels–Alder reaction.

3.3. Modhephene

Modhephene (**5**) was the first isolated natural product shown to possess the [3.3.3]propellane skeleton. It was isolated for the first time in 1978 from *Isocoma wrightii* by Zalkov et al.[122,123] and has inspired a total of 19 syntheses, of which 13 are formal. The different approaches can be classified into five different key reaction types, namely acid-catalyzed rearrangements, thermal rearrangements, photochemical rearrangements, anionic cyclizations and radical cyclizations (Scheme 31). Because of the number of syntheses published, only the key ring-forming steps of each synthesis will be shown. The syntheses of modhephene until 1996 have appeared as a subsection in a review on polyquinane natural products.[124] There have also been two accounts of approaches towards modhephene.[125,126]



Scheme 30. Retrosynthetic analysis of a colombiasin A model study.

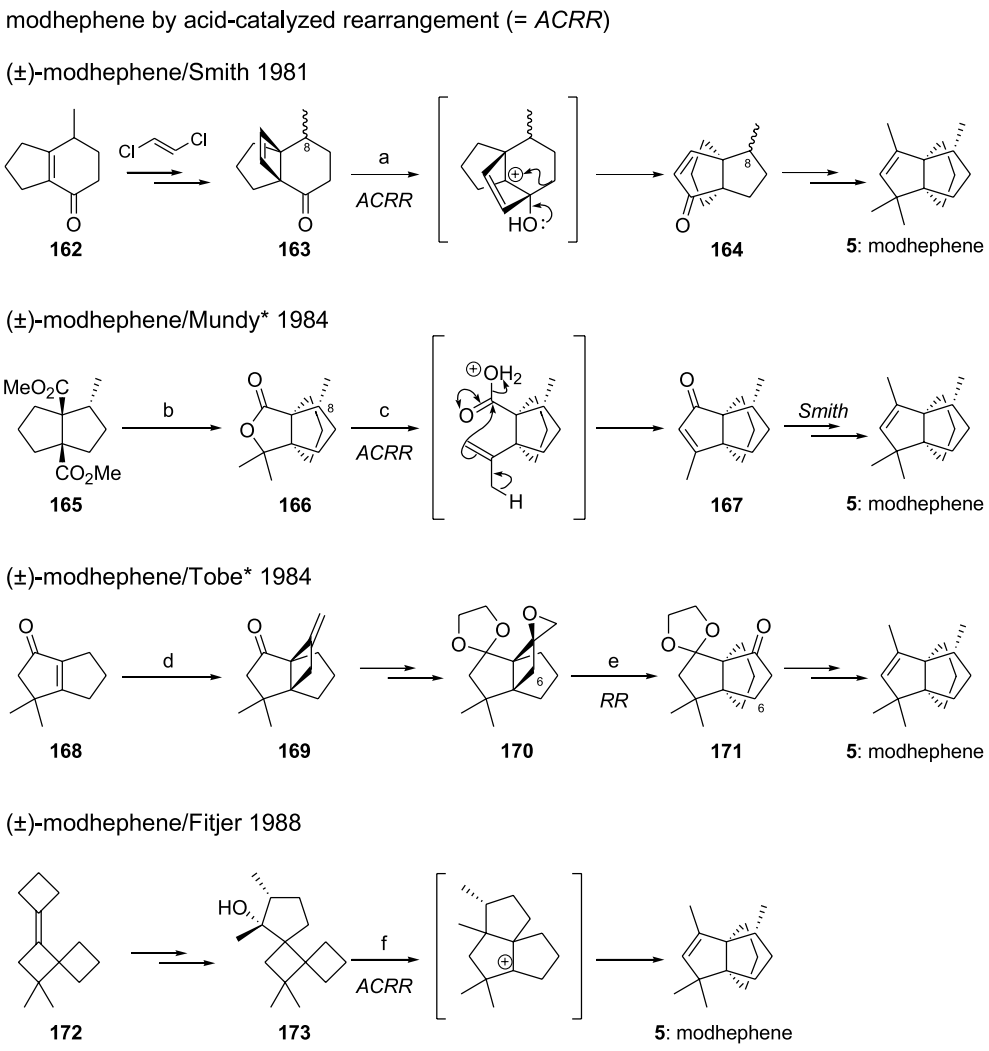


Scheme 31. Classification of different approaches towards modhephene by key reaction.

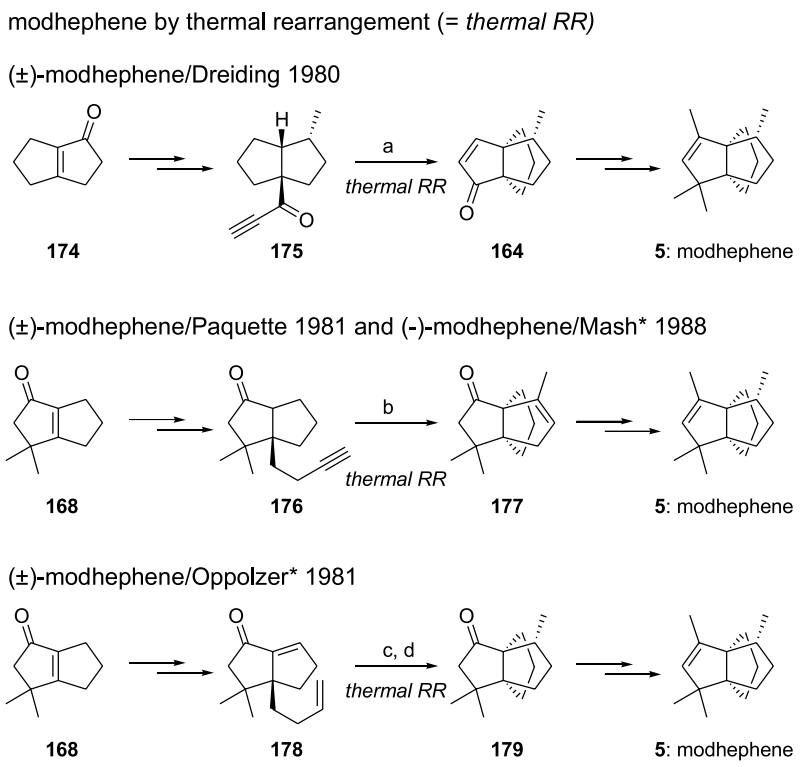
The stereoselective installation of the C_8-methyl group has been one of the greatest challenges in the synthesis of modhephene, further complicated by difficulties in the separation of diastereomers.

3.3.1. Acid-catalyzed rearrangement. Smith and Jerris published one of the first total syntheses of modhephene (**5**) (Scheme 32) in 1981 (others followed shortly; three different total syntheses were published in 1981 and another in 1980).[127,128] They utilized Cargill's work on acid-catalyzed rearrangements of β,γ-unsaturated ketones[129] to perform the key ring-forming step, namely **163**→**164**. Ketone **163** was synthesized from enone **162** via a [2+2] photochemical cyclization, which, unfortunately, gave only a ca. 1.3:1 ratio of the C_8-methyl group with the major diastereomer being the desired isomer.

In 1984, Wilkening and Mundy published a formal total synthesis of modhephene (**5**)[130] relying on a phosphorus pentoxide–methanesulfonic acid-catalyzed rearrangement (**166**→**167**).[131] Catalytic hydrogenation was used to obtain



Scheme 32. (a) *p*-TsOH, PhH, reflux, 4 h, 64%, α-Me:β-Me 57:43; (b) MeLi, THF, rt, overnight, 80%; (c) MsOH, P_2O_5, 85 °C, 44 h, 22%; (d) allene, CH_2Cl_2, −78 °C, 94%, 4.5:1 selectivity; (e) LiBr, HMPA, PhH, 60 °C, 86%, 10:1 selectivity. (f) *p*-TsOH, PhH, 70 °C, 20 min, 34%.



Scheme 33. (a) 620 °C, 14 Torr, 1 h, 95%, 2:1:1 mixture of products; (b) decalin, 360 °C, 4 h, 85%; (c) tol, 250 °C, 16 h, 76%; (d) H_2, Pd/C, EtOH, rt, 12 h, 98%.

the correct stereochemistry at C_8 selectively. They also published a total synthesis of modhephene,[132] but, later, the Curran group (see Scheme 36) found that there was a discrepancy with the assignment of a common intermediate (see Ref. 47 in the paper).[133] Since Wilkening and Mundy only had mass spectral data of modhephene, this casts doubt over the identity of the products in the total synthesis.‡

The key step in the formal synthesis by the Tobe group[135] is not an acid-catalyzed rearrangement, but a very efficient lithium bromide–HMPA-catalyzed[136] epoxide–carbonyl rearrangement[137–140] of epoxide **170**. Since the rearrangement is chelation controlled, the less substituted carbon (C_6) migrates with good selectivity. The precursor to epoxide **170**, olefin **169**, is made from the readily available enone **168**. It is interesting to note the similarity of this strategy to the Tobe group synthesis of marasmic acid (**3**) in Scheme 4.

Fitjer et al. have published a short and original synthesis of modhephene via an acid-catalyzed cascade rearrangement of alcohol **173**.[141–143] The group has also studied the absolute configuration and optical rotation of modhephene by resolving an intermediate and preparing both (−)- and (+)-modhephene separately.[144]

‡ The Curran group also reports[133] (see Ref. 70 in the paper) that there has been a misassignment of spectra of the two diastereomers of ketone **164** (Scheme 32) in the Smith paper.[128] Wilkening and Mundy used a sample provided by Smith to prove the identity of **164** and thus the product of their catalytic hydrogenation. Kraus and Shi[134] claim that the misassignment derives from the mix-up in Smith's data, but, in fact, the Mundy assignment was based on comparison with an authentic sample of **164** from Professor Smith. It remains unclear whether Professor Smith had sent the wrong diastereomer of **164** to Wilkening and Mundy or if there is a genuine error with the chemistry.

3.3.2. Thermal rearrangement. Thermal rearrangement has been the strategy of choice for four different groups who have synthesized modhephene (Scheme 33). Karpf and Dreiding utilized an α-alkynone cyclization of alkyne **175** to construct the [3.3.3]propellane ring framework.[145,146] While the reaction gives a good yield, it also gives two other products in a 2:1:1 ratio that can be separated later. They arrive at the same intermediate as Smith and Jerris (**164**, see Scheme 32), but, since the publications were received within 8 days by the editorial staff of the journals, the syntheses can be considered independent.

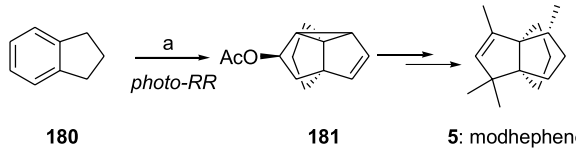
Schostarez and Paquette used an intramolecular ene reaction of acetylene **176** to form the modhephene ring system in good yield in their short synthesis of modhephene.[147,148] Seven years later, Mash et al. published the first total synthesis of (−)-modhephene,[149–151] where a chiral auxiliary-based strategy was used to synthesize enantiomerically enriched acetylene **176** (78% ee). From intermediate **176** onwards, they follow the footsteps of Schostarez and Paquette.

The Oppolzer group has published two different routes to **177** (see Scheme 32), a common intermediate to the Dreiding and Smith syntheses.[152,153] Of these, the latter is significantly shorter. The key transformation in their synthesis was the ene reaction of **178**, which produced propellane **179** selectively.

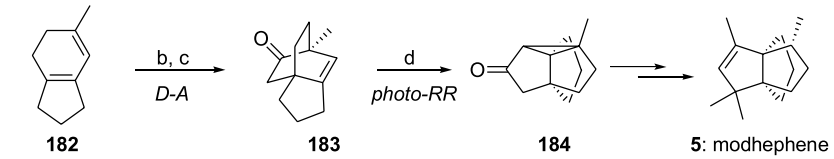
3.3.3. Photochemical rearrangement. A photochemical rearrangement has been the basis of three different syntheses of modhephene (Scheme 34), in the first of which, by Wender and Dreyer, indan (**180**) and vinyl acetate were

modhephene by photochemical rearrangement (= *photo-RR*)

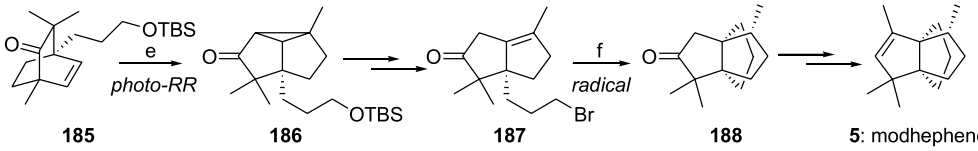
(±)-modhephene/Wender 1982



(±)-modhephene/Mehta 1985



(±)-modhephene/Uyehara* 1996



Scheme 34. (a) hν, Vycor, vinyl acetate, cyclohexane, 35 h, 21%; (b) 1-chloroacrylonitrile, tol, 80 °C, 16 h, 43%, 4:1 regioselectivity; (c) $Na_2S\cdot 9H_2O$, EtOH, 60 °C, 6 h, 58%; (d) hν, acetone, 45 min, 47%, 15:1 selectivity; (e) hν, acetone, rt, 1.5 h, 91%; (f) Bu_3SnH, AIBN, PhH, reflux, 2 h, 42%.

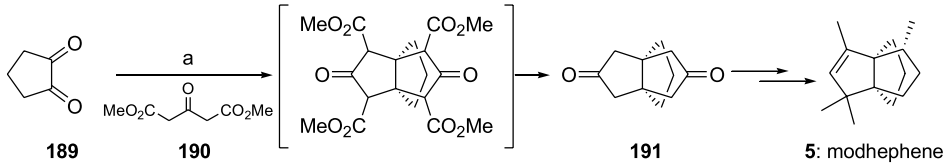
irradiated with Vycor-filtered light to produce a complex mixture from which acetate **181** was isolated in 21% yield.[154] This arene–olefin meta cycloaddition established the [3.3.3]propellane structure and the remaining steps were used to incorporate the required methyl groups into the molecule.

In their synthesis of modhephene,[155–157] Mehta and Subrahmanyam relied on a Diels–Alder reaction (**182**→**183**) followed by an oxa-di-π-methane rearrangement (**183**→**184**).

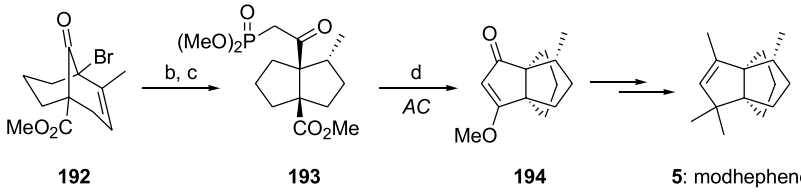
The Uyehara group also chose an oxa-di-π-methane

modhephene by anionic cyclization (= *AC*)

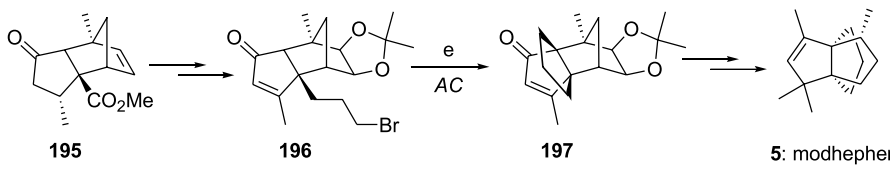
(±)-modhephene/Cook 1983



(±)-modhephene/Kraus* 1991



(±)-modhephene/Suri* 1993



Scheme 35. (a) **190**, pH 5 aqueous buffer, rt, several days, then H_3O^+, heat; (b) $(MeO)_2P(O)Me$, *n*-BuLi, −78 °C, 15 min, then **192**, −78 °C→rt, 1 h, 49%; (c) $[Ir(COD)(PCy_3)(py)]PF_6$, H_2, CH_2Cl_2, rt, 15 h; (d) KH, 18-crown-6, PhH, reflux, 6 h, 33% over two steps; (e) LDA, HMPA, THF, −70 °C, 79%.

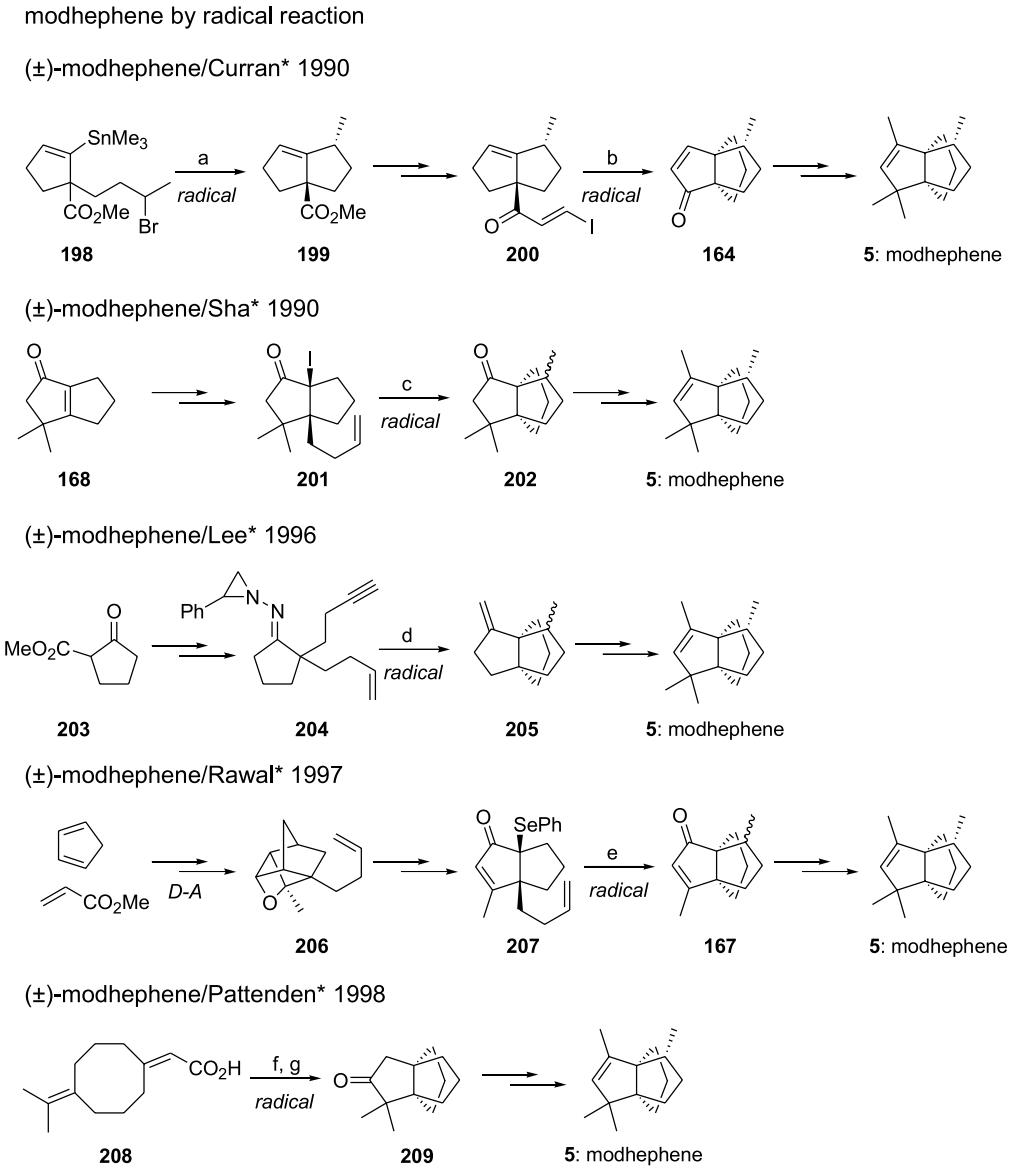
rearrangement to construct the ring system of modhephene.[158,159] Irradiation of bicycle **185** for 1.5 h provided the tricyclic ketone **186** in 91% yield. To form the third five-membered ring, ketone **186** was elaborated to bromide **187**, which cyclized to give propellane **188** in the presence of tributyltin hydride and AIBN.

3.3.4. Anionic cyclization. An anionic cyclization has been employed by three research groups during their synthesis of modhephene (Scheme 35). Cook and co-workers[160] decided to use the Weiss reaction,[161,162] that is, the reaction between dimethyl 3-ketoglutarate (**190**) and diketone **189**, to construct the [3.3.3]propellane system.

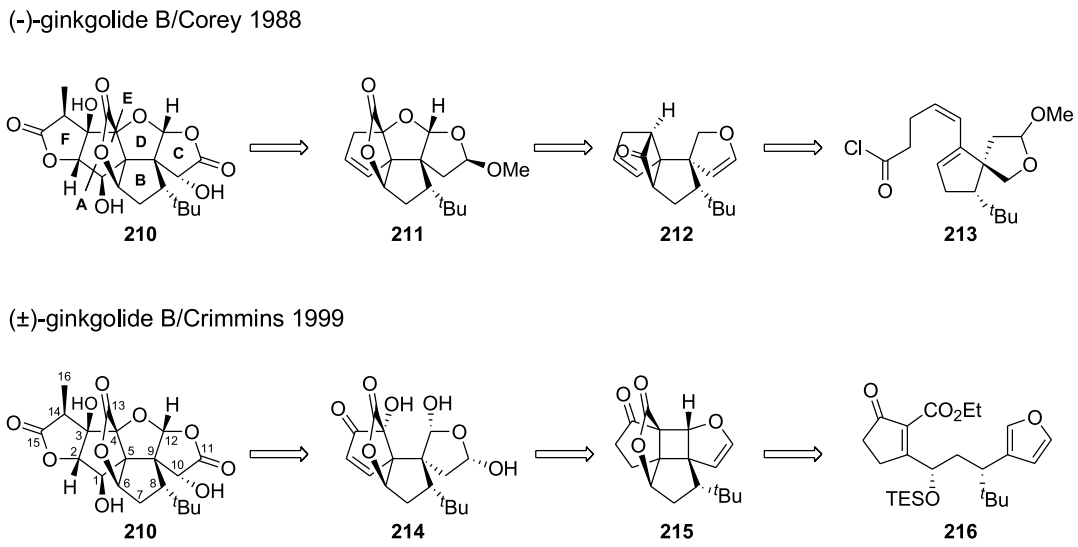
Kraus and Shi have published a formal total synthesis of modhephene utilizing a rearrangement of bridgehead bromide **192** with the anion of dimethyl methyl phosphonate followed by a potassium hydride-mediated cyclization of the resulting phosphonate **193**.[134,163]

Suri's original, but lengthy, approach towards modhephene involved an intramolecular enolate alkylation of bromide **196** to provide the [3.3.3]propellane ring system.[164]

3.3.5. Radical reaction. Radical cyclizations have been the most popular method to make the [3.3.3]propellane ring system in modhephene and have been the key reaction in six different total syntheses. In addition to the syntheses shown in Scheme 36, the Uyehara group has also utilized radical cyclization to build the third unsubstituted ring of modhephene (see Scheme 34, **187**→**188**). The Curran group has published two different radical-based syntheses of modhephene. The first was a formal total synthesis with sequential radical cyclizations[133] and the second used a tandem transannular radical cyclization.[165] Because their first route was significantly shorter and more effective (11 steps and 16% overall yield vs 21 steps and 6% overall yield), it will be discussed here. Their strategy hinged on closing two of the three five-membered rings with a radical



Scheme 36. (a) Bu_3SnH, AIBN, PhH, reflux, 10 h, 90%; (b) Bu_3SnH, AIBN, DPPE, PhH, reflux, 7 h, 88%; (c) Bu_3SnH, AIBN, PhH, 85%, α:β 4:1; (d) Bu_3SnH, AIBN, PhH, 8 h, then SiO_2, 74%, α:β>9:1; (e) Bu_3SnH, AIBN, PhH, −78 °C, α:β 6:1; (f) 2-(*o*-IC_6H_4)CH_2CH_2SH, DCC, DMAP, CH_2Cl_2, 85%; (g) Bu_3SnH, AIBN, PhH, reflux, 59%.



Scheme 37. Retrosynthetic analysis of ginkgolide B.

reaction. Vinylstannane **198** cyclized smoothly to give the *trans* ester **199**, which was further elaborated to give the vinyl iodide **200**, which, in turn, provided the enone **164** in 88% yield.

In their formal total synthesis of modhephene, Sha et al. also used an intramolecular radical cyclization.[166] Their substrate, iodo-olefin **201**, afforded a 4:1 selectivity of the desired α-C_8 diastereomer when exposed to the standard tributyltin hydride/AIBN conditions.

Lee et al. have published a short and successful tandem radical cyclization approach towards modhephene.[167] Cyclization of *N*-aziridinyl imine **204** provided >90% selectivity for the desired exocyclic olefin **205**. Recently, they have published another tandem radical cyclization route to modhephene.[168]

Dvorak and Rawal synthesized their intramolecular radical cyclization substrate in an interesting manner.[169] They used a Diels–Alder reaction followed by a Paterno-Büchi reaction to access oxetane **206**. The hidden diquinane unit present in **206** was revealed by a direct fragmentation of **206** followed by oxidation of the remaining alcohol and selenation to give enone **207**. Then, exposure of **207** to tributyltin hydride and AIBN provided propellane **167** in a 6:1 selectivity as the major product.

The latest synthesis of modhephene was published by De Boeck and Pattenden.[170,171] Their approach called for the construction of an eight-membered ring that was then cyclized by virtue of an α-ketenyl radical intermediate into propellane **209**.

4. Propellanes with lactones

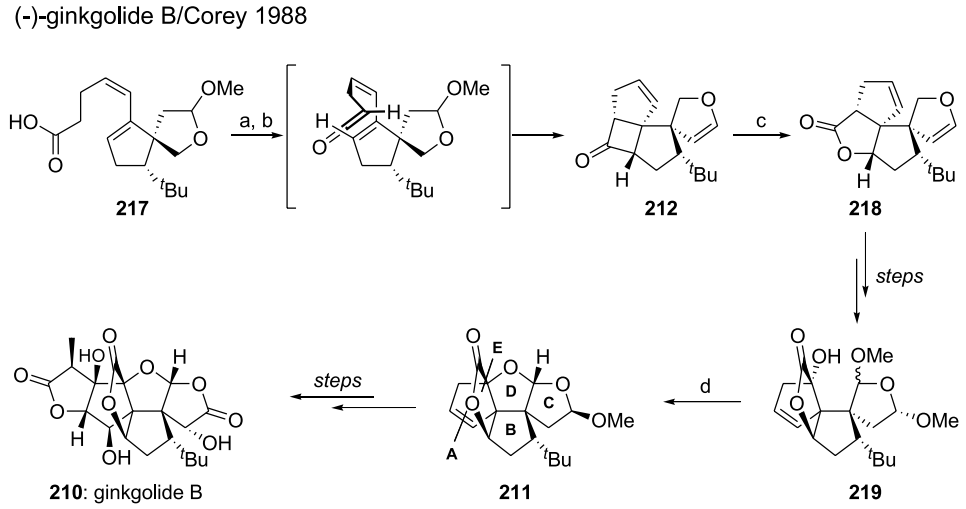
4.1. Ginkgolide B

Ginkgolide B (**210**) is a complex polyoxygenated and polycyclic natural product isolated from the extracts of *Ginkgo biloba*.[172–174] It is the most active platelet-activating factor (PAF) antagonist isolated from ginkgo extracts. This synthetically challenging molecule has been the target of two different total syntheses, by the groups of Corey[175,176] and Crimmins (Scheme 37).[177–179] The key ring-forming transformation in the Corey group synthesis is an internal ketene–olefin cycloaddition, whereas the Crimmins group relied on a [2+2] photocycloaddition.

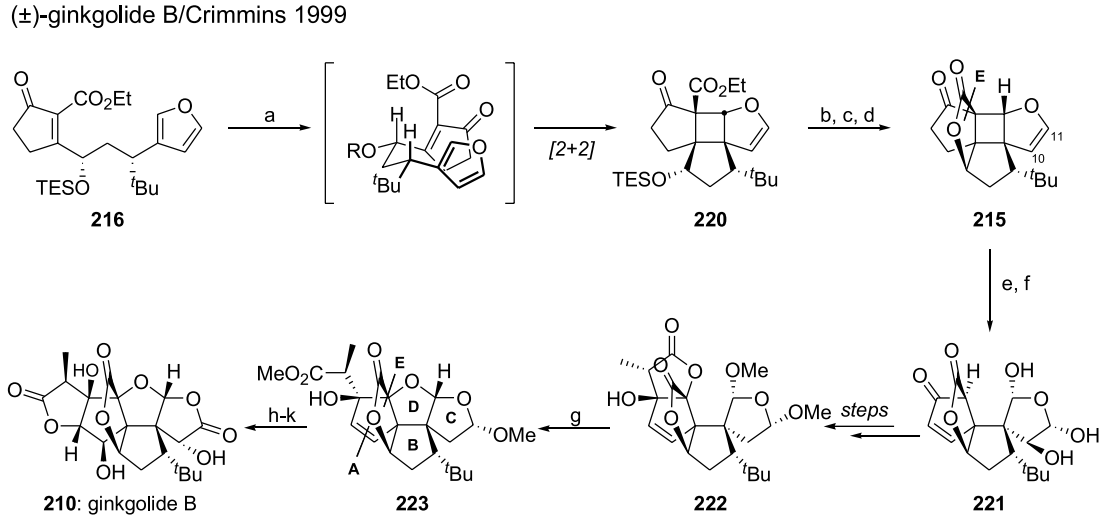
The first ring-forming step in the Corey group synthesis[§] of ginkgolide B[175,176] was an internal ketene–olefin cycloaddition (Scheme 38).[181,182] Treatment of acid **217** with oxalyl chloride gave the corresponding acid chloride (**213**, see Scheme 37), which was then eliminated with tributylamine to form the ketene. The ketene immediately underwent cycloaddition followed by elimination of the anomeric methoxy group to give the tetracyclic ketone **212**. Baeyer-Villiger oxidation by triphenylmethyl hydroperoxide then produced lactone **218**, which was further elaborated into the more highly oxidized lactone **219**. Treatment of **219** with acid then provided the ABCDE ring fragment of ginkgolide B (**211**), that also contains a [3.3.3]propellane structure. Ten further steps afforded (−)-ginkgolide B (**210**).

The first key ring-forming transformation in the Crimmins group's synthesis of ginkgolide B (Scheme 39)[177–179] was a [2+2] photocycloaddition of enoate **216**, which proceeded with remarkable efficiency and stereoselectivity to give a single cycloadduct **220** in quantitative yield. The E ring of ginkgolide B was then closed via a sequence of silyl deprotection, mesylation and acid-catalyzed cyclization. Next, the cyclobutane ring was opened with a retroaldol fragmentation via a one-pot selenylation–elimination sequence followed by epoxidation of the C_{10}–C_{11} double bond to furnish the dialdehyde hydrate **221**. Several steps later, the dilactone **222** was ready for the closure of ring D. Treatment of dilactone **222** with camphorsulfonic acid gave the ABCDE ring fragment **223**. Four more steps were required to deliver (±)-ginkgolide B (**210**).

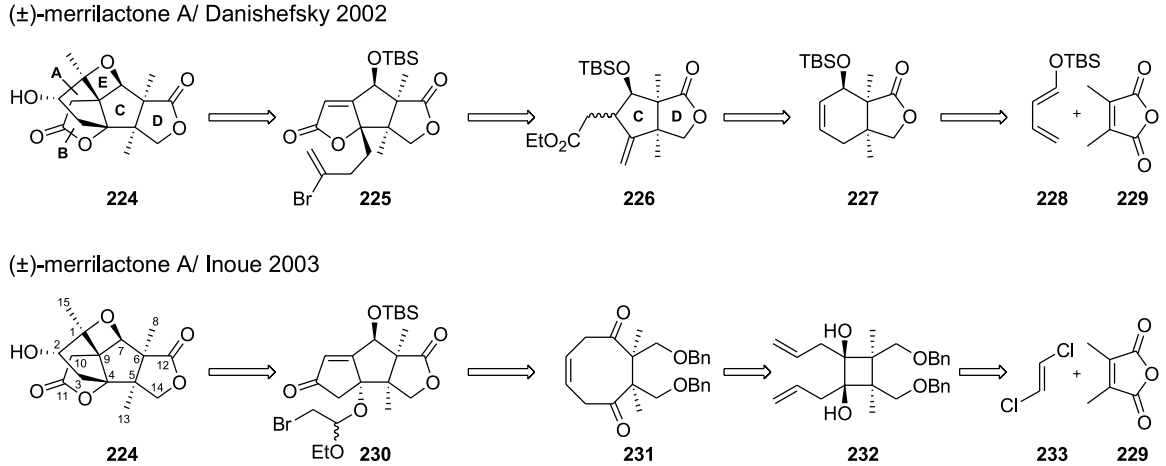
[§] The Corey group syntheses of ginkgolide A, B and bilobalide have been thoroughly analyzed in Professor Corey's Robert Robinson lecture.[180]



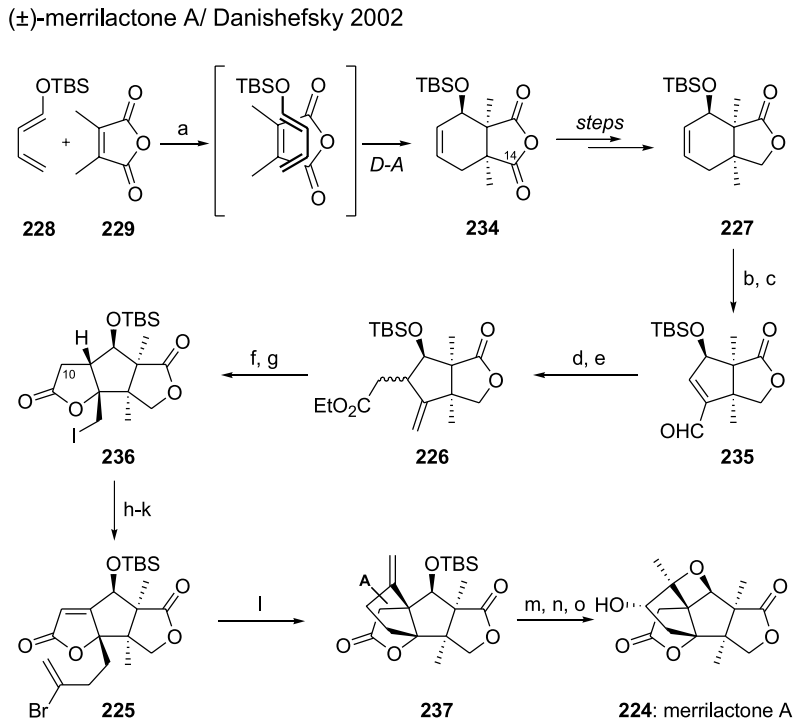
Scheme 38. (a) $(COCl)_2$, PhH, rt, 2 h, then (b) *n*-Bu_3N, tol, reflux, 3 h, 71–89% over two steps; (c) Ph_3COOH, 8:1 acetone:1 N NaOH, −30 °C, 2 h, 86%; *steps*; (d) CSA, CH_2Cl_2, rt, 24 h (75% over two steps).



Scheme 39. (a) hν, >350 nm, hexanes, rt, 17 h, quant.; (b) 5% HF, MeCN, 0 °C→rt, 1 h; (c) MsCl, Et_3N, CH_2Cl_2, 0 °C, 45 min; (d) 4 Å MS, EtOH, reflux, 26 h, then H_2O, reflux, 8 h, then PPTS, PhH, reflux, 16 h, 63% from **220**; (e) PhSeCl, HCl, EtOAc, rt, 1 h, then $NaIO_4$, H_2O, THF, 2 h, rt; 78%; (f) DMDO, acetone, H_2O, 8 h, rt, then *p*-TsOH, rt, 15 h, 94%; *steps*; (g) CSA, MeOH, reflux, 18 h, 88%; (h) PPTS, pyr, PhCl, reflux, 4 h, 85%; (i) $VO(acac)_2$, TBHP, 4 Å MS, CH_2Cl_2, rt, 4 days, then *p*-TsOH, rt, 2 h, 81%; (j) DMDO, acetone, H_2O, rt, 20 h; (k) Br_2, NaOAc, H_2O, AcOH, rt, 20 h, 52% over two steps.



Scheme 40. Retrosynthetic analysis of merrilactone A.



Scheme 41. (a) Symm-collidine, Methylene Blue, mesitylene, 165 °C, 2.5 days, 74%; *steps*; (b) O_3, CH_2Cl_2, MeOH, −78 °C, then PPh_3, →rt; (c) $Bn_2NH\cdot TFA$, PhH, 63 °C, 9 h, 94% over two steps; (d) $NaBH_4$, CH_2Cl_2, MeOH, −78 °C→rt, quant.; (e) $MeC(OEt)_3$, PivOH, mesitylene, 135 °C, 24 h, 92%, α:β 1:1.8; (f) LiOH, MeOH, H_2O, rt 12 h; (g) I_2, $NaCHO_3$, THF, rt, 12 h, then separation, 59% over two steps; (h) allyl$SnBu_3$, AIBN, PhH, 85 °C, 4.5 h, 75%; (i) LHMDS, TMSCl, THF, −78 °C, 30 min, then PhSeCl, →rt, 1.5 h; (j) PhSeBr, MeCN, rt, 30 min; (k) O_3, CH_2Cl_2, −78 °C, then 1-hexene, then NEt_3, PhH, reflux, 30 min, 77% over three steps; (l) Bu_3SnH, AIBN, PhH, 85 °C, 1.5 h, 90%; (m) $TsOH\cdot H_2O$, PhH, reflux, 3 h, 90%; (n) *m*-CPBA, CH_2Cl_2, rt, 2 days, α:β 3.5:1; (o)[188] $TsOH\cdot H_2O$, CH_2Cl_2, rt, 1 day, 71% over two steps.

4.2. Merrilactone A

The pentacyclic sesquiterpene dilactone merrilactone A (**224**), isolated from *Illicim merrilianum* in 2000,[183] has already been the target of two total syntheses, by the Danishefsky group in 2002,[¶,184] and by the Inoue group a year later (Scheme 40).[186] The Danishefsky group had envisaged a radical cyclization to form the A ring and an allyl-lactonization to form the B ring. The CD ring fragment **226** could be formed with a ring cleavage–reclosure sequence of the Diels–Alder adduct **227**. The Inoue group strategy called for desymmetrization of *meso*-diketone **231** through an intramolecular aldol reaction. Mehta and Singh recently published their approach to the ABCD ring system of merrilactone A.[187]

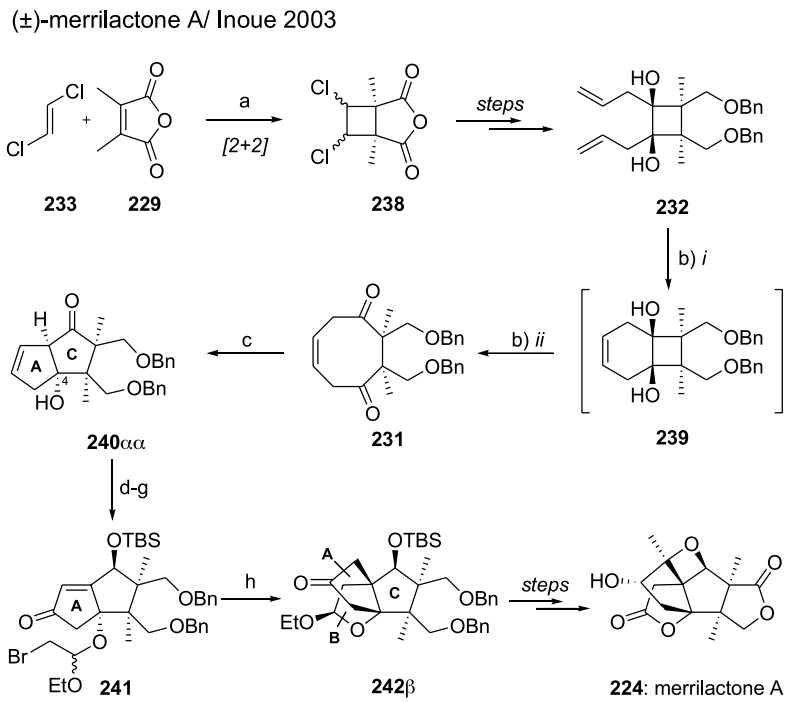
The merrilactone A synthesis by Birman and Danishefsky commences with a Diels–Alder reaction of diene **228** and dimethylmaleic anhydride (**229**) (Scheme 41).[184] After reducing the C_{14}-carbonyl (merrilactone A numbering) regioselectively, ozonolysis effected the opening of the six-membered ring. This was followed by an aldol condensation to close the five-membered ring to give enal **235**. Reduction of the aldehyde paved the way for the Johnson ortho ester variant of the Claisen rearrangement, which provided a 1:1.8 mixture of esters (**226**), which was subsequently hydrolyzed. Iodolactonization and chromatographic separation then gave the pure minor iodide **236**, which was allylated to prepare for the A ring cyclization. Selenylation at C_{10}, bromoselenylation of the terminal vinyl group and oxidative deselenylation afforded the cyclization precursor **225**. Exposure of the vinyl bromide **225** to tributyltin hydride and AIBN then effected the formation of the [3.3.3]propellane **237**. Isomerization of the exocyclic double bond, epoxidation and acid-catalyzed homo-Payne rearrangement according to the procedure of Fukuyama and co-workers[188] produced (±)-merrilactone A (**224**) in 71% yield over two steps.

The first step in the Inoue group's synthesis towards merrilactone A (Scheme 42) was a [2+2] photocycloaddition between 1,2-dichloroethene (**233**) and dimethylmaleic anhydride (**229**).[186] The side chains of the symmetrical diol **232** were installed so that a ring-closing metathesis could be performed. This was followed by opening of the four-membered ring to give diketone **231**. Exposure of **231** to LHMDS provided the AC ring fragment **240**αα together with the A ring diastereomer **240**ββ in a 3.1:1 ratio, respectively. A two-carbon side chain was attached to the C_4-hydroxyl of ring A (merrilactone A numbering) to allow the B-ring cyclization to be performed. With the enoate **241** in hand, cyclization with tributyltin hydride and triethylborane gave the ABC ring fragment **242** of merrilactone A. Another 13 steps were required to finish the synthesis of (±)-merrilactone A (**224**).

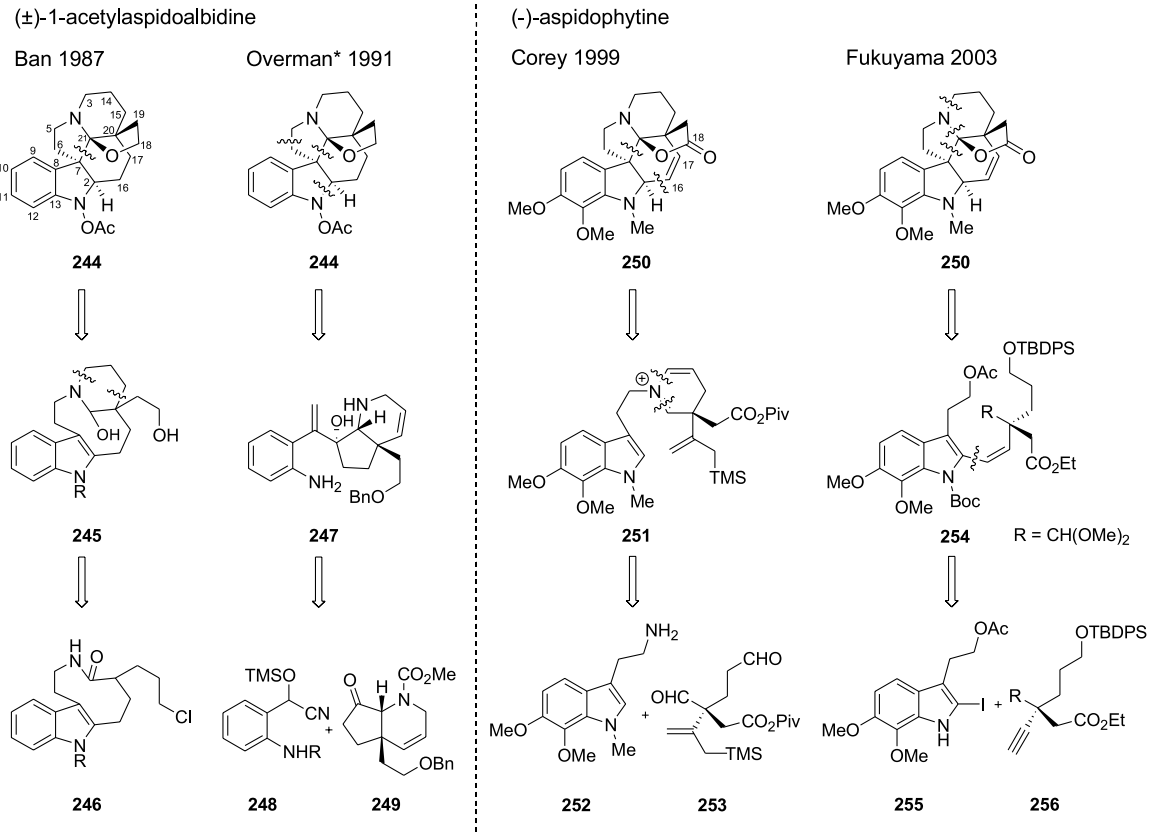
5. Indole alkaloids

Indole alkaloids are a large group of nitrogen-containing natural products. The scope of this review covers the

¶ The Danishefsky group has recently published an enantioselective approach to intermediate **236** (Scheme 41).[185]

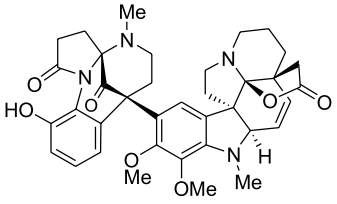


Scheme 42. (a) hν, Benzophenone, acetone, rt, 3 h; *steps*; (b) i. $(PCy_3)_2Cl_2Ru{=}CHPh$, CH_2Cl_2, reflux, 14 h, then ii. $Pb(OAc)_4$, rt, 95%; (c) LHMDS, THF, −78 °C, 1 h, 64% **240**αα, 22% **240**ββ; (d) *m*-CPBA, CH_2Cl_2, rt, 4 h, 81%; (e) DBU, CH_2Cl_2, −30 °C, 1 h, 81%; (f) IBX, DMSO, rt, 30 min, 94%; (g) Br_2, ethyl vinyl ether, CH_2Cl_2, −78 °C, 15 min, then sm, *N*,*N*-dimethylaniline, →rt, 1 day, 62%, 4:1 selectivity; (h) Bu_3SnH, BEt_3/O_2, tol, rt, 30 min, 57% **242**β, 16% **242**α.



Scheme 43. Retrosynthetic analyses of 1-acetoxyaspidoalbidine and aspidophytine.

syntheses of four of them, namely 1-acetylaspidoalbidine, aspidophytine, kopsanone and lapidilectine B, because these natural products can also be classified as propellanes. They have been further divided into two groups on the basis of the similarities in their structures (Schemes 43 and 48).



243: haplophytine

5.1. 1-Acetylaspidoalbidine and aspidophytine

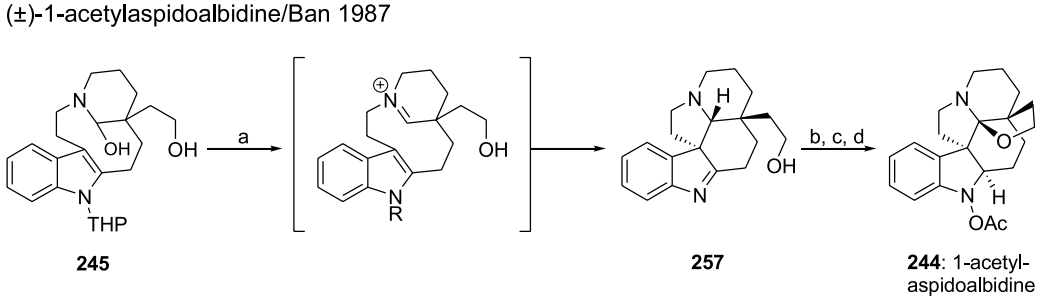
1-Acetylaspidoalbidine (**244**) was isolated in 1963 from *Vallesia dichotoma* Ruiz Et Pav[189] and the structure was proposed originally by Walser and Djerassi in 1964.[190] Several syntheses of the molecule have been reported in the literature, by the groups of Ban[191–193] and Overman (Scheme 43).[194] The Ban group has considerable experience in this area and confirmed the structure of 1-acetylaspidoalbidine by total synthesis in 1975.[193] The route described here is the latest of their total syntheses of this compound. The Overman group's synthesis is based on a tandem aza-Cope-Mannich process with the disconnections shown in Scheme 43.

The structure of aspidophytine (**250**) differs from 1-acetylaspidoalbidine (**244**) only in the degree of unsaturation at the C_{16}–C_{17} bond and at C_{18} and in the substitution of the aromatic ring. Aspidophytine is actually a degradation product of haplophytine (**243**), which was isolated from *Haplophyton cimicidum* in Mexico.[195,196] Aspidophytine has inspired two total syntheses to date by the Corey[197] and Fukuyama[198,199] groups. The retrosynthetic analyses of these syntheses (Scheme 43) show the same basic disconnections, but the order of realization is different.

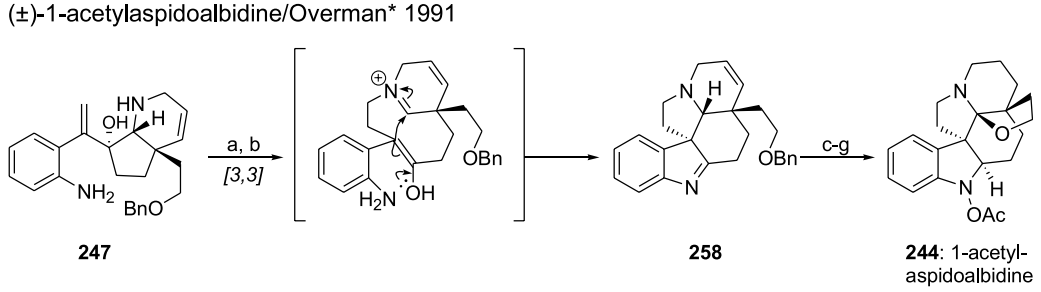
The key step in the 1-acetylaspidoalbidine synthesis by the Ban group is the acid-catalyzed transannular cyclization of diol **245** to the pentacyclic alcohol **257**, only three steps from the natural product itself (Scheme 44).[191–193] As the ultimate step, mercury(II) acetate effects the final cyclization to give the [4.4.3]propellane structure.[200]

The key ring-forming transformation in the formal total synthesis of 1-acetylaspidoalbidine by the Overman group[194] is the aza-Cope rearrangement-Mannich cyclization sequence that was developed in the group (Scheme 45).[109] The treatment of aminoalcohol **247** with paraformaldehyde effects an imine formation, which is followed by an aza-Cope [3,3]-sigmatropic rearrangement–Mannich cyclization under acidic conditions to provide pentacycle **258**. The five following steps then conclude the synthesis of (±)-1-acetylaspidoalbidine (**244**).

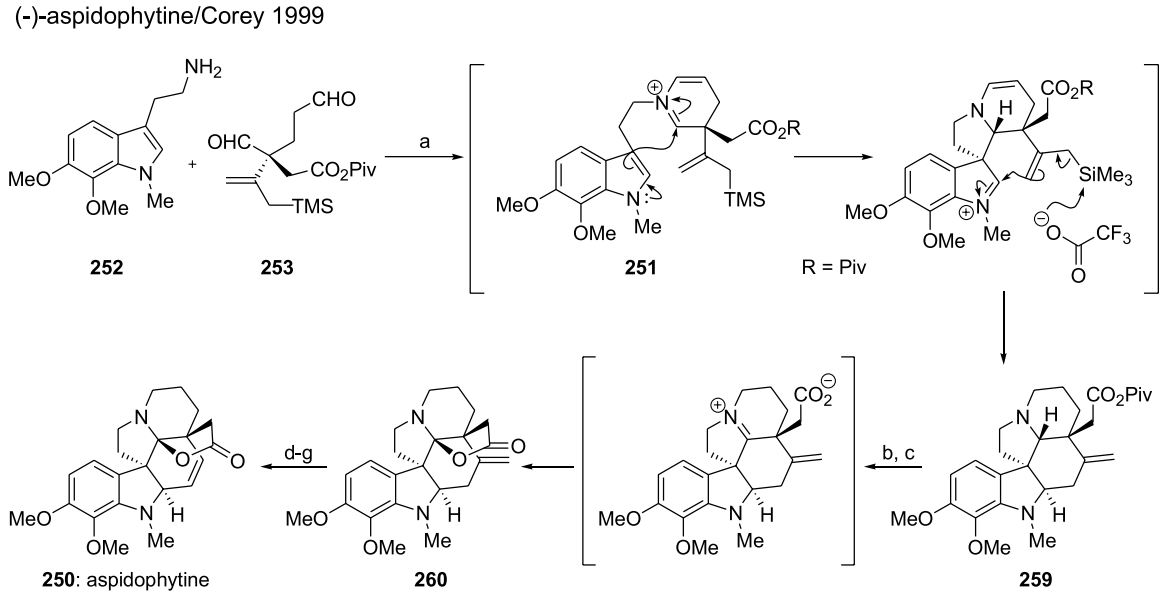
The Corey group devised the concise and convergent synthesis of aspidophytine **250** shown in Scheme 46.[197] The key transformation was an acid-catalyzed cascade cyclization of the tryptamine derivative **252** and dialdehyde **253**, which forms the pentacyclic core of the molecule. Then, after having hydrolyzed the pivalate of **259**, potassium



Scheme 44. (a) 10% HCl, THF, 0 °C, 30 min, 70%; (b) $LiAlH_4$; (c) acetylation; (d) $Hg(OAc)_2$, 5% AcOH, 65–70 °C, 7 h, 64%.



Scheme 45. (a) $(CH_2O)_n$, Na_2SO_4, tol, rt, 24 h, quant.; (b) CSA, Na_2SO_4, PhH, reflux, 2.5 h; (c) $LiAlH_4$, THF, 0 °C, 2 h, 67% over two steps; (d) Na, NH_3, THF, −70 °C, quant.; (e) Pd/C, HCO_2NH_4, EtOH, reflux, 1 h, 98%; (f) Ac_2O; [(g)[193] $Hg(OAc)_2$, 5% AcOH, 65–70 °C, 7 h, 64%, reaction not performed, formal total synthesis].



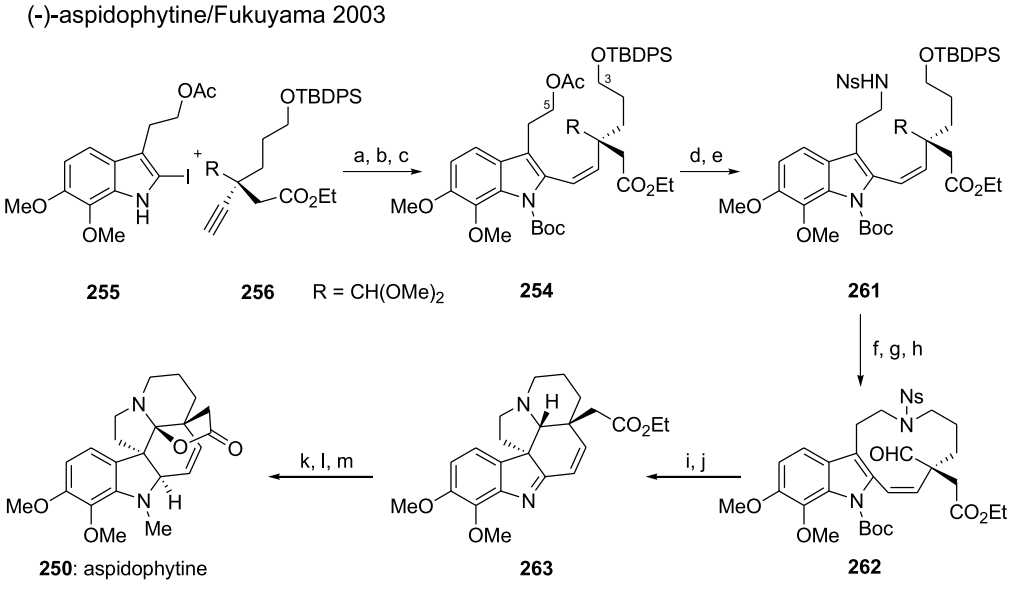
Scheme 46. (a) MeCN, rt, 5 min, then TFAA, 0 °C, 2 h, then $NaBH_3CN$, 0 °C→rt, 30 min, 66%; (b) NaOH, EtOH, 75 °C, 20 h, 88%; (c) $K_3Fe(CN)_6$, $NaHCO_3$, *t*-BuOH/H_2O 1:2, rt, (fast), 92%; (d) OsO_4, DMAP, *t*-BuOH/H_2O 1:2, rt, 5–10 min, then Na_2SO_3; (e) $Pb(OAc)_4$, AcOH, CH_2Cl_2, −20 °C, 5–10 min, 71% over two steps; (f) KHMDS, THF, −78 °C, 30 min, then $PhNTf_2$, 54%; (g) $Pd(PPh_3)_4$, Bu_3SnH, THF, rt, 1 h, 86%.

ferricyanide effected an oxidative lactonization to the [4.4.3]propellane **260**. An oxidative cleavage of the exocyclic double bond in **260** followed by enol triflate formation and treatment with tributyltin hydride provided (−)-aspidophytine (**250**).

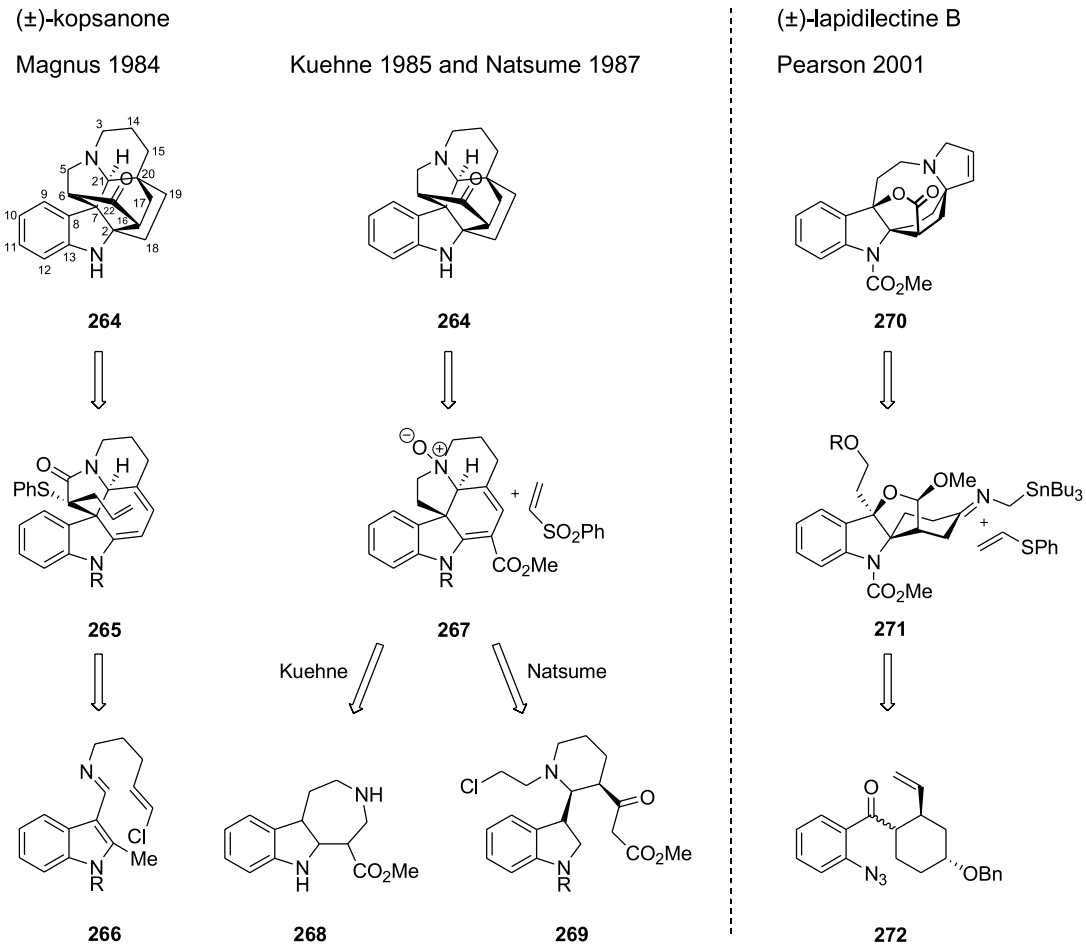
A Sonogashira coupling between iodoindole **255** and acetylene **256** is the first step towards forming the propellane ring system of aspidophytine in the Fukuyama group's synthesis (Scheme 47).[198,199] The coupled product was Boc protected and the triple bond was reduced selectively to olefin **254**. Changing the C_5 substituent to a nosylate-activated nitrogen enabled the formation of the 11-membered ring (**262**) once the C_3-alcohol was deprotected. Removing the nosylate and exposure of the product to trifluoroacetic acid furnished the pentacycle **263** in 56% yield over two steps. Conversion of the imine of **263** into the corresponding *N*-methylindole derivative followed by lactone formation to close the last remaining ring provided (−)-aspidophytine (**250**).

5.2. Kopsanone and lapidilectine B

The kopsane alkaloids have been known since 1890,[201] but their structures remained unknown until the 1960s. Two total syntheses and one formal total synthesis have been

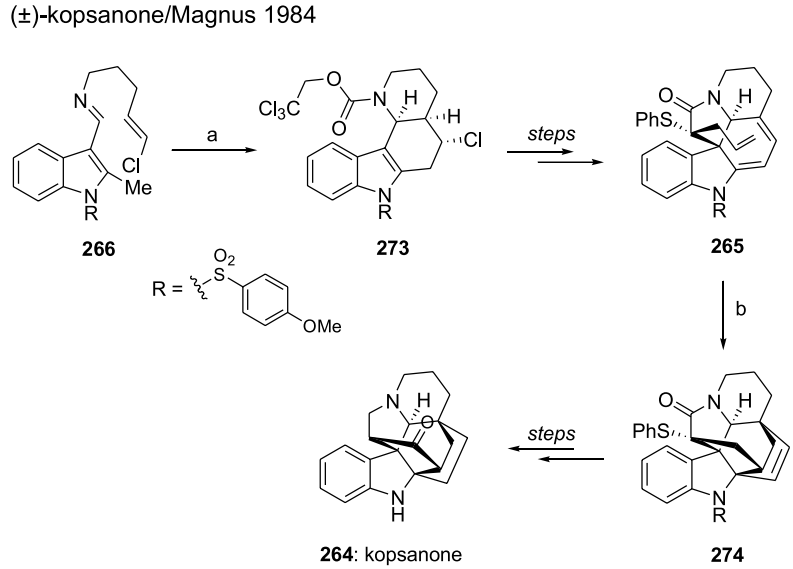


Scheme 47. (a) $Pd(PPh_3)_4$, CuI, EtN_3, 70 °C, 2 h, 78%; (b) Boc_2O, DMAP, CH_3CN, rt, 15 min, 94%; (c) Pd/C, H_2, EtOH, rt, 3.5 h, 97%; (d) K_2CO_3, H_2O, MeOH, rt, 1 h, 96%; (e) *o*-$NsNH_2$, PPh_3, PhH, DEAD, rt, 5 min, 93%; (f) TBAF, THF, rt, 1 h, 93%; (g) PPh_3, DEAD, PhH, rt, 5 min, 92%; (h) TMSBr, CH_2Cl_2, 70 °C, 15 min, then pH 7.0 buffer, 92%; (i) PhSH, Cs_2CO_3, MeCN, 55 °C, 20 min; (j) TFA, Me_2S, CH_2Cl_2, rt, 5 min, then pH 7.8 buffer, EtOAc, 5 °C, 30 min, 56% over two steps; (k) HCHO, pH 7.0 buffer, MeOH, H_2O, $NaBH_3CN$, −70 °C, 30 min, →rt, 2 h, 67%; (l) NaOH, EtOH, 70 °C, 2.5 h, then HCl, 5 °C, 52%; (m) $K_3Fe(CN)_6$, $NaHCO_3$, *t*-BuOH/H_2O 1:2, 5 °C→rt, 10 min, 56%.

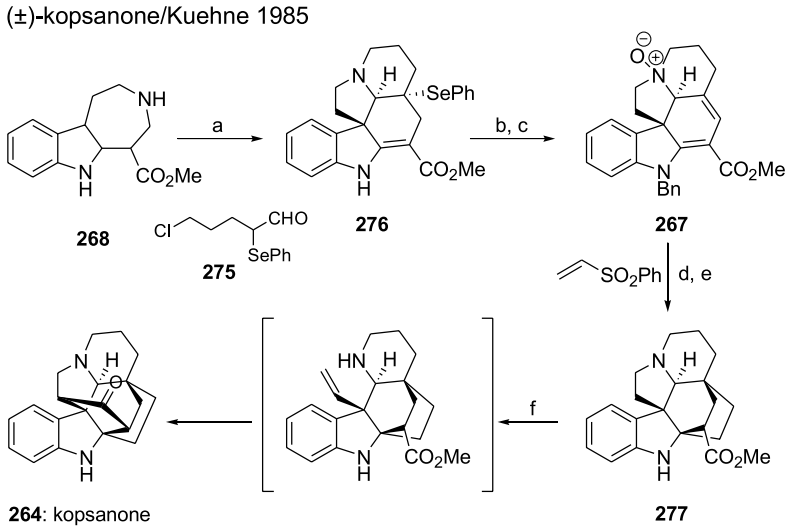


Scheme 48. Retrosynthetic analyses of kopsanone and lapidilectine B.

published for kopsanone (**264**), a member of this group (Scheme 48). The syntheses of the Natsume[202,203] and Kuehne[204] groups use the same methods to install the [4.3.3]propellane ring system, so only the earlier synthesis from the Kuehne group is discussed here in detail (see Scheme 48). The Kerr group has also published an approach towards kopsane alkaloids.[205] A key step in the first published synthesis of kopsanone by Magnus et al. is the intramolecular Diels–Alder reaction of **265**.[206,207] The Kuehne group synthesis is based on a Diels–Alder reaction between diene **267** and phenyl vinyl sulfone.[204]



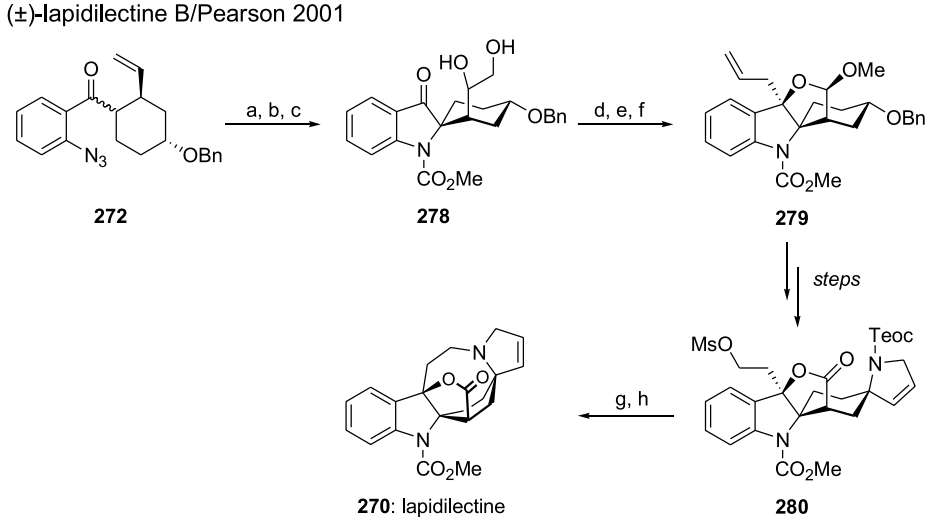
Scheme 49. (a) Cl_3CCH_2OCOCl, *i*-Pr_2NEt, PhCl, 0→120 °C, 40 min, then 120 °C, 8 h, 50%; *steps*; (b) 95–100 °C, PhH, 4 h, 81%.



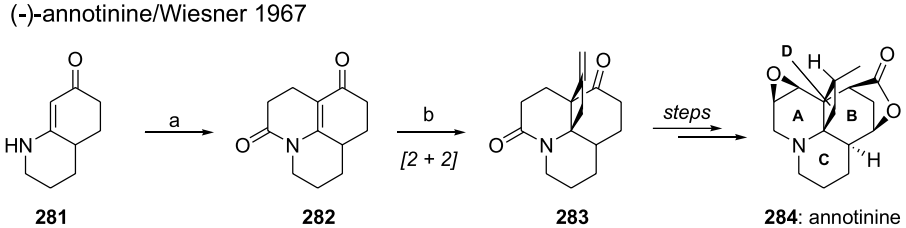
Scheme 50. (a) **275**, H_3BO_3, CH_2Cl_2, reflux, 12 h, 33%; (b) BnBr, NaH, DMF, rt, 30 min, 86%; (c) *m*-CPBA, CH_2Cl_2, −78 °C, 79%; (d) phenyl vinyl sulfone, PhH, 100 °C, 16 h, 57%; (e) Raney-Ni, EtOH, reflux, 3 h, 67%; (f) MeOH, 210 °C, 36 h, 88%.

Lapidilectine B (**270**) was isolated from *Kopsia lapidilecta* in 1992[208] and was synthesized by the group of Pearson (Scheme 48).[209,210] The main features of the synthesis of this polycyclic indole alkaloid include a Smalley azido-enolate cyclization to form the indoxy core of the molecule.

The synthesis of the propellane moiety of kopsanone by Magnus et al. began with the treatment of the vinyl chloride **266** with trichloroethyl chloroformate and gave tetracycle **273** in 50% yield (Scheme 49).[206,207] After installing the diene portion and the allylic side chain, the stage was set for the intramolecular Diels–Alder reaction of **265**, which provided the [4.3.3]propellane **274** in 81% yield. Six more steps were required to finish the first total synthesis of (±)-kopsanone (**264**).



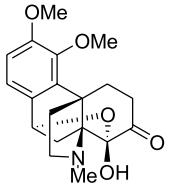
Scheme 51. (a) KOH, *i*-PrOH, 15 °C, 1 h, (68% over two steps), 2.2:1 selectivity; (b) *t*-BuLi, $ClCO_2Me$, THF, −10 °C, 30 min, 89%; (c) OsO_4, NMO, acetone, rt, overnight, 82%, 6:1 selectivity; (d) allylMgBr, THF, −40 °C→rt, overnight, 90%; (e) $NaIO_4$, pH 7 buffer, THF, 0 °C→rt, overnight; (f) CSA, MeOH, 1 h, rt, 59% over two steps; *steps*; (g) TFA, CH_2Cl_2, rt, 30 min; (h) *i*-Pr_2NEt, MeCN, rt, 2 h, then 60 °C, 10 h, 76% over two steps.



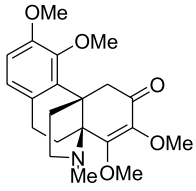
Scheme 52. (a) Acrylic acid, 135 °C, 2 h, 63%; (b) allene, hν, −70 °C, 20 h, 54%.

In the Kuehne group synthesis of kopsanone (Scheme 50),[204] the key pentacyclic intermediate **276** came from an adaptation of a biomimetic secodine cyclization that they had investigated extensively.[211] Benzylation of the indole nitrogen and oxidative elimination of the selenyl group afforded *N*-oxide **267**, which was ready for the Diels–Alder cyclization. Heating of diene **267** with phenyl vinyl sulfone effected the formation of hexacycle **277** after reduction of the double bond and the sulfone with Raney nickel. Finally, heating of ester **277** to 210 °C for 36 h provided (±)-kopsanone (**264**).

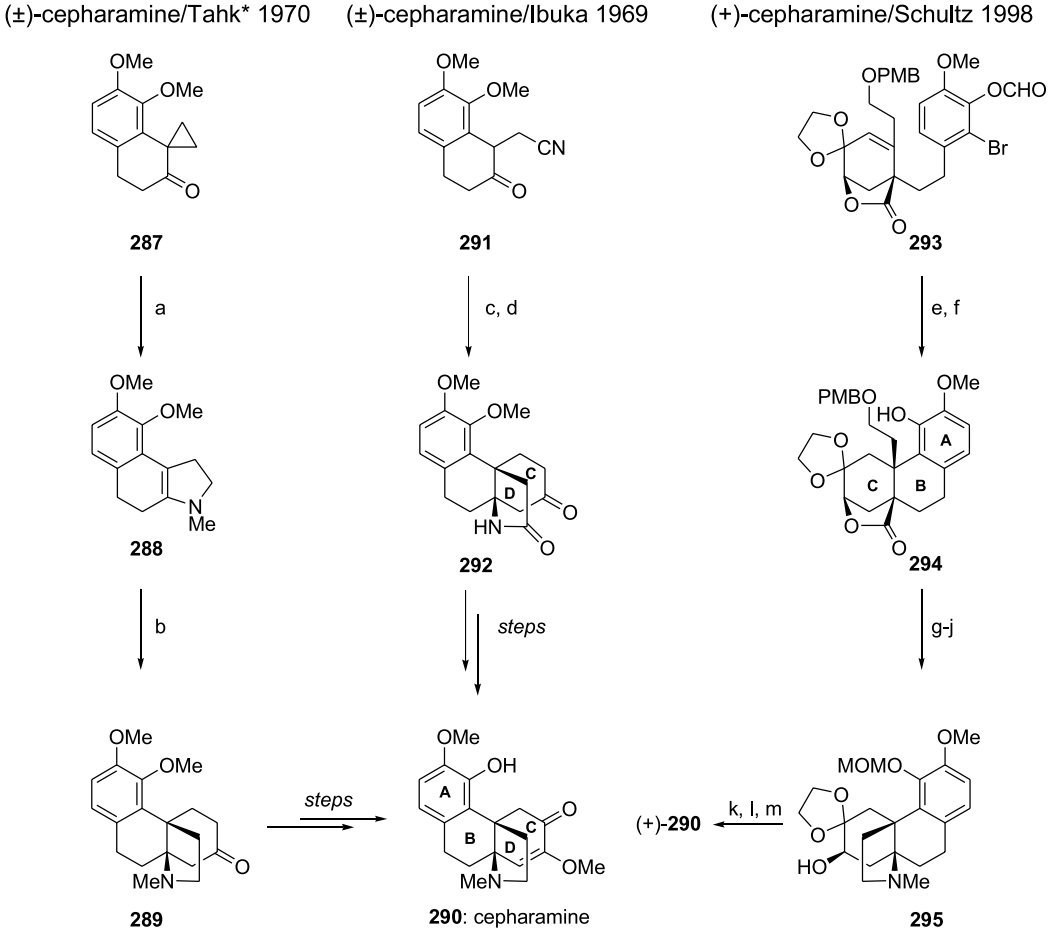
Treatment of azide **272** with potassium hydroxide is the first ring-forming step in the lapidilectine B synthesis by Pearson et al. (Scheme 51).[209,210] The Smalley cyclization provided an indole derivative that was then protected at the nitrogen and dihydroxylated to give diol **278** in a 6:1 selectivity. Allylation of the ketone followed by oxidative cleavage of the diol and treatment with camphorsulfonic acid furnished methyl acetal **279**. After closing the pyrroline ring with a cycloaddition (see Scheme 48), mesylate **280** was ready for the final eight-membered ring closure. Removing the Teoc-group from the pyrroline nitrogen and treatment of the remaining salt with diisopropylethylamine provided (±)-lapidilectine B (**270**).



285: methaphanine



286: hasubanonine



Scheme 53. (a) $MeNH_2$, CaO, PhH, 100–110 °C, 7 days, 69%; (b) MVK, rt, 1 h, then AcOH, 40→−78 °C, evacuated to 125 mmHg, →70 °C, 5 h, 20%; (c) MVK, NaOH, MeOH, reflux, 45 min; (d) Na, EtOH, reflux, 4 h, 50% over two steps; (e) Bu_3SnH, AIBN, PhH, 80 °C, 10 h; (f) K_2CO_3, MeOH, THF, H_2O, rt, overnight, 56% over two steps; (g) NaH, MOMCl, THF, reflux, 15 h, 99%; (h) $NaNH_2$, NH_3, THF, −30 °C→rt, 2 h; (i) NaOMe, Br_2, MeOH, THF, −78 °C, 1 h, →reflux, 1 h, 93% over two steps; (j) $LiAlH_4$, THF, reflux, 22 h, 99%; (k) $(COCl)_2$, DMSO, CH_2Cl_2, −78→10 °C, 1 h, then Et_3N, −10 °C→rt, 30 min, 85%; (l) KH, 18-crown-6, DMF, 0 °C→rt, 30 min, then MeI, rt, 15 h, 65%; (m) *p*-TsOH, acetone, H_2O, 60 °C, 39 h, 97%.

6. Other alkaloids

Other than the indole alkaloids covered in Section 5, the syntheses of four additional alkaloids with the propellane ring structure have appeared in the literature. Two of these, namely cepharamine and metaphanine, share a common ring structure called the hasubanan skeleton and will be discussed together in Section 6.2. Three research groups have reported on their synthetic efforts towards the hasubanan skeleton and these will also be briefly discussed in the same Section.

6.1. Annotinine

The same research group that reported the total synthesis of the *Lycopodium* alkaloid annotinine (**284**) in 1967[212–215] deduced the structure of the molecule during the years 1956–1957 (Scheme 52).[216] Their synthesis starts with a condensation of acrylic acid and vinylogous amide **281** to provide the [2+2] photochemical cycloaddition substrate **282**. The cycloaddition of **282** with allene proceeded smoothly to give the ABCD ring system of annotinine (**283**). Several more steps, including an optical resolution, were required to finish the synthesis of (−)-annotinine (**284**).

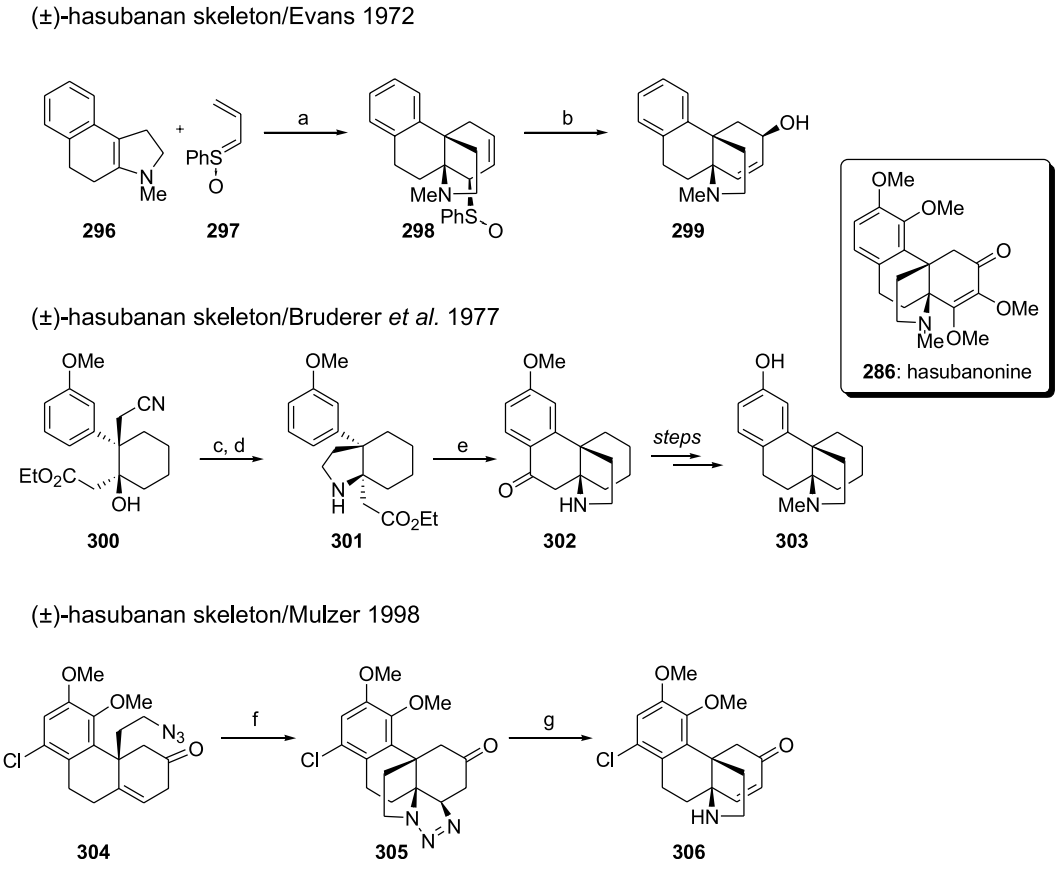
6.2. Cepharamine and the hasubanan skeleton

Three total syntheses of cepharamine (**290**), an alkaloid isolated from *Stephania cepharantha* Hayata in 1966, have been published (Scheme 53).[217] The Ibuka group published the first total synthesis in 1969,[218,219] then the Tahk group published in 1970[220] and Schultz in 1998.[221] Ibuka et al. have also published a synthesis of methaphanine (**285**)[222,223] and hasubanonine (**286**),[224,225] but, since these syntheses use the same methodology to form the [4.3.3]propellane ring system as in their synthesis of cepharamine, they will not be discussed here in more detail.

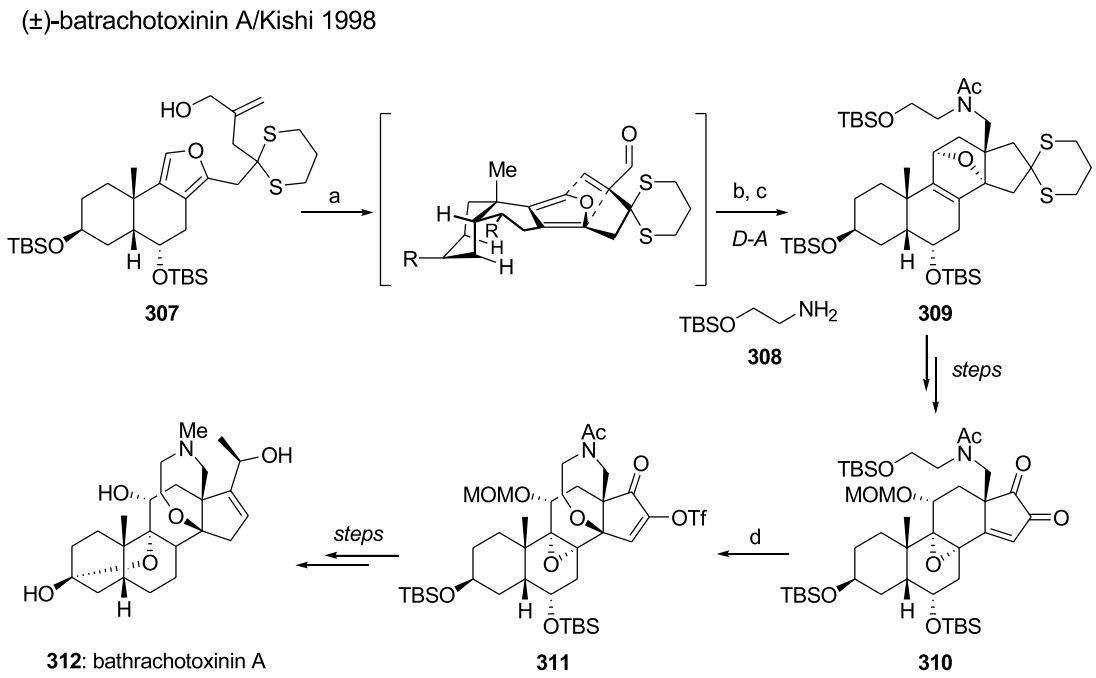
In the Ibuka group synthesis of cepharamine (**290**) (Scheme 53),[218,219] the key ring-forming step was the formation of the CD ring system. Exposure of nitrile **291** to methyl vinyl ketone (MVK) followed by treatment with sodium ethoxide provided ketoamide **292** in 50% yield.

In the formal total synthesis of cepharamine by the Tahk group (Scheme 53),[220] the CD ring system was formed by treatment of cyclopropyl ketone **287** with methylamine to afford the ring expansion product **288**. Annulation with methyl vinyl ketone then completed the ring system of cepharamine (**289**).

The Schultz group strategy for the synthesis of cepharamine differs significantly from the previous syntheses (Scheme 53).[221] The key step in their synthesis was a radical cyclization of **293** followed by hydrolysis of the formate ester to give the ABC ring fragment **294**. After an MOM protection and a Hofmann-type rearrangement to transform the five-membered lactone into a six-membered lactam ring,



Scheme 54. (a) MeCN, 70 °C, 24 h; (b) $Na_2S·9H_2O$, MeOH, 65 °C, 8 h; (c) $POCl_3$, pyr, reflux, 5 h; (d) NH_3, Raney-Ni, H_2, EtOH, 80 °C, 38% over two steps; (e) HCl, H_2O, reflux, 1 h, 83%; (f) PhH, reflux, 1 h, 76%; (g) pyr, reflux, 5 h, 73%.



Scheme 55. (a) Mn_2O, CH_2Cl_2, rt, 1 h; then filtration; then CH_2Cl_2, rt, 12 h; (b) **308**, AcOH, 4 Å MS, PhH, rt, 3 h; then $NaCNBH_3$, MeOH, 0 °C, 15 min; (c) Ac_2O, pyr, rt, overnight, 76% over three steps, >25:1 selectivity; steps; (d) TASF, THF/DMF 10:1, 0 °C→rt, 1 h; then $PhNTf_2$, Et_3N, 30 min, rt, 95%.

treatment with lithium aluminium hydride provided alcohol **295**. This alcohol was then transformed into (+)-cepharamine ((+)-**290**), the unnatural enantiomer of the molecule, in three steps.

The synthetic efforts towards the hasubanan skeleton are summarized in Scheme 54. The key transformation in the Evans group approach is a Diels–Alder reaction between tetrahydrobenzindole **296** and sulfoxide **297** to give tetracycle **298**.[226–228] Bruderer et al. used a dehydration to achieve the closure of the five-membered pyrroline ring (**300**→**301**). An acid-catalyzed cyclization then furnished tetracycle **302**.[229] The latest synthesis of the hasubanan skeleton by the Mulzer group features an intramolecular 1,3-dipolar cycloaddition of **304** followed by a subsequent elimination of N_2 to give aminoenone **306**.[230]

6.3. Bathrachotoxinin A

Bathrachotoxinin A (**312**) is a unique steroidal alkaloid that possesses several interesting structural features. It has been synthesized partially from steroid precursors by Wehrli and co-workers.[231,232] A total synthesis was published by the Kishi group in 1998 (Scheme 55).[233] The synthesis features an *exo*-selective intramolecular Diels–Alder reaction (step a) and an oxy-Michael addition (step d).

7. Summary

The syntheses of propellane-containing natural products have been reviewed with the emphasis being on natural products, which contain a three-membered ring as part of the propellane structure. Propellanes are a well-established structural motif that can be found in diverse natural products. This makes their total synthesis a challenging task, because no generally applicable method can be utilized in the syntheses. However, the Diels–Alder reaction arises as one of the more commonly applied ring-forming reactions among these syntheses.[34]

For the cyclopropane ring-containing natural products described here, only two different methods for the formation of the cyclopropane ring have been applied. Potential new avenues for synthesizing [*m.n*.1]propellane-containing natural products could involve both inter- and intramolecular cyclopropanation reactions.

Acknowledgements

We thank the Finnish Cultural Foundation, Finnish Academy of Sciences, Helsinki University of Technology and National Technology Agency (TEKES) in Finland for financial support and Dr. Melanie Clarke for checking the language of the manuscript.

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Tetrahedron 61 (2005) 8809–8820

Conformational studies of *N*-carbomethoxy-2-alkoxyindolenines by dynamic NMR, crystallography, and molecular mechanics

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Received 13 April 2005; revised 5 July 2005; accepted 8 July 2005

Abstract—The synthesis of *N*-carbomethoxy-2-alkoxyindolenines and the transformation to their tautomeric indoles is reported. Variable-temperature ^{1}H NMR studies of these 2-alkoxyindolenines evidenced dynamic processes involving two low-energy *E* and *Z* equilibrating conformers around the N–C(=O) carbamate bond, for which the barriers ($\Delta G^{\neq}$) between the two conformations are in the order of 12.5–13.9 kcal/mol, as deduced from computational NMR line shape simulations. The rotational barrier decreases as the bulkiness of the alkoxyl group increases, with the *E* conformer being always more stable. Molecular mechanics calculations evidenced a preferred *quasi*-axial position of the alkoxyl group in the five-membered ring as the steric effect increases, in agreement with X-ray diffraction studies.

1. Introduction

Previous studies on natural occurring and synthetic indoles bearing a *N*-carbamate group evidenced the equilibrium conformers arising from hindered rotation about the N–C(=O) bond.[1] A similar dynamic process has also been observed for *N*-acetyl- and *N*-formyl- substituted indole and indoline derivatives.[2] However, in contrast to the efforts invested to quantify and understand the barrier to rotation about the N–C(=O) bond in amides, very few examples on carbamates are described.[3] Since the rotational free energies ($\Delta G^{\neq}$) are largely governed by steric and electronic effects provided by substituents around the N–C(=O) bond,[2d] the present study was undertaken to examine the role that hydroxyl, alkoxyl, and acetyloxyl substituents, adjacent to the N-atom, play on the N–C(=O) rotational barrier for indolenines **3**, **4a–d**, **6** and for indoles **5a–d**. The results obtained from variable temperature ^{1}H NMR spectroscopy were compared with molecular mechanics calculations,[4] while computational line shape simulations allow determining $\Delta G^{\neq}$ values, which are in the 12.5–13.9 kcal/mol range for the N–C(=O) bond rotation. In addition, X-ray diffraction studies were performed on single crystals of indolylbromomalonate **2**, 2-alkoxyindolylidenes **4b–d**, 2-alkoxyindoles **5a–c**, and 2-acetoxyindolylidene **6**.

2. Results and discussion

2.1. Synthesis

We recently reported[5] a one-pot practical method for the preparation of 2-hydroxyindolenine **3** by oxidation of indolylmalonate **1** with bromine through a supposed 2-bromoindolenine, which proved to be unstable for chromatographic purification on silica gel, and therefore, hydrolysis of the crude product was accomplished to give **3**. The suspected intermediate could now be isolated, characterized by spectral means, and its structure confirmed by single crystal X-ray diffraction analysis as indolylbromomalonate **2** (Scheme 1, Fig. 1, Table 3), allow clarifying the course of the reaction. It is known[6] that in 3-substituted indoles the C–H bond alpha to the five-membered ring could be activated by Lewis acids, to carry out acetylations. With this precedent, a plausible mechanism for the C–Br bond formation in **2**, via activation of the C(8)–H bond, is depicted in Scheme 2. Thus, addition of bromine to indolylmalonate **1** would afford the bromonium

Keywords: Dynamic NMR; X-ray analysis; Alkoxyindolenines; Anomeric effect.
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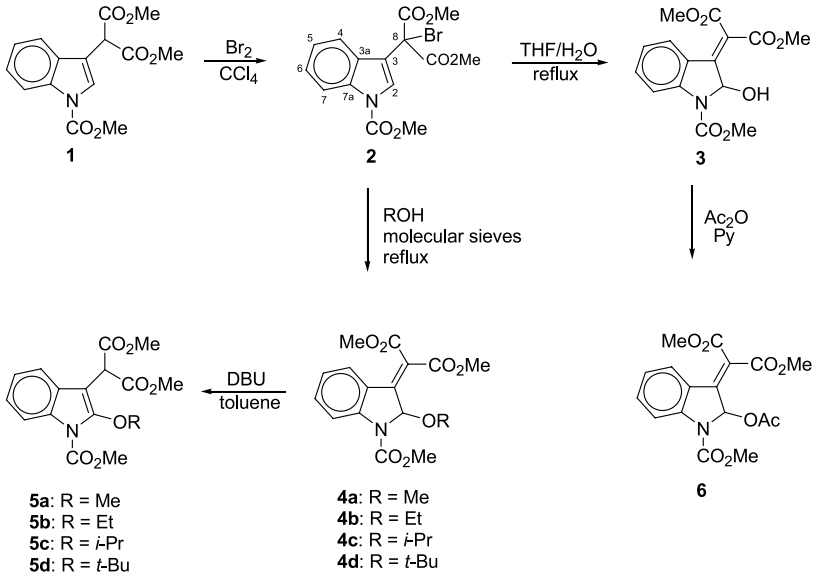
doi:10.1016/j.tet.2005.07.023



Scheme 1.

ion **7**, followed by H(8)-abstraction generating a labile 2-bromoindolenine **8**, which after allylic rearrangement provides stable indolylbromomalonate **2**. The driving force for this tautomerization is given by restoration of the aromaticity of the heterocycle. Finally, addition of water to indolylbromomalonate **2** affords 2-hydroxyindolenine **3**.

Having established the identity of the intermediate as indolylbromomalonate **2**, we were attracted by their synthetic potential. Careful study of its reactivity towards nucleophilic reagents, such as alcohols, could allow the synthesis of 2-alkoxyindoenines, which are scarcely documented.[7,8] Previously, 2-methoxyindolenines have been prepared by Wittig–Horner–Emmons olefination of 2-methoxyindol-3-ones[7] and by treatment of a 2-hydroxyindolenine with methanol in the presence of anhydrous HCl.[8] In the first case, some limitations exist for the substrate to be olefined, due to the instability of the produced indolenines, which could isomerize to indoles under the reaction conditions.

Formation of 2-alkoxyindenines **4a–d** is herein described by treatment of indolylbromomalonate **2** with alcohols. When **2** was reacted with MeOH in the presence of molecular sieves, 2-methoxyindolenine **4a** was isolated in 76% yield. In a similar way, we were able to convert **2** to the corresponding 2-alkoxyindolenines **4b–d** by treatment with EtOH, *i*-PrOH and *t*-BuOH, respectively, in 64–89% yield. The tautomeric 2-alkoxyindoles[8] **5a–d** were prepared from the corresponding 2-alkoxyindolenines **4a–d** using 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU)[9] in refluxing toluene for 2 h, in 52–81% yield. In addition, the 2-hydroxyindolenine **3** was

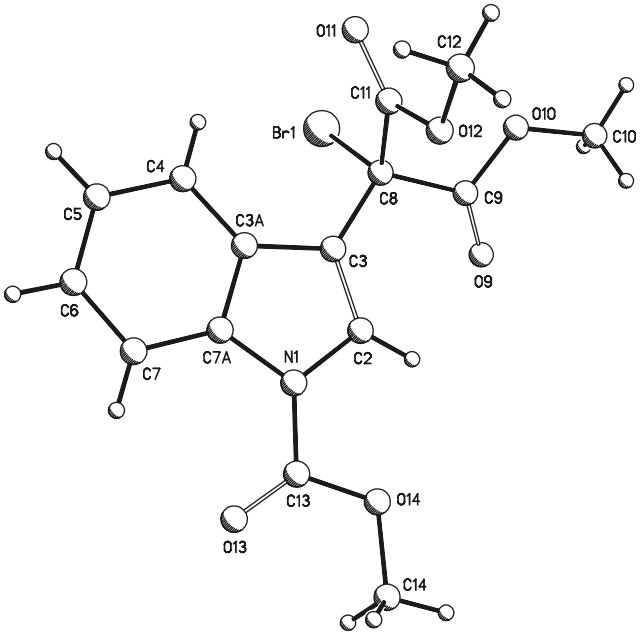
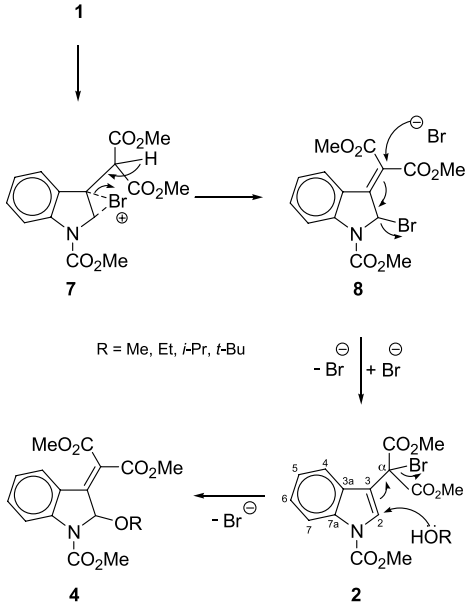


Figure 1. X-ray diffraction structure of **2**.



Scheme 2.

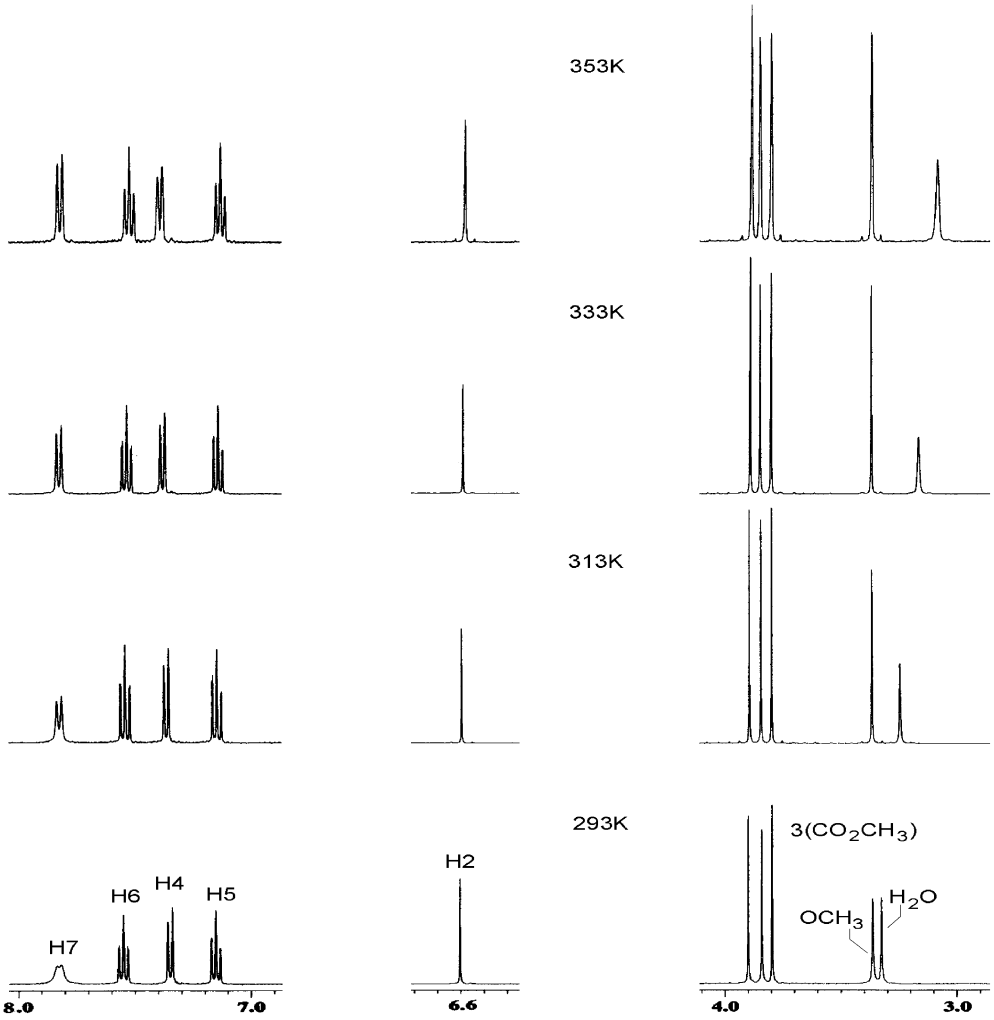


Figure 2. Variable temperature 400 MHz ^{1}H NMR spectra of **4a** measured in DMSO-d_6 solutions.

converted to its acetylated derivative **6**, in 81% yield, when treated with Ac_2O/pyridine.

2.2. ^{1}H NMR evaluation

The room temperature (293 K) ^{1}H NMR spectra of 2-hydroxyindolenine **3**, 2-alkoxyindolenines **4a–d**, 2-alkoxyindoles **5a–d**, and 2-acetoxyindolenine **6**, recorded in DMSO-d_6 solutions, exhibited pronounced differences. Thus, extensive line broadening of aromatic H-7, the *N*-carbomethoxyl and 2-alkoxyl group signals were observed for **3** and **4a–d**, whereas indole tautomers **5a–d** and 2-acetoxyindolenine **6** gave sharp lines. Heating solutions of **3** and **4a–d** above 313 K (DMSO-d_6) collapsed the broad resonances into sharp signals, while cooling to 233 K (CD_2Cl_2) allowed observation of signals owing to two conformers, as is illustrated in Figures 2 and 3 for **4a**. The broadness of the H-7, –NCO_2Me and –OMe signals for **4a** suggests the presence of conformational isomers arising from slow rotation around the N–CO_2Me bond,[3b,c] giving rise to the two mayor *E* and *Z* conformers denoted by the torsion angle C(2)–N(1)–C═O (Scheme 3). The broadness of these proton signals also indicates that rotation around the N–CO_2Me bond is the dominant dynamic process. The conformational *E*/*Z* ^{1}H NMR assignment of indolenines **3** and **4a–d** was based on the use of the H-7 resonance as a starting point, since its characteristic broad signal appears at higher frequency.[3b,c] For instance, in the case of **4a**, irradiation of H-7 at 7.82 ppm collapsed the triple doublet at 7.54 ppm to a double doublet, which was then assigned to H-6. Hence the doublet at 7.35 ppm and the triplet at 7.14 ppm were assigned to H-4 and H-5, respectively. At 213 K (Fig. 3) two well resolved doublets for H-7, with different intensities, appear at 8.01 and 7.67 ppm, which were assigned to the *E* and *Z* conformers, respectively. Thus, in all cases the H-7 signal of the major conformer was shifted 0.30–0.36 ppm to lower fields relative to the minor isomer (Table 1), indicating that H-7 of the major conformer lays in the deshielding current of the carbonyl carbamate group and therefore, corresponds to the *E* conformer.[2c,d]

Observation of Figure 3 reveals that the H-4 signal for both conformers is shifted to high fields when the temperature is decreased. This is rationalized in terms of the anisotropic effect exerted by one of the carbonyl groups at H-4.[3e] This effect could be greater when the temperature is decreased due to the slow rate of rotation of the group.

The observed *E*/*Z* ratio was determined by integration of the respective H-7 peaks in the ^{1}H NMR spectra at 213 K, and

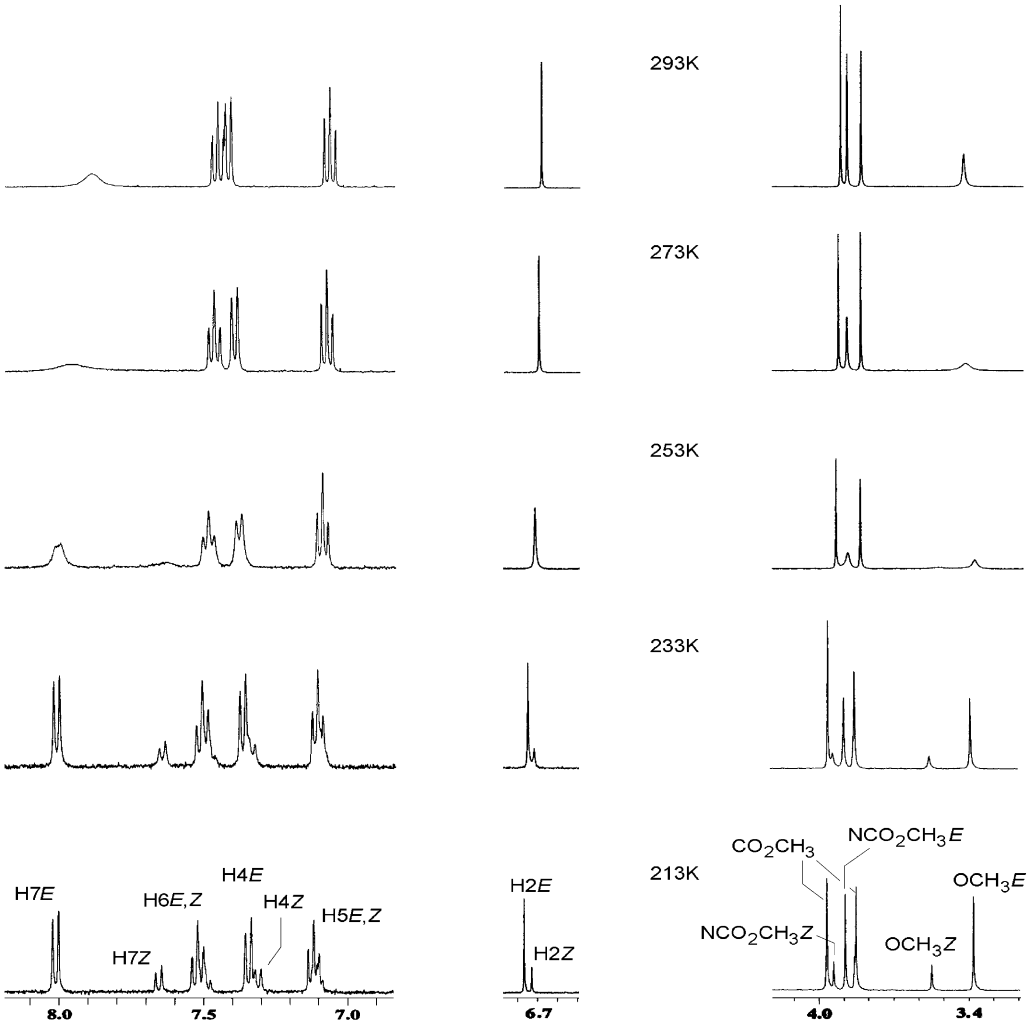
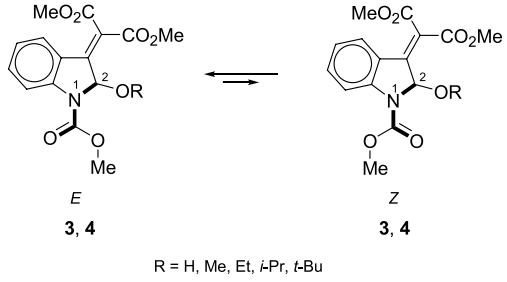


Figure 3. Variable temperature 400 MHz ^{1}H NMR spectra of **4a** measured in CD_2Cl_2 solutions.



Scheme 3. *E* and *Z* conformers for indolenines **3** and **4**.

varied from 1:0.32 for **3** to 1:0.84 for **4d** (R=*t*-Bu) (Table 2). From these values, the free energy difference ΔG^0 of the *E*/*Z* conformers in CD_2Cl_2 solutions were calculated using the Eyring equation. Inspection of the data in Table 2 reveals that the proportion of *Z* rotamers increase with the increment of the steric bulkiness of the alkoxy group at the 2-position. Thus, for the sterically bulkier 2-*tert*-butoxyindolenine **4d**, the ΔG^0 value for the *E*/*Z* rotamers was 0.07 kcal/mol, while for less hindered **3** it was 0.48 kcal/mol.

Table 1. Chemical shifts (ppm) and coupling constants (Hz) of indolenines **3** and **4a–d** in the *E* and *Z* conformations at 213 K in CD_2Cl_2 solution

Compound	H-7	H-6	H-4	H-5	H-2	CO_2Me	NCO_2Me	OCH_n
3*E*	8.03, 8.0	7.53, 8.0	7.27, 7.6	7.14, 7.7	6.66, 3.3	3.98, 3.86	3.89	
4a*E*	8.01, 8.0	7.52, 8.0	7.35, 7.9	7.11, 7.9	6.77	3.96, 3.85	3.89	3.37
4b*E*	8.00, 8.0	7.51, 8.0	7.36, 7.7	7.11, 7.8	6.75	3.96, 3.84	3.88	3.82–3.65
4c*E*	7.97, 8.0	7.52, 8.0	7.35, 7.9	7.12, 7.8	6.89	3.96, 3.84	3.88	4.02, 6.1
4d*E*	7.88, 8.4	7.51, 8.3	7.35, 7.8	7.14, 7.9	7.01	3.94, 3.83	3.89	
3*Z*	7.67, 8.3	7.51, 8.3	7.29, 7.7	7.11, 7.9	6.71, 3.7	3.98, 3.86	3.96	
4a*Z*	7.66, 8.4	7.50, 8.4	7.31, 7.8	7.10, 7.9	6.73	3.96, 3.86	3.94	3.54
4b*Z*	7.65, 8.6	7.49, 8.6	7.32, 7.8	7.10, 7.9	6.74	3.96, 3.84	3.92	3.82–3.65
4c*Z*	7.63, 8.4	7.50, 8.3	7.32, 7.8	7.12, 7.9	6.90	3.96, 3.84	3.91	4.16–4.18, 6.2
4d*Z*	7.58, 8.4	7.49, 8.4	7.34, 7.7	7.14, 7.9	7.00	3.94, 3.83	3.86	

Table 2. *E*/*Z* ratio, free energy differences ΔG^0 and barriers to internal rotation ($\Delta G^{\neq}$) of indolenines **3** and **4a–d**

Compound	*E*/*Z* ratio[a]	ΔG^0 (kcal/mol)[b]	$\Delta G^{\neq}$ (kcal/mol)	
3	1:0.32	0.48	$E \rightarrow Z$	14.5
			$Z \rightarrow E$	13.9
4a	1:0.30	0.51	$E \rightarrow Z$	13.8
			$Z \rightarrow E$	13.2
4b	1:0.32	0.48	$E \rightarrow Z$	13.7
			$Z \rightarrow E$	13.1
4c	1:0.41	0.38	$E \rightarrow Z$	13.6
			$Z \rightarrow E$	13.3
4d	1:0.84	0.07	$E \rightarrow Z$	12.6
			$Z \rightarrow E$	12.5

[a] Determined by ^{1}H NMR integration of the H-7 signal.
[b] Estimated ΔG^0 values from $-RT \ln K$ (at 213 K, in CD_2Cl_2), K=[major isomer]/[minor isomer].

2.3. Quantitative measurements

The dynamic behavior of the restricted rotation about the N–CO_2Me bond for the *E* and *Z* rotamers was quantitatively studied for indolenines **3** and **4a–d** using variable-temperature ^{1}H NMR spectroscopy in CD_2Cl_2 solution. At 213 K the ^{1}H NMR spectrum of the aromatic region of **4a** (Fig. 4) shows two sets of signals for all the protons, revealing that at this temperature the interconversion of the two conformers is slow. On raising the temperature (233 K) the signals for both conformers broaden, they coalesce around 253 K and finally (at 273) yield one set of sharp signals for H-4 to H-6, while the H-7 signal remains broad, even at room temperature. Computer line-shape simulation[10] of the ^{1}H NMR spectra of **3** and **4a–d** allowed determination of the kinetic constants (k) for the N–CO_2Me bond rotation at various temperatures, as is shown in Figure 4 for **4a**, from which the activation free energy ($\Delta G^{\neq}$) given in Table 2 was obtained[11]. As one can see for **4a** at 293 K, there is almost free rotation since k=450 s^{-1}, whereas at 213 K it is 0 s^{-1}. At intermediate temperatures of 233 and 273 K values for k of 2 and 170 s^{-1}, respectively, were found, from which an interconversion barrier ($\Delta G^{\neq}$) of 13.5±0.3 kcal/mol was calculated. As

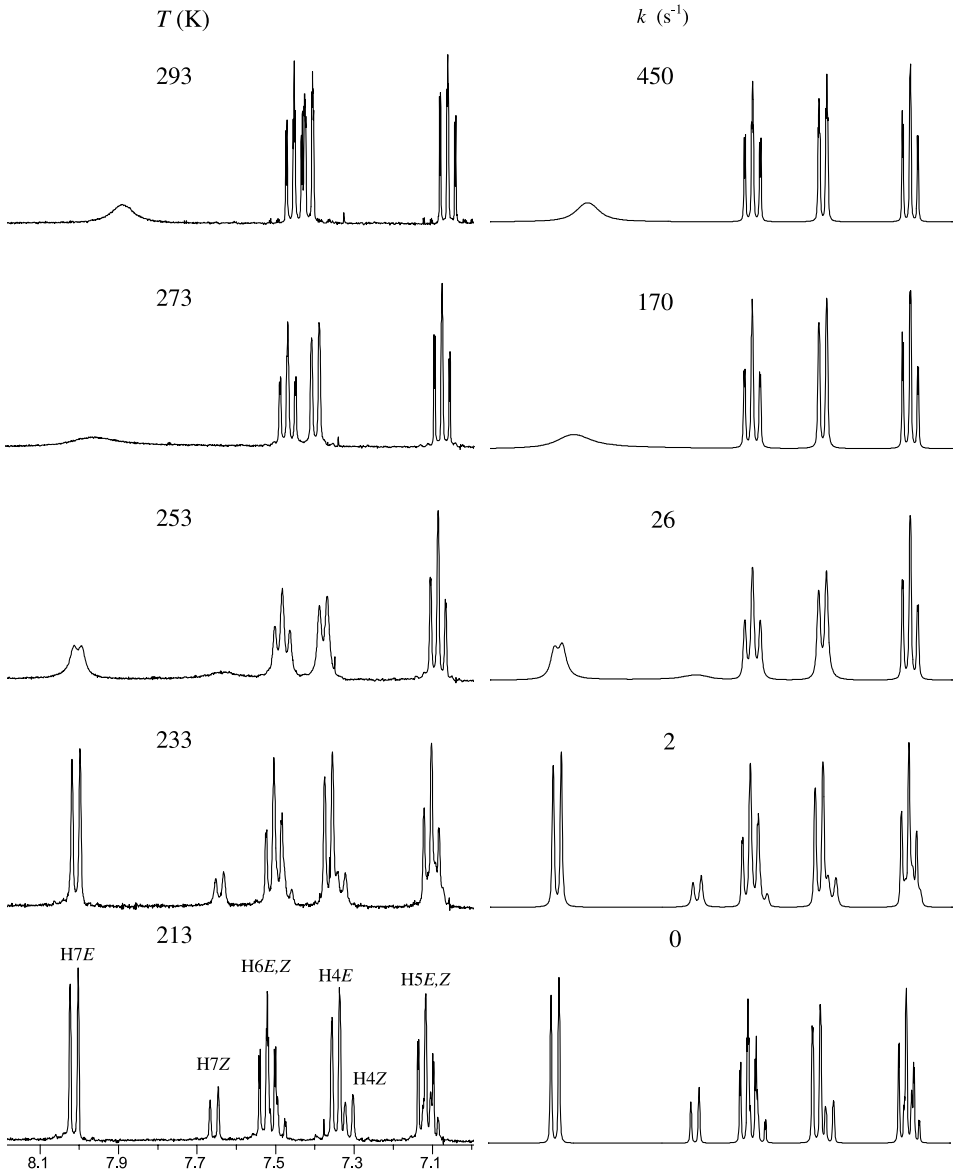


Figure 4. Temperature dependence (left) for the aromatic ^{1}H NMR signals (400 MHz in CD_2Cl_2) of **4a** and computer simulation (right) obtained with the indicated rate constants (k).

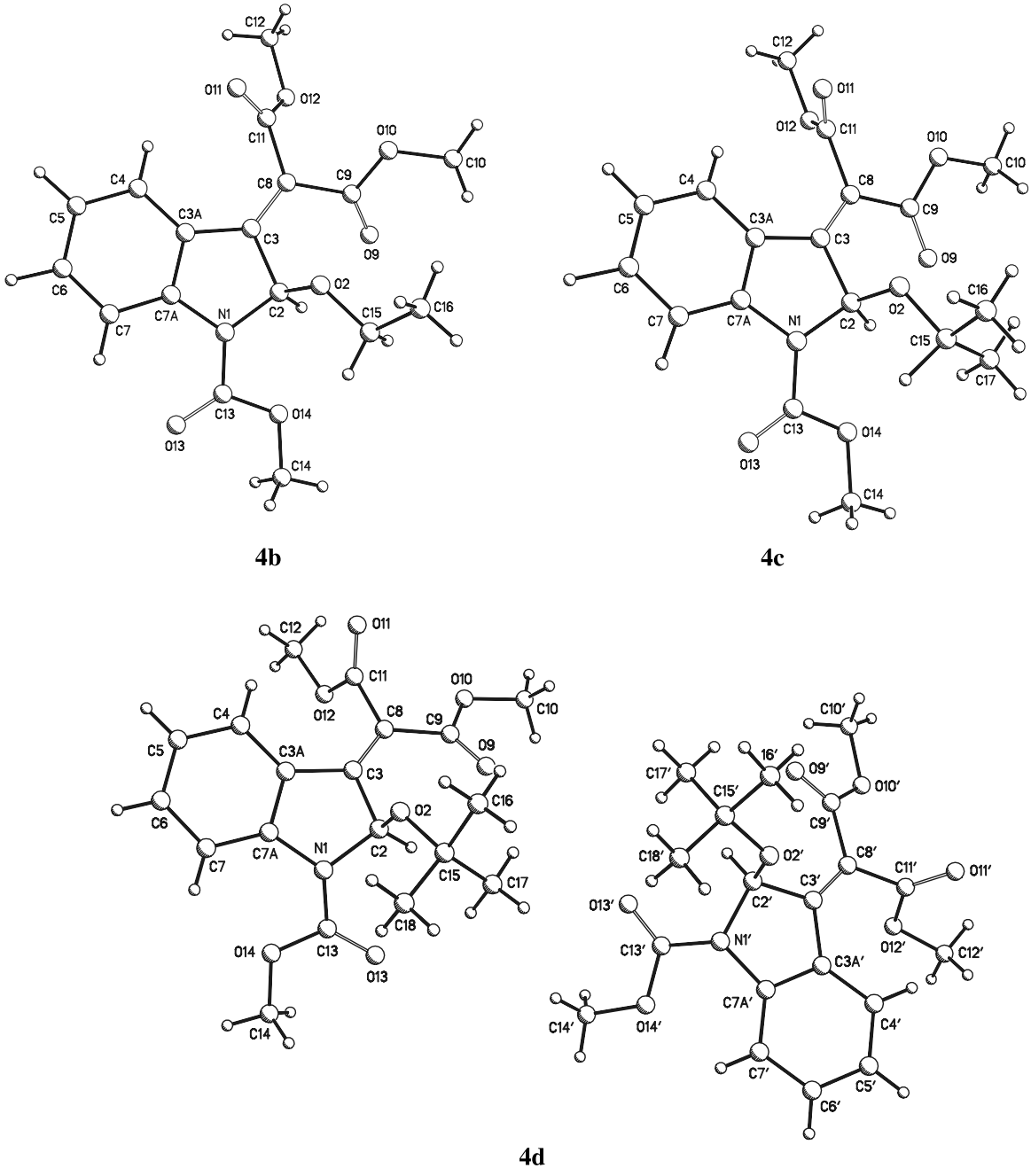


Figure 5. X-ray diffraction structures of **4b–d**.

often observed in conformational processes,[12] the free energy of activation is independent of temperature within the experimental errors, thus, suggesting a negligible $\Delta S^{\neq}$ value.

As evidenced from Table 2, the steric effect exerted by the alkoxy substituent at C-2 plays an important role in the rotation about the N–CO_2Me bond. A relatively low barrier to rotation is observed on going from –OH to –O*t*-Bu, since the rotation barrier for sterically bulkier –O*t*-Bu substituent was found to be about 1.4 kcal/mol lower than that of 2-hydroxyindolenine **3** (Table 2).

2.4. X-ray crystallography

Compounds **4b–d**, **5a–c**, and **6** gave single crystals suitable for X-ray diffraction analysis. The corresponding structures are shown in Figures 5 and 6, while the lattice constants and relevant crystal data are reported in Table 3. From the drawings in Figure 5 it can be seen that the *E* conformation is preferred in the crystalline state for **4b** and **4c**, while conformer *Z* is preferred for **4d**. It is worthnoting that the X-ray crystal structure of **4d** reveals a racemic crystal,[13] arising from a racemate of chiral objects packed in pairs of enantiomers related to each other by improper symmetry elements.

The sum of bond angles around the N atom is 356.7, 358.5, and 355.2° for **4b**, **4c**, and **4d**, respectively, suggesting that the geometry of the nitrogen atom is not totally planar according to the degree of pyramidalization, defined as

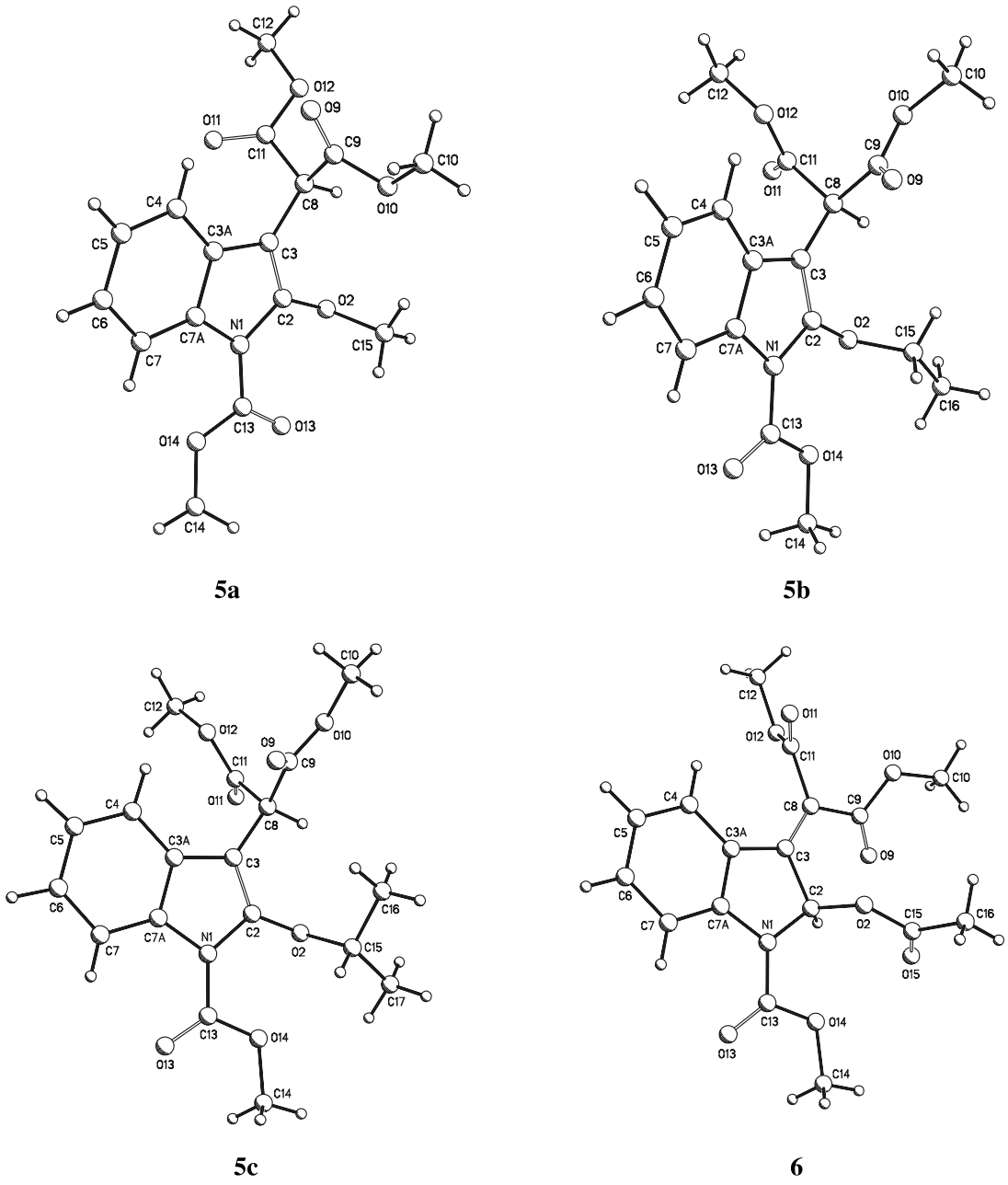


Figure 6. X ray diffraction structures of **5a–c** and **6**.

$360° - \Sigma(R–N–R)$,[14] which results in 3.3, 1.5, and 4.8 for **4b**, **4c** and **4d**, respectively.

From the solid state results it is evident that, on going from **4b** to **4d**, the five membered ring adopts a preferred enveloped conformation in which C-2 is the flap, as is indicated from the dihedral angles C(2)–C(3)–C(3a)–C(7a) and C(2)–N(1)–C(7a)–C(3a) (Table 4). In other words, a higher steric effect of the –OR group increases its preference for the *quasi*-axial position, and from this tendency one could infer that the –OR and $–CO_2Me$ groups could be separated enough from each other to decrease the steric repulsion and therefore, both *E* and *Z* conformers could exist in almost the same ratio, as seems to be the case for **4d** (Table 2). According to this result, H-2 would change from a *quasi*-axial to a *quasi*-equatorial position on going from **3** to **4d**, and as a consequence, this proton should lay in the deshielding region of the exocyclic double bond, as evidenced by the dihedral angle H(2)–C(2)–C(3)–C(8) given in Table 4. Since for the *E* conformer in compounds **3** and **4a–d** the relative chemical shifts order of H-2 in CD_2Cl_2 solutions at 213 K is **3 < 4a < 4b < 4c < 4d** (Table 1), it is evident that an increase of the steric effect of the –OR group is associated to its preference for the *quasi*-axial position.

The *quasi*-axial preference for the alkoxy substituent at C-2 in **4** might be attributed to an anomeric effect[15] present in the O(2)–C(2)–N(1) moiety. The $n \rightarrow \sigma^*$ orbital interactions involving the oxygen lone pair of the alkoxy group and the C(2)–N(1) vacant anti-bonding orbital could also play an important role in the phenomenon. Besides, it is

Table 3. X-ray data collection and processing parameters for **4b–d**, **5a–c**, and **6**

Compound	**2**	**4b**	**4c**	**4d**	**5a**	**5b**	**5c**	**6**
Formula	$C_{15}H_{14}O_6NBr$	$C_{17}H_{19}O_7N$	$C_{18}H_{21}O_7N$	$C_{19}H_{23}O_7N$	$C_{16}H_{17}O_7N$	$C_{17}H_{19}O_7N$	$C_{18}H_{21}O_7N$	$C_{17}H_{17}O_8N$
Size (mm^3)	0.66×0.40×0.12	0.53×0.39×0.32	0.26×0.48×0.29	0.22×0.36×0.48	0.28×0.54×0.31	0.36×0.23×0.29	0.43×0.55×0.50	0.34×0.23×0.29
Crystal system	Monoclinic	Monoclinic	Triclinic	Monoclinic	Monoclinic	Triclinic	Triclinic	Monoclinic
Space group	$P2_1/c$	$P2_1/n$	$P1$ (bar)	$P2_1/c$	$P2_1/c$	$P1$ (bar)	$P1$ (bar)	$P2_1/n$
a (Å)	13.917(1)	7.7377(6)	8.7937(5)	19.682(3)	7.7516(9)	8.039(1)	8.5297(5)	11.613(2)
b (Å)	8.157(1)	15.516(1)	11.0847(6)	11.665(2)	13.414(2)	9.908(2)	10.0004(6)	8.665(1)
c (Å)	15.451(1)	14.405(1)	11.2999(6)	18.610(3)	15.747(2)	11.814(2)	11.8842(7)	17.792(3)
α (°)	90	90	113.662(1)	90	90	66.856(4)	110.514(1)	90
β (°)	111.726(2)	96.522(2)	100.276(1)	113.44(1)	100.960(3)	76.985(5)	105.660(1)	106.614(4)
γ (°)	90	90	106.107(1)	90	90	88.342(5)	93.695(2)	90
V ($Å^3$)	1629.4(2)	1718.3(2)	915.21(9)	3920.1(1)	1607.6(3)	841.2(3)	899.87(9)	1715.80
D_{calcd} ($g\ cm^{-3}$)	1.57	1.35	1.32	1.28	1.39	1.38	1.34	1.41
Z	4	4	2	8	4	2	2	4
μ (mm^{-1})	2.55	0.11	0.10	0.10	0.11	0.11	0.10	0.11
T (K)	298	297	295	298	295	295	295	293
$2\theta_{range}$ (°)	1.58–26.00	1.94–25.99	2.08–25.99	1.13–26.05	2.01–25.86	1.93–26.01	1.93–26.01	1.88–25.10
Total reflections	10851	11200	6143	25513	10847	3215	6012	10364
Unique reflections	3188	3377	3568	7719	1140	2824	3524	3032
R_{int} (%)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Observed reflections	1825	2207 $I\geq 4\sigma(I)$	1973 $I\geq 4\sigma(I)$	2708 $I\geq 4\sigma(I)$	1140 $I\geq 4\sigma(I)$	1846 $I\geq 4\sigma(I)$	2355 $I\geq 4\sigma(I)$	1850 $I\geq 4\sigma(I)$
Parameters	212	255	260	496	222	243	260	253
R (%), R_w (%)	3.8, 8.2	3.8, 10.1	6.1, 15.9	5.3, 9.3	5.2, 14.2	7.4, 21.1	5.0, 13.9	6.3, 16.2
ρ_{max} (e $Å^{-3}$)	0.36	0.16	0.34	0.27	0.55	0.48	0.20	0.23
CCDC no.	276294	276295	276296	276297	276298	276299	276300	276301

Table 4. Experimental (X-ray) and calculated (MMFF) torsion angle (ϕ, °) for **4a–d**

Compound	**4b**		**4c**		**4d**	
Torsion angle	X-ray	MMFF	X-ray	MMFF	X-ray	MMFF
C(2)–C(3)–C(3a)–C(7a)	−5.3	−8.2	−7.5	−10.8	−17.0	−19.1
C(2)–N(1)–C(7a)–C(3a)	7.3	7.6	6.5	8.7	14.6	12.8
H(2)–C(2)–C(3)–C(8)	−54.2	−48.0	−51.9	−43.0	−36.3	−24.8
O(2)–C(2)–N(1)–C(13)	−97.0	−84.0	−92.5	−85.0	−115.1	−90.3

worthnoting that the higher *quasi*-axial preference of the alkoxy substituent at C-2 in **4** increases the pyramidalization of the N atom, and therefore, the delocalization of the N-lone pair with the carbonyl double bond decreases, causing a decrease in the barrier to rotation and therefore, a faster *E*/*Z* interconversion on going from **4d** to **4a** and **3** (Table 2).

Regarding tautomer **5a**, the preferred conformation in the solid state is *Z* (Fig. 5), while for compounds **5b**, **5c**, and **6** the *E* conformer is preferred, in agreement with the results obtained in DMSO-d_6. The five-membered ring in these compounds is planar. For **6** the dihedral angles C(2)–C(3)–C(3a)–C(7a) and C(2)–N(1)–C(7a)–C(3a) are −2.96 and 2.47°, respectively. The geometry of the N atom is trigonal planar since the sum around this atom is 359.4°. The torsion angle C(7a)–N(1)–C(13)–O(13) is −3.6°, which means a delocalization of the nitrogen lone pair with the C═O group.

2.5. Theoretical barriers

In order to find whether a possible correlation could be established between the preferred conformation determined experimentally and the relative stabilities for indolenines **3** and **4a–d**, the energy characteristics for their *E* and *Z* conformers were calculated. Their theoretical and relative energies were estimated from molecular mechanics[16] (MMFF94[4]) and are reported in Table 5. The results obtained from this method match very well with those obtained for the *E*/*Z* ratio of conformers observed in the ^1H NMR spectra in CD_2Cl_2 solutions at 213 K (Fig. 3 and Table 2). In addition, the theoretical barrier to rotation of the N–CO_2CH_3 bond in **3** and **4a–d** were calculated at the MMFF level by the dihedral drive option in the Spartan 04 program (Fig. 7). The $\Delta G^{\neq}$ values for **3** and **4a–d**, obtained from Figure 7, show that conformers *E* and *Z* in **3** and **4a–d**

Table 5. Theoretical MMFF energies (ΔH_f) and relative energies (E_{rel}) for the conformational minima of the *E* and *Z* conformers of **3** and **4a–d**

Compound	Conformer	ΔH_f (kcal/mol)	E_{rel} (kcal/mol)
3	*E*	−72.2	0
	Z	−71.9	0.3
4a	*E*	−57.0	0
	Z	−56.5	0.5
4b	*E*	−59.5	0
	Z	−59.0	0.5
4c	*E*	−54.8	0
	Z	−54.2	0.6
4d	*E*	−41.3	0
	Z	−40.7	0.6

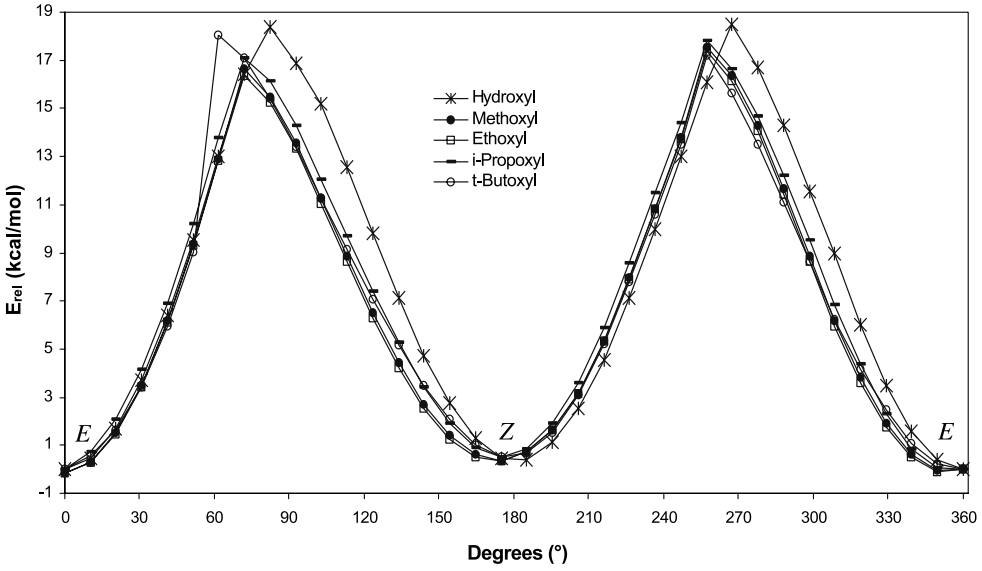


Figure 7. Conformational profiles for **3** and **4a–d** obtained at the molecular mechanics (MMFF94) level of theory. The dihedral angle is defined by C(7a)–N(1)–C(13)–O(13). The minima correspond to the *E* and *Z* conformers.

can be interconverted by overcoming energy barriers of 18.3, 16.6, 16.4, 17.1 and 17.2 kcal/mol, respectively. The energy of the barriers confirm the dependence of the *quasi*-axial position of the alkoxy group at C-2, essentially being determined by the steric interaction between the alkoxy moiety and the carbonyl group of the carbamate. Rotation of the N–CO_2Me bond implies pyramidalization of the nitrogen atom as n–$p^*_{C=O}$-delocalization is progressively decreased, until it disappears completely at a twist of ca. 90° (270°), where the lone pair lies in the nodal plane of the π-system of the carbonyl group. As can be seen in Figure 7, the 90° (270°) rotamer represents a higher energy conformation due to the steric repulsion between the CO_2CH_3 and the OR groups.

The values (Table 4) for the torsion angles C(2)–C(3)–C(3a)–C(7a), C(2)–N(1)–C(7a)–C(3a), H(2)–C(2)–C(3)–C(8), and O(2)–C(2)–N(1)–C(13), calculated by molecular mechanics for **4b–d**, follow the same tendency than those obtained from X-ray diffraction analysis.

3. Conclusions

The synthesis of several indolenines and their transformation to the corresponding indole derivatives was carried out. From the temperature dependence study of the ^{1}H NMR spectra of **4a–d**, as well as the crystallographic and computational analysis, we conclude that two factors can influence the preferred *E* conformation in these indolenines: (a) the coplanarity of the N–CO_2CH_3 group with the aromatic system; (b) the steric interaction of the 2-alkoxyl substituent and the carbomethoxyl group.

4. Experimental

4.1. General experimental procedures

Column chromatography was carried out using Merck silica gel 60 (230–400 mesh).[17] Analytical thin layer chromatography was performed on silica gel 60 F_{254} coated aluminum sheets (0.25 mm thickness) with a fluorescent indicator. Visualization was accomplished with UV light (254 nm). Melting points were determined on a Thermolyne apparatus and are uncorrected. IR spectra were recorded on a Perkin Elmer 2000 FT-IR spectrophotometer. EIMS were obtained on a Hewlett Packard 5989A spectrometer. The ^{1}H and ^{13}C NMR spectra were obtained on a JEOL Eclipse 400 spectrometer working at 400 and 100 MHz, respectively, using DMSO-d_6, CD_2Cl_2 or $CDCl_3$ as the solvent. Low temperature ^{1}H NMR studies were carried out using diluted solutions (3 mg/mL) of **3** and **4a–e** in CD_2Cl_2. Chemical shifts are reported in ppm downfield from tetramethylsilane. Microanalytical determinations were performed using a Perkin Elmer Series II 2400 apparatus. Single crystal X-ray diffraction studies were done on a Bruker Smart 6000 CCD diffractometer.

4.1.1. Dimethyl (1-carbomethoxy-3-indolyl)bromomalonate 2. To a solution of indole **1** (1.64 mmol) in CCl_4 (25 mL) was added Br_2 (3.26 mmol, 167 μL) at once and the resulting mixture was stirred at room temperature for 2 h. The mixture was treated with a saturated solution of $NaHSO_3$ (30 mL) and stirred during 20 min. The aqueous phase was separated and extracted with CH_2Cl_2 (50 mL), and the combined organic layer was washed with brine (2× 30 mL), dried over Na_2SO_4, filtered and evaporated under reduced pressure to dryness to afford **2** as a yellow oil, which solidified on standing (0.617 g, 98%), mp 75–77 °C. Recrystallization from EtOAc/hexane gave colorless crystals, mp 87–89 °C; R_f 0.28 (EtOAc/hexane 2:3). IR (KBr) ν_{max} 3009, 2955, 1742, 1459, 1444 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 8.18 (1H, d, J=8.1 Hz, H-7), 8.10 (1H, s, H-2), 7.61 (1H, d, J=7.6 Hz, H-4), 7.32 (1H, td, J=8.1, 1.1 Hz, H-6), 7.25 (1H, td, J=7.6, 1.1 Hz, H-5), 3.95 (3H, s, NCO_2CH_3), 3.82 (6H, s, $2CO_2CH_3$). ^{13}C NMR ($CDCl_3$) δ 166.1 (2C=O ester), 150.8 (C=O carbamate), 135.6 (C-7a), 127.5 (C-3a), 125.4 (C-2), 125.3 (C-6), 123.2 (C-5), 120.4 (C-4), 116.1 (C-3), 115.3 (C-7), 57.3 (C-8), 54.3 ($2CO_2CH_3$), 54.0

(NCO_2CH_3). EIMS *m/z* (relative intensity) 385 (M^+, 51), 383 (51), 326 (72), 324 (73), 186 (100). HRMS *m/z* 383.0008 (M^+, $C_{15}H_{14}BrNO_6$ requires 383.0004). Anal. Calcd for $C_{15}H_{14}BrNO_6$: C, 46.90; H, 3.67; N, 3.65. Found: C, 46.63; H, 3.70; N, 3.64.

4.2. General procedure for the preparation of 2-alkoxyindolenines 4a–d

Indole **2** (0.63 g, 1.64 mmol) was dissolved in 20 mL of the appropriate alcohol (MeOH, EtOH, *i*-PrOH or *t*-BuOH), 6.5 g of molecular sieves (3 Å) were added, and the resulting mixture was stirred under reflux for 40 min. The mixture was allowed to cool to the room temperature, filtered and the volatiles were evaporated under vacuum. The resulting crude product was purified by silica gel column chromatography, eluting with EtOAc/hexane (2:3, v/v), to give the corresponding indolenines **4a–d**.

4.2.1. Dimethyl (1-carbomethoxy-2-methoxy-3-indolylidene)malonate 4a. This compound was obtained as colorless crystals (0.488 g, 89%), mp 111–113 °C (EtOAc/Et_2O/hexane); R_f 0.32 (EtOAc/hexane 1:3). IR ($CHCl_3$) ν_{max} 2954, 1725, 1471, 1441, 1385 cm^{-1}. ^1H NMR (DMSO-d_6) δ 7.82 (1H, br d, J=7.6 Hz, H-7), 7.54 (1H, td, J=7.6, 0.9 Hz, H-6), 7.35 (1H, d, J=7.7 Hz, H-4), 7.14 (1H, t, J=7.7 Hz, H-5), 6.60 (1H, s, H-2), 3.90 (3H, s, CO_2CH_3), 3.84 (3H, s, NCO_2CH_3), 3.80 (3H, s, CO_2CH_3), 3.35 (3H, br s, OCH_3). ^{13}C NMR (DMSO-d_6) δ 165.5 and 163.0 (C=O ester), 152.5 (C=O carbamate), 146.8 (C-3), 145.2 (C-7a), 133.9 (C-6), 124.4 (C-4), 123.7 (C-5), 122.5 (C-3a), 119.6 (C-8), 115.7 (C-7), 88.6 (C-2), 56.0 (OCH_3), 53.1 (CO_2CH_3), 53.0 (NCO_2CH_3) 52.7 (CO_2CH_3). EIMS *m/z* (relative intensity) 335 (M^+, 40), 303 (100), 276 (44), 105 (48). Anal. Calcd for $C_{16}H_{17}O_7N$: C, 57.31; H, 5.11; N, 4.18. Found: C, 57.17; H, 5.09; N, 4.48.

4.2.2. Dimethyl (1-carbomethoxy-2-ethoxy-3-indolylidene)malonate 4b. This compound was obtained as colorless crystals (0.505 g, 89%), mp 87–88 °C (EtOAc/Et_2O/hexane); R_f 0.34 (EtOAc/hexane 1:3). IR ($CHCl_3$) ν_{max} 2955, 1726, 1472, 1441, 1385 cm^{-1}. ^1H NMR (DMSO-d_6) δ 7.81 (1H, br d, J=7.6 Hz, H-7), 7.52 (1H, t, J=7.6 Hz, H-6), 7.37 (1H, d, J=7.9 Hz, H-4), 7.13 (1H, t, J=7.9 Hz, H-5), 6.62 (1H, s, H-2), 3.90 (3H, s, CO_2CH_3), 3.77–3.68 (2H, m, CH_2), 3.84 (3H, s, NCO_2CH_3), 3.80 (3H, s, CO_2CH_3), 1.05 (3H, t, J=7.2 Hz, CH_3). ^{13}C NMR (DMSO-d_6) δ 165.5 and 163.1 (C=O ester), 152.5 (C=O carbamate), 147.2 (C-3), 145.1 (C-7a), 133.8 (C-6), 124.5 (C-4), 123.6 (C-5), 122.5 (C-3a), 119.5 (C-8), 115.8 (C-7), 88.1 (C-2), 64.9 (OCH_2), 53.0 (CO_2CH_3), 52.9 (NCO_2CH_3), 52.5 (CO_2CH_3), 15.3 (CH_3). EIMS *m/z* (relative intensity) 349 (M^+, 43), 304 (82), 288 (100), 257 (86), 230 (42), 59 (79). Anal. Calcd for $C_{17}H_{19}O_7N$: C, 58.45; H, 5.48; N, 4.01. Found: C, 58.00; H, 5.38; N, 3.87.

4.2.3. Dimethyl (1-carbomethoxy-2-isopropoxy-3-indolylidene)malonate 4c. This compound was obtained as pale yellow crystals (0.507 g, 85%), mp 82–84 °C (EtOAc/Et_2O/hexane); R_f 0.36 (EtOAc/hexane 1:3). IR ($CHCl_3$) ν_{max} 2957, 1726, 1471, 1441, 1385 cm^{-1}. ^1H NMR (DMSO-d_6) δ 7.79 (1H, br d, J=7.1 Hz, H-7), 7.51 (1H, t, J=7.1 Hz, H-6), 7.37 (1H, d, J=7.9 Hz, H-4), 7.14 (1H, t, J=7.9 Hz, H-5), 6.76 (1H, s, H-2), 3.99 (1H, m, CH); 3.90 (3H, s, CO_2CH_3), 3.83 (3H, s NCO_2CH_3), 3.79 (3H, s, CO_2CH_3), 1.12 (3H, d, J=6.1 Hz, CH_3), 1.01 (3H, d, J=6.1 Hz CH_3). ^{13}C NMR (DMSO-d_6) δ 165.5 and 163.1 (C=O ester), 152.6 (C=O carbamate), 147.5 (C-3), 145.0 (C-7a), 133.6 (C-6), 124.5 (C-4), 123.8 (C-5), 123.1 (C-3a), 119.5 (C-8), 116.3 (C-7), 86.2 (C-2), 70.1 (OCH), 53.0 (CO_2CH_3), 52.9 (NCO_2CH_3), 52.5 (CO_2CH_3), 23.5 and 22.1 ($2CH_3$). EIMS *m/z* (relative intensity) 363 (M^+, 25), 304 (100), 276 (27), 257 (86), 230 (41), 59 (49). Anal. Calcd for $C_{18}H_{21}O_7N$: C, 59.50; H, 5.82; N, 3.85. Found: C, 59.17; H, 5.81; N, 3.49.

4.2.4. Dimethyl (1-carbomethoxy-2-terbutoxy-3-indolylidene)malonate 4d. This compound was obtained as colorless crystals (0.393 g, 64%), mp 96–98 °C (EtOAc/Et_2O/hexane); R_f 0.42 (EtOAc/hexane 1:3). IR ($CHCl_3$) ν_{max} 2956, 1727, 1469, 1440, 1369 cm^{-1}. ^1H NMR (DMSO-d_6) δ 7.69 (1H, br d, J=7.8 Hz, H-7), 7.51 (1H, t, J=7.8 Hz, H-6), 7.38 (1H, d, J=7.7 Hz, H-4), 7.16 (1H, t, J=7.8 Hz, H-5), 6.87 (1H, s, H-2), 3.88 (3H, s, CO_2CH_3), 3.83 (3H, s, NCO_2CH_3), 3.79 (3H, s, CO_2CH_3), 1.24 (9H, s, $C[CH_3]_3$). ^{13}C NMR (DMSO-d_6) δ 165.5 and 162.8 (C=O ester), 152.5 (C=O carbamate), 149.2 (C-3), 144.8 (C-7a), 133.3 (C-6), 124.6 (C-4), 124.2 (C-3a), 124.1 (C-5), 119.1 (C-8), 117.6 (C-7), 83.5 (C-2), 76.2 ($C[CH_3]_3$), 53.0 (CO_2CH_3), 52.9 (NCO_2CH_3), 52.6 (CO_2CH_3), 28.1, ($C[CH_3]_3$). EIMS *m/z* (relative intensity) 377 (M^+, 9), 304 (54), 288 (38), 257 (100), 230 (88) 59 (70). Anal. Calcd for $C_{19}H_{23}O_7N_1$: C, 60.47; H, 6.14; N, 3.71. Found: C, 60.34; H, 6.10; N, 3.65.

4.3. General procedure for the preparation 2-alkoxyindoles 5a–d

To a solution of the appropriate indolenine **4a–d** (0.60 mmol) in toluene (10 mL) was added 180 μL (1.2 mmol) of DBU and the resulting mixture was stirred under reflux for 2 h. The mixture was allowed to cool to the room temperature and washed with a saturated solution of NH_4Cl (2×10 mL), dried over Na_2SO_4, filtered and evaporated under reduced pressure to give a yellow oil, which was purified by silica gel column chromatography, eluting with EtOAc/hexane (1:3, v/v), to afford the corresponding indoles **5a–d**.

4.3.1. Dimethyl (1-carbomethoxy-2-methoxy-3-indolyl) malonate 5a. This compound was obtained as colorless crystals (0.105 g, 52%), mp 108–110 °C (EtOAc/Et_2O/hexane); R_f 0.28 (EtOAc/hexane 1:3). IR ($CHCl_3$) ν_{max} 2954, 1725, 1471, 1441, 1385 cm^{-1}. ^1H NMR (DMSO-d_6) δ 8.03 (1H, d, J=8.2 Hz, H-7), 7.44 (1H, d, J=7.4 Hz, H-4), 7.29 (1H, td, J=8.2, 1.2 Hz, H-6), 7.23 (1H, td, J=7.4, 1.0 Hz, H-5), 5.11 (1H, s, H-8), 4.01 (3H, s, OCH_3), 3.90 (3H, s, NCO_2CH_3), 3.70 (6H, s, $2CO_2CH_3$). ^{13}C NMR (DMSO-d_6) δ 167.9 (2C=O ester), 150.2 (C=O carbamate), 149.3 (C-2), 130.8 (C-7a), 125.8 (C-3a), 123.7 (C-6), 123.1 (C-5), 119.3 (C-4), 114.8 (C-7), 98.5 (C-3), 63.9 (OCH_3), 54.0 (NCO_2CH_3), 52.7 ($2CO_2CH_3$), 46.7 (C-8). EIMS *m/z* (relative intensity) 335 (M^+, 45), 276 (100), 200 (34), 59 (54). Anal. Calcd for $C_{16}H_{17}O_7N$: C, 57.31; H, 5.11; N, 4.18. Found: C, 57.35; H, 5.07; N, 4.16.

4.3.2. Dimethyl (1-carbomethoxy-2-ethoxy-3-indolyl)

malonate 5b. This compound was obtained as colorless crystals (0.118 g, 57%), mp 133–134 °C (EtOAc/Et_2O/hexane); R_f 0.38 (EtOAc/hexane 1:3). IR ($CHCl_3$) ν_{max} 2957, 1741, 1461, 1440 cm^{-1}. ^1H NMR (DMSO-d_6) δ 8.02 (1H, d, J=7.8 Hz, H-7), 7.43 (1H, d, J=7.6 Hz, H-4), 7.28 (1H, td, J=7.8, 1.4 Hz, H-6), 7.22 (1H, td, J=7.6, 1.1 Hz, H-5), 5.06 (1H, s, H-8), 4.13 (2H, q, J=7.1 Hz, CH_2), 4.00 (3H, s, NCO_2CH_3), 3.69 (6H, s, $2CO_2CH_3$), 1.33 (3H, t, J=7.1 Hz, CH_3). ^{13}C NMR (DMSO-d_6) δ 167.8 (2C=O ester), 150.2 (C=O carbamate), 148.2 (C-2), 130.9 (C-7a), 125.8 (C-3a), 123.5 (C-6), 122.9 (C-5), 119.3 (C-4), 114.7 (C-7), 98.6 (C-3), 72.6 (CH_2), 53.9 (NCO_2CH_3), 52.5 ($2CO_2CH_3$), 46.9 (C-8), 14.6 (CH_3). EIMS m/z (relative intensity) 349 (M^+, 39), 289 (56), 257 (88), 230 (100), 158 (56), 59 (41). Anal. Calcd for $C_{17}H_{19}O_7N$: C, 58.45; H, 5.48; N, 4.01. Found: C, 58.47; H, 5.41; N, 3.48.

4.3.3. Dimethyl (1-carbomethoxy-2-isopropoxy-3-indolyl)malonate 5c. This compound was obtained as colorless crystals (0.176 g, 81%), mp 113–114 °C (EtOAc/Et_2O/hexane); R_f 0.45 (EtOAc/hexane 1:3). IR ($CHCl_3$) ν_{max} 2981, 2956, 1740, 1461, 1439 cm^{-1}. ^1H NMR (DMSO-d_6) δ 8.02 (1H, dd, J=7.9, 1.0 Hz, H-7), 7.41 (1H, dd, J=8.0, 1.5 Hz, H-4), 7.27 (1H, td, J=7.9, 1.5 Hz, H-6), 7.22 (1H, td, J=8.0, 1.0 Hz, H-5), 4.94 (1H, s, H-8), 4.50 (1H, sep, J=6.2 Hz, OCH), 4.00 (3H, s, NCO_2CH_3), 3.68 (6H, s, $2CO_2CH_3$), 1.26 (6H, d, J=6.2 Hz $2CH_3$). ^{13}C NMR (DMSO-d_6) δ 167.9 (2C=O ester), 150.4 (C=O carbamate), 146.7 (C-2), 131.2 (C-7a), 125.9 (C-3a), 123.4 (C-6), 123.0 (C-5), 119.5 (C-4), 114.8 (C-7), 99.4 (C-3), 79.3 (OCH), 53.9 (NCO_2CH_3), 52.6 ($2CO_2CH_3$), 47.3 (C-8), 21.3 ($2CH_3$). EIMS m/z (relative intensity) 363 (M^+, 7), 321 (30), 289 (64), 257 (100), 230 (90), 158 (33). Anal. Calcd for $C_{18}H_{21}O_7N_1$: C, 59.50; H, 5.82; N, 3.85. Found: C, 59.39; H, 5.80; N, 3.84.

4.3.4. Dimethyl (1-carbomethoxy-2-terbutoxy-3-indolyl) malonate 5d. This compound was obtained as colorless crystals (0.160 g, 71%), mp 119–121 °C (EtOAc/Et_2O/hexane); R_f 0.41 (EtOAc/hexane 1:3). IR ($CHCl_3$) ν_{max} 2956, 1739, 1461, 1439 cm^{-1}. ^1H NMR (DMSO-d_6) δ 7.95 (1H, d, J=8.1 Hz, H-7), 7.42 (1H, ddd, J=7.5, 1.0, 0.8 Hz, H-4), 7.27 (1H, td, J=8.1, 1.0 Hz, H-6), 7.21 (1H, td, J=7.5, 1.0 Hz, H-5), 4.94 (1H, s, H-8), 3.98 (3H, s, NCO_2CH_3), 3.68 (6H, s, $2CO_2CH_3$), 1.32 (9H, s, $C(CH_3)_3$). ^{13}C NMR (DMSO-d_6) δ 167.9 (2C=O ester), 150.9 (C=O carbamate), 145.3 (C-2), 131.3 (C-7a), 125.8 (C-3a), 123.5 (C-6), 122.8 (C-5), 119.8 (C-4), 114.1 (C-7), 100.9 (C-3), 86.7 ($OC(CH_3)_3$), 53.7 (NCO_2CH_3), 52.7 ($2CO_2CH_3$), 47.8 (C-8), 27.8 ($OC[CH_3]_3$). EIMS m/z (relative intensity) 377 (M^+, 4), 321 (38), 259 (81), 257 (100), 230 (83), 186 (31). Anal. Calcd for $C_{19}H_{23}O_7N_1$: C, 60.47; H, 6.14; N, 3.71. Found: C, 60.18; H, 6.08; N, 3.52.

4.3.5. Dimethyl (1-carbomethoxy-2-acetoxy-3-indolylidene)malonate 6. A solution of 0.2 g (0.62 mmol) of indolenine **3** in 5 mL of pyridine was treated with 2 mL (21.2 mmol) of acetic anhydride, and stirred at room temperature for 18 h. The mixture was poured over 20 g of ice and extracted with 50 mL of EtOAc. The organic phase was washed with an aqueous 10% solution of HCl (2×15 mL), water, a saturated solution of $NaHCO_3$ (2×15 mL) and water, dried over anhydrous Na_2SO_4 and evaporated to dryness under vacuum to afford a yellow oil, which was purified by silica gel column chromatography eluting with EtOAc/hexane (2:3, v/v) to give **6** as pale yellow crystals (0.223 g, 99%), mp 119–121 °C ($CHCl_3$/hexane); R_f 0.19 (EtOAc/hexane 1:3). IR ($CHCl_3$) ν_{max} 3016, 1725, 1632, 1600 cm^{-1}. ^1H NMR (DMSO-d_6) δ 7.87 (1H, d, J=8.1 Hz, H-7), 7.61 (1H, s, OCH), 7.55 (1H, td, J=8.1, 1.1 Hz, H-6), 7.45 (1H, d, J=7.8 Hz, H-4), 7.18 (1H, td, J=7.8, 1.0 Hz, H-5), 3.90 (6H, 2s, $2CO_2CH_3$), 3.79 (3H, s, NCO_2CH_3). ^{13}C NMR (DMSO-d_6) δ 166.9 (C=O, acetyl) 165.0 and 162.8 (2C=O ester), 151.4 (C=O carbamate), 145.1 (C-3), 144.9 (C-7a), 133.8 (C-6), 124.6 (C-4), 123.9 (C-5), 122.2 (C-3a), 120.4 (C-8), 115.2 (C-7), 80.8 (C-2), 53.1, 53.0, and 52.9 ($3OCH_3$), 20.5 (CH_3). EIMS m/z (relative intensity) 363 (M^+, 30), 304 (22), 289 (31), 262 (100), 257 (51). Anal. Calcd for $C_{17}H_{17}O_8N$: C, 56.20; H, 4.72; N, 3.86. Found: C, 56.07; H, 4.69; N, 3.74.

4.4. X-ray diffraction analysis of 2, 4b–d, 5a–c, and 6

Single crystals of **2** were grown by slow crystallization from AcOEt/hexane, those of **4b–d** and **5a–c** from EtOAc–Et_2O–hexane, and those of **6** from $CHCl_3$/hexane. A total of 1321 frames were collected at a scan width of 0.3° and an exposure time of 10 s/frame, using Mo radiation (λ=0.7073 Å). These data were processed with the SAINT software package, provided by the diffractometer manufacturer, by using a narrow-frame integration algorithm. An empirical absorption correction was applied. The structures for compounds **2**, **4b**,**c**, **5a–c** and **6** were solved by direct methods using the SHELXS-97[18] program while for **4d**, the SIR02[19] software was used. Both programs are included in the WINGX VI.6[20] package. The structural refinement was carried out by full-matrix least squares on F^2. The non-hydrogen atoms were treated anisotropically, and the hydrogen atoms, included in the structure factor calculation, were refined isotropically. Atomic coordinates, bond lengths, bond angles, anisotropic thermal parameters, hydrogen coordinates, calculated and observed structure factors, and torsion angles are in deposit at the Cambridge crystallographic data center. The deposition numbers are included in Table 3.

Acknowledgements

This research was partially supported by CONACYT (México) grant I35601-E. We thank Q.F.B. Angelina Hernández (CINVESTAV-IPN) for the X-ray analysis support.

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Tetrahedron 61 (2005) 8821–8829
Tetrahedron

Concept and synthetic approach for a kilogram scale synthesis of octa-D-arginine amide nonahydrochloride salt

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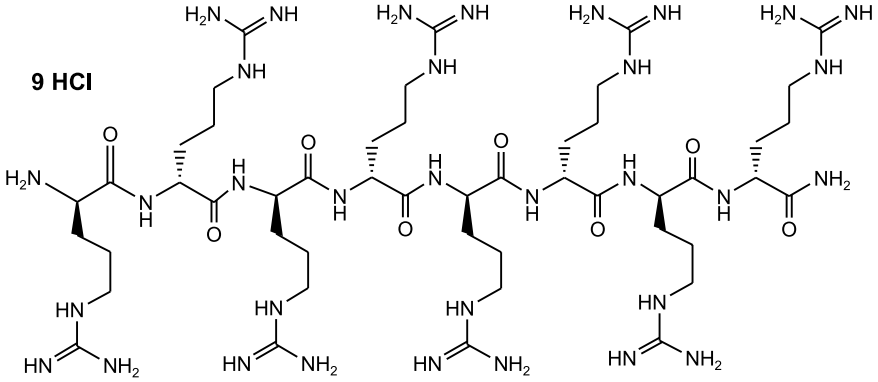
Received 4 April 2005; revised 1 July 2005; accepted 8 July 2005

Available online 28 July 2005

Abstract—Oligomers of arginine, such as octa-D-arginine amide, are excellent transporters for active drugs through cell membranes and tissue. The synthesis of octa-D-arginine amide, as the nonahydrochloride salt, was approached via a solution phase synthetic route involving the preparation of an octa-D-ornithine intermediate, which was then converted into the desired octa-D-arginine compound through a guanidinylation step. The multi-step synthesis was carried out at pilot scale, resulting in the preparation of 700 g of the target molecule. No chromatographic purification was needed at any step of the process.

1. Introduction

Timing and development of scalable processes are critical to the successful production of a peptide-based drug.[1] Although solid phase synthesis is a common approach used to prepare oligopeptides, this method can have limitations, such as the high cost of excess reagents and protected amino acids, the high volume of solvents used, potential limited scalability, and the usual requirement for a chromatographic purification once the peptide is cleaved from the resin. A solution phase synthesis appeared to be a more attractive approach for the preparation of our target molecule, octa-D-arginine amide nonahydrochloride salt[2–11] (**6**) (Scheme 1) in terms of robustness, scalability, and the potential to minimize labor-intensive purification. The limited availability and cost of protected D-arginine derivatives led us to explore the development of a synthesis in which D-ornithine is substituted for the D-arginine residues. A per-guanidinylation step would then



Scheme 1. Octa-D-arginine amide hydrochloride.

Keywords: Amino acids and derivatives; Arginine; Guanidinylation; Peptide transport; Peptide process development; Minimal isolation peptide synthesis.
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doi:10.1016/j.tet.2005.07.037

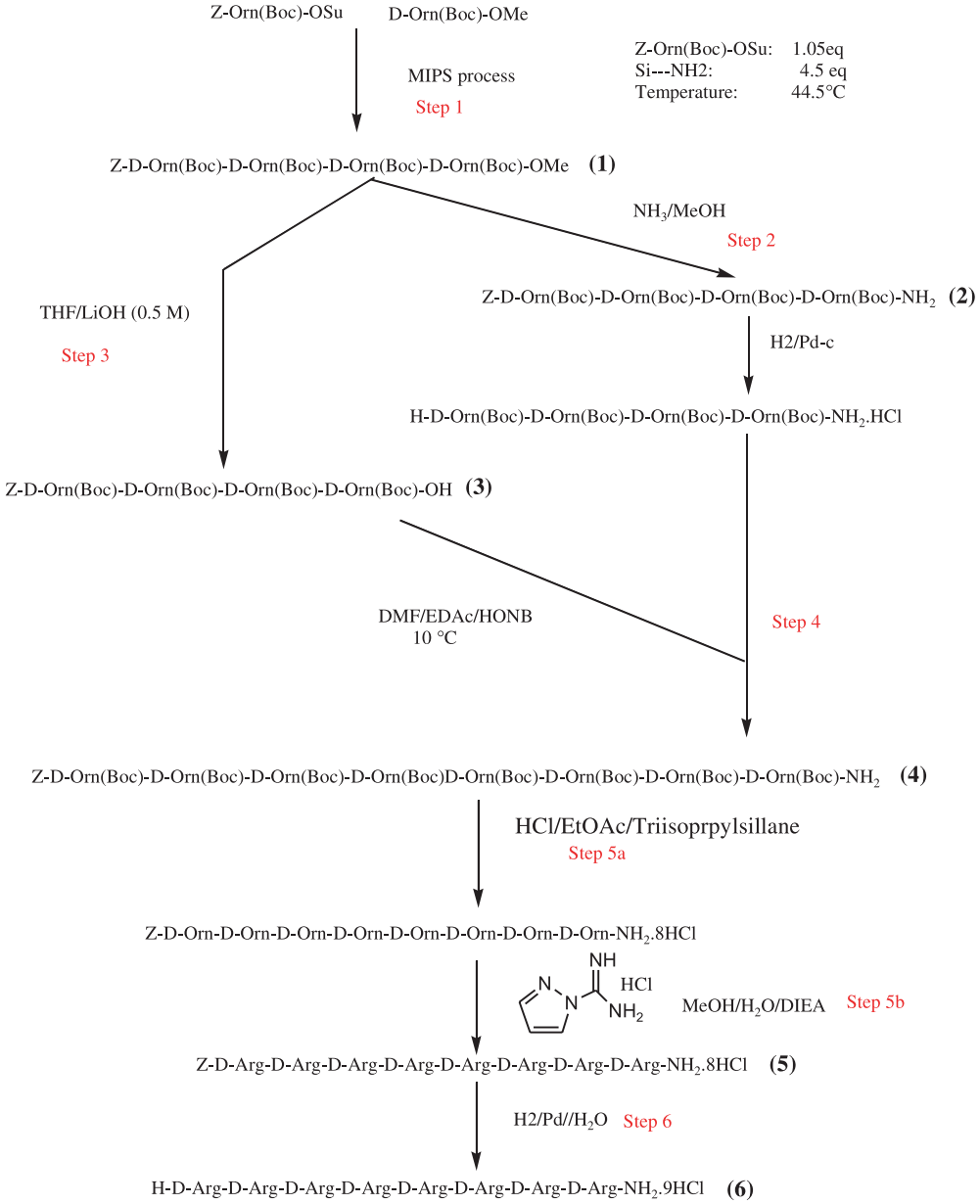
be used for conversion of the 8 ornithine residues to the corresponding arginine residues.[12–18]

The synthetic process, as described in Scheme 2, resulted in the preparation of 0.7 kg of the desired compound, isolated as a hydrochloride salt (**6**). The main features of the process were the synthesis of a key crystalline tetrapeptide ester intermediate (**1**) with only one isolation, followed by the preparation of two crystalline tetrapeptide intermediates (**2**) and (**3**) from the common tetrapeptide ester (**1**). Coupling of the N-terminal deprotected (**2**) with C-terminal deprotected (**3**) gave the fully protected octa-D-ornithine intermediate (**4**), which upon global removal of 8 amine-protecting groups followed by global guanidinylation of the free amines in one step gave the desired D-arginine residues. No chromatographic purification was used at any step of the process. At the final step a simple activated carbon treatment of the desired octa-D-arginine amide hydrochloride salt (**6**) in water was sufficient for purification.

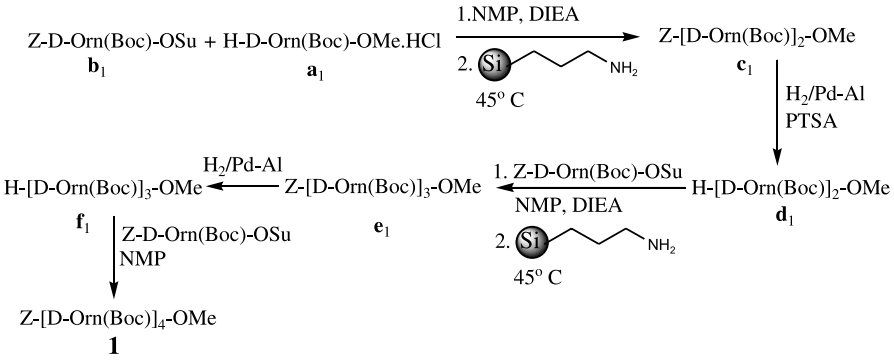
2. Results and discussion

In step 1, *Z*-[D-Orn(Boc)]$_4$-OMe (**1**) (Scheme 3), was prepared following a continuous process (MIPS, minimal isolation peptide synthesis) to minimize the isolation steps of the intermediate dipeptide and tripeptide.

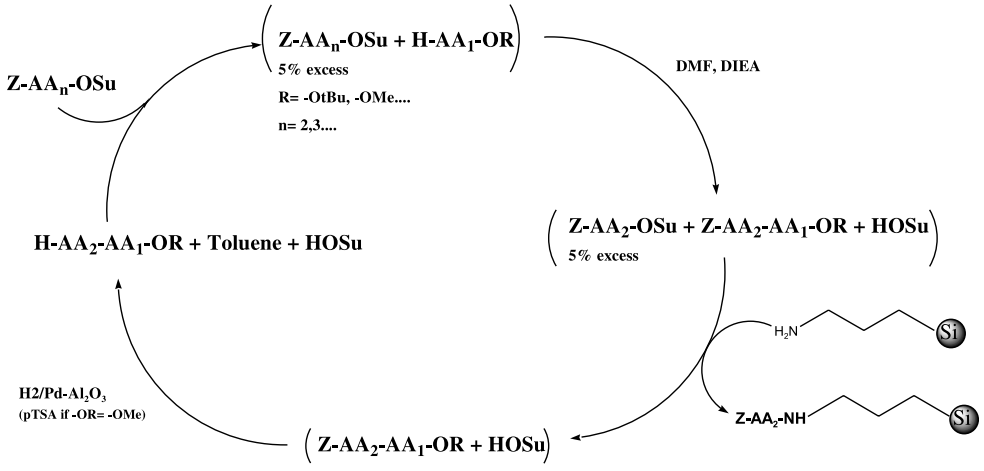
The general procedure for conducting the MIPS process is described in Scheme 4 below. Excess *N*-benzyloxycarbonyl-protected amino acid *N*-hydroxysuccinimide ester[19] (*Z*-AA-OSu) is used to drive the acylation to completion. Residual *Z*-AA-OSu is captured/scavenged by the addition of amine-derivatized insoluble resin or silicagel,[20,21] which



Scheme 2. Octa-D-arginine amide—overall process.



Scheme 3. M.I.P.S process step 1. *Z*-[D-Orn(Boc)$_4$-OMe.



Scheme 4. MIPS process cycle.

is then removed from the reaction mixture by filtration. Subsequent *N*-deprotection of the soluble peptide by hydrogenolysis completes one MIPS cycle. The process can be repeated ($n-1$) times for a peptide of (n) residues in length (Scheme 4).

In the present case, the hydrochloride salt of H-D-Orn(Boc)-OMe (**a$_1$**) was coupled with a 5% molar excess of the preactivated *N*-hydroxysuccinimide ester *Z*-D-Orn(Boc)-OSu (**b$_1$**), in *N*-methylpyrrolidinone in the presence of DIEA, to form *Z*-[D-Orn(Boc)]$_2$-OMe (**c$_1$**). Once the coupling was complete, the excess of *Z*-D-Orn(Boc)-OSu was scavenged with an aminosilica gel, which was then removed by filtration. The remaining solution was then hydrogenated in the presence of *p*-toluenesulfonic acid, giving H-[D-Orn(Boc)]$_2$-OMe·*p*TSA (**d$_1$**). The same process described above was repeated to obtain the tripeptide intermediate, *Z*-[D-Orn(Boc)]$_3$-OMe (**e$_1$**). The solution was carried through an additional hydrogenolysis/coupling reaction. No scavenging reaction was necessary for removal of the excess active ester at this stage. The NMP solution of the fully-protected tetrapeptide (**1**) was diluted with isopropyl acetate and the resulting mixture was washed with aqueous solutions, followed by distillation of the organic layer. The fully-protected tetrapeptide was then crystallized from methanol/isopropylacetate/heptane.

To proceed to step 2 (Scheme 2), half of step 1, *Z*-[D-Orn(Boc)]$_4$-OMe (**1**) was converted to the corresponding C-terminal amide, *Z*-[D-Orn(Boc)]$_4$-NH$_2$ (**2**), via treatment with a solution of anhydrous ammonia in methanol. The ammonolysis was performed at a temperature of no more than 10 °C in order to minimize racemization and impurity formation, which were observed at room temperature. Following distillation of the ammonia, the material was isolated by crystallization from methanol/isopropyl acetate/heptane.

Saponification of the second half of step 1 material led to *Z*-[D-Orn(Boc)]$_4$-OH (**3**) (step 3; Scheme 2). The saponification was carried out with lithium hydroxide in a mixture of tetrahydrofuran and water. The temperature was maintained at no more than 10 °C to control racemization of the C-terminal residue, which was observed at room temperature. The reaction was then quenched by adjusting to pH 4 with aqueous HCl solution. The product was extracted into isopropyl acetate. After a series of aqueous washes, the organic layer was distilled and *Z*-[D-Orn(Boc)]$_4$-OH (**3**) was crystallized from methanol/isopropylacetate/heptane.

These conditions of saponification were chosen in order to avoid pre-mature removal of the benzyloxycarbonyl protecting group under basic aqueous conditions. A summary of the different conditions tried during lab studies is reported in Table 1.

Table 1. Conditions examined for removal of methyl ester

	Saponification	Temperature (°C)	*Z*-[D-Orn(Boc)]$_4$-OH HPLC peak area (%)
NMP/NaOH (1 N) (1.3 equiv)	Not complete	0	35
MeOH/NaOH (1 N) (1.3 equiv)	Not complete	0	95
NMP/LiOH (1 N) (1.3 equiv)	Complete (slow overnight)	0	35
MeOH/LiOH (1 N) (1.3 equiv)	Complete (slow overnight)	0	95
THF/LiOH (1 N) (1.3 equiv)	Complete (4 h)	0	99.7
THF/LiOH (0.5 N) (1.5 equiv)	Complete (4 h)	10	99.9

Catalytic hydrogenation of *Z*-[D-Orn(Boc)]$_4$-NH_2 (**2**) was carried out in dimethylformamide in the presence of 5% palladium on alumina (35 psi, 25 °C). Complete removal of the benzyloxycarbonyl group went smoothly to afford a dimethylformamide solution of the tetrapeptide H-[D-Orn(Boc)]$_4$-NH_2.

The free amine solution was coupled with *Z*-[D-Orn(Boc)]$_4$-OH (**3**) in the presence of *N*-ethyl-*N*-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDAc·HCl) and *N*-hydroxy-5-norbornene-2,3-dicarboximide (HONB)[22,23] to obtain the fully protected octapeptide, *Z*-[D-Orn(Boc)]$_8$-NH_2 (**4**), which precipitated from the reaction mixture by addition of water. The crude, protected octapeptide was isolated by filtration. The wet cake was redissolved in a mixture of acetone and water and the desired product was precipitated by additional water. This re-precipitation procedure resulted in an increase in purity from 89.5 to 92.5%.

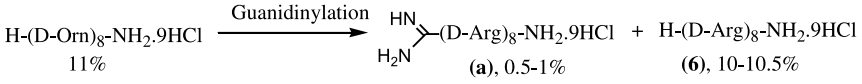
HONB was chosen as a coupling auxiliary because of the low levels of racemization (0.3%) that were observed in lab studies on this segment condensation. Other coupling conditions that were attempted for the condensation of *Z*-[D-Orn(Boc)]$_4$-OH with H-[D-Orn(Boc)]$_4$-NH_2 are summarized in Table 2.

The deprotection of *Z*-[D-Orn(Boc)]$_8$-NH_2 (**4**) was accomplished with HCl in ethyl acetate in the presence of triisopropylsilane as a scavenger at ambient temperature. This is a solid-to-solid reaction. After completion of the reaction, the product was filtered and dried under a nitrogen atmosphere.

Guanidinylation of *Z*-D-(Orn)$_8$-NH_2·8HCl was achieved with 1-pyrazole-1-carboxamidine hydrochloride salt[5–17,18] and diisopropylethylamine in methanol–water. Upon reaction completion, isopropyl alcohol was added to precipitate the product, *Z*-(D-Arg)$_8$-NH_2·8HCl (**5**). While most of the diisopropylethylamine hydrochloride salt (DIEA·HCl), formed during the guanidinylation was removed, some of this salt (3.5 wt%) remained trapped with the solids. *Z*-(D-Arg)$_8$-NH_2 (**5**) was precipitated from a methanol solution by addition of isopropanol to give product with trace (0.01 wt%) DIEA·HCl.

The intermediate at step 4, *Z*-[D-Orn(Boc)]$_8$-NH_2 (**4**), was obtained with a purity of >92 pa%, as judged by HPLC. Several impurities formed during step 5, that is the synthesis of the protected octa-arginine amide *Z*-(D-Arg)$_8$-NH_2 (**5**). The impurities are primarily attributed to the global-Boc removal step. A summary of these impurities, which were then carried to the guanidinylation step and indentified by LC/MS, is reported below:

1. Partial removal of *Z* protecting group during the Boc removal step. During the guanidinylation reaction step, about 10% of final product, H-(D-Arg)$_8$-NH_2 (**6**), was observed, as well an over-guanidinylated (**a**) side product (0.5–1%) (Scheme 5).



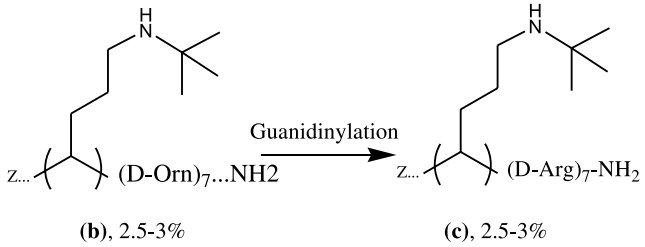
Scheme 5. Over guanidinylated impurity.

2. During the removal of the Boc protecting groups, a mixture of mono-*t*-butylated impurities was observed (**b**). These impurities were then carried to the next step, resulting in the formation of a partially guanidinylated impurity (**c**) at the same level as (**b**). An investigation to improve the Boc deprotection reaction conditions resulted in a mixture of HCl/EtOAc/triisopropylsilane showing the most promise. Under these conditions, the level of *t*-butylated impurities was decreased from 8 to 2.5% (Scheme 6).

Table 2. Racemization study during segment condensation

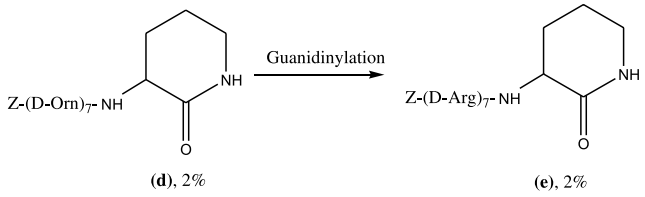
	Temperature (°C)	Racemization level of *Z*-DDDLDDDD-NH_2 [D=D-Orn(Boc)- and L=L-Orn(Boc)] (%)
DMF/EDAc in water/HOBt·H_2O	−10	1.1
DMF/EDAc/HOBt	−10	0.7
DMF/EDAc/HOBt	0	0.9
DMF/EDAc/HOBt	Room temperature	3.1
DMF/EDAc in water/HONB	10	0.3
DMF/EDAc/HONB	10	0.3

The level of racemization was measured by HPLC.



Scheme 6. *tert*-Butylation side reaction.

3. During the deprotection, lactam formation occurred at the C-terminal ornithine residue (**d**), which led to the formation of a partially guanidinylated impurity (**e**) (Scheme 7).



Scheme 7. Lactam formation.

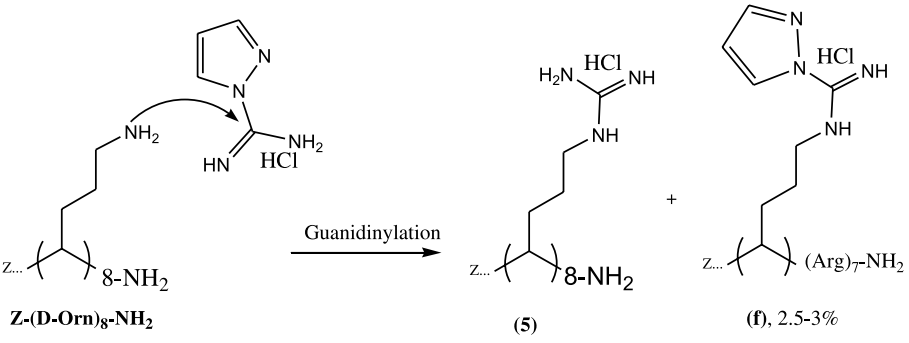
4. Impurity (**f**) formed during the guanidinylation. Formation of this impurity is attributed to ammonia serving as the leaving group in place of pyrazole. This side reaction was minimized when the guanidinylation reaction was carried out in methanol/water as the reaction solvent rather than a *N*-methylpyrrolidinone/water mixture (Scheme 8).

Hydrogenolysis of Z-(D-Arg)$_8$-NH$_2$ was carried out in H_2O with 10 wt% of 5% Pd/Al_2O_3 in the presence of 1 equiv HCl. The crude product solution was subjected to two carbon treatments to effect purification. Among the eight types of carbon evaluated, L3S provided adequate purification with best recovery. Laboratory experiments showed that two carbon treatments (1.8×loading for each) gave product at 94 pa% and an overall recovery of 65%. The pyrazole·HCl by-product from the guanidinylation step was also effectively removed. A summary of these experiments is reported in Tables 3 and 4.

The solution was then concentrated to a volume of 8 L by reverse osmosis and the final product was isolated by lyophilization.

3. Conclusion

A solution phase synthesis of H-(D-Arg)$_8$-NH$_2$·9HCl (**6**) was developed and demonstrated at the kilogram scale. The minimum isolation process for the preparation of tetrapeptide (**1**) was an efficient approach to this key intermediate. Conversion of the fully protected octa-D-ornithine intermediate (**4**) to the protected octa-D-arginine intermediate (**5**) avoided the need to develop what would be a more costly and complicated process if protected D-arginine was the starting material. Given the need and opportunity, the process as described herein could be used for the preparation of larger quantities of octa-D-arginine amide hydrochloride.



Scheme 8. Guanidinylation side reaction.

Table 3. Carbon treatment of H-(D-Arg)$_8$-NH$_2$·9HCl[a]

	H-(D-Arg)$_8$-NH$_2$·9HCl HPLC peak area (%)	H-(D-Arg)$_8$-NH$_2$·9HCl recovery (%)
L3S	89.4	91
5SC/G	88.5	79
ENO-PC	85.7	70
CPL	85.9	71
5% Pd/C	86.2	90
10% Pd/C	86.4	91
Darco G60	89.5	85

[a] Crude H-(D-Arg)$_8$-NH$_2$·9HCl at 77.2 peak area % was used for the study.

Table 4. Carbon purification using L3S[a]

	HPLC peak area (%)	Pyrazole peak area (%)	Recovery (%)
1st Carbon treatment	90.73	1.63	81.6
2nd Carbon treatment	93.62	0.15	80.5

[a] Crude H-(D-Arg)$_8$-NH$_2$·9HCl at 79.9% peak area % and pyrazole at 6.1% peak area % was used for the study.

4. Experimental

4.1. General

All starting materials were obtained from commercial suppliers and used as received. Ultrapure aminosilica gel was obtained from SiliCycle, Inc, Que., Canada. Carbon treatment was performed with carbon L3S from CECA S.A (France). Triisopropylsilane, 99%, was obtained from Johnson Mathey. All reactions were performed under an atmosphere of nitrogen. Analytical thin layer chromatography was performed on MERCK 5×10 cm SG-60F 250 μ precoated silicagel plates. Visualization was accomplished with iodine vapor. RP-HPLC analysis were performed on Agilant 100 Series HPLC, using analytical columns Chromolith RP-18e, 100×4.6 mm; for compounds **1**, **2**, **3** and Water Symmetry300 C18, 250×4.6 mm, 5 μm; for compounds **4**, **5**, **6**; with UV detection (λ=205 nm). The products were eluted utilizing a solvent gradient [*A*= water:acetonitrile:perchloric acid (850:150:1); *B*=water:-acetonitrile:perchloric acid (150:850:1); for compounds **1**, **2**, **3** and *A*=0.1% perchloric acid in water; *B*=0.1% perchloric acid in water/acetonitrile 5:95%; for compounds **4**, **5**, **6**]. The reverse osmosis was performed on a Millipore unit with a Nanomax-50 membrane (1.8″ cartridge). The lyophilization was performed with an FTS tray freeze dryer. NMR spectra were measured on a Varian UI500 magnetic resonance spectrometer (^{1}H NMR spectra at 500 MHz, ^{13}C NMR spectra at 125 MHz). Mass spectra were collected with an Agilent Series 1100 LC/MS using electrospray (ES-MS). The column was Technikrom Kromasil KR100-5C18-250A, 250×4.6 nm, (λ=205 nm); elution with *A*= 0.1% trifluoroacetic acid in water; *B*=0.1% trifluoroacetic acid in acetonitrile. For compounds **1**, **2**, **3**, and **4**, the mass reported corresponds to the structure with the loss of a Boc protecting group (100), which is common with ES-MS.

4.1.1. Preparation of *Z*-[D-Orn(Boc)]$_4$-OMe (1). *Coupling stage for Z*-[D-Orn(Boc)]$_2$-OMe (**c$_1$**). To a reactor were charged successively 2.0 kg of H-D-Orn(Boc)-OMe·HCl (**a$_1$**) and 3.4 kg of *Z*-D-Orn(Boc)-OSu (**b$_1$**) (5% molar excess). *N*-Methylpyrrolidinone (27.0 kg) was charged to the same reactor to dissolve the solids under agitation at an internal temperature of 20±5 °C. Diisopropylethylamine (1.0 kg) was then charged to the reactor and the mixture was stirred for 2–3 h. An in process sample was taken for TLC and HPLC analysis. Analytical results indicated that no starting material, H-D-Orn(Boc)-OMe·HCl (**a$_1$**), was detected and the reaction mixture was used directly in the next step.

Scavenging stage for Z-[D-Orn(Boc)]$_2$-OMe (**c$_1$**). To a reactor was charged 1.2 kg of Si-amine (amine-derivatized insoluble silicagel), then the coupling reaction mixture from above was charged to the same reactor. *N*-Methylpyrrolidinone (5.0 kg) was used as a rinse. The mixture was stirred at 45±5 °C. An in process sample was pulled after 12 h for HPLC analysis. The disappearance of *Z*-D-Orn(Boc)-OSu was monitored by HPLC. Analytical results indicated that no starting material, *Z*-D-Orn(Boc)-OSu, was detected. The temperature was adjusted to 20±5 °C and the reaction mixture was filtered through a filter pot to a hydrogenolysis vessel, which was previously charged with 1.5 kg of *p*-toluenesulfonic acid and 0.25 kg of 5% palladium on alumina. Two rinses with *N*-methylpyrrolidinone (4.5 kg) were used, then the mixture carried to the next step.

*Hydrogenolysis stage, H-[D-Orn(Boc)]$_2$-OMe·pTSA (**d$_1$**).* The solution was hydrogenated at approximately 40 psi while maintaining the temperature at 20±5 °C. Every 2 h the reactor was subjected to 2 vent/purge cycles to remove CO_2 generated from the *Z* group hydrogenolysis. After 6 h, an in process sample was pulled to monitor the reaction by HPLC. Analytical result showed the reaction was complete (*Z*-[D-Orn(Boc)]$_2$-OMe (**c$_1$**)=0.0%).

Coupling stage for Z-[D-Orn(Boc)]$_3$-OMe (**e$_1$**). The hydrogenated solution was filtered through a filter pot from the hydrogenolysis vessel to a reactor, which was previously charged with 3.3 kg of *Z*-D-Orn(Boc)-OSu. *N*-Methylpyrrolidinone (4.5 kg) was used as a rinse of the hydrogenolysis vessel and the filter pot. The solution was mixed for 15 min to dissolve the solids, then diisopropylethylamine (1.1 kg) was charged to the reactor and the mixture was stirred at an internal temperature of 20±5 °C. An in process sample was taken after 4 h for HPLC analysis. Analytical results indicated that no starting material, H-[D-Orn(Boc)]$_2$-OMe (**d$_1$**), was detected.

Scavenging stage for Z-[D-Orn(Boc)]$_3$-OMe (**e$_1$**). To a reactor was charged 1.4 kg of Si-amine, then the coupling reaction mixture was charged to the same reactor. *N*-Methylpyrrolidinone (5.0 kg) was used as a rinse. The mixture was stirred at 45±5 °C. An in process sample was pulled after 36 h for HPLC analysis. The disappearance of *Z*-D-Orn(Boc)-OSu was monitored by HPLC. Analytical results indicated that 0.67% of *Z*-D-Orn(Boc)-OSu was detected and the reaction was called complete. The temperature was adjusted to 20±5 °C and the reaction mixture was filtered through a filter pot to a hydrogenation vessel, which was previously charged with 0.3 kg of 5% palladium on alumina. Two rinses with *N*-methylpyrolidinone (4.5 kg) were used and the mixture was carried to the next step.

*Hydrogenolysis stage, H-[D-Orn(Boc)]$_3$-OMe (**f$_1$**).* The solution was hydrogenated at approximately 40 psi while maintaining the temperature at 20±5 °C. After 2 h the reactor was subjected to 2 vent/purge cycles to remove CO_2 generated from the *Z* group hydrogenolysis. After 3 h, an in process sample was pulled to monitor the reaction by HPLC. The analytical result showed the reaction was complete (*Z*-[D-Orn(Boc)]$_3$-OMe (**e$_1$**)=0.0%).

Coupling stage for Z-[D-Orn(Boc)]$_4$-OMe (**1**). The hydrogenated solution was filtered through a filter pot from the

hydrogenolysis vessel to a reactor, which was previously charged with 3.24 kg of *Z*-D-Orn(Boc)-OSu. *N*-Methylpyrrolidinone (4.5 kg) was used as a rinse of the hydrogenolysis vessel and the filter pot. The mixture was stirred at an internal temperature of 20±5 °C. An in process sample was taken after 3 h for HPLC analysis. Analytical results indicated that no starting material, H-[D-Orn(Boc)]$_3$-OMe (**f$_1$**), was detected.

The reaction mixture was diluted with 116 kg of isopropyl acetate and the resulting solution was washed with purified water (89 kg). The organic layer was then distilled under vacuum at a jacket temperature of 45 °C to a volume of approximately 40 L. To the reactor was charged 11 kg of methanol and the mixture was stirred at about 40 °C until all solids dissolved. The temperature was then adjusted to about 20 °C and 124 kg isopropyl acetate was charged to the reactor. Crystalline solids formed and after 6 h heptane (18 kg) was charged to the reactor to complete the crystallization. Solids were filtered and rinsed with isopropyl acetate (30 kg). The wet cake was dried under vacuum at 50 °C for 8 h (sample tested for loss on drying showed 0% LOD) to give 5.26 kg (72%) of **1**, with a purity of 95.4% by HPLC. MS 924 $[M+H-(Boc)]^+$. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.35 (s, 36H), 1.39 (m, 8H), 1.45–1.69 (m, 8H), 2.89 (m, 8H), 3.60 (s, 3H), 3.97 (td, J=8.54, 4.73 Hz, 1H), 4.21–4.25 (m, 3H), 4.96–5.01 (m, J=12.50 Hz, 1H), 5.00–5.04 (m, J=12.70 Hz, 1H), 6.72–6.76 (m, 4H), 7.31–7.36 (m, 5H), 7.39 (m, 1H, N), 7.85–7.88 (m, 2H), 8.20 (d, J=7.17 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 28.2, 29.3–29.6, 39.3–39.5, 51.7, 51.6, 51.9–52.0, 54.4, 65.4, 77.4, 127.6–128.3, 137.0, 155.5–155.6, 155.9, 171.6, 171.2, 171.7, 173.3. HRMS (EI) *m/z* 1023.5953 (calcd for $C_{49}H_{83}N_8O_{15}$ 1023.5972).

4.1.2. Preparation of *Z*-[D-Orn(Boc)]$_4$-NH$_2$ (2). To a reactor were charged 2.5 kg of *Z*-[D-Orn(Boc)]$_4$-OMe (**1**) and 22 kg of methanol. The mixture was stirred at 45 °C until solids were dissolved. The temperature was then adjusted to 5 °C and 7.5 kg of anhydrous ammonia was charged to the reactor while maintaining the temperature at no more than 15 °C. At the end of the addition, the temperature was adjusted to 10 °C and the solution was mixed. An in process sample was pulled after 43 h for HPLC analysis. Analytical results indicated that 0.9% starting material, *Z*-[D-Orn(Boc)]$_4$-OMe (**1**), remained and the reaction was called complete (in-process limit for *Z*-[D-Orn(Boc)]$_4$-OMe (**1**) was set at 1.0%).

The temperature was adjusted to 20 °C and the contents of the reactor were distilled under vacuum at this temperature for about 40 min. The distillation was then carried out at 45 °C under vacuum until the mixture reached 8 L. To the reactor was charged 5 kg of methanol and the mixture was stirred at a 45 °C until solids dissolved. The temperature was then adjusted to 20 °C and 54 kg of isopropyl acetate was charged to the reactor. The mixture was stirred for 12 h to crystallize *Z*-[D-Orn(Boc)]$_4$-NH$_2$ (**2**). Heptane (14 kg) was charged to the reactor to complete the crystallization. After 3 h, the solids were filtered and rinsed with a mixture of methanol (1.5 kg)/isopropyl acetate (17 kg)/heptane (4.3 kg). The wetcake was dried under vacuuum at 50 °C for 8 h (LOD 0%) to give 1.92 kg (76%) of product, with a purity of 97% by HPLC. MS 910 $[M+H-(Boc)]^+$. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.35 (s, 36H), 1.36 (m, 8H), 1.46–1.61 (m, 8H), 2.89 (m, 8H), 3.98 (td, J=8.54, 4.73 Hz, 1H), 4.21–4.25 (m, 3H), 4.97–5.01 (m, J=12.50 Hz, 1H), 5.01–5.05 (m, J=12.50 Hz, 1H), 6.73–6.76 (m, 4H), 7.01–725 (s, 2H), 7.31–7.36 (m, 5H), 7.39 (m, 1H), 7.73 (d, J=7.63 Hz, 1H), 7.91 (d, J=7.78 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 25.9–26.1, 28.5, 29.3–29.6, 39.5, 51.9, 52.2–52.3, 54.4, 65.4, 77.4, 127.6–128.3, 136.9, 155.5, 155.9, 171.1, 171.5, 171.8, 173.3. HRMS (EI) *m/z* 1008.5967 (calcd for $C_{48}H_{82}N_9O_{14}$ 1008.5976).

4.1.3. Preparation of *Z*-[D-Orn(Boc)]$_4$-OH (3). To a reactor were charged 2.5 kg of *Z*-[D-Orn(Boc)]$_4$-OMe (**1**), tetrahydrofuran (22 kg), and purified water (6.7 kg). The contents of the reactor were mixed at room temperature until solids dissolved. The internal temperature was adjusted to 10 °C and 7.33 kg of 0.5 M lithium hydroxide solution was charged. The solution was mixed while maintaining the temperature at no more than 15 °C. An in process sample was pulled after 2 h for HPLC analysis. Analytical results indicated that 1.0% starting material, *Z*-[D-Orn(Boc)]$_4$-OMe (**1**), remained and the reaction was called complete. While maintaining the internal temperature at no more than 15 °C, 37.5 kg of 0.1 N HCl solution was charged to the reactor, resulting in a solution at pH<4. The reaction mixture was diluted with 30 kg of isopropyl acetate. The aqueous layer was separated and was back-extracted with 30 kg of isopropyl acetate. The organic layers were combined and washed with purified water (30 kg). The organic layer was then distilled under vacuum at 45 °C to a volume of approximately 15 L. To the reactor was charged 5 kg of methanol and the mixture was stirred at 45 °C until solids dissolved. The temperature was then adjusted to 20 °C and 52.5 kg of isopropyl acetate was charged to the reactor. The mixture was stirred for 12 h to effect crystallization of *Z*-[D-Orn(Boc)]$_4$-OH (**3**). Heptane (20.4 kg) was charged to the reactor to complete the crystallization. After 3 h the solids were filtered and rinsed with a mixture of methanol (1.2 kg)/isopropyl acetate (14 kg)/heptane (6 kg). The wet cake was dried under vacuum at 50 °C for 8 h to an LOD of 0% (limit 2%), to give 1.84 kg (77%) of product, with a purity of 97.2% by HPLC analysis. MS 909 $[M+H-(Boc)]^+$. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.35 (s, 36H), 1.36 (m, 8H), 1.47–1.62 (m, 8H), 2.89 (m, 8H), 3.98 (m, 1H), 4.14 (m, 1H), 4.24–4.27 (m, 2H), 4.96–5.01 (m, J=12.50 Hz, 1H), 5.01–5.06 (m, J=12.50 Hz, 1H), 6.72–6.77 (m, 4H), 7.30–7.35 (m, 5H), 7.38 (m, 1H), 7.85–7.89 (m, 2H), 8.03 (m, 1H), 12.54 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 25.9–26.1, 28.3, 28.5–29.6, 39.5, 51.7, 51.9–52.1, 54.4, 65.4, 77.4, 127.7–128.3, 137.0, 155.6, 155.9, 171.3, 171.4, 171.8, 173.3. HRMS (EI) *m/z* 1009.5796 (calcd for $C_{48}H_{81}N_8O_{15}$ 1009.5816).

4.1.4. Preparation of *Z*-[D-Orn(Boc)]$_8$-NH$_2$ (4). To a reactor were charged 1.75 kg of *Z*-[D-Orn(Boc)]$_4$-NH$_2$ (**2**), 0.175 kg of palladium on alumina, and dimethylformamide (30 kg). The solution was then hydrogenated at 40 psi and 20 °C. An in-process sample was pulled after 3 h for HPLC analysis. Analytical results indicated that no starting material, *Z*-[D-Orn(Boc)]$_4$-NH$_2$ (**2**), was detected and the reaction was called complete. The catalyst was removed by

filtration and the filter cake was washed with dimethylformamide (15 kg).

To a second reactor were charged 1.67 kg of *Z*-[D-Orn(Boc)]$_4$-OH (**3**), 0.34 kg of HONb and the hydrogenated solution from above. The mixture was stirred until all solids dissolved, then the internal temperature was adjusted to 10 °C. To the same reactor was added a solution of EDAc·HCl (0.50 kg) in purified water (0.50 kg). The mixture was stirred at 10 °C. An in process sample was pulled after 6 h for HPLC analysis. Analytical results indicated that 0.33% starting material, *Z*-[D-Orn(Boc)]$_4$-OH (**3**), remained and the reaction was called complete. The internal temperature was adjusted to 20 °C. Under high agitation, 48 kg of purified water was charged to the mixture. The solution was stirred for 1 h. The precipitate was filtered and rinsed with a mixture of dimethylformamide (5 kg) and water (5 kg), followed by a rinse with 10 kg of water. The material was blown dry with nitrogen for 2 h, then the wet cake was recharged to the same reactor, followed by the addition of acetone (42 kg) and purified water (16 kg). The mixture was stirred until solids were dissolved, then 31 kg of purified water was slowly charged to the reactor. The mixture was stirred for 1 h. The precipitate was filtered and rinsed with a mixture of acetone (5 kg) and water (6 kg). The material was blown dry with nitrogen for 2 h, then the wet cake was dried under vacuum at 50 °C to an LOD of 0%. Upon completion of isolation and drying, 2.29 kg (71%) was obtained, with a purity of 92.5% (HPLC area %). MS/ES 1765 $[M+H-(Boc)]^+$. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.35 (s, 72H), 1.36 (m, 16H), 1.62–1.46 (m, 16H), 2.89 (m, 16H), 3.98 (m, 1H), 4.14 (m, 1H), 4.21 (m, 6H), 4.97–5.01 (m, J=12.50 Hz, 1H), 5.01–5.05 (m, J=12.50 Hz, 1H), 6.71–6.76 (m, 8H), 7.01–7.25 (s, 2H), 7.31–7.35 (m, 5H), 7.40 (m, 1H), 7.72 (m, 1H), 7.89 (m, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 25.9–26.1, 28.2, 29.4–29.6, 39.5, 52.0, 52.2, 52.4, 65.4, 77.4, 127.6–128.2, 136.9, 155.5, 156.0, 171.1, 171.4, 173.3. HRMS (EI) *m/z* for $[M+2H]^{2+}$ 933.0669 (calcd for $C_{88}H_{153}N_{17}O_{26}$ 933.0659).

4.1.5. Preparation of *Z*-(D-Arg)$_8$-NH$_2$·8HCl (5). To a reactor were charged 2.5 kg of *Z*-[D-Orn(Boc)]$_8$-NH$_2$ (**4**) and a mixture of ethyl acetate (60 kg)/anhydrous HCl (7.3 kg)/triisopropylsilane (10.6 kg). The slurry was stirred at 20 °C. An in process sample was pulled after 90 min for HPLC analysis. Analytical results indicated that no starting material, *Z*-[D-Orn(Boc)]$_8$-NH$_2$ (**4**), was detected. The solids were filtered and rinsed with ethyl acetate (30 kg), then the wet-cake was blown dry with nitrogen for 12 h to give *Z*-(D-Orn)$_8$-NH$_2$·8HCl (2.5 kg). The dried *Z*-(D-Orn)$_8$-NH$_2$·8HCl was charged to a reactor, followed by 3.4 kg of 1*H*-pyrazole-1-carboxamidine hydrochloride, water (2.7 kg), and methanol (16.2 kg). The mixture was stirred until solids dissolved. Diisopropylethylamine (4.2 kg) was charged and the mixture was stirred. An in process sample was pulled after 4 h for HPLC analysis. Analytical results indicated that no starting material, *Z*-(D-Orn)$_8$-NH$_2$·8HCl, was detected. The reaction mixture was slowly poured into 144 kg of isopropyl alcohol under high agitation to precipitate the product. The reactor was rinsed with 10 kg of methanol and the rinse was charged to the IPA slurry. After 20 min, the precipitate was filtered and rinsed with isopropyl alcohol (30 kg). The wet cake was dried under vacuum at 40 °C for 8 h to give 2.1 kg of product. The product was dissolved in methanol (16.5 kg). The solution was slowly poured into 63 kg of isopropyl alcohol under high mixing. The reactor was rinsed with methanol (1.6 kg) and the rinse was added to the IPA slurry, the precipitate was filtered and rinsed with isopropyl alcohol (8 kg). The wet cake was dried under vacuum at 55 °C for 12 h to give 1.44 kg (69%) product with a purity of 77% by HPLC. MS 701 $[M/2+H]^+$ (as free base), 467 $[M/3+H]^+$ (as free base). ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.51 (m, 16H), 1.59–1.71 (m, 16H), 3.13 (m, 16H), 4.17 (m, 1H,), 4.27 (m, 7H), 4.97–5.01 (m, J=12.50 Hz, 1H), 5.01–5.06 (m, J=12.50 Hz, 1H), 7.14–7.51 (s, 2H), 7.31–7.36 (m, 5H) 7.03–7.47 (m, 24H), 7.50 (m, 1H), 7.84 (m, 8H), 8.22 (m, 7H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 25.0–25.1, 28.9–29.0, 40.3–40.4, 52.1, 52.1–52.5, 65.5, 127.7–128.4, 136.9, 157, 171.4–171.5, 171.8, 173.4. HRMS (EI) *m/z* for $[M+3H]^{3+}$ 467.6313 (calcd for $C_{56}H_{108}N_{33}O_{10}$ 467.6314).

4.1.6. Preparation of H-(D-Arg)$_8$-NH$_2$·9HCl (6). To a reactor were charged 2.0 kg of *Z*-(D-Arg)$_8$-NH$_2$·8HCl (**5**), 0.2 kg of 5% palladium on alumina, water (25 kg), and 0.115 kg of 12 N hydrochloric acid. The mixture was then hydrogenated at 30 psi and 20 °C. An in process sample was pulled after 4 h for HPLC analysis. Analytical results indicated that no starting material, *Z*-(D-Arg)$_8$-NH$_2$·8HCl (**5**), was detected. The catalyst was filtered and washed with water (25 kg). The hydrogenated solution was charged to a reactor followed by a rinse with water (3 kg). L3S activated carbon (2.4 kg) was charged and the mixture was stirred at ambient temperature. After 4 h the mixture was filtered and the carbon was washed with water (3×3 kg).

The combined filtrates were transferred to a reactor followed by a water rinse (3 kg). To the same reactor was charged 1.4 kg of L3S activated carbon and the mixture was stirred at ambient temperature. After 4 h the mixture was filtered and the carbon was washed with water (3×3 kg). The combined filtrates were concentrated by reverse osmosis from a volume of approximately 45–8 L. The concentrated solution was then frozen and lyophilized to give H-(D-Arg)$_8$-NH$_2$·9HCl (0.7 kg) with a purity of 94% by HPLC. MS 634 $[M/2+H]^+$ (as free base), 623 $[M/3+H]^+$ (as free base). ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.51 (m, 16H), 1.60–1.71 (m, 16H), 3.13 (m, 16H), 4.17 (m, 1H), 4.27 (m, 7H), 7.04–7.49 (m, 24H), 7.50 (m, 2H), 7.14–7.52 (s, 2H), 7.84–7.93 (m, 8H), 8.02 (m, 1H), 8.23 (m, 4H), 8.33 (m, 1H), 8.76 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 24.2, 25.0, 28.8–29.0, 40.3–40.4, 52.1–52.5, 157.0, 169.8, 171.1, 171.2, 171.5, 173.4. HRMS (EI) *m/z* for $[M+3H]^{3+}$ 422.9527 (calcd for $C_{48}H_{102}N_{33}O_8$ 422.9524).

Acknowledgements

We thank Ian Marsden for his critical ^{1}H NMR analysis.

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Tetrahedron 61 (2005) 8830–8835
Tetrahedron

Synthesis and absolute configuration of annuionone A

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Received 2 June 2005; revised 6 July 2005; accepted 7 July 2005

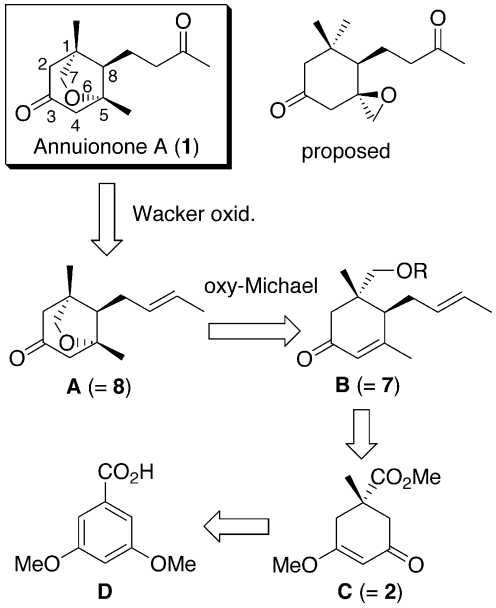
Available online 26 July 2005

Abstract—Synthesis of both enantiomers of annuionone A (**1**), an allelopathic agent isolated from *Helianthus annuus* (sunflower), was accomplished. The absolute configuration of the naturally occurring **1** was determined to be 1*S*,5*R*,8*R*.

1. Introduction

Annuionone A and its relatives (annuionones B–G) have been isolated from *Helianthus. annuus* (sunflower) by Macías and his co-workers as allelopathic agents.[1] Although, the initially proposed structure of annuionones A was structurally unique ionone-type bisnorsesquiterepenes with an *exo*-epoxide moiety,[1a] the correct structure has been confirmed by our racemate synthesis to be **1** as depicted in Scheme 1.[2,3] However, the absolute configurations of the naturally occurring **1** and other annuionones have still remained unsolved. Thus, we initiated the synthesis of optically active annuionone A (**1**) to determine its absolute stereochemistry. Herein, we report the synthesis of both enantiomers of **1** in detail.

Our synthetic plan for **1** is illustrated in Scheme 1. The target compound **1** was obtainable by Wacker oxidation of the intermediate **A**. For construction of the 6-oxabicyclo-[3.2.1]octane framework of **A**, we adopted oxy-Michael reaction as an appropriate methodology. The intermediate **B** should be available from the known compound **C**, which was prepared from **D**. This synthetic plan was completely based on our racemate synthesis.[2] Thus, the remaining problem was how to prepare the optically active form. In other words, there was a need to carry out optical resolution or asymmetric reaction at an appropriate stage.



Scheme 1. Structure of annuionone A (**1**) and its synthetic plan.

2. Results and discussion

First, the known ketoester **2** was prepared according to the reported procedure.[4] The chemoselective reduction of the ester group of **2** was achieved by treatment with LDA and then DIBAL, furnishing **3** (86%). The hydroxyl group of **3** was protected as the *t*-butyldimethylsilyl (TBS) ether to give **4** (91%). For installation of the 3-oxobutyl side-chain, we first attempted homoallylation of **4**, because a homoallyl group was thought to be convertible into a 3-oxobutyl group with ease by Wacker oxidation.[5] Unfortunately, however, the conversion of **4** into **5** was not so successful. Even after an in-depth study of the reaction conditions, the observed

Keywords: Sesquiterpene; Allelochemical; Absolute configuration; Optical resolution.
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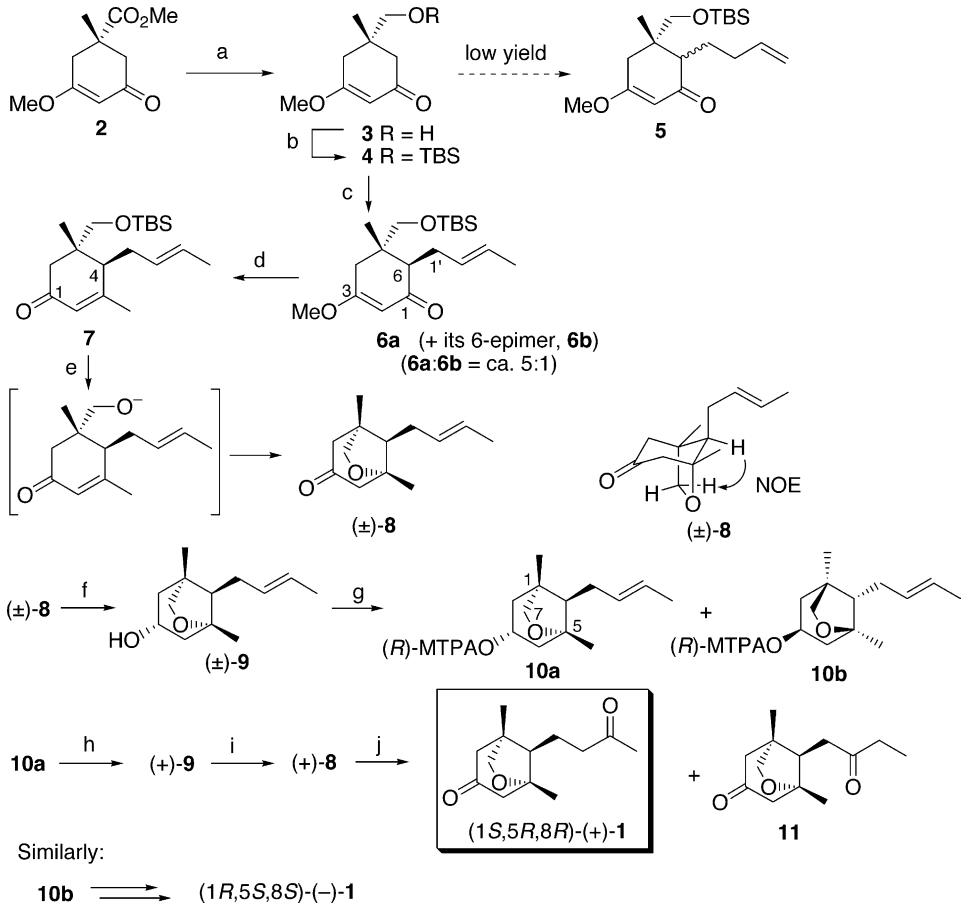
doi:10.1016/j.tet.2005.07.017

best yield was only 18% under the following conditions: LDA, THF, HMPA, 4-iodo-1-butene, −78 °C to room temperature. Thus, we then turned to crotylation of **4**, which was performed by treatment with LDA and crotyl bromide in the presence of HMPA, furnishing a chromatographically inseparable mixture of **6a** and **6b** (90%; **6a**:**6b**=ca. 5:1, based on ^{1}H NMR analysis). Although, it could be easily deduced that the major diastereomer should be the β-isomer (**6a**) by consideration of the steric factor, its relative configuration could not be determined by NOE studies, because the signals assigned as 6- and 1′-H were poorly resolved. At this stage, we have also found that **6a** contained its (*Z*)-isomer (~10%),[6] that was derived from geometrically impure crotyl bromide (commercially available). However, we continued our synthesis without further purification, because we already knew that the (*Z*)-isomer was inseparable but would not impede our tasks all through the steps. From now on, thus, the presence of (*Z*)-isomer is not mentioned to avoid difficulty of description and also unnecessary confusion. Treatment with MeLi and the following work-up could convert **6a/b** into a mixture of **7** and its 4-epimer (85%). At this stage, diastereomers with regard to C-4 were separated by careful chromatography to give the diastereomerically pure **7**.[7] After removal of the TBS protecting group of **7**, subsequent intramolecular oxy-Michael addition took place to give the desired adduct (±)-**8** (86%). The relative configuration of **8** was confirmed by the observation of NOE as depicted in Scheme 2.

As mentioned before, there was a need to secure an optically active intermediate. We envisioned adopting optical resolution. For the purpose of conventional optical resolution, (±)-**8** was converted into the corresponding secondary alcohol (±)-**9** by diastereoselective reduction with L-Selectride® (78%). Our first attempts to resolve (±)-**9** by enzymatic resolution did not succeed, even after screening of over ten hydrolytic enzymes such as Lipase PS, Lipase AK, Chirazyme L-2, etc. The next attempt was the so-called classical resolution using a chiral derivatizing reagent. To our delight, we found that the corresponding (*R*)-α-methoxy-α-(trifluoromethyl)phenylacetates [(*R*)-MTPA esters, **10a/b**] were chromatographically separable.[8] By careful chromatography, we obtained the less polar diastereomer **10a** (41%) and the more polar **10b** (40%), respectively. Application of a modified Mosher's method[9] made it possible to determine the absolute configurations of **10a/b**, because the crucial $\Delta\delta$ (**10a**−**10b**) values were clearly observed in 1-Me, 5-Me and 7-H as shown in Figure 1. The obtained diastereomerically pure **10a** was subjected to reductive cleavage of the resolving auxiliary to furnish (+)-**9** (76%). This was then oxidized with *o*-iodoxybenzoic acid (IBX)[10] to give (+)-**8** (86%). Finally,



Scheme 2. Synthesis of both enantiomers of **1**. Reagents and conditions: (a) LDA, THF, DIBAL, toluene (86%); (b) TBSCl, imidazole, DMF (91%); (c) LDA, THF, crotyl bromide, HMPA (90%); (d) MeLi, Et_2O, satd aq NH_4Cl (85%); (e) TBAF, THF (86%); (f) L-Selectride®, THF, −78 °C (78%); (g) (*S*)-MTPACl, DMAP, pyridine, 40 °C (41% for **10a** and 40% for **10b**); (h) $LiAlH_4$, THF (76%); (i) IBX, DMSO (86%); (j) $PdCl_2$, CuCl, O_2, aq DMF (64% for **1** and 6% for **11**).

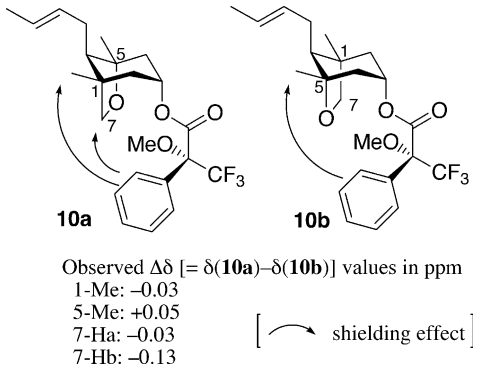


Figure 1. Absolute configurations of **10a/b**.

Wacker oxidation[5] of (+)-**8** was followed by SiO_2 column chromatography to furnish (1*S*,5*R*,8*R*)-(+)-**1** (64%), $[\alpha]_D^{22}$ + 16.9 (c=0.50 in $CHCl_3$), {lit.[1a] $[\alpha]_D^{25}$ +12.3 (c=0.4 in $CHCl_3$)}, and its regioisomer **11** (6%). The various spectral data of synthetic (1*S*,5*R*,8*R*)-(+)-**1** are in good accord with those of the natural product. Similarly, **10b** was also converted into (1*R*,5*S*,8*S*)-(−)-**1**, $[\alpha]_D^{23}$ −17.6 (c 0.35 in $CHCl_3$). Therefore, the absolute configuration of the naturally occurring annuionone A (**1**) was determined as 1*S*,5*R*,8*R*.

3. Conclusion

In conclusion, we have accomplished the first synthesis of both enantiomers of annuionone A (**1**), which enabled us to determine the absolute configuration of naturally occurring annuionones A to be 1*S*,5*R*,8*R*. Furthermore, it can be easily deduced that other annuionones such as B, E and F must have the same absolute configuration.

4. Experimental

4.1. General

IR spectra were measured on a Shimadzu IR-408 spectrophotometer. ^{1}H NMR spectra were recorded at 300 MHz on a JEOL JNM-AL300 spectrometer. The peak for $CHCl_3$ in $CDCl_3$ (at δ 7.26) was used for the internal standard. Chemical shifts are reported in ppm on the δ scale and J-values are given in Hz. ^{13}C NMR spectra were recorded at 75 MHz on a JEOL JNM-AL300 spectrometer. The peak for $CDCl_3$ (at δ 77.0) was used for the internal standard. Optical rotations were taken with a HORIBA SEPA-300 polarimeter. Mass spectra were measured with a JEOL JMS-SX102A spectrometer. Column chromatography was carried out on Kanto Chemical Co. Inc. Silica Gel 60 N (spherical, neutral, 63–210 μm). Flash chromatography was carried out on Kanto Chemical Co. Inc. Silica Gel 60 N (spherical, neutral, 40–50 μm). Analytical HPLC was carried out with a Shimadzu LC-10A system. TLC analyses were performed on Merck silica gel plates 60 F_{254}.

4.1.1. 5-Hydroxymethyl-3-methoxy-5-methyl-2-cyclohexen-1-one (3). *n*-BuLi (1.59 M in hexane; 3.8 mL, 6.0 mmol) was added to a solution of (*i*-Pr)$_2$NH (0.97 mL, 6.6 mmol) in anhydrous THF (6 mL) at 0 °C under Ar. After stirring for 30 min at 0 °C, a solution of **2** (0.98 g, 4.9 mmol) in anhydrous THF (10 mL) was added at −78 °C. After stirring for 30 min with warming to −15 °C gradually, DIBAL (1.5 M in toluene; 7.4 mL, 11 mmol) was added at −78 °C. After the mixture was stirred for 1 h at room temperature, it was quenched with MeOH and saturated aqueous Rochelle's salt, and extracted with EtOAc. The organic layer was dried with $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel chromatography to give **3** (0.73 g, 86%) as a colorless oil: IR (neat) 3400 (s, O–H), 1650, 1630, 1600 (each s, C=O, C=C) cm^{-1}; ^{1}H NMR δ 1.02 (s, 3H, 5-Me), 2.09 (dd, J= 1.2, 16.5 Hz, 1H, 6-H), 2.10 (dd, J=1.2, 17.4 Hz, 1H, 4-H), 2.42 (d, J=16.5 Hz, 1H, 6-H), 2.60 (d, J=17.4 Hz, 1H, 4-H), 2.75 (br s, 1H, OH), 3.40 (s, CH_2OH, 2H), 3.68 (s, OMe, 3H), 5.34 (s, 1H, 2-H); ^{13}C NMR δ 22.6, 37.1, 37.4, 45.4, 55.8, 69.5, 101.0, 177.2, 199.5; HRMS (EI) *m/z* calcd for $C_9H_{14}O_3$: 170.0943, found 170.0939.

4.1.2. 5-*t*-Butyldimethylsilyloxymethyl-3-methoxy-5-methyl-2-cyclohexen-1-one (4). Imidazole (0.86 g, 13 mmol) and TBSCl (1.9 g, 13 mmol) were added to a solution of **3** (1.44 g, 8.46 mmol) in DMF (15 mL) at room temperature. After stirring overnight, the reaction mixture was quenched with saturated aqueous NH_4Cl and extracted with EtOAc. The organic layer was washed with water and brine, dried with $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel chromatography to give **4** (2.18 g, 91%) as a colorless oil: IR (neat) 1660 (s, C=O), 1610 (s, C=C), 1255 (m, SiMe) cm^{-1}; ^{1}H NMR δ 0.01 (s, 3H, SiMe), 0.02 (s, 3H, SiMe), 0.87 (s, 9H, Bu^t), 0.98 (s, 3H, 5-Me), 2.01 (dd, J=1.5, 17.1 Hz, 1H, 4-H), 2.04 (dd, J=1.5, 16.5 Hz, 1H, 6-H), 2.44 (d, J= 16.5 Hz, 1H, 6-H), 2.65 (d, J=17.1 Hz, 1H, 4-H), 3.30 (d, J=9.9 Hz, 1H, CH_2OTBS), 3.35 (d, J=9.9 Hz, 1H, CH_2OTBS), 3.68 (s, 3H, OMe), 5.34 (br s, 1H, 2-H); ^{13}C NMR δ −5.65, −5.61, 18.2, 22.5, 25.8, 37.1, 37.7, 45.6, 55.7, 69.9, 101.0, 177.0, 199.4; HRMS (EI) *m/z* calcd for $C_{15}H_{28}O_3Si$: 284.1808, found 284.1798.

4.1.3. (5*S,6*S**,2′*E*)-6-(2′-Butenyl)-5-*t*-butyldimethylsilyloxymethyl-3-methoxy-5-methyl-2-cyclohexen-1-one (6a).** *n*-BuLi (1.58 M in hexane; 10.4 mL, 16.4 mmol) was added to a solution of (*i*-Pr)$_2$NH (2.5 mL, 18 mmol) in anhydrous THF (20 mL) at 0 °C under Ar. After stirring for 30 min at 0 °C, a solution of **4** (3.62 g, 12.7 mmol) in anhydrous THF (20 mL) was added at −78 °C. After stirring for 30 min with warming to −15 °C gradually, a solution of crotyl bromide (Aldrich; 2.64 g, 16.6 mmol) in anhydrous HMPA (5 mL) was added at −15 °C. After the mixture was stirred for 30 min with warming to room temperature gradually, the reaction mixture was quenched with saturated aqueous NH_4Cl and extracted with EtOAc. The organic layer was washed with water and brine, dried with $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel chromatography to give a mixture of **6a** and **6b** (3.90 g, 90%; **6a**:**6b**=ca. 5:1) as a colorless oil.

Compound **6a**: IR (neat) 1660 (s, C=O), 1620 (s, C=C), 1255 (m, SiMe) cm^{-1}; ^{1}H NMR δ 0.01 (s, 3H, SiMe), 0.02 (s, 3H, SiMe), 0.87 (s, 12H, Bu^t, 5-Me), 1.61 (d, 3H, J= 6.0 Hz, 4′-H$_3$), 1.99 (d, 1H, J=17.4 Hz, 4-H), 2.01–2.37 (m, 3H, 6-H, 1′-H$_2$), 2.71 (d, 1H, J=17.4 Hz, 4-H), 3.19 (d,

1H, $J=9.9$ Hz, CH_2OTBS), 3.52 (d, 1H, $J=9.9$ Hz, CH_2OTBS), 3.66 (s, 3H, OMe), 5.29 (s, 1H, 2-H), 5.35–5.54 (m, 2H, 2′-, 3′-H); ^{13}C NMR δ −5.6, 17.9, 18.2, 18.7, 25.8, 25.8, 28.4, 37.2, 40.9, 51.6, 55.5, 68.4, 100.6, 125.5, 130.3, 175.1, 201.5; HRMS (EI) *m/z* calcd for $C_{19}H_{34}O_3Si$: 338.2277, found 338.2279.

Compound **6b**: ^{1}H NMR (only clearly observed peaks) δ 1.06 (s, 5-Me), 2.44 (d, $J=17.4$ Hz, 4-H), 3.21 (d, $J=9.9$ Hz, CH_2OTBS), 5.27 (s, 2-H).

4.1.4. (4*S,5*S**,2′*E*)-4-(2′-Butenyl)-5-*t*-butyldimethylsilyloxymethyl-3,5-dimethyl-2-cyclohexen-1-one (7).** MeLi (1.20 M in Et_2O; 18.3 mL, 15.3 mmol) was added to a solution of **6** (1.85 g, 5.48 mmol) in anhydrous Et_2O (10 mL) at 0 °C under Ar. After stirring overnight with warming to room temperature gradually, the reaction mixture was quenched with saturated aqueous NH_4Cl and extracted with EtOAc. The organic layer was washed with water and brine, dried with $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel chromatography to give a diastereomeric mixture of **7** and its α-isomer (1.50 g, 85%). This mixture was then carefully separated by flash chromatography to afford pure **7** (0.53 g, 30%) and the remaining diastereomeric mixture (0.72 g, 41%).

Compound **7**: IR (neat) 1670 (s, C=O), 1630 (s, C=C), 1260 (m, SiMe) cm^{-1}; ^{1}H NMR δ −0.03 (s, 3H, SiMe), −0.02 (s, 3H, SiMe), 0.86 (s, 9H, Bu^t), 1.02 (s, 3H, 5-Me), 1.62 (d, $J=5.7$ Hz, 3H, 4′-H_3), 1.95 (s, 3H, 3-Me), 2.12 (d, $J=17.7$ Hz, 1H, 6-H), 2.30 (d, $J=17.7$ Hz, 1H, 6-H), 2.27–2.45 (m, 3H, 4-H, 1′-H_2), 3.22 (d, $J=9.6$ Hz, 1H, CH_2OTBS), 3.34 (d, $J=9.6$ Hz, 1H, CH_2OTBS), 5.33–5.52 (m, 2H, 2′-, 3′-H), 5.86 (s, 1H, 2-H); ^{13}C NMR δ −5.7, −5.6, 17.9, 18.2, 21.9, 24.4, 25.8, 32.3, 41.0, 43.5, 44.8, 69.4, 126.0, 127.0, 128.8, 164.5, 199.2; HRMS (EI) *m/z* calcd for $C_{19}H_{34}O_2Si$: 322.2328, found 322.2336.

4.1.5. (1*S,5*R**,8*R**,2′*E*)-8-(2′-Butenyl)-1,5-dimethyl-6-oxabicyclo[3.2.1]octan-3-one (±)-8.** TBAF (1.0 M in THF; 7.4 mL, 7.4 mmol) was added to a solution of **7** (2.00 g, 6.20 mmol) in THF (20 mL). After stirring for 4 h at room temperature, the reaction mixture was quenched with saturated aqueous NH_4Cl and extracted with EtOAc. The organic layer was washed with water and brine, dried with $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel chromatography to give (±)-**8** (1.11 g, 86%) as a colorless oil: IR (neat) 1710 (s, C=O) cm^{-1}; ^{1}H NMR δ 1.05 (s, 3H, 1-Me), 1.28 (s, 3H, 5-Me), 1.67 (d, $J=5.1$ Hz, 3H, 4′-H_3), 1.78 (t, $J=7.2$ Hz, 1H, 8-H), 2.02–2.40 (m, 6H, 2-, 4-, 1′-H_2), 3.56 (dd, $J=2.4$, 7.8 Hz, 1H, 7-H), 3.62 (d, $J=7.8$ Hz, 1H, 7-H), 5.38–5.57 (m, 2H, 2′-, 3′-H); ^{13}C NMR δ 17.8, 20.6, 24.9, 28.8, 43.1, 48.7, 49.5, 53.0, 78.3, 83.2, 126.8, 129.1, 209.7; HRMS (EI) *m/z* calcd for $C_{13}H_{20}O_2$: 208.1463, found 208.1466.

4.1.6. (1*S,3*R**,5*R**,8*R**,2′*E*)-8-(2′-Butenyl)-1,5-dimethyl-6-oxabicyclo[3.2.1]octan-3-ol (±)-9.** L-Selectride® (1.02 M in THF; 0.60 mL, 0.61 mmol) was added to a solution of (±)-**8** (0.11 g, 0.53 mmol) in anhydrous THF (4 mL) at −78 °C under Ar. After stirring for 30 min at −78 °C, the reaction mixture was quenched with saturated aqueous NH_4Cl and extracted with EtOAc. The organic layer was washed with water and brine, dried with $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel chromatography to give (±)-**9** (87 mg, 78%) as a colorless oil: IR (neat) 3400 (s, O–H) cm^{-1}; ^{1}H NMR δ 0.93 (s, 3H, 5-Me), 1.23 (s, 3H, 1-Me), 1.50 (t, $J=7.5$ Hz, 1H, 8-H), 1.61–1.91 (m, 5H, 2-, 4-H_2, 1′-H), 1.63 (br d, $J=5.7$ Hz, 3H, 4′-H_3), 2.06–2.15 (m, 1H, 1′-H), 3.43 (d, $J=9.0$ Hz, 1H, OH), 3.45 (dd, $J=2.4$, 7.5 Hz, 1H, 7-H), 3.88–3.95 (m, 1H, 3-H), 3.93 (d, $J=7.5$ Hz, 2H, 7-H), 5.30–5.49 (m, 2H, 2′-, 3′-H); ^{13}C NMR δ 17.7, 20.9, 25.9, 28.5, 39.2, 39.5, 42.1, 53.1, 66.3, 76.8, 84.5, 125.9, 129.7; HRMS (EI) *m/z* calcd for $C_{13}H_{22}O_2$: 210.1620, found 210.1621.

4.1.7. (1*S*,3*R*,5*R*,8*R*,2′*E*)-8-(2′-Butenyl)-1,5-dimethyl-6-oxabicyclo[3.2.1]octan-3-yl (*R*)-α-methoxy-α-(trifluoromethyl)phenylacetate (10a) and (1*R*,3*S*,5*S*,8*S*,2′*E*)-8-(2′-butenyl)-1,5-dimethyl-6-oxabicyclo[3.2.1]octan-3-yl (*R*)-α-methoxy-α-(trifluoromethyl)phenylacetate (10b). (*S*)-MTPACl (0.41 mL, 2.2 mmol) and DMAP (89 mg, 0.73 mmol) were added to a solution of (±)-**9** (154 mg, 0.732 mmol) in pyridine (10 mL) at 40 °C under Ar. After stirring for 5 days at 40 °C, the reaction mixture was quenched with water and extracted with EtOAc. The organic layer was washed with water and brine, dried with $MgSO_4$, and concentrated under reduced pressure. The residue was purified by flash silica gel chromatography to give the more polar **10a** (129 mg, 41%) and the less polar **10b** (126 mg, 40%).

Compound **10a**: $[\alpha]_D^{25}$ +82.8 ($c=1.02$, $CHCl_3$): IR (neat) 1740 (s, C=O) cm^{-1}; ^{1}H NMR δ 0.90 (s, 3H, 1-Me), 1.21 (s, 3H, 5-Me), 1.48–1.53 (m, 2H, 2-, 8-H), 1.64 (d, $J=5.7$ Hz, 3H, 4′-H_3), 1.70–1.94 (m, 4H, 2-, 1′-H, 4-H_2), 2.05–2.14 (m, 1H, 1′-H), 3.32 (dd, $J=2.4$, 7.8 Hz, 1H, 7-H), 3.62 (d, $J=7.8$ Hz, 1H, 7-H), 3.64 (s, 3H, OMe), 5.25–5.52 (m, 3H, 3-, 2′-, 3′-H), 7.36–7.41 (m, 3H, Ar), 7.64–7.69 (m, 2H, Ar); ^{13}C NMR δ 17.8, 21.5, 25.5, 28.5, 36.0, 37.1, 41.3, 53.1, 55.6, 71.3, 75.8, 81.6, 84.1 (q, $J=27.4$ Hz), 123.4 (q, $J=287.9$ Hz), 126.1, 127.2, 128.3, 129.4, 129.6, 132.7, 166.1; HRMS (EI) *m/z* calcd for $C_{23}H_{29}F_3O_4$: 426.2018, found 426.2020.

Compound **10b**: $[\alpha]_D^{22}$ +1.57 ($c=1.02$, $CHCl_3$): IR (neat) 1740 (s, C=O) cm^{-1}; ^{1}H NMR δ 0.93 (s, 3H, 1-Me), 1.16 (s, 3H, 5-Me), 1.48–1.70 (m, 3H, 2-, 4-, 8-H), 1.63 (d, $J=4.8$ Hz, 3H, 4′-H_3), 1.81–1.96 (m, 3H, 2-, 4-, 1′-H), 2.03–2.13 (m, 1H, 1′-H), 3.35 (dd, $J=2.4$, 7.5 Hz, 1H, 7-H), 3.51 (s, 3H, OMe), 3.75 (d, $J=7.5$ Hz, 1H, 7-H), 5.29–5.47 (m, 3H, 3-H, 2′-, 3′-H), 7.37–7.42 (m, 3H, Ar), 7.53–7.60 (m, 2H, Ar); ^{13}C NMR δ 17.8, 21.5, 25.5, 28.5, 36.4, 37.2, 41.4, 53.1, 55.2, 71.2, 76.0, 81.5, 84.5 (q, $J=27.4$ Hz), 123.3 (q, $J=287.9$ Hz), 126.1, 127.8, 128.4, 129.5, 129.7, 131.8, 166.2; HRMS (EI) *m/z* calcd for $C_{23}H_{29}F_3O_4$: 426.2018, found 426.2020.

4.1.8. Determination of the diastereomeric purities of 10a and 10b. The diastereomeric purities of **10a/b** were estimated by HPLC analysis [column: Kanto Mightysil Si 60 250-3.0 column (5 μm); solvent: hexane/EtOAc=50:1; flow rate: 1.0 mL/min; detection: at 254 nm]: **10a** t_R/min 26.8 (~100%, **10a**), 38.0 (~0%, **10b**). The diastereomeric purity of **10a** was estimated to be ~100% de: **10b** t_R/min

26.8 (0.35%, **10a**), 38.0 (99.65%, **10b**). The diastereomeric purity of **10b** was estimated to be 99.3% de.

4.1.9. (1*S*,3*R*,5*R*,8*R*)-8-(2′-Butenyl)-1,5-dimethyl-6-oxabicyclo[3.2.1]octan-3-ol (+)-9. $LiAlH_4$ (11 mg, 0.29 mmol) was added to a solution **10a** (117 mg, 0.274 mmol) in anhydrous THF (3 mL). After stirring for 1 h at room temperature, the reaction mixture was quenched with 1 M HCl and extracted with EtOAc. The organic layer was washed with water and brine, dried with $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel chromatography to give (+)-**9** (44 mg, 76%) as a colorless oil: $[\alpha]_D^{25}$ +10.4 (c=0.88, MeOH); HRMS (EI) m/z calcd for $C_{13}H_{22}O_2$: 210.1620, found 210.1624. NMR and IR spectra of (+)-**9** were identical to those of (±)-**9**.

4.1.10. (1*R*,3*S*,5*S*,8*S*)-8-(2′-Butenyl)-1,5-dimethyl-6-oxabicyclo[3.2.1]octan-3-ol (−)-9. In the same manner as described above, **10b** (115 mg, 0.269 mmol) was converted to (−)-**9** (41 mg, 71%): $[\alpha]_D^{23}$ −9.45 (c=0.81, MeOH); HRMS (EI) m/z calcd for $C_{13}H_{22}O_2$: 210.1620, found 210.1609. NMR and IR spectra of (−)-**9** were identical to those of (±)-**9**.

4.1.11. (1*S*,5*R*,8*R*)-8-(2′-Butenyl)-1,5-dimethyl-6-oxabicyclo[3.2.1]octan-3-one (+)-8. IBX (65 mg, 0.23 mmol) was added to a solution (+)-**9** (44 mg, 0.21 mmol) in DMSO (5 mL). After stirring for 1.5 h at room temperature, the reaction mixture was quenched with water and filtered through Celite. The filtrate was and diluted with EtOAc. The organic layer was washed with water and brine, dried with $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel chromatography to give (+)-**8** (37 mg, 86%) as a colorless oil: $[\alpha]_D^{23}$ +12.0 (c=0.94, $CHCl_3$); HRMS (EI) m/z calcd for $C_{13}H_{20}O_2$: 208.1463, found 208.1456. NMR and IR spectra of (+)-**8** were identical to those of (±)-**8**.

4.1.12. (1*R*,5*S*,8*S*)-8-(2′-Butenyl)-1,5-dimethyl-6-oxabicyclo[3.2.1]octan-3-one (−)-8. In the same manner as described above, (−)-**9** (41 mg, 0.19 mmol) was converted to (−)-**8** (34 mg, 85%): $[\alpha]_D^{24}$ −12.8 (c=0.68, $CHCl_3$); HRMS (EI) m/z calcd for $C_{13}H_{20}O_2$: 208.1463, found 208.1457. NMR and IR spectra of (−)-**8** were identical to those of (±)-**8**.

4.1.13. (1*S*,5*R*,8*R*)-1,5-Dimethyl-8-(3′-oxobutyl)-6-oxabicyclo[3.2.1]octan-3-one (+)-1. $PdCl_2$ (4 mg, 0.02 mmol) and CuCl (18 mg, 0.18 mmol) were added to a mixture of DMF and water (7:1; 0.5 mL). After stirring for 40 min under oxygen at room temperature, a solution of (+)-**8** (37 mg, 0.18 mmol) in a mixture of DMF and water (7:1; 1 mL) was added, and the reaction mixture was stirred for 3 days at room temperature. It was then quenched with 1 M HCl and extracted with EtOAc. The organic layer was washed with saturated aqueous $NaHCO_3$, water and brine, dried with $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel chromatography to give (+)-**1** (25 mg, 64%) and its regioisomer **11** (2 mg, 6%).

Compound (+)-**1**: $[\alpha]_D^{22}$ +16.9 (c=0.50, $CHCl_3$): IR (neat) 1720 (s, C=O) cm^{-1}; ^1H NMR δ 1.07 (s, 3H, 1-Me), 1.31 (s, 3H, 5-Me), 1.58–1.72 (m, 2H, 1′-H_2), 1.76–1.88 (m, 1H, 8-H), 2.18 (s, 3H, 4′-H_3), 2.24 (br s, 1H, 4-H), 2.35–2.43 (m, 3H, 2-H_2, 4-H), 2.63–2.68 (m, 2H, 2′-H_2), 3.55 (dd, J=2.7, 8.1 Hz, 1H, 7-H), 3.62 (d, J=7.8 Hz, 1H, 7-H); ^{13}C NMR δ 18.5, 20.7, 24.9, 30.1, 42.6, 43.4, 48.5, 49.3, 53.0, 78.2, 83.4, 207.5, 209.0; HRMS (EI) m/z calcd for $C_{13}H_{20}O_3$: 224.1412, found 224.1402.

Compound **11**: ^1H NMR δ 0.99 (s, 3H, 1-Me), 1.11 (t, J=7.5 Hz, 3H, 4′-H_3), 1.24 (s, 3H, 5-Me), 2.27–2.64 (m, 9H, 2-, 4-, 1′-H_2, 4′-H), 3.64–3.70 (m, 2H, 7-H_2).

4.1.14. (1*R*,5*S*,8*S*)-1,5-Dimethyl-8-(3′-oxobutyl)-6-oxabicyclo[3.2.1]octan-3-one (−)-1. In the same manner as described above, (−)-**8** (34 mg, 0.16 mmol) was converted to (−)-**1** (17 mg, 48%): $[\alpha]_D^{23}$ −17.6 (c=0.35, $CHCl_3$); HRMS (EI) m/z calcd for $C_{13}H_{20}O_3$: 224.1412, found 224.1412. NMR and IR spectra of (−)-**1** were identical to those of (+)-**1**.

Acknowledgements

We thank Professor Emeritus Kenji Mori (The University of Tokyo) for giving us the opportunity to start this project. We also thank Dr. T. Tashiro (RIKEN) for his generous support of this work. This work was financially supported by Grant-in-Aid for Scientific Research from Japan Society for the Promotion of Science.

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Tetrahedron 61 (2005) 8836–8847
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New developments in the synthesis of pyrrolizidinone-based dipeptide isosteres

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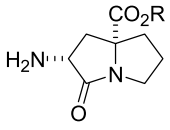
Received 25 May 2005; revised 28 June 2005; accepted 7 July 2005

Abstract—1,3-Dipolar cycloaddition of acrylamide with the cyclic nitrone derived from proline *tert*-butyl ester has been employed in the synthesis of bicyclic Gly-(*s-cis*)Pro isosteres suitably protected for the Fmoc-based solid phase peptide synthesis. (*R*)-1-Phenylethylamine was introduced as chiral auxiliary to resolve racemic intermediates and obtain enantiopure compounds. Using methacrylamide as dipolarophile, the analogous Ala-Pro mimetics have been prepared in racemic form, whereas the same strategy applied to methyl itaconate failed to give the corresponding Asp-Pro mimetic.

1. Introduction

Severely constrained dipeptides able to induce a folding in peptide chains are useful building blocks for synthesizing peptides with a reduced conformational freedom.[1] Various bicyclic lactams with the nitrogen at the bridgehead position have been used as scaffolds of rigid dipeptide surrogates able to stabilize or fix the active conformation of peptides.[2] Recently we reported the design and synthesis of new members of this class of compounds, the Gly-(*s-cis*)Pro turn mimetic (GPTM) **1** and its enantiomer, that are able to mimic the two central residues of a β-VI turn according to theoretical and experimental conformational studies.[3]



R = H: **1** (GPTM)
R = Me: **2** (GPTM-OMe)
R = ^{t}Bu: **3** (GPTM-O^{t}Bu)

The synthetic approach to **1** consists of the 1,3-dipolar cycloaddition (1,3-DC) of nitrone **4** with acrylamide followed by catalytic hydrogenation of isoxazolidines **5a** and **5b** that directly afford the bicyclic lactams **7a** and **7b**, respectively, by reductive N–O cleavage and intramolecular transamidation (Scheme 1). Next, both hydroxy esters **7** are converted into the methyl ester **2** through standard stereospecific functional group interconversion reactions.

The optically pure GPTM-OMe (2*R*,7a*R*)-**2** and (2*S*,7a*S*)-**2** were obtained through the separation of diastereomeric intermediates such as Mosher's ester,[4] or derivatives of enantiopure phenylethylamine.[3b] The suitability of GPTMs as building blocks in peptide synthesis was proved by coupling them either at the *N*-terminus or *C*-terminus with natural amino acids. One of the major problems of using dipeptide isosteres **2** is the difficulty of cleaving a methyl ester in general peptide synthesis. A *tert*-butyl ester is of much wider use and utility. In this paper, we report the synthesis of the new O-*t*Bu protected isostere GPTM-O*t*Bu **3**, that revealed not a simple protecting group change, and some preliminary studies toward the extension of the synthetic strategy to 2-substituted dipeptide isosteres.

2. Results and discussion

Nitrone **8** was synthesized by oxidation of proline *tert*-butyl ester through a modification of the method reported for the methyl derivative **4**.[5] In particular, the one pot two-step oxidation of the secondary amine to nitrone was more conveniently carried out using, in sequence, two different oxidation systems. The cyclic amino ester was liberated from the commercial hydrochloride salt and directly oxidised to the corresponding hydroxylamine by methyltrioxorhenium/hydrogen peroxide (MTO/H_2O_2). As soon as the amine was consumed, the crude reaction mixture of

Keywords: Nitrone; 1,3-Dipolar cycloaddition; Lactam; Constrained amino acid; Peptidomimetic.
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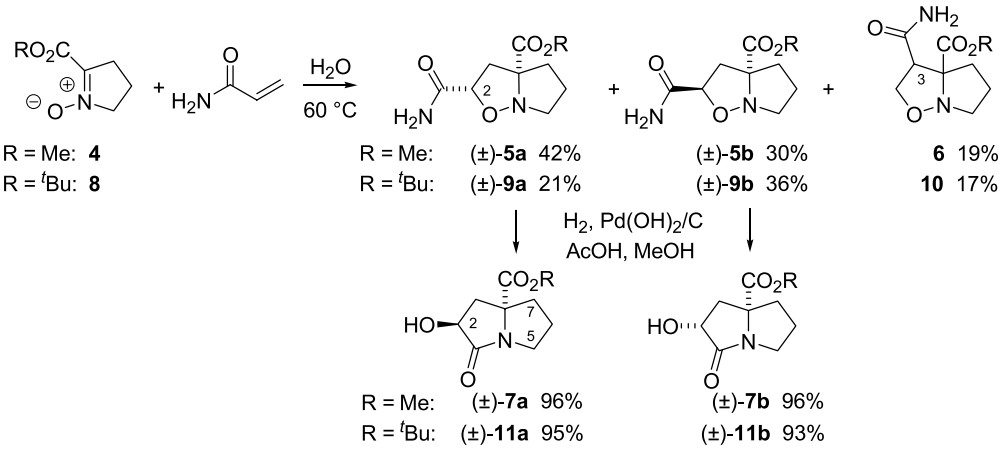
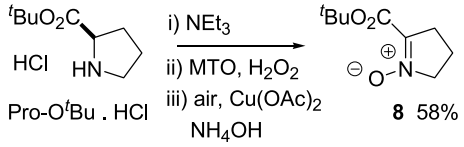
doi:10.1016/j.tet.2005.07.020



Scheme 1.

1-hydroxyproline was added with copper(II) diacetate and concentrated aqueous ammonia solution, and bubbled with air. Under this conditions, nitrone **8** was obtained regioselectively in 58% overall yield after purification by chromatography on silica gel (Scheme 2).



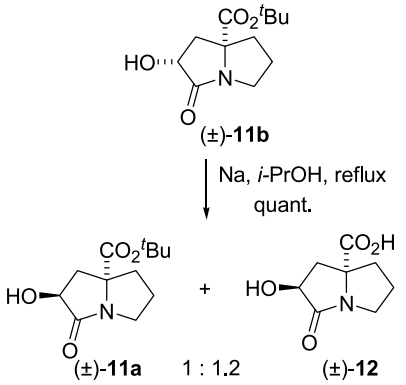
Scheme 2.

The treatment of nitrone **8** with acrylamide (2 equiv) in water at 60 °C for 14 h afforded a mixture of adducts **9a**, **9b** and **10** in 1.2:2.1:1 ratio and 74% overall yield (Scheme 1). Compared with the analogue cycloaddition of nitrone **4** with acrylamide[3b] the reaction showed a similar regioselectivity in favor of the 2-substituted adducts, but an opposite *exo*/*endo* diastereoselectivity, slightly in favor of the *endo* adduct **9b**. This is likely caused by the more sterically demanding *tert*-butyl moiety, which disfavors the *exo* approach of the dipolarophile.

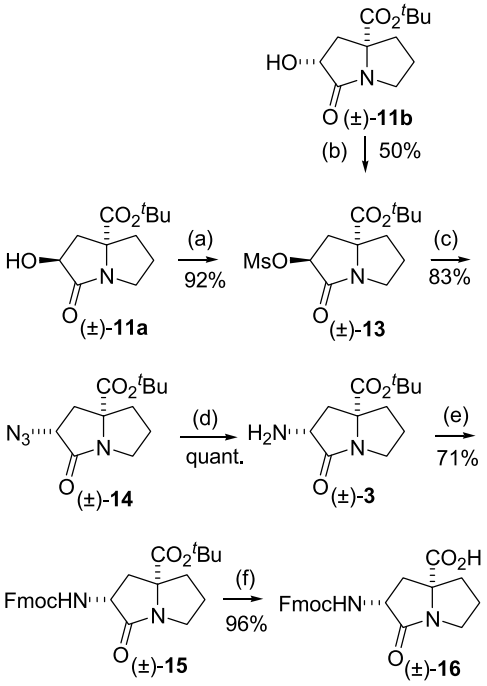
Like the methyl analogues, the *trans*- and *cis*-pyrrolizidinones **11a** and **11b** were smoothly obtained from the *exo* and *endo* adduct **9a** and **9b**, respectively, by hydrogenation in the presence of a catalytic amount of $Pd(OH)_2$ and 10 mol equiv of AcOH (93–95% yield) (Scheme 1).

The synthesis of cis amine ester **3** from the trans hydroxy ester **11a** required an inversion of configuration at C-2, whereas an overall retention was necessary in the case of the cis isomer **11b**.

The isomerization of the cis alcohol **11b** to the thermodynamically more stable trans isomer **11a** was tested under different reaction conditions. A complete C-2 epimerization of **11b** could be achieved with 2 equiv of sodium in refluxing 2-propanol (Scheme 3). This was only partial with methyl ester **7b**.[3b] Unfortunately, a partial hydrolysis of the *tert*-butyl ester could not be avoided and a significant amount of the trans hydroxy acid **12** was obtained along



Scheme 3.



Scheme 4. (a) MsCl, NEt_3, CH_2Cl_2. (b) MsOH, DEAD, TPP, THF. (c) NaN_3, DMF. (d) Raney-Ni, MeOH. (e) FmocCl, $NaHCO_3$, acetone/H_2O. (f) TFA.

with **11a**. Therefore, a double S_N2 reaction sequence was preferred to convert **11b** to the GPTM-O*t*Bu.

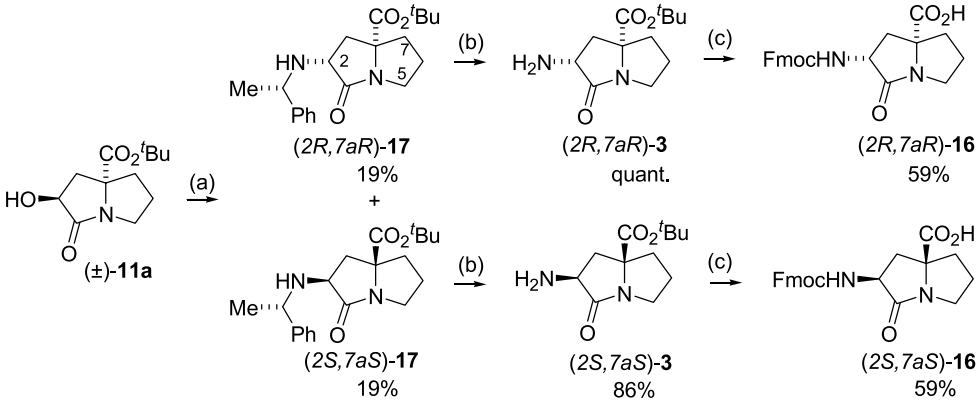
The trans mesylate **13** was selectively prepared from **11a** and **11b** with MsCl under standard conditions and with MsOH under Mitsunobu conditions,[6] respectively (Scheme 4).

Nucleophilic displacement of the mesylate group with NaN_3 followed by reduction of the azido group by Raney-Ni, yielded the racemic amine **3**. *N*-Protection with FmocCl followed by *tert*-butyl ester hydrolysis under acidic conditions afforded the Fmoc-GPTM-OH **16**, suitable for the Fmoc-based solid phase peptide synthesis (SPPS), in 57% yield with respect to the mesylate **13** (Scheme 4). Unfortunately, mesylate **13** did not react with the chiral 1-phenylethylamine: the more reactive triflate was necessary for this purpose (Scheme 5).

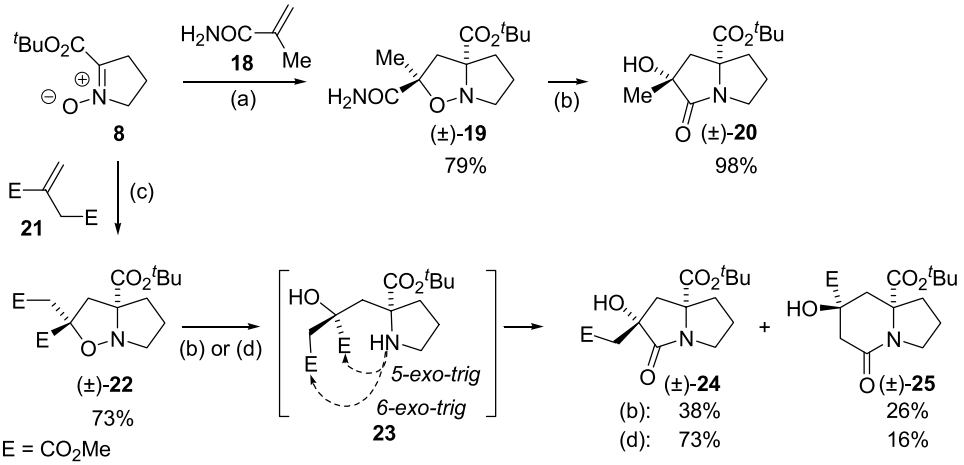
The absolute configuration of C-2 and C-7a stereocenters in amines **17** was established by comparison of their ^1H NMR spectra with those of the analogue methyl derivatives.[3b,7] The assignment was also indirectly validated by comparison of the sign of the optical rotation of (2*S*,7a*S*)-**16** with the same compound derived from (2*S*,7a*S*)-**2**.[3b,4]

The synthesis of optically pure **16** was completed via removal of the chiral auxiliary by hydrogenolysis of **17** followed by the *N*-protection/CO_2H-deprotection sequence as previously described for the racemic compound (Scheme 5).

The crucial role of amino acid side chains in peptide chemistry and bioactivity prompted us to investigate prospective approaches to constrained Xaa-Pro dipeptide mimetics forced in the *s-cis* configuration by a methylene bridge between the two amino acidic α carbons, namely 2-substituted GPTMs. To this end, two different variations in our strategy are possible. The side chain can be added to the C-2 of the GPTM through alkylation in analogy with the procedures reported for C-2 alkylation of amino esters[8] or, alternatively, a dipolarophile incorporating the desired chain in a suitable position can be used in the cycloaddition with the cyclic nitrones **4** or **8**. This second strategy, which allows the introduction of the side chain from the beginning of the synthesis, appeared particularly appealing and was tested. Methacrylamide (**18**) and dimethylitaconate (**21**) were chosen as model compounds of 2-substituted acrylates, and their cycloadditions with nitrone **8** followed by elaboration of the cycloadducts were investigated. According to our synthetic methodology, the dipolarophiles **18**



Scheme 5. (a) (i) Tf_2O, Py, CH_2Cl_2; (ii) (*S*)-$PhMeCHNH_2$, CH_2Cl_2. (b) H_2, $Pd(OH)_2/C$, MeOH. (c) (i) FmocOSu, $NaHCO_3$, acetone/H_2O; (ii) TFA.



Scheme 6. (a) H_2O, 60 °C, 12 h. (b) $Pd(OH)_2/C$ (cat), H_2, AcOH (10 mol equiv), MeOH. (c) neat, 42 °C, 2.5 h. (d) $Pd(OH)_2/C$ (cat), H_2, MeOH.

and **21** should afford Ala-(*s-cis*)-Pro and Asp-(*s-cis*)-Pro mimetics, respectively.

The cycloadditions of nitrone **8** with **18** and **21** were completely regio- and diastereoselective affording one single cycloadduct. In particular, the sole isoxazolidine **19** was obtained in 79% yield by heating a mixture of **8** and **18** (2 equiv) in water at 60 °C for 12 h, whereas neat **8** and **21** (1.1 equiv) afforded adduct **22** in 73% yield by heating at 42 °C for 2.5 h (Scheme 6).

The structures of adducts **19** and **22** were elucidated by spectroscopic means and the relative configuration, arising from a (Me/CH_2E)-*exo* approach of the dipolarophile, was assigned by analogy with related 1,3-DC.[9] Indirect confirmation of the identity of isoxazolidine **19** was achieved by X-ray crystallography of the corresponding methyl ester obtained by cycloaddition of methacrylamide (**18**) with nitrone **4** (Fig. 1).

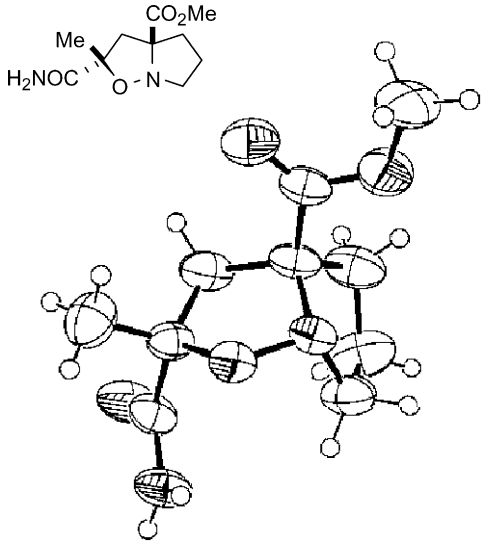
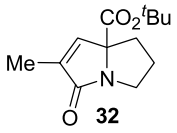


Figure 1. ORTEP drawing from the X-ray crystal structure of cycloadduct of methacrylamide (**18**) and nitrone **4**.[10]

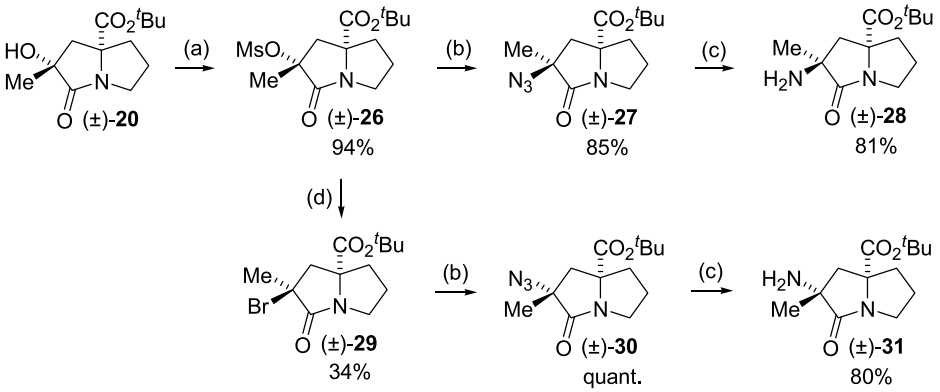
Analogously to the 2-monosubstituted adducts **9**, the pyrrolo[1,2-*b*]isoxazolidine **19** afforded pyrrolizidinone **20** in high yield (98%) by $Pd(OH)_2$ catalyzed hydrogenation in the presence of 10 mol equiv of AcOH (Scheme 6). Under the same conditions, **22** gave a ca. 1.5:1 mixture of pyrrolizidinone **24** and indolizidinone **25** through the competitive 5- and 6-*exo-trig* cyclizations, respectively, of the intermediate amino diester **23**. It was found that the 5-*exo-trig* pathway becomes more important in the absence of AcOH. In this case, **24** and **25** were obtained in ca. 4.5:1 ratio and 89% overall yield.

The tertiary alcohol **20** was easily converted into the amino ester **28** through the usual three-step procedure (Scheme 7). Despite the more sterically congested substrate, the insertion of the azide group smoothly occurred at room temperature with complete inversion of the configuration, and only a small amount of the elimination product **32** was detected in the crude product mixture.

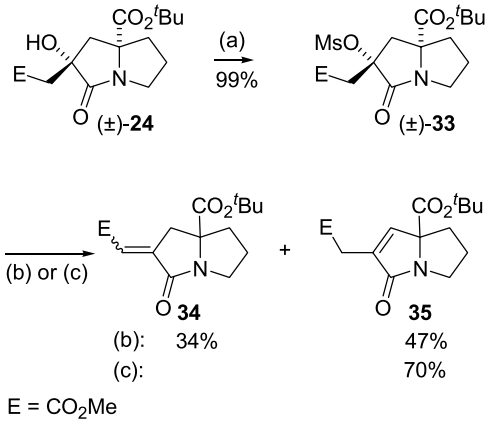


The direct S_N2 substitution of the hydroxy group of **20** was attempted with various halogenating agents, but all the methods failed to give the desired compound. The treatment of mesylate **26** with iodide and bromide salts afforded the unsaturated compound **32** as the main or exclusive product, with the exception of KBr under phase transfer conditions, which gave a 1:1 mixture of **29** and **32**. The 2-bromo-pyrrolizidinone **29** smoothly reacted with NaN_3 to give **30**, which in turn, was reduced with Raney-Ni to afford the amino ester **31** (Scheme 7).

Both **28** and **31** contain the backbone of dipeptide Ala-Pro fixed in the *s-cis* configuration. The isomer **31**, which presents both the amino and carboxylic groups on the *exo* face of the bicyclic system is likely able to induce a β-turn when incorporated in peptides, whereas **28** prevents the folding. The synthesis of **28** and **31** proved that 2-alkyl GPTMs can be prepared starting from cyclic nitrones and 2-alkylacrylates. An advantage of employing methacrylates as dipolarophiles is the complete regio- and stereoselectivity of the cycloaddition step. On the other hand, the tendency of 2-alkyl-2-hydroxy-pyrrolizidin-3-one derivatives to undergo elimination precludes the use of very good leaving groups such as triflate that are necessary to resolve the racemic intermediates by the synthesis of diasteromeric



Scheme 7. (a) MsCl, NEt_3. (b) NaN_3, DMF. (c) Ra-Ni, MeOH. (d) KBr (5 equiv), $(C_{12}H_{25})Me_3N^+Br^-$ (1 equiv), CH_2Cl_2, H_2O.



Scheme 8. (a) MsCl, NEt_3, 0 °C, 30 min. (b) NEt_3, CH_2Cl_2, rt. (c) NEt_3, CH_3CN, 60 °C, 1 h.

amino esters. Accordingly, it will be necessary to apply a different strategy to synthesize enantiopure 2-alkyl GPTM.

The mesylation of itaconate derivative **24** at 0 °C afforded **33** in quantitative yield (Scheme 8). The presence of the electron withdrawing group dramatically increases the propensity to undergo elimination so that the reaction of **33** with NaN_3 afforded exclusively the elimination product **35**.

The behaviour of **33** under mild basic conditions was also tested. For example, the treatment of mesylate **33** with an excess of triethylamine at room temperature for 1 h gave a 1.4:1 mixture of the elimination products **34** and **35** bearing the double bond in *exo*- and *endo*-cyclic position, respectively (81% overall yield) (Scheme 8). The same reaction performed at 60 °C afforded exclusively **35** in 70% yield. Likely, compound **35** is the thermodynamic product in a thermal equilibrium.

3. Conclusion

An extension of the synthetic strategy to new dipeptide isosteres with a pyrrolizidinone skeleton was accomplished. New racemic constrained Ala-(*s-cis*)Pro mimetics and enantiomerically pure GPTMs orthogonally protected for the solid phase peptide synthesis were synthesized starting from proline *tert*-butyl ester and acrylamides and their incorporation in selected peptides is now in progress in our laboratories. The same synthetic sequence applied to itaconate as dipolarophile afforded only a 5-oxo-2,3-dihydro-1*H*-pyrrolizine-7a(5*H*)-carboxylate derivative.

4. Experimental

4.1. General

All the reactions requiring anhydrous conditions were carried out under nitrogen and the solvents were appropriately dried before use. NMR spectra were recorded in $CDCl_3$ (unless otherwise stated) and the data are reported in δ (ppm) from TMS. Multiplicity of the ^{13}C NMR was determined by means of APT and HMQC experiments. In mass spectra relative percentages are shown in brackets. R_f values refer to TLC on 0.25 mm silica gel plates.

4.1.1. *tert*-Butyl 3,4-dihydro-2*H*-pyrrole-5-carboxylate 1-oxide (8). 35% H_2O_2 (0.32 mL, 3.63 mmol) was added dropwise to a solution of proline *tert*-butyl ester (623 mg, 3.63 mmol) in MeOH (9 mL) at 0 °C. MTO was added in portions of 0.1% mol until the disappearance of the amino ester was indicated by TLC analysis (AcOEt–MeOH = 10:1) (usually 3–4 additions are necessary). The mixture was allowed to warm to rt and then, 33% NH_4OH (0.90 mL) and $Cu(OAc)_2$ (106 mg, 0.59 mmol) were added. Air was bubbled into the solution for 15 min, then the solvent was removed under reduced pressure. The reaction mixture was added with saturated $NaHCO_3$ (12 mL) and extracted with $CHCl_3$ (3×20 mL). The combined organic extracts were dried over Na_2SO_4, filtered and evaporated to give crude nitrone **8** (751 mg) as a green oil. Purification by chromatography on silica gel (AcOEt–MeOH = 10:1) afforded pure **8** (393 mg, 58%) as a yellow oil.

Compound **8**. R_f = 0.20 (AcOEt); ^1H NMR (200 MHz) δ 4.18 (dt, J = 8.1, 1.8 Hz, 1H; 2-H_a), 4.13 (dt, J = 8.4, 2.0 Hz, 1H; 2-H_b), 3.00 (dt, J = 7.3, 2.0 Hz, 1H; 4-H_a), 2.97 (dt, J = 7.7, 1.8 Hz, 1H; 4-H_b), 2.14 (p, J = 7.9 Hz, 2H; 3-H), 1.52 (s, 9H; CMe_3); ^{13}C NMR (50 MHz) δ 158.3 (s; CO_2tBu), 134.8 (s; C-5), 82.3 (s; CMe$_3$), 66.5 (t; C-2), 29.8 (t; C-4), 27.9 (q, 3C; CMe_3), 16.4 (t; C-3); IR ($CDCl_3$) ν = 2983, 2934, 2246, 1721, 1690, 1555, 1370, 1237, 1153 cm^{-1}; MS (EI): m/z = 185 (6, M^+), 129 (26), 11 (99), 85 (46); $C_9H_{15}NO_3$ (185.22): Calcd C 58.36, H 8.16, N 7.56; found: C 57.97, H 8.20, N 7.50.

4.1.2. *tert*-Butyl (2*S,3a*R**)-2-(aminocarbonyl)tetrahydropyrrolo[1,2-*b*]isoxazole-3a(4*H*)-carboxylate (9a), *tert*-Butyl (2*R**,3a*R**)-2-(aminocarbonyl)tetrahydropyrrolo[1,2-*b*]isoxazole-3a(4*H*)-carboxylate (9b) and *tert*-butyl 3-(aminocarbonyl)tetrahydropyrrolo[1,2-*b*]isoxazole-3a(4*H*)-carboxylate (10).** A suspension of acrylamide (158 mg, 2.22 mmol) and nitrone **8** (199 mg, 1.08 mmol) in water (0.5 mL) was heated at 60 °C for 15 h. After concentration, the mixture of the three cycloadducts **9a**, **9b** and **10** was separated by chromatography on silica gel (CH_2Cl_2–MeOH = 30:1) to obtain the pure isoxazolidines **9a** (58 mg, 21%), **9b** (100 mg, 36%) and **10** (46 mg, 17%) as white solids.

Compound **9a**. R_f = 0.35 (CH_2Cl_2–MeOH = 15:1); mp 94–95 °C (iPr_2O); ^1H NMR (200 MHz) δ 6.91 (br s, 1H; NH), 5.57 (br s, 1H; NH), 4.52 (dd, J = 8.8, 4.4 Hz, 1H; 2-H), 3.40–3.30 (m, 1H; 6-H_a), 3.22–3.12 (m, 1H; 6-H_b), 3.10 (dd, J = 13.2, 4.8 Hz, 1H; 3-H_a), 2.54 (dd, J = 13.2, 8.8 Hz, 1H; 3-H_b), 2.28–2.18 (m, 1H; 4-H_a), 2.05–1.74 (m, 3H; 4-H_b, 5-H), 1.44 (s, 9H; CMe_3); ^{13}C NMR (50 MHz) δ 178.6, 174.5 (s; CO_2tBu, $CONH_2$), 82.0, 78.0 (s; C-3$_a$, CMe$_3$), 77.0 (d; C-2), 57.5 (t; C-6), 44.3, 36.0 (t; C-3, C-4), 27.8 (q, 3C; CMe_3), 24.3 (t; C-5); IR (CDCl3) ν = 3515, 3398, 2981, 1726, 1690, 1572, 1370, 1158, 1107, 1059 cm^{-1}; MS (EI): m/z = 257 (0.3, MH^+), 255 [1, $(M-H)^+$], 155 (75), 138 (29), 110 (38), 82 (20), 57 (100); $C_{12}H_{20}N_2O_4$ (256.30): Calcd C 56.23, H 7.87, N 10.93; found: C 56.31, H 7.64, N 11.07.

Compound **9b**. R_f=0.32 (CH_2Cl_2–MeOH=15:1); mp 100–102 °C (*i*Pr_2O); ^{1}H NMR (200 MHz) δ 6.26 (br s, 1H; NH), 5.87 (br s, 1H; NH), 4.50 (dd, J=9.2, 7.4 Hz, 1H; 2-H), 3.39–3.30 (m, 1H; 6-H_a), 3.22–3.12 (m, 1H; 6-H_b), 3.19 (dd, J=13.0, 7.5 Hz, 1H; 3-H_a), 2.27–2.06 (m, 1H; 4-H_a), 2.23 (dd, J=12.8, 9.6 Hz, 1H; 3-H_b), 1.98–1.83 (m, 3H; 4-H_b, 5-H), 1.46 (s, 9H; C*Me*$_3$); ^{13}C NMR (50 MHz) δ 172.7, 171.4 (s; *C*O_2tBu, $CONH_2$), 82.0, 78.4 (s; C-3$_a$, *C*Me_3), 77.5 (d; C-2), 57.4 (t; C-6), 43.9, 35.0 (t; C-3, C-4), 27.8 (q, 3C; C*Me*$_3$), 24.2 (t; C-5); IR ($CDCl_3$) ν=3519, 3402, 2981, 1725, 1693, 1573, 1370, 1159, 1117 cm^{-1}; MS (EI): m/z=257 (0.4, MH^+), 255 [1, $(M-H)^+$], 155 (100), 138 (53), 110 (57), 82 (55), 57 (100); $C_{12}H_{20}N_2O_4$ (256.30): Calcd C 56.23, H 7.87, N 10.93; found: C 55.98, H 7.64, N 11.21.

Compound **10**. R_f=0.58 (CH_2Cl_2–MeOH=15:1); mp 98–100 °C (Et_2O); ^{1}H NMR (200 MHz) δ 6.85 (br s, 1H; NH), 5.49 (br s, 1H; NH), 4.31 (t, J=9.1 Hz, 1H; 3-H), 4.06 (dd, J=9.0, 7.7 Hz, 1H; 2-H_a), 3.81 (dd, J=9.2, 7.7 Hz, 1H; 2-H_b), 3.46–3.37 (m, 1H; 6-H_a), 3.25–3.12 (m, 1H; 6-H_b), 2.09–1.92 (m, 4H; 4-H, 5-H), 1.50 (s, 9H; C*Me*$_3$); ^{13}C NMR (50 MHz) δ 174.0, 171.3 (s; *C*O_2tBu, $CONH_2$), 83.2, 78.5 (s; C-3$_a$, *C*Me_3), 67.0 (t; C-2), 56.8 (t; C-6), 33.9 (d; C-3), 32.6 (t; C-4), 27.8 (q, 3C; C*Me*$_3$), 24.7 (t; C-5); IR (KBr) ν=3641, 3123, 2985, 1740, 1685, 1604, 1396, 1257, 1119, 838, 658 cm^{-1}; MS (EI): m/z=255 [1, $(M-H)^+$], 167 (40), 156 (5), 110 (14), 91 (100), 83 (17), 77 (16), 57 (96); $C_{12}H_{20}N_2O_4$ (256.30): Calcd C 56.23, H 7.87, N 10.93; found: C 56.33, H 8.02, N 10.87.

4.1.3. *tert*-Butyl (2*R,7a*R**)-2-hydroxy-3-oxotetrahydro-1*H*-pyrrolizine-7a(5*H*)-carboxylate (11b).** A mixture of the isoxazolidine **9b** (529 mg, 2.07 mmol) and acetic acid (1.2 mL, 20.66 mmol) in MeOH (21.0 mL) was hydrogenated over 20% $Pd(OH)_2$/C (108 mg, 10% mol) overnight at atmospheric pressure. The reaction mixture was then filtered and concentrated. The resulting colorless oil was dissolved in CH_2Cl_2, stirred in the presence of K_2CO_3 and Na_2SO_4 and filtered, yielding the alcohol **11b** (465 mg, 93%) as a white solid, which was used in the next step without further purification.

Compound **11b**. R_f=0.33 (CH_2Cl_2–MeOH=15:1); mp 128–130 °C (*i*Pr_2O); ^{1}H NMR (400 MHz) δ 4.39 (dd, J=7.0, 1.4 Hz, 1H; 2-H), 3.64 (dt, J=11.7, 8.0 Hz, 1H; 5-H_a), 3.30 (ddd, J=11.7, 9.0, 4.3 Hz, 1H; 5-H_b), 2.52 (dd, J=14.3, 1.4 Hz, 1H; 1-H_a), 2.42 (ddd, J=12.7, 7.0, 2.7 Hz, 1H; 7-H_a), 2.24 (dd, J=14.4, 7.0 Hz, 1H; 1-H_b), 2.20–2.12 (m, 2H; 6-H), 1.70 (br s, 1H; OH), 1.59 (dm, J=12.7 Hz, 1H; 7-H_b), 1.52 (s, 9H; C*Me*$_3$); ^{13}C NMR (100 MHz) δ 173.6, 173.1 (s; *C*O_2tBu, C-3), 82.7 (s; *C*Me_3), 75.1 (d; C-2), 72.9 (s; C-7$_a$), 41.3 (t; C-5), 40.0 (t; C-1), 35.7 (t; C-7), 27.8 (q, 3C; C*Me*$_3$), 26.0 (t; C-6); IR (KBr) ν=3256, 2978, 1742, 1703, 1450, 1367, 1155, 1122, 852, 645 cm^{-1}; MS (EI): m/z=239 (1), 140 (14), 111 (15), 100 (19), 86 (45), 72 (80), 58 (100); $C_{12}H_{19}NO_4$ (241.28): Calcd C 59.73, H 7.94, N 5.81; found: C 59.38, H 7.49, N 5.66.

4.1.4. *tert*-Butyl (2*S,7a*R**)-2-hydroxy-3-oxotetrahydro-1*H*-pyrrolizine-7a(5*H*)-carboxylate (11a).** Following the previous procedure, alcohol **11a** (564 mg, 95%) was obtained from isoxazolidine **9a** (629 mg, 2.46 mmol) as a white solid.

Compound **11a**. R_f=0.32 (AcOEt); mp 125–126 °C (*i*Pr_2O); ^{1}H NMR (200 MHz) δ 4.69 (t, J=8.9 Hz, 1H; 2-H), 3.70 (dt, J=11.7, 7.7 Hz, 1H; 5-H_a), 3.53 (br s, 1H; OH), 3.15 (dt, J=11.7, 5.8 Hz, 1H; 5-H_b), 3.00 (dd, J=12.6, 7.5 Hz, 1H; 1-H_a), 2.49–2.37 (m, 1H; 6-H_a), 2.14–1.98 (m, 1H; 6-H_b), 1.90 (dd, J=12.6, 10.4 Hz, 1H; 1-H_b), 1.78–1.60 (m, 2H; 7-H), 1.46 (s, 9H; C*Me*$_3$); ^{13}C NMR (50 MHz) δ 174.6, 171.9 (s; C-3, *C*O_2tBu), 82.4 (s; *C*Me_3), 72.5 (d; C-2), 70.0 (s; C-7$_a$), 42.0 (t; C-5), 41.7 (t; C-1), 36.0 (t; C-7), 27.8 (q, 3C; C*Me*$_3$), 25.2 (t; C-6); IR (KBr) ν=3351, 2982, 1732, 1674, 1428, 1149, 1110, 624 cm^{-1}; MS (EI): m/z=242 (2, MH^+), 140 (100), 112 (98), 105 (16), 97 (12), 84 (60), 57 (84); $C_{12}H_{19}NO_4$ (241.28): Calcd C 59.73, H 7.94, N 5.81; found: C 60.12, H 7.89, N 6.08.

4.1.5. *tert*-Butyl (2*S,7a*R**)-2-[(methoxysulfonyl)oxy]-3-oxotetrahydro-1*H*-pyrrolizine-7a(5*H*)-carboxylate (13).** *Procedure A*. Freshly distilled triethylamine (1.3 mL, 9.54 mmol) and methanesulfonyl chloride (0.37 mL, 4.77 mmol) were added to a solution of alcohol **11a** (766 mg, 3.18 mmol) in CH_2Cl_2 under nitrogen atmosphere, at 0 °C. After 1 h, the solution was diluted with CH_2Cl_2, washed in turn with water and brine and dried over Na_2SO_4. The filtrate was concentrated under reduced pressure to give crude mesylate **13** (931 mg, 92%) as a white solid, which was used in the next step without further purification.

Procedure B. Methanesulfonic acid (24 μL, 0.36 mmol) was added to a solution of alcohol **11b** (44 mg, 0.18 mmol) and triphenylphosphine (144 mg, 0.55 mmol) in dry THF (0.5 mL) under nitrogen atmosphere and the reaction flask was dipped in an oil bath at 40 °C. After diethylazodicarboxylate (86 μL, 0.55 mmol) was added, the reaction mixture was stirred at 40 °C under nitrogen overnight. Purification of the crude product by chromatography on silica gel (petroleum ether–AcOEt=2:1) afforded pure **13** (29 mg, 50%) as a white solid along with recovered starting material **11b** (5 mg, 89% conversion).

Compound **13**. R_f=0.40 (petroleum ether–AcOEt=1:1); mp 125–127 °C (*i*Pr_2O); ^{1}H NMR (400 MHz) δ 5.55 (t, J=9.1 Hz, 1H; 2-H), 3.70 (dt, J=11.7, 7.8 Hz, 1H; 5-H_a), 3.22–3.13 (m, 1H; 5-H_b), 3.09 (dd, J=13.0, 7.9 Hz, 1H; 1-H_a), 2.50 (ddd, J=12.7, 7.0, 3.3 Hz, 1H; 7-H_a), 2.16 (dd, J=13.1, 10.2 Hz, 1H; 1-H_b), 2.15–2.03 (m, 2H; 6-H), 1.68 (dt, J=12.7, 9.6 Hz, 1H; 7-H_b), 1.47 (s, 9H; C*Me*$_3$); ^{13}C NMR (100 MHz) δ 170.9, 168.0 (s; C-3, *C*O_2tBu), 83.1 (s; *C*Me_3), 79.5 (d; C-2), 69.8 (s; C-7$_a$), 41.9 (t; C-5), 39.9 (t+q, 2C; C-1, SCH_3), 35.8 (t; C-7), 27.8 (q, 3C; C*Me*$_3$), 25.1 (t; C-6); IR (KBr) ν=2977, 1727 (br), 1376, 1359, 1219, 1173, 1154, 980, 844, 766 cm^{-1}; MS (EI): m/z=320 (3, MH^+), 218 (32), 122 (100), 112 (19), 94 (10), 79 (27), 58 (76); $C_{13}H_{21}NO_6S$ (319.37): Calcd C 48.89, H 6.63, N 4.39; found: C 48.47, H 6.27, N 4.57.

4.1.6. *tert*-Butyl (2*R,7a*R**)-2-azido-3-oxotetrahydro-1*H*-pyrrolizine-7a(5*H*)-carboxylate (14).** Compound **13** (365 mg, 1.14 mmol) was dissolved in DMF (3.8 mL) and the solution was added with NaN_3 (112 mg, 1.72 mmol). The reaction mixture was stirred overnight at 40 °C, then it was allowed to cool to rt, diluted with CH_2Cl_2, treated dropwise with 10% aqueous HCl solution and vigorously stirred for 15 min. The separated organic phase was washed

sequentially with 5% $NaHCO_3$, H_2O and brine, dried over Na_2SO_4 and concentrated. The azide **14** (251 mg, 83%) was obtained as a white solid after crystallization from *n*-hexane.

Compound **14**. R_f=0.53 (petroleum ether–AcOEt=1:1); mp 61–62 °C (*n*-hexane); ^{1}H NMR (400 MHz) δ 4.23 (dd, J=7.5, 1.2 Hz, 1H; 2-H), 3.70 (dt, J=11.7, 7.8 Hz, 1H; 5-H_a), 3.34 (ddd, J=11.7, 8.3, 5.0 Hz, 1H; 5-H_b), 2.57 (dd, J=14.2, 1.2 Hz, 1H; 1-H_a), 2.33 (ddd, J=12.7, 6.6, 3.4 Hz, 1H; 7-H_a), 2.22 (dd, J=14.2, 7.5 Hz, 1H; 1-H_b), 2.21–2.14 (m, 2H; 6-H), 1.68 (dt, J=12.7, 9.7 Hz, 1H; 7-H_b), 1.53 (s, 9H; CMe_3); ^{13}C NMR (50 MHz) δ 171.9, 169.1 (s; C-3, CO_2tBu), 82.5, 72.9 (s; C-7$_a$, CMe$_3$), 64.2 (d; C-2), 41.6 (t; C-5), 37.8 (t; C-1), 36.4 (t; C-7), 27.8 (q, 3C; CMe_3), 25.9 (t; C-6); IR (KBr) ν=2970, 2116, 1734, 1700, 1412, 1370, 1155, 1119, 847, 695 cm^{-1}; MS (EI): m/z=207 (12), 165 (43), 137 (32), 81 (31), 73 (12), 57 (100); $C_{12}H_{18}N_4O_3$ (266.30): Calcd C 54.12, H 6.81, N 21.04; found: C 54.09, H 6.23, N 20.37 (the best analysis out of several carried out on pure samples according to mp and NMR spectra).

4.1.7. *tert*-Butyl (2*R,7a*R**)-2-amino-3-oxotetrahydro-1*H*-pyrrolizine-7a(5*H*)-carboxylate (3).** A water suspension of Raney-Ni was added to a solution of azide **14** (197 mg, 0.74 mmol) in MeOH (15 mL). The reaction mixture was stirred at rt for 1 h and then filtered through a short pad of silica gel. Evaporation of the solvent under reduced pressure afforded amine **3** (741 mg, colorless oil, quantitative yield), which was directly used in the next step without further purification.

Compound **3**. ^{1}H NMR (200 MHz) δ 4.72 (br s, 1H; N*H*H), 3.61 (dt, J=11.7, 8.1 Hz, 2H; 5-H), 3.19 (ddd, J=11.5, 9.2, 4.4 Hz, 1H; 2-H), 2.46–2.27 (m, 2H; 1-H_a, 7-H_a), 2.14–2.04 (m, 4H; 1-H_b, 6-H, N*H*H), 1.61–1.52 (m, 1H; 7-H_b), 1.47 (s, 9H; CMe_3).

4.1.8. *tert*-Butyl (2*R,7a*R**)-2-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}-3-oxotetrahydro-1*H*-pyrrolizine-7a(5*H*)-carboxylate (15).** Racemic amine **3** (45 mg, 0.19 mmol) was dissolved in CH_2Cl_2 (1.0 mL) and H_2O (0.5 mL) and treated sequentially with $NaHCO_3$ (47 mg, 0.56 mmol) and FmocCl (73 mg, 0.28 mmol). The reaction mixture was stirred at rt overnight and washed in turn with water and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated. Purification of the crude residue by chromatography on silica gel (Et_2O) afforded the pure dipeptide isostere **15** (62 mg, 71%) as a white solid.

Compound **15**. R_f=0.31 (Et_2O); mp 152–154 °C; ^{1}H NMR (400 MHz) δ 7.66 (d, J=7.3 Hz, 2H; fluorenyl-), 7.48 (d, J=7.3 Hz, 2H; fluorenyl-), 7.30 (t, J=7.4 Hz, 2H; fluorenyl-), 7.21 (t, J=7.4 Hz, 2H; fluorenyl-), 5.46 (br s, 1H; NH), 4.42–4.29 (m, 3H; 2-H, OCH_2CH), 4.12 (t, J=6.3 Hz, 1H; OCH$_2$C*H*), 3.56 (dt, J=11.5, 8.2 Hz, 1H; 5-H_a), 3.15 (dt, J=12.2, 3.4 Hz, 1H; 5-H_b), 2.38–2.24 (m, 3H; 1-H, 7-H_a), 2.07–1.93 (m, 2H; 6-H), 1.54–1.43 (m, 1H; 7-H_b), 1.38 (s, 9H; CMe_3); ^{13}C NMR (50 MHz) δ 172.6, 171.6, 155.8 (s; C-3, CO_2tBu, CO_2CH_2), 143.8 (s; fluorenyl-), 143.7 (s; fluorenyl-), 141.2 (s, 2C; fluorenyl-), 127.6 (d, 2C; fluorenyl-), 127.0 (d, 2C; fluorenyl-), 125.0 (d, 2C; fluorenyl-), 119.8 (d, 2C; fluorenyl-), 82.7, 72.5 (s; C-7$_a$, CMe$_3$), 67.0 (t; OCH_2CH), 56.3 (d; C-2), 47.0 (d; OCH$_2$*C*H), 41.7 (t; C-5), 38.1 (t; C-1), 35.1 (t; C-7), 28.0 (q, 3C; CMe_3), 25.7 (t; C-6); IR ($CDCl_3$) ν=3429, 2981, 1709 (br), 1511, 1451, 1370, 1227, 1155, 1044 cm^{-1}; MS (EI): m/z=463 (0.04, MH$^+$), 361 (4), 220 (19), 205 (74), 178 (100), 166 (26), 122 (36), 91 (23), 57 (85); $C_{27}H_{30}N_2O_5$ (462.54): Calcd C 70.11, H 6.54, N 6.06; found: C 70.31, H 6.69, N 6.19.

4.1.9. *tert*-Butyl (2*R,7a*R**)-2-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}-3-oxotetrahydro-1*H*-pyrrolizine-7a(5*H*)-carboxylic acid (16).** TFA (0.42 mL) was added dropwise to compound **15** (32 mg, 0.07 mmol) at 0 °C, until **15** was completely dissolved. The solution was stirred for 1 h at rt. All volatiles were removed under reduced pressure to give acid **16** (27 mg, 96%) as a colorless glass, which was solidified by addition of Et_2O.

Compound **16**. Mp 191–194 °C (Et_2O–*n*-hexane=1:1); ^{1}H NMR (400 MHz, CD_3OD) δ 7.81 (d, J=7.5 Hz, 2H; fluorenyl-), 7.66 (d, J=7.4 Hz, 2H; fluorenyl-), 7.40 (t, J=7.4 Hz, 2H; fluorenyl-), 7.33 (t, J=7.4 Hz, 2H; fluorenyl-), 4.36–4.34 (m, 1H; 2-H), 4.36 (d, J=6.6 Hz, 2H; OCH_2CH), 4.23 (t, J=6.7 Hz, 1H; OCH$_2$C*H*), 3.64 (dt, J=10.8, 8.0 Hz, 1H; 5-H_a), 3.31–3.28 (m, 1H; 5-H_b), 2.62 (dd, J=14.2, 4.9 Hz, 1H; 1-H_a), 2.42–2.30 (m, 2H; 1-H_b, 7-H_a), 2.21–2.03 (m, 2H; 6-H), 1.76–1.68 (m, 1H; 7-H_b); ^{13}C NMR (50 MHz) δ 176.5, 175.1, 158.1 (s; C-3, CO_2H, CO_2CH_2), 145.1 (s, 2C; fluorenyl-), 142.4 (s, 2C; fluorenyl-), 128.7 (d, 2C; fluorenyl-), 128.1 (d, 2C; fluorenyl-), 126.1 (d, 2C; fluorenyl-), 120.8 (d, 2C; fluorenyl-), 72.8 (s; C-7$_a$), 68.1 (t; OCH_2CH), 56.2 (d; C-2), 47.7 (d; OCH$_2$*C*H), 43.7 (t; C-5), 36.4 (t; C-1), 36.1 (t; C-7), 26.0 (t; C-6); IR (KBr) ν=3411, 2967, 1728, 1635, 1512, 1217, 762, 742 cm^{-1}; MS (EI): m/z=406 (0.04, M$^+$), 178 (100), 165 (48), 89 (20), 76 (25); $C_{23}H_{22}N_2O_5$ (406.43): Calcd C 67.97, H 5.46, N 6.89; found: C 67.61, H 5.52, N 6.86.

4.1.10. *tert*-Butyl (2*R*,7a*R*)-3-oxo-2[(1-phenylmethyl)amino]tetrahydro-1*H*-pyrrolizine-7a(5*H*)-carboxylate [(2*R*,7a*R*)-17] and *tert*-butyl (2*S*,7a*S*)-3-oxo-2[(1-phenylmethyl)amino]tetrahydro-1*H*-pyrrolizine-7a(5*H*)-carboxylate [(2*S*,7a*S*)-17]. A solution of alcohol **11a** (116 mg, 0.48 mmol) and pyridine (65 μL, 0.64 mmol) in CH_2Cl_2 (2.0 mL) was added dropwise to a solution of trifluoromethanesulfonic anhydride (110 μL, 0.67 mmol) in CH_2Cl_2 (2.0 mL) at 0 °C and under nitrogen atmosphere. The reaction mixture was stirred at 0 °C until TLC analysis (AcOEt) showed the disappearance of the starting reagent **11a** (30 min), then was rapidly filtered through silica gel and concentrated. The residue oil was diluted with CH_2Cl_2 (1.0 mL) and added to a solution of (*S*)-α-methylbenzylamine (180 μL, 1.44 mmol) in CH_2Cl_2 (1.0 mL) at 0 °C. The reaction mixture was allowed to warm to rt during 1 h, and then concentrated. The crude mixture was separated by chromatography on silica gel (petroleum ether–AcOEt=2:1) to give the two diastereomeric enantiopure amines (2*R*,7a*R*)-**17** (32 mg, 19%) and (2*S*,7a*S*)-**17** (32 mg, 19%) as colorless oils.

Compound (2*S*,7a*S*)-**17**. R_f=0.20; $[\alpha]_D^{20}$ −2.3 (*c* 0.8, $CHCl_3$); ^{1}H NMR (200 MHz) δ 7.40–7.20 (m, 5H; Ph), 4.18 (q, J=6.6 Hz, 1H; NC*H*CH$_3$), 3.60 (dt, J=11.7, 8.1 Hz, 1H; 5-H_a), 3.26 (dd, J=8.1, 2.6 Hz, 1H; 2-H), 3.21–

3.11 (m, 1H; 5-H_b), 2.29 (ddd, J=12.3, 6.4, 3.1 Hz, 1H; 7-H_a), 2.14 (dd, J=13.9, 2.6 Hz, 1H; 1-H_a), 2.08–1.94 (m, 3H; 1-H_b, 6-H), 1.50–1.39 (m, 1H; 7-H_b), 1.47 (s, 9H; CMe_3), 1.31 (d, J=6.5 Hz, 3H; NCHCH_3); ^{13}C NMR (50 MHz) δ 175.3, 173.2 (s; CO_2tBu, C-3), 145.0 (s; Ph), 128.3 (d, 2C; Ph), 127.2 (d, 2C; Ph), 126.9 (d; Ph), 82.0, 72.5 (s; C-7a, CMe$_3$), 60.8, 56.3 (d; NCHCH$_3$, C-2), 41.5 (t; C-5), 39.0, 36.1 (t; C-1, C-7), 27.9 (q, 3C; CMe_3), 25.7 (t; C-6), 24.3 (q; NCH$_3$); IR ($CDCl_3$) ν=2979, 1724, 1685, 1394, 1370, 1156, 1095 cm^{-1}; MS (EI): m/z=345 (5, MH$^+$), 281 (72), 274 (95), 262 (100), 105 (38), 83 (20), 57 (100); $C_{20}H_{28}N_2O_3$ (344.45): Calcd C 69.74, H 8.19, N 8.13; found: C 69.43, H 8.27, N 8.06.

Compound (2*R*,7a*R*)-**17**. R_f=0.14; $[\alpha]_D^{20}$ −75.0 (*c* 0.5, $CHCl_3$); ^{1}H NMR (200 MHz) δ 7.35–7.18 (m, 5H; Ph), 3.91 (q, J=6.6 Hz, 1H; NCHCH$_3$), 3.62 (dt, J=11.5, 7.9 Hz, 1H; 5-H_a), 3.33 (dd, J=7.6, 2.7 Hz, 1H; 2-H), 3.27–3.15 (m, 1H; 5-H_b), 2.46 (dd, J=13.6, 2.6 Hz, 1H; 1-H_a), 2.24 (ddd, J=12.1, 5.9, 4.0 Hz, 1H; 7-H_a), 2.10–1.98 (m, 3H; 1-H_b, 6-H), 1.58–1.46 (m, 1H; 7-H_b), 1.44 (s, 9H; CMe_3), 1.35 (d, J=6.6 Hz, 3H; NCHCH_3); ^{13}C NMR (50 MHz) δ 174.8, 173.1 (s; CO_2tBu, C-3), 144.5 (s; Ph), 128.5 (d, 2C; Ph), 127.0 (d; Ph), 126.6 (d, 2C; Ph), 81.9, 72.6 (s; C-7$_a$, CMe$_3$), 60.2, 55.6 (d; C-2, NCHCH$_3$), 41.9 (t; C-5), 37.1, 35.9 (t; C-1, C-7), 27.8 (q, 3C; CMe_3), 25.7 (t; C-6), 23.8 (q; NCH$_3$); IR ($CDCl_3$) ν=2979, 1723, 1685, 1457, 1370, 1157, 1099 cm^{-1}; MS (EI): m/z=343 [0.4, (M−H)$^+$], 120 (26), 105 (45), 97 (26), 83 (43), 69 (42), 57 (100); $C_{20}H_{28}N_2O_3$ (344.45): Calcd C 69.74, H 8.19, N 8.13; found: C 69.60, H 8.14, N 8.02.

4.1.11. (2*R*,7a*R*)-*tert*-Butyl 2-{[(9*H*-fluoren-9-ylmethoxy) carbonyl]amino}-3-oxotetrahydro-1*H*-pyrrolizine-7a(5*H*)-carboxylate [(2*R*,7a*R*)-15]. Amine (2*R*,7a*R*)-**17** (62 mg, 0.18 mmol) was dissolved in MeOH (5.0 mL) and hydrogenated overnight in a Parr apparatus at 40 atm in the presence of 20% Pd(OH)$_2$/C (19 mg, 20% mol). After the reaction mixture was filtered and evaporated, the *tert*-butyl (2*R*,7a*R*)-2-amino-3-oxotetrahydro-1*H*-pyrrolizine-7a(5*H*)-carboxylate [(2*R*,7a*R*)-**3**] was obtained in quantitative yield (43 mg) as a colorless oil, which was used in the next step without further purification. The enantiopure amine (2*R*,7a*R*)-**3** (43 mg, 0.18 mmol) was dissolved in acetone (1.0 mL) and treated sequentially with $NaHCO_3$ (47 mg, 0.56 mmol) and FmocOSu (79 mg, 0.23 mmol). The reaction mixture was stirred overnight, then the salts were filtered off, and the resulting solution was diluted with Et_2O and washed three times with water. The organic phase was dried over Na_2SO_4 and concentrated. The crude residue was purified by chromatography on silica gel (Et_2O) affording pure (2*R*,7a*R*)-**15** (51 mg, 61%) as a white solid, which showed identical physical and spectroscopic properties with those of racemic **15**, except for optical rotation.

Compound (2*R*,7a*R*)-**15**. $[\alpha]_D^{22}$ −24.1 (*c* 0.9, $CHCl_3$).

4.1.12. (2*S*,7a*S*)-*tert*-Butyl 2-{[(9*H*-fluoren-9-ylmethoxy) carbonyl]amino}-3-oxotetrahydro-1*H*-pyrrolizine-7a(5*H*)-carboxylate [(2*S*,7a*S*)-15]. Following the procedure for the preparation of (2*R*,7a*R*)-**15**, *tert*-butyl (2*S*,7a*S*)-2-amino-3-oxotetrahydro-1*H*-pyrrolizine-7a(5*H*)-carboxylate [(2*S*,7a*S*)-**3**] was obtained as a colorless oil in 86% yield (68 mg) starting from amine (2*S*,7a*S*)-**17** (114 mg, 0.33 mmol) and in turn converted to (2*S*,7a*S*)-**15** (80 mg, 61%, white solid).

Compound (2*S*,7a*S*)-**15**. $[\alpha]_D^{22}$ +25.2 (*c* 1.0, $CHCl_3$).

4.1.13. *tert*-Butyl (2*R*,7a*R*)-2-{[(9*H*-fluoren-9-ylmethoxy) carbonyl]amino}-3-oxotetrahydro-1*H*-pyrrolizine-7a(5*H*)-carboxylic acid [(2*R*,7a*R*)-16]. Acid (2*R*,7a*R*)-**16** was obtained from the corresponding *tert*-butyl ester (2*R*,7a*R*)-**15** following the same procedure as described for racemic **16**.

Compound (2*R*,7a*R*)-**16**. 96% Yield; $[\alpha]_D^{22}$ −18.4 (*c* 0.42, MeOH).

4.1.14. *tert*-Butyl (2*S*,7a*S*)-2-{[(9*H*-fluoren-9-ylmethoxy) carbonyl]amino}-3-oxotetrahydro-1*H*-pyrrolizine-7a(5*H*)-carboxylic acid [(2*S*,7a*S*)-16]. Acid (2*S*,7a*S*)-**16** was obtained from the corresponding *tert*-butyl esters (2*S*,7a*S*)-**15** following the same procedure as described for racemic **16**.

Compound (2*S*,7a*S*)-**16**. 96% Yield; $[\alpha]_D^{22}$ +19.5 (*c* 0.12, MeOH).

4.1.15. *tert*-Butyl (2*R,3a*R**)-2-(aminocarbonyl)-2-methyltetrahydropyrrolo[1,2-*b*]isoxazole-3a(4*H*)-carboxylate (19).** Methacrylamide **18** (250 mg, 2.94 mmol) was added to a solution of nitrone **8** (272 mg, 1.47 mmol) in water (455 μL) and the reaction mixture was stirred at 60 °C for 12 h. After solvent evaporation, the purification of the crude product by chromatography on silica gel (CH_2Cl_2–MeOH=30:1) afforded pure isoxazolidine **19** (314 mg, 79%) as a white solid.

Compound **19** R_f=0.34; mp 118–120 °C; ^{1}H NMR (400 MHz) δ 6.39 (br s, 1H; NH), 6.28 (br s, 1H; NH), 3.32 (ddd, J=12.9, 7.1, 4.2 Hz, 1H; 6-H_a), 3.18 (ddd, J=12.8, 8.5, 7.0 Hz, 1H; 6-H_b), 2.91 (d, J=13.4 Hz, 1H; 3-H_a), 2.57 (d, J=13.2 Hz, 1H; 3-H_b), 2.18–2.11 (m, 1H; 4-H_a), 2.07–1.95 (m, 1H; 5-H_a), 1.92–1.80 (m, 2H; 4-H_b, 5-H_b), 1.48 (s, 3H; CH_3), 1.43 (s, 9H; CMe_3); ^{13}C NMR (50 MHz) δ 176.7, 171.7 (s; CO_2tBu, CONH$_2$), 83.7, 81.9, 78.8 (s; C-2, C-3$_a$, CMe$_3$), 57.0 (t; C-6), 49.1, 35.6 (t; C-3, C-4), 27.8 (q, 3C; CMe_3), 24.4 (q; CH_3), 24.1 (t; C-5); MS (EI): m/z=270 (1, M$^+$), 169 (100), 125 (50), 110 (7), 82 (57); IR (KBr) ν=3387, 2974, 1733, 1665, 1156, 668 cm^{-1}; $C_{13}H_{22}N_2O_4$ (270.16): Calcd C 57.76, H 8.20, N 10.36; found: C 57.31, H 8.25, N 10.70.

4.1.16. *tert*-Butyl (2*R,7a*R**)-2-hydroxy-2-methyl-3-oxotetrahydro-1*H*-pyrrolizine-7a(5*H*)-carboxylate (20).** The solution of isoxazolidine **19** (1.20 g, 4.50 mmol) and acetic acid (2.6 mL, 45.0 mmol) in MeOH (43.0 mL) was hydrogenated over 20% Pd(OH)$_2$/C (156 mg, 5% mol) for 15 h at atmospheric pressure. The reaction mixture was then filtered and concentrated. The resulting colorless oil was dissolved in CH_2Cl_2, and stirred in the presence of K_2CO_3 and Na_2SO_4. The filtrate was concentrated under reduced pressure to yield the alcohol **20** (1.12 g, 98%) as a white solid, which was used in the next step without further purification.

Compound **20**. R_f=0.27 (AcOEt–petroleum ether=2:1); mp 90–92 °C (*i*Pr_2O); ^{1}H NMR (400 MHz) δ 3.61 (dt, J= 11.7, 8.1 Hz, 1H; 5-H_a), 3.42 (br s, 1H; OH), 3.29–3.22 (m, 1H; 5-H_b), 2.67 (d, J=13.8 Hz, 1H; 1-H_a), 2.37 (ddd, J= 12.6, 7.0, 2.8 Hz, 1H; 7-H_a), 2.16–2.08 (m, 2H; 6-H), 1.92 (d, J=13.8 Hz, 1H; 1-H_b), 1.64–1.51 (m, 1H; 7-H_b), 1.48 (s, 9H; C*Me*$_3$), 1.39 (s, 3H; CH_3); ^{13}C NMR (50 MHz) δ 174.2, 173.4 (s; *C*O_2*t*Bu, C-3), 82.6, 79.3, 70.7 (s; C-2, C-7$_a$, *C*Me_3), 46.9 (t; C-1), 41.2, 35.9 (t; C-5, C-7), 27.8 (q, 3C; C*Me*$_3$), 25.7 (t; C-6), 23.6 (q; CH_3); MS (EI): *m*/*z*=154 (55), 126 (62), 84 (100), 67 (5); IR (KBr) ν=3310, 2981, 1717, 1683, 1447, 1163, 849, 628 cm^{-1}; $C_{13}H_{21}NO_4$ (255.31): Calcd C 61.16, H 8.29, N 5.49; found: C 61.20, H 8.58, N 5.28.

4.1.17. *tert*-Butyl (2*R,7a*R**)-2-methyl-2-[(methylsulfonyl)oxy]-3-oxotetrahydro-1*H*-pyrrolizine-7a(5*H*)-carboxylate (26).** Freshly distilled triethylamine (204 μL, 1.48 mmol) and methanesulfonyl chloride (69 μL, 0.89 mmol) were added to a solution of alcohol **20** (150 mg, 0.59 mmol) in CH_2Cl_2 (1.0 mL) under nitrogen atmosphere, at 0 °C. After 12 h, the solution was diluted with CH_2Cl_2, washed in turn with water and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure to give crude **26** (184 mg, 94%) as a white solid, which was used in the next step without further purification.

Compound **26**. R_f=0.29 (AcOEt–petroleum ether=1:1); mp 102–105 °C (*i*Pr_2O); ^{1}H NMR (200 MHz) δ 3.63 (dt, J= 12.1, 7.7 Hz, 1H; 5-H_a), 3.39–3.27 (m, 1H; 5-H_b), 3.28 (d, J=15.0 Hz, 1H; 1-H_a), 2.99 (s, 3H; SCH_3), 2.28–2.10 (m, 3H; 6-H, 7-H_a), 1.97 (d, J=14.6 Hz, 1H; 1-H_b), 1.70 (s, 3H; CH_3), 1.65–1.54 (m, 1H; 7-H_b), 1.44 (s, 9H; C*Me*$_3$); ^{13}C MNR (50 MHz) δ 171.4, 168.2 (s; *C*O_2*t*Bu, C-3), 92.5, 82.5, 70.3 (s; C-2, C-7a, *C*Me_3), 45.9 (t; C-5), 42.1 (t; C-1), 39.7 (q; SCH_3), 36.5 (t; C-7), 27.7 (q, 3C; C*Me*$_3$), 25.3 (t; C-6), 21.9 (q; *C*H_3); MS (EI): *m*/*z*=333 (0.1, M^+), 232 (19), 136 (100), 83 (9), 57 (52); IR (KBr) ν=3434, 3010, 1727, 1716, 1355, 1168, 889, 531 cm^{-1}; $C_{14}H_{23}NO_6S$ (333.40): Calcd C 50.43, H 6.95, N 4.20; found: C 50.63, H 6.97, N 3.95.

4.1.18. *tert*-Butyl (2*S,7a*R**)-2-azido-2-methyl-3-oxotetrahydro-1*H*-pyrrolizine-7a(5*H*)-carboxylate (27) and *tert*-butyl-6-methyl-5-oxo-2,3-dihydro-1*H*-pyrrolizine-7a(5*H*)-carboxylate (32).** Compound **26** (60 mg, 0.18 mmol) was dissolved in DMF (522 μL) and NaN_3 (47 mg, 0.72 mmol) was added. The reaction mixture was stirred at rt for 12 h, then diluted with CH_2Cl_2, treated dropwise with 10% aqueous HCl solution and vigorously stirred for 15 min. The separated organic phase was washed sequentially with 5% $NaHCO_3$, H_2O and brine, dried over Na_2SO_4 and concentrated. The crude mixture containing the major product **27** and traces of **32** was purified by chromatography on silica gel (AcOEt–petroleum ether= 1:3) to afford the pure azide **27** (43 mg, 85%) as a white solid.

Compound **27**. R_f=0.51; mp 55–56 °C (*i*Pr_2O); ^{1}H NMR (400 MHz) δ 3.74 (dt, J=11.3, 7.8 Hz, 1H; 5-H_a), 3.25 (ddd, J=11.4, 9.3, 4.1 Hz, 1H; 5-H_b), 2.68 (d, J=13.4 Hz, 1H; 1-H_a), 2.33 (ddd, J=11.7, 7.4, 2.7 Hz, 1H; 7-H_a), 2.18–2.01 (m, 2H; 6-H), 2.06 (d, J=13.4 Hz, 1H; 1-H_b), 1.74–1.66 (m, 1H; 7-H_b), 1.57 (s, 3H; CH_3), 1.50 (s, 9H; C*Me*$_3$); ^{13}C NMR (50 MHz) δ 172.8, 172.3 (s; *C*O_2*t*Bu, C-3), 82.6, 69.7, 67.2 (s; C-2, C-7a, *C*Me_3), 44.7 (t; C-5), 42.0, 37.1 (t; C-1, C-7), 27.8 (q, 3C; C*Me*$_3$), 25.0 (t; C-6), 21.4 (q; CH_3); MS (EI): *m*/*z*=151 (13), 123 (41), 95 (39), 57 (37); IR (KBr) ν=2978, 2117, 1737, 1709, 1416, 1230 cm^{-1}; $C_{13}H_{20}N_4O_3$ (280.32): Calcd C 55.70, H 7.19, N 19.99; found: C 55.39, H 7.06, N 20.48.

Compound **32**. R_f=0.27 (AcOEt–petroleum ether=1:3); mp 73–75 °C (*i*Pr_2O); ^{1}H NMR (400 MHz) δ 6.83 (s, 1H; 7-H), 3.59 (dt, J=11.1, 8.1 Hz, 1H; 3-H_a), 3.35 (ddd, J= 11.3, 7.6, 4.0 Hz, 1H; 3-H_b), 2.30–2.22 (m, 3H; 1-H_a, 2-H), 1.85 (d, J=1.2 Hz, 3H; CH_3), 1.50–1.40 (m, 1H; 1-H_b), 1.45 (s, 9H; C*Me*$_3$); ^{13}C NMR (100 MHz) δ 175.5, 169.5 (s; *C*O_2*t*Bu, C-5), 141.1 (d; C-7), 136.0 (s; C-6), 82.5, 77.0 (s; C-7a, *C*Me_3), 42.2 (t; C-3), 33.9 (t; C-1), 28.0 (q; CH_3), 27.9 (q, 3C; C*Me*$_3$), 11.1 (t; C-2); MS (EI): *m*/*z*=237 (0.4, M^+), 136 (53), 57 (100); IR (KBr) ν=3077, 2982, 1717, 1683, 1373, 1156, 848, 508 cm^{-1}; $C_{13}H_{19}NO_3$ (237.29): Calcd C 65.80, H 8.07, N 5.90; found: C 65.91, H 8.30, N 6.18.

4.1.19. *tert*-Butyl (2*S,7a*R**)-2-amino-2-methyl-3-oxotetrahydro-1*H*-pyrrolizine-7a(5*H*)-carboxylate (28).** A water suspension of Raney-Ni was added to a solution of azide **27** (109 mg, 0.39 mmol) in MeOH (8.0 mL) and the reaction mixture was stirred at rt for 2 h. After filtration through a short pad of Celite and evaporation of the solvent, purification by chromatography on silica gel [CH_2Cl_2–MeOH (1% NH_3)=30:1] afforded amine **28** (80 mg, 81%) as a white solid.

Compound **28**. R_f=0.29; mp 72–73 °C (*i*Pr_2O); ^{1}H NMR (400 MHz) δ 3.64 (dt, J=10.9, 7.8 Hz, 1H; 5-H_a), 3.20 (ddd, J=11.2, 8.9, 4.2 Hz, 1H; 5-H_b), 2.72 (d, J=13.3 Hz, 1H; 1-H_a), 2.26 (ddd, J=12.6, 7.2, 3.1 Hz, 1H; 7-H_a), 2.06–1.96 (m, 2H; 6-H), 1.86 (d, J=13.3 Hz, 1H; 1-H_b), 1.71 (br s, 2H; NH), 1.67–1.59 (m, 1H; 7-H_b), 1.44 (s, 9H; C*Me*$_3$), 1.25 (s, 3H; CH_3); ^{13}C NMR (50 MHz) δ 177.8, 172.9 (s; *C*O_2*t*Bu, C-3), 82.0, 69.1, 61.0 (s; C-2, C-7a, *C*Me_3), 47.7 (t; C-5), 41.8, 37.6 (t; C-1, C-7), 27.7 (q, 3C; C*Me*$_3$), 25.3 (q; CH_3), 24.9 (t; C-6); MS (EI): *m*/*z*=254 (2, M^+), 197 (7), 153 (51), 126 (100), 85 (54), 55 (51); IR (KBr) ν=2980, 2931, 1726, 1687, 1371 cm^{-1}; $C_{13}H_{22}N_2O_3$ (254.16): Calcd C 61.39, H 8.72, N 11.01; found: C 61.56, H 8.95, N 11.17.

4.1.20. *tert*-Butyl (2*S,7a*R**)-2-bromo-2-methyl-3-oxotetrahydro-1*H*-pyrrolizine-7a(5*H*)-carboxylate (29).** A 0.45 M solution of KBr (54 mg, 0.45 mmol) in water was added to a solution of **26** (30 mg, 0.09 mmol) in CH_2Cl_2 (1 mL). Then, *N*,*N*,*N*-trimethyl-1-dodecanaminium bromide (28 mg, 0.09 mmol) was added and the reaction mixture was stirred at rt for 2 days. Evaporation of the solvent and purification by chromatography on silica gel (AcOEt–petroleum ether=1:3) afforded the pure bromide **29** (10 mg, 34%, white solid) and the elimination product **32** (7 mg).

Compound **29**. R_f=0.48; mp 69–71 °C; ^{1}H NMR (200 MHz) δ 3.74 (dt, J=11.3, 7.8 Hz, 1H; 5-H_a), 3.35–3.22 (m, 1H; 5-H_b), 3.04 (d, J=13.9 Hz, 1H; 1-H_a), 2.80 (d, J=13.9 Hz, 1H; 1-H_b), 2.30 (ddd, J=11.7, 6.6, 2.1 Hz, 1H; 7-H_a), 2.18–2.01 (m, 2H; 6-H), 1.90 (s, 3H; CH_3), 1.83–1.73 (m, 1H; 7-H_b), 1.48 (s, 9H; C*Me*$_3$); ^{13}C NMR (50 MHz) δ 172.1, 171.9 (s; *C*O_2*t*Bu, C-3), 82.7, 70.7, 61.9 (s; C-2,

C-7a, *C*Me$_3$), 49.4 (t; C-5), 42.8, 36.3 (t; C-1, C-7), 29.5 (q; CH$_3$), 27.8 (q, 3C; C*Me*$_3$), 25.0 (t; C-6); MS (EI): *m*/*z*=318 (M$^+$, 3), 218 (71), 216 (100), 138 (29), 136 (75), 109 (34), 56 (44); IR (KBr) ν=2984, 1726, 1408, 1366, 1156, 842 cm^{-1}; $C_{13}H_{20}BrNO_3$ (318.21): Calcd C 49.07, H 6.34, N 4.40; found: C 49.35, H 6.29, N 4.26.

4.1.21. *tert*-Butyl (2*R,7a*R**)-2-azido-2-methyl-3-oxotetrahydro-1*H*-pyrrolizine-7a(5*H*)-carboxylate (30).** Bromide **29** (23 mg, 0.07 mmol) was dissolved in DMF (200 μL) and NaN_3 (19 mg, 0.29 mmol) was added. The reaction mixture was stirred at rt for 12 h, then the excess of NaN_3 was filtered off eluting with AcOEt. Evaporation of the solvent yielded azide **30** (20 mg, quantitative yield) as a waxy solid, which was used for the next step without further purification.

Compound **30**. R_f=0.65 (AcOEt); ^{1}H NMR (400 MHz) δ 3.67 (dt, *J*=11.6, 7.7 Hz, 1H; 5-H$_a$), 3.36 (dt, *J*=11.6, 6.5 Hz, 1H; 5-H$_b$), 2.77 (d, *J*=13.6 Hz, 1H; 1-H$_a$), 2.26 (dm, *J*=12.5 Hz, 1H; 7-H$_a$), 2.19–2.12 (m, 2H; 6-H), 1.86 (d, *J*=13.6 Hz, 1H; 1-H$_b$), 1.67 (dt, *J*=12.5, 9.4 Hz, 1H; 7-H$_b$), 1.53 (s, 3H; C*H*$_3$), 1.51 (s, 9H; C*Me*$_3$); ^{13}C NMR (50 MHz) δ 172.0, 170.1 (s; *C*O$_2$*t*Bu, C-3), 82.3, 70.7, 68.9 (s; C-2, C-7a, *C*Me$_3$), 45.8 (t; C-5), 41.6, 36.6 (t; C-1, C-7), 27.8 (q, 3C; C*Me*$_3$), 25.7 (t; C-6), 20.2 (q; CH$_3$); MS (EI): *m*/*z*=281 (1, MH$^+$), 253 (1), 178 (15), 57 (100); IR (CDCl$_3$) ν=3677, 3331, 2981, 2110, 1705 (br), 1417, 1370, 1231, 1156 cm^{-1}.

4.1.22. *tert*-Butyl (2*R,7a*R**)-2-amino-3-oxotetrahydro-1*H*-pyrrolizine-7a(5*H*)-carboxylate (31).** A water suspension of Raney-Ni was added to a solution of azide **30** (20 mg, 0.07 mmol) in MeOH (1.4 mL) and the reaction mixture was stirred at rt for 2 h. After filtration through a short pad of Celite and evaporation of the solvent, purification by chromatography on silica gel [CH$_2$Cl$_2$–MeOH (1% NH$_3$)=30:1] afforded amine **31** (14 mg, 80%) as a pale yellow oil.

Compound **31**. R_f=0.16; ^{1}H NMR (200 MHz) δ 3.60 (dt, *J*=11.7, 8.0 Hz, 1H; 5-H$_a$), 3.26–3.13 (m, 1H; 5-H$_b$), 2.47 (d, *J*=13.9 Hz, 1H; 1-H$_a$), 2.47–2.38 (m, 1H; 7-H$_a$), 2.07–1.98 (m, 2H; 6-H), 1.99 (d, *J*=13.9 Hz, 1H; 1-H$_b$), 1.87 (br s, 2H; NH), 1.59–1.47 (m, 1H; 7-H$_b$), 1.46 (s, 9H; C*Me*$_3$), 1.27 (s, 3H; CH$_3$); ^{13}C NMR (50 MHz) δ 177.4, 173.3 (s; *C*O$_2$*t*Bu, C-3), 82.4, 70.5, 61.2 (s; C-2, C-7a, *C*Me$_3$), 46.8 (t; C-5), 41.2, 36.6 (t; C-1, C-7), 27.9 (q, 3C; C*Me*$_3$), 25.6 (t; C-6), 25.5 (q; CH$_3$); MS (EI): *m*/*z*=255 (0.3, MH$^+$), 239 (11), 182 (31), 136 (100), 124 (64), 85 (53), 58 (53); IR (CDCl$_3$) ν=2980, 2933, 1710, 1684, 1370, 1156, 1141 cm^{-1}; $C_{13}H_{22}N_2O_3$ (254.16): Calcd C 61.39, H 8.72, N 11.01; found: C 61.67, H 8.61, N 11.05.

4.1.23. *tert*-Butyl (2*S,3a*R**)-2-methyl-2-(2-methoxy-2-oxoethyl)tetrahydropyrrolo[1,2-*b*]isoxazole-2,3a-(4*H*) dicarboxylate (22).** Nitrone **8** (299 mg, 1.62 mmol) and dimethyl itaconate **21** (277 mg, 1.78 mmol) were heated at 42 °C for 2.5 h. The crude product was purified by chromatography on silica gel (petroleum ether–AcOEt=5:1) to yield the sole adduct **22** (403 mg, 73%) as a colorless oil.

Compound **22**. R_f=0.24; ^{1}H NMR (400 MHz) δ 3.79 (s, 3H; OMe), 3.66 (s, 3H; OMe), 3.47 (ddd, *J*=13.6, 6.9, 3.4 Hz, 1H; 6-H$_a$), 3.14 (A part of an AB system, *J*=16.6 Hz, 1H; C*H*HCO$_2$Me), 3.15–3.09 (m, 1H; 6-H$_b$), 3.06 (d, *J*=13.5 Hz, 1H; 3-H$_a$), 3.02 (B part of an AB system, *J*=16.4 Hz, 1H; C*H*HCO$_2$Me), 2.71 (d, *J*=13.5 Hz, 1H; 3-H$_b$), 2.27–2.13 (m, 2H; 4-H$_a$, 5-H$_a$), 2.01–1.95 (m, 1H; 4-H$_b$), 1.90–1.80 (m, 1H; 5-H$_b$), 1.48 (s, 9H; C*Me*$_3$); ^{13}C NMR (50 MHz) δ 171.2, 170.9, 170.1 (s; *C*O$_2$*t*Bu, *C*O$_2$Me, *C*O$_2$Me), 82.2, 81.7, 78.6 (s; C-2, C-3a, *C*Me$_3$), 56.8 (t; C-6), 52.5 (q; OMe), 51.4 (q; OMe), 48.2 (t; C-3), 40.7 (t; *C*H$_2$CO$_2$Me), 35.7 (t; C-4), 27.5 (q, 3C; C*Me*$_3$), 24.0 (t; C-5); IR (CDCl$_3$) ν=2982, 2955, 1735 (br), 1602, 1438, 1370, 1148 cm^{-1}; MS (EI): *m*/*z*=343 (0.6, M$^+$), 242 (85), 168 (26), 110 (24), 59 (50), 57 (100); $C_{16}H_{25}NO_7$ (343.37): Calcd C 55.97, H 7.34, N 4.08; found: C 56.16, H 7.42, N 4.21.

4.1.24. *tert*-Butyl (2*S,7a*R**)-2-hydroxy-2-(2-methoxy-2-oxoethyl)-3-oxotetrahydro-1*H*-pyrrolizine-7a(5*H*)-carboxylate (24) and *tert*-butyl (7*R**,8a*R**)-7-hydroxy-7-(2-methoxy-2-oxoethyl)-5-oxohexahydroindolizine-8a(1*H*)-carboxylate (25).** Adduct **22** (515 mg, 1.5 mmol) was dissolved in MeOH (15 mL) and 20% Pd(OH)$_2$/C (10% mol) was added. The mixture was stirred under H$_2$ atmosphere overnight and the catalyst was filtered. The solvent was removed under reduced pressure to give a mixture of alcohols **24** and **25** in 4.5:1 ratio. The separation by chromatography on silica gel (AcOEt–petroleum ether=2:1, then AcOEt) afforded alcohols **24** (349 mg, 73%) and **25** (78 mg, 16%) as colorless solids. When the same reaction was performed under acidic conditions (10 equiv of acetic acid), a 1.5:1 mixture of alcohols **24** and **25** was obtained.

Compound **24**. R_f=0.16 (AcOEt–petroleum ether=2:1); mp 93–95 °C (*i*Pr$_2$O); ^{1}H NMR (400 MHz) δ 4.02 (br s, 1H; OH), 3.71 (s, 3H; OMe), 3.63 (dt, *J*=11.7, 8.0 Hz, 1H; 5-H$_a$), 3.27 (ddd, *J*=11.6, 8.4, 4.8 Hz, 1H; 5-H$_b$), 2.78 (A part of an AB system, *J*=16.6 Hz, 1H; C*H*HCO$_2$Me), 2.72 (d, *J*=13.9 Hz, 1H; 1-H$_a$), 2.69 (B part of an AB system, *J*=16.4 Hz, 1H; C*H*HCO$_2$Me), 2.35 (ddd, *J*=12.5, 6.7, 3.3 Hz, 1H; 7-H$_a$), 2.15 (d, *J*=13.9 Hz, 1H; 1-H$_b$), 2.17–2.09 (m, 2H; 6-H), 1.65 (dt, *J*=12.5, 9.6 Hz, 1H; 7-H$_b$), 1.48 (s, 9H; C*Me*$_3$); ^{13}C NMR (100 MHz) δ 173.2, 172.6, 171.9 (s; C-3, *C*O$_2$*t*Bu, *C*O$_2$Me), 82.6, 79.7, 71.0 (s; C-2, C-7a, *C*Me$_3$), 51.9 (q; OMe), 45.3 (t; C-1), 41.5 (t; C-5) 39.8 (t; *C*H$_2$CO$_2$Me), 35.8 (t; C-7), 27.8 (q, 3C; C*Me*$_3$), 25.8 (t; C-6); IR (KBr) ν=3492, 2986, 1730, 1714, 1694, 1437, 1420, 1368, 1251, 1219, 1158, 1127, 1118 cm^{-1}; MS (EI): *m*/*z*=314 (4, MH$^+$), 212 (81), 194 (100), 152 (44), 110 (64), 84 (20), 59 (13), 57 (36); $C_{15}H_{23}NO_6$ (313.35): Calcd C 57.50, H 7.40, N 4.47; found: C 57.11, H 7.22, N 4.29.

Compound **25**. R_f=0.09 (AcOEt–petroleum ether=2:1); mp 144–146 °C (*i*Pr$_2$O); ^{1}H NMR (400 MHz) δ 4.00 (br s, 1H; OH), 3.78 (s, 3H; OMe), 3.78–3.68 (m, 1H; 3-H$_a$), 3.56–3.50 (m, 1H; 3-H$_b$), 2.98 (d, *J*=14.4 Hz, 1H; 6-H$_a$ or 8-H$_a$), 2.70 (d, *J*=15.4 Hz, 1H; 6-H$_a$ or 8-H$_a$), 2.48 (dd, *J*=15.6, 1.8 Hz, 1H; 6-H$_b$ or 8-H$_b$), 2.43–2.37 (m, 1H; 1-H$_a$), 2.03 (dd, *J*=14.6, 1.6 Hz, 1H; 6-H$_b$ or 8-H$_b$), 2.00–1.86 (m, 2H; 1-H$_b$, 2-H$_a$), 1.82–1.72 (m, 1H; 2-H$_b$), 1.44 (s, 9H; C*Me*$_3$); ^{13}C NMR (100 MHz) δ 174.6, 172.0, 167.2 (s; C-5,

CO_2tBu, CO_2Me), 82.4, 73.7, 67.2 (s; C-7, C-8a, CMe_3), 53.2 (q; OMe), 44.9 (t; C-3), 42.4, 42.1 (t; C-6, C-8), 39.7 (t; C-1), 27.7 (q, 3C; CMe_3), 21.2 (t; C-2); IR (KBr) ν=3353, 2981, 1724 (br), 1644 (br), 1449, 1433, 1302, 1273, 1157 cm^{-1}; MS (EI): m/z=314 (43, MH^+), 258 (12), 212 (78), 194 (100), 162 (23), 110 (20), 84 (22), 57 (26); $C_{15}H_{23}NO_6$ (313.35): Calcd C 57.50, H 7.40, N 4.47; found: C 57.47, H 7.47, N 4.31.

4.1.25. *tert*-Butyl (2*S,7a*R**)-2-(2-methoxy-2-oxoethyl)-2-[(methylsulfonyl)oxy]-3-oxotetrahydro-1*H*-pyrrolizine-7a(5*H*)-carboxylate (33).** Alcohol **24** (100 mg, 0.32 mmol) was dissolved in dry CH_2Cl_2 (0.3 mL) and methanesulfonyl chloride (37 μL, 0.48 mmol) and triethylamine (66 μL, 0.48 mmol) were added dropwise at 0 °C. The mixture was stirred at 0 °C for 30 min and then, diluted with CH_2Cl_2. The organic layer was washed in turn with H_2O and brine, dried over Na_2SO_4 and filtered. The solvent was removed under reduced pressure to give mesylate **33** (124 mg, 99%) as a colorless oil, which was used in the next step without further purification.

Compound **33**. R_f=0.33 (AcOEt–petroleum ether=2:1); ^{1}H NMR (400 MHz) δ 3.70 (dt, J=11.5, 7.7 Hz, 1H; 5-H_a), 3.67 (s, 3H; OMe), 3.39 (ddd, J=11.5, 9.0, 4.1 Hz, 1H; 5-H_b), 3.32 (d, J=1.76 Hz, 2H; CH_2CO_2Me), 3.28 (d, J=15.2 Hz, 1H; 1-H_a), 3.06 (s, 3H; SCH_3), 2.56 (d, J=15.0 Hz, 1H; 1-H_b), 2.28–2.06 (m, 3H; 7-H_a, 6-H), 1.89–1.81 (m, 1H; 7-H_b), 1.48 (s, 9H; CMe_3); ^{13}C NMR (100 MHz) δ 171.8, 169.7, 167.4 (s; C-3, CO_2tBu, CO_2Me), 91.5, 82.6, 70.8 (s; C-2, C-7a, CMe_3), 52.0 (q; OMe), 42.7 (t; C-5), 42.3 (t; C-1) 39.9 (q; SCH_3), 38.4 (t; CH_2CO_2Me), 36.2 (t; C-7), 27.8 (q, 3C; CMe_3), 25.5 (t; C-6); IR ($CDCl_3$) ν=2981, 1730, 1714, 1439, 1369, 1355, 1342, 1232, 1180, 1156, 1121 cm^{-1}; MS (EI): m/z=391 (0.06, M^+), 290 (2), 194 (41), 162 (100).

4.1.26. *tert*-Butyl 6-(2-methoxy-2-oxoethyl)-5-oxo-2,3-dihydro-1*H*-pyrrolizine-7a(5*H*)-carboxylate (35). Crude mesylate **33** (124 mg, 0.32 mmol) was directly treated with triethylamine (133 μL, 0.96 mmol) in CH_3CN (0.4 mL) at 60 °C with 1 h. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography (AcOEt–petroleum ether=1:1) to yield pure **35** (66 mg, 70%) as a colorless glass. When the reaction was performed at rt, a 1.4:1 mixture of **35** and **34** was obtained.

Compound **34**. ^{1}H NMR (400 MHz, selection of signals) δ 6.58 (t, J=2.9 Hz, 1H; CHCO_2Me), 3.79 (s, 3H; OMe), 3.83–3.75 (m, 1H; 5-H_a), 3.62 (AX part of an ABX system,, J=20.1, 2.3 Hz, 1H; 1-H_a), 3.40–3.35 (m, 1H; 5-H_b), 3.11 (BX part of an ABX system, J=20.3, 3.3 Hz, 1H; 1-H_b), 2.50 (ddd, J=12.5, 7.4, 1.6 Hz, 1H; 7-H_a), 2.20–2.08 (m, 2H; 6-H), 1.60–1.40 (m, 1H; 7-H_b), 1.48 (s, 9H; CMe_3); ^{13}C NMR (100 MHz) δ 171.5, 167.0, 166.4 (s; C-3, CO_2tBu, CO_2Me), 150.5 (s; C-2), 119.1 (d; CHCO_2Me), 82.6, 71.7 (s; C-7a, CMe_3), 51.7 (q; OMe), 42.2 (t; C-5), 35.4 (t; C-1) 33.8 (t; C-7), 27.7 (q, 3C; CMe_3), 25.1 (t; C-6).

Compound **35**. R_f=0.29; ^{1}H NMR (400 MHz) δ 7.17 (t, J=1.6 Hz, 1H; 7-H), 3.70 (s, 3H; OMe), 3.60 (td, J=11.3, 8.3 Hz, 1H; 3-H_a), 3.36 (ddd, J=11.3, 7.6, 4.6 Hz, 1H; 3-H_b), 3.33 (AX part of an ABX system, J=18.2, 1.4 Hz, 1H; CHHCO_2Me), 3.27 (BX part of an ABX system, J=18.2, 1.6 Hz, 1H; CHHCO_2Me), 2.36–2.24 (m, 3H; 1-H_a, 2-H), 1.55–1.44 (m, 1H; 1-H_b), 1.45 (s, 9H; CMe_3); ^{13}C NMR (100 MHz) δ 174.0, 170.3, 168.9 (s; C-5, CO_2tBu, CO_2Me), 143.7 (d; C-7), 82.8, 77.5 (s; C-7a, CMe_3), 52.1 (q; OMe), 42.2 (t; C-3), 33.9 (t; C-1) 30.6 (t; CH_2CO_2Me), 28.6 (t; C-2), 27.8 (q, 3C; CMe_3); IR ($CDCl_3$) ν=2981, 1736 (br), 1691 (br), 1438, 1370, 1225, 1153, 1107 cm^{-1}; MS (EI): m/z=296 (3, MH^+), 194 (33), 135 (71), 106 (18), 57 (100); $C_{15}H_{21}NO_5$ (295.33): Calcd C 61.00, H 7.17, N 4.74; found: C 60.91, H 7.53, N 4.65.

Acknowledgements

Authors thank the Ministry of Instruction, University and Research (MIUR, Italy) for financial support (projects PRIN 2002031849 and 2004031072, and FIRB 2001-RBNE017F8N). Ente Cassa di Risparmio di Firenze is acknowledged for granting a 400 MHz NMR spectrometer to the Department of Organic Chemistry. Dr. C. Faggi is acknowledged for X-rays determination. Mrs. B. Innocenti and Mr. M. Passaponti are acknowledged for technical support.

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Tetrahedron 61 (2005) 8848–8854

Synthesis and reactions of 3-alkylsulfanyl-1,3-dihydro-2,1-benzisothiazole 2,2-dioxides

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Received 19 May 2005; revised 28 June 2005; accepted 7 July 2005

Available online 26 July 2005

Abstract—Alkyl- and arylsulfanylation of 1,3-dihydro-2,1-benzisothiazole 2,2-dioxides (benzosultams) **1a–c** and pyridosultam **1d** with dialkyl and diaryl disulfides provides dithioacetals of 2-aminobenzaldehydes **6–13**. 1,3-Dimethylbenzosultam **19** with disulfides forms 3-alkyl(aryl)sulfanyl-1,3-dimethylbenzosultams **20–22** that undergo thermal extrusion of SO_2 followed by a [1,5] sigmatropic hydrogen shift in the intermediate aza-*ortho*-xylylene leading to 1-arylvinyl sulfides **24–26**. Tandem alkylation–sulfanylation of benzo- and pyridosultams **1a–d** with 4-bromobutyl thiocyanate gives tetrahydrothiopyrano-spiro-benzosultams **27–30** that, after extrusion of SO_2 and [1,5] hydrogen shift, form 2-aryl-5,6-dihydro-4*H*-thiopyrans **32–35**. Alkylation of pyridosultam **1d** with 3-chloropropyl thiocyanate leads directly to 2-pyrido-3,4-dihydrothiophene derivative **37**.

1. Introduction

Thermal extrusion of sulfur dioxide from 2,1-benzisothiazoline 2,2-dioxides (benzosultams)[1] provides 6-methylenecyclohexa-2,4-dien-1-imines (*ortho*-quinone methide imines, aza-*ortho*-xylylenes).[2] These reactive 1-azadienes undergo Diels–Alder reaction leading to 1,2,3,4-tetrahydroquinoline derivatives,[3] undergo [1,5] sigmatropic hydrogen shift leading to Schiff bases or 2-vinylanilines,[4] or add nucleophiles to form 2-aminobenzyl derivatives.[5,6] We were interested in how replacement of the hydrogen atoms in the exocyclic methylene group with heteroatoms would influence the reaction course of aza-*ortho*-xylylenes. In one of our previous papers, we described the extrusion of SO_2 from 3,3-dichlorobenzosultams and trapping of the generated 6-(dichloromethylene)cyclohexa-2,4-dien-1-alkylimines with amines.[7]

In this paper, we present results of our studies on the introduction of alkyl-(or aryl) thio substituents into the 3-position of benzosultams and the use of the obtained products as precursors of practically unknown aza-*ortho*-xylylenes bearing alkylsulfanyl substituents in the exocyclic methylene group.

Keywords: Benzosultams; Spiro compounds; Alkylsulfanylation; Extrusion; [1,5] Sigmatropic hydrogen shift; Aza-*ortho*-xylylenes; Dihydrothiopyrans; Phase-transfer catalysis; Sulfides; Disulfides.
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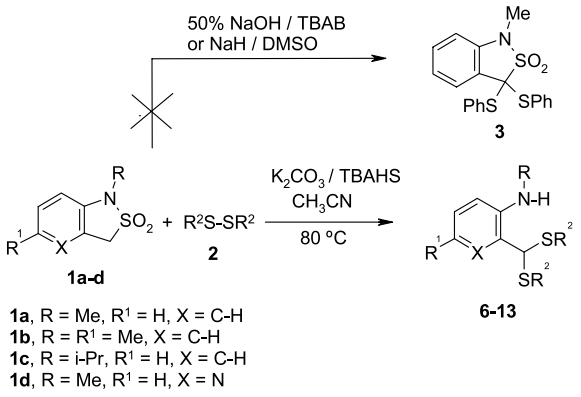
doi:10.1016/j.tet.2005.07.015

2. Results and discussion

Methods of introduction of thio substituents into position-α to electron-withdrawing groups deal with reactions of the corresponding carbanions with sulfanylating agents, such as *S*-alkyl methanethiosulfonates,[8–10] dialkyl disulfides,[11] or organic thiocyanates.[12] Numerous reactions of this type were performed under phase-transfer catalysis conditions employing both solid–liquid[10] and liquid–liquid[12] systems. Direct monosulfanylation of α-sulfonyl carbanions with dimethyl disulfide in the presence of sodium hydride in DMSO has also been described.[13]

Our attempts to employ these base-solvent systems to obtain 3-(phenylsulfanyl)benzosultam under analogous conditions failed. In the reaction of equimolar amounts of benzosultam **1a** and diphenyl disulfide in the presence of NaH in DMSO, the starting materials were completely consumed and a complex inseparable mixture of products, probably originating from mono- and di-sulfenylated benzosultams, was formed.

Attempts to obtain 3,3-di(phenylsulfanyl)benzosultam **3** from benzosultam **1a** and 2 mol of diphenyl disulfide under standard phase-transfer catalysis conditions [50% NaOH, tetrabutylammonium bromide (TBAB)] were also unsuccessful (Scheme 1). Similar results were obtained using other base-solvent systems such as NaH in DMSO or *t*-BuOK in DMF. In these instances GC–MS analysis of the reaction mixture has revealed presence of products whose molecular mass corresponded to an addition of diphenyl



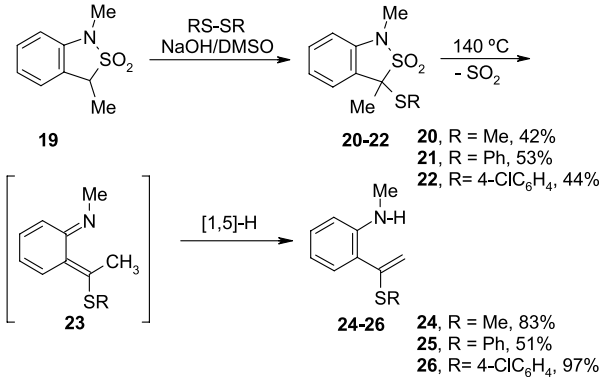
Product	R	R^1	X	R^2	Yield (%)
6	Me	H	C-H	Me	32
7	Me	H	C-H	Ph	37
8	Me_2CH	H	C-H	Ph	79
9	Me	Me	C-H	Ph	55
10	Me	Me	C-H	Me	50
11	Me_2CH	H	C-H	Me	54
12	Me	H	N	Me	54
13	Me	H	N	Ph	34

Scheme 1.

disulfide to the intermediate azaxylylene. Results of these attempts suggested low thermal stability of the expected mono- and/or bis-sulfenylated products. When this reaction was run in the presence of solid K_2CO_3 and tetrabutylammonium hydrogensulfate (TBAHS) as a phase-transfer catalyst in boiling acetonitrile (80 °C), TLC analysis has revealed the presence of one product. The reaction was complete in 24 h, and the dithioacetal of 2-aminobenzaldehyde **6** was isolated in 37% yield. Other benzosultams reacted similarly giving the dithioacetals **7**–**13** in moderate to good yields.

Plausible routes for the formation of these products are shown in the Scheme 2. The first one (**A**) involves extrusion of SO_2 from the initially formed 3-methylthio derivative **14** to form azaxylylene **15**, which intercepts the alkylthiolate anion generated from the disulfide. Another route (**B**) may follow heterolytic fission of the C–SO_2 bond leading to a zwitterionic species **16** isomeric to benzoxathiazine **17**. Both **16** and **17** can intercept a thiolate anion, with a consecutive elimination of SO_2 to form dithioacetal **6**. Both of these routes involve thermal instability of the intermediate monosulfanylated products. The reported thermal decomposition of relatively unstable α-alkylthiobenzyl sulfones occurred at temperature 130–170 °C.[13]

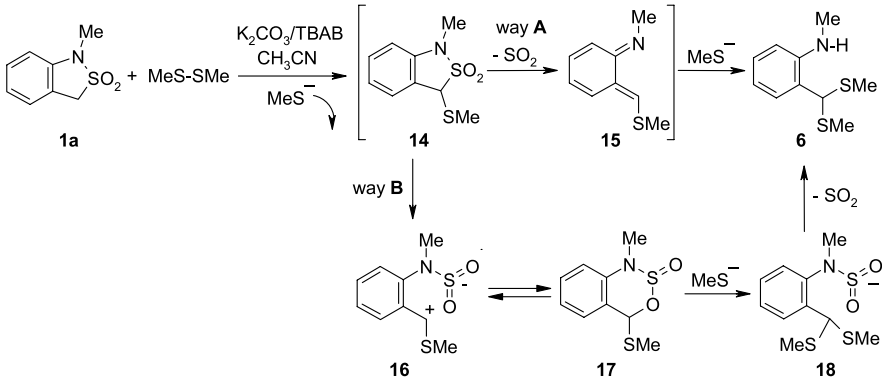
The alkyl- and arylsulfanylation of the tertiary carbanion of 1,3-dimethylbenzosultam **19** with dimethyl and diaryl disulfides in the presence of solid powdered NaOH in dimethylsulfoxide proceeded smoothly, and the expected 3-methylsulfanyl- and 3-arylsulfanylbenzosultams (**20**–**22**) were obtained (Scheme 3). Heating of 3-methyl-3-sulfanyl derivatives in boiling chlorobenzene (140 °C) results in extrusion of SO_2 and the intermediate aza-*ortho*-xylylenes **23** undergo [1,5]-sigmatropic hydrogen shift to give 1-arylvinyl sulfides **24**–**26** in good yields.



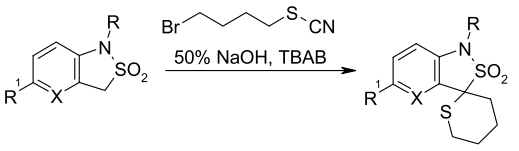
Scheme 3.

We next, investigated alkylation–sulfanylation of the sultams **1a**–**d** with 4-bromobutyl thiocyanate under phase-transfer catalysis conditions employing 50% aqueous NaOH and tetrabutylammonium bromide (TBAB). The expected spiro compounds **25**–**28** were obtained in good yields. Thermal extrusion of SO_2 from the spiro compounds proceeded smoothly and the intermediate azaxylylenes **31** underwent [1,5] sigmatropic hydrogen shift to form novel 6-aryl-3,4-dihydro-2*H*-thiopyrans **32**–**35** in good yields (Scheme 4).

Attempts to obtain analogous spiro compounds, spiro-2-thietanes and spiro-2-tetrahydrothiophenes, in reactions of benzosultams **1a**–**c** with 2-chloroethyl and 3-chloropropyl thiocyanates were unsuccesful. These reactions led to complex mixtures of products, from which isolation of expected spiro compounds proved impossible. Only in the



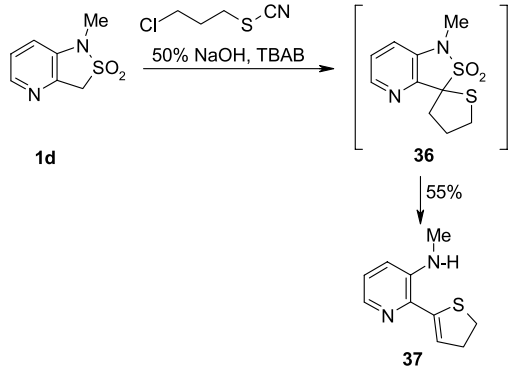
Scheme 2.



R	R^1	X	No.	Yield (%)	No.	Yield (%)
Me	H	C-H	**27**	63	**32**	95
Me	Me	C-H	**28**	53	**33**	91
Me_2CH	H	C-H	**29**	73	**34**	98
Me	H	N	**30**	34	**35**	73

Scheme 4.

case of alkylation of pyridosultam **1d** with 3-chloropropyl thiocyanate dihydrothiophene derivative **37** was obtained in moderate yield (Scheme 5). No intermediate spiro sultam **36** was isolated. This is probably due to an elimination of SO_2 facilitated by an antiperiplanar configuration of the C–H and C–SO_2 bonds in the intermediate spiro compound **36**. Similar elimination was observed earlier during alkylation of pyridosultam **1d** with 1,2-bis(bromomethyl)benzene.[14]



Scheme 5.

3. Conclusion

In conclusion, the reaction course of the 2,1-benzisothiazoline 2,2-dioxides (benzosultams) with sulfanylating agents depends on the structure of both starting materials. The alkylsulfanylation of benzosultams with dialkyl and diaryl disulfides in the presence of potassium carbonate in acetonitrile leads directly to 2-aminobenzaldehyde dithioacetal derivatives. 3-Alkylbenzosultams undergo 3-alkyl-(aryl)sulfanylation to form stable 3-alkyl-3-sulfanyl derivatives, that can transform into 1-arylvinyl sulfides via thermal extrusion of SO_2 followed by a [1,5] hydrogen shift in the intermediate aza-*ortho*-xylylene. Spirobenzosultams obtained in the reaction of benzosultams with 1,ω-haloalkyl thiocyanates transform into 2-aryldihydrothiopyrans and 2-aryldihydrothiophenes.

4. Experimental

4.1. General

Melting points are uncorrected. ^{1}H and ^{13}C NMR and spectra were obtained on a Varian Mercury 400 BB (400 and 100 MHz, respectively) instrument in $CDCl_3$ with TMS as internal standard. Coupling constants *J* are given in Hz. IR spectra were recorded with Perkin Elmer 2000 FTIR instrument. Mass spectra (electron impact, 70 eV) were obtained on AMD 604 (AMD Intectra GmbH, Germany) instrument. HRMS were measured in the presence of perfluorokerosene as the reference compound. Elemental analyses were obtained using a Elementar Vario EL III instrument. Column chromatography was performed using silica gel 240–400 mesh (Merck). Benzosultams **1a–c** and **19** were obtained from the corresponding *N*-alkyl-2-chloro-*N*-(alkanesulfonyl)anilines following the known procedures.[15,16] Pyridosultam **1d** was prepared according to the earlier described procedure.[14]

4.2. Reactions of benzosultams with disulfides. General procedure

Sultam **1a–d** (1 mmol), disulfide **2** (1.1 mmol), K_2CO_3 (0.5 g, 4 mmol) and tetrabutylammonium hydrogensulfate (34 mg, 0.1 mmol) were heated in acetonitrile (5 mL) at reflux under an argon atmosphere until the starting material disappeared (6–40 h, TLC control). The reaction mixture was then poured into water (50 mL), and product was extracted with dichloromethane (3×25 mL). The combined extracts were washed with water, dried with Na_2SO_4 and evaporated. Products were purified by column chromatography (silica gel–hexane/ethyl acetate 2:1 for **6–11** or hexane/ethyl acetate 1:2 for **12** and **13**). The following products were obtained:

4.2.1. {2-[Bis-(methylsulfanyl)methyl]phenyl}methylamine (6). Oil. ^{1}H NMR (400 MHz): δ=2.08 (s, 6H), 2.89 (s, 3H), 4.50 (br s, 1H), 4.89 (s, 1H), 6.71 (d, *J*=8.1 Hz, 1H), 6.73 (ddd, *J*=7.6, 7.4, 1.1 Hz, 1H), 7.23 (ddd, *J*=8.1, 7.4, 1.5 Hz, 1H), 7.30 (dd, *J*=7.6, 1.5 Hz, 1H). ^{13}C NMR (100 MHz): δ=14.99, 30.70, 53.65, 111.09, 116.65, 121.48, 128.70, 129.01, 146.76. IR (KBr, cm^{-1}) ν: 3395, 2980, 2915, 2813, 1724, 1657, 1602, 1581, 1512, 1462, 1425, 1315, 1273, 1171, 1063, 1044, 961, 748. MS (EI 70 eV, *m/z*, %): 213 (M^+, 9), 166 (70), 150 (7), 118 (100), 91 (34). HRMS for $C_{10}H_{15}NS_2$ calcd 213.0646, found 213.0656. Elemental analysis for $C_{10}H_{15}NS_2$ (213.36) calcd C 56.30, H 7.09, N 6.56, found C 56.34, H 6.95, N 6.50.

4.2.2. {2-[Bis-(phenylsulfanyl)methyl]phenyl}methylamine (7). Oil. ^{1}H NMR (400 MHz): δ=2.93 (s, 3H), 4.60 (br s, 1H), 5.54 (s, 1H), 6.58 (dt, *J*=7.4, 1.0 Hz, 1H), 6.71 (d, *J*=7.4 Hz, 1H), 7.16–7.24 (m, 8H), 7.31–7.35 (m, 4H). ^{13}C NMR (100 MHz): δ=30.93, 58.21, 111.27, 117.09, 122.14, 127.69, 128.80, 129.16, 129.33, 132.15, 134.51, 146.60. IR (KBr) ν: 3363, 3072, 3057, 2813, 1656, 1603, 1580, 1510, 1479, 1463, 1438, 1425, 1315, 1170, 1086, 1066, 1024, 742, 689. MS (EI 70 eV, *m/z*, %): 337 (M^+, 1), 228 (57), 150 (4), 118 (100), 91 (21). HRMS for $C_{20}H_{19}NS_2$ calcd 337.0959, found 337.0961. Elemental

analysis for $C_{20}H_{19}NS_2$ (337.49) calcd C 71.18, H 5.67, N 4.15, found C 71.27, H 5.61, N 4.21.

4.2.3. {2-[Bis-(phenylsulfanyl)methyl]phenyl}isopropylamine (8). Oil. ^{1}H NMR (400 MHz): δ=1.29 (d, J=6.2 Hz, 6H), 3.72 (sept, J=6.2 Hz, 1H), 4.35 (br s, 1H), 5.48 (s, 1H), 6.54 (dt, J=7.4, 1.1 Hz, 1H), 6.71 (d, J=8.0 Hz, 1H), 7.14 (ddd, J=8.3, 7.4, 1.5 Hz, 1H), 7.20–7.26 (m, 7H), 7.32–7.36 (m, 4H). ^{13}C NMR (100 MHz): δ=22.98, 44.14, 58.55, 112.25, 116.48, 122.00, 127.65, 128.80, 129.16, 129.37, 132.12, 134.80, 144.88. IR (KBr) ν: 3353, 3059, 2965, 2928, 1601, 1582, 1511, 1479, 1461, 1438, 1383, 1365, 1316, 1259, 1176, 1086, 1067, 1052, 1025, 742, 689. MS (EI 70 eV, *m/z*, %): 365 (M^+, 0.5), 256 (42), 162 (3), 146 (100), 131 (24), 110 (4). HRMS for $C_{22}H_{23}NS_2$ calcd 365.1272, found 365.1288. Elemental analysis for $C_{22}H_{23}NS_2$ (365.53) calcd C 72.28, H 6.34, N 3.83, found C 72.25, H 6.46, N 3.83.

4.2.4. {2-[Bis-(phenylsulfanyl)methyl]-4-methylphenyl}methylamine (9). Oil. ^{1}H NMR (400 MHz): δ=2.14 (s, 3H), 2.89 (s, 3H), 4.40 (br s, 1H), 5.53 (s, 1H), 6.62 (d, J=8.1 Hz, 1H), 7.00 (dd, J=8.1, 2.1 Hz, 1H), 7.03 (br s, 1H), 7.19–7.26 (m, 6H), 7.30–7.36 (m, 4H). ^{13}C NMR (100 MHz): δ=20.26, 31.13, 57.87, 111.49, 122.49, 127.58, 128.77, 129.36, 129.60, 129.74, 131.99, 134.70, 144.35. IR (KBr) ν: 3366, 3057, 2917, 2873, 2810, 1653, 1616, 1579, 1513, 1478, 1438, 1310, 1227, 1168, 1086, 1066, 1024, 943, 808, 739, 689. MS (EI 70 eV, *m/z*, %): 351 (M^+, 2), 242 (86), 164 (4), 132 (100), 117 (27), 105 (13). HRMS for $C_{21}H_{21}NS_2$ calcd 351.1115, found 351.1123.

4.2.5. {2-[Bis-(methylsulfanyl)methyl]-4-methylphenyl}methylamine (10). Oil. ^{1}H NMR (400 MHz): δ=2.09 (s, 6H), 2.26 (s, 3H), 2.87 (s, 3H), 4.87 (s, 1H), 6.63 (d, J=8.2 Hz, 1H), 7.04 (dd, J=8.2, 1.8 Hz, 1H), 7.12 (d, J=1.8 Hz, 1H). ^{13}C NMR (100 MHz): δ=15.02, 20.43, 30.97, 53.60, 111.39, 121.81, 125.92, 129.18, 129.41, 144.47. IR (KBr) ν: 3392, 3361, 2979, 2915, 2809, 1654, 1615, 1578, 1514, 1433, 1311, 1266, 1171, 960, 807. MS (EI 70 eV, *m/z*, %): 227 (12), 180 (65), 164 (7), 132 (100), 117 (33), 105 (16), 91 (4). HRMS for $C_{11}H_{17}NS_2$ calcd 227.0802, found 227.0795. Elemental analysis for $C_{11}H_{17}NS_2$ (227.37) calcd C 58.10, H 7.53, N 6.16, found C 58.11, H 7.43, N 6.22.

4.2.6. {2-[Bis-(methylsulfanyl)methyl]phenyl}isopropylamine (11). Oil. ^{1}H NMR (400 MHz): δ=1.26 (d, J=6.3 Hz, 6H), 2.07 (s, 6H), 3.69 (sept, J=6.3 Hz, 1H), 4.29 (br s, 1H), 4.86 (s, 1H), 6.66 (ddd, J=7.4, 7.4, 1.1 Hz, 1H), 6.72 (br d, J=8.1 Hz, 1H), 7.18 (ddd, J=8.1, 7.4, 1.5 Hz, 1H), 7.28 (dd, J=7.4, 1.5 Hz, 1H). ^{13}C NMR (100 MHz): δ=14.97, 28.86, 44.07, 53.84, 112.17, 116.08, 121.13, 128.86, 129.06, 145.06. IR (KBr) ν: 3378, 2967, 2916, 2869, 1601, 1582, 1513, 1458, 1435, 1383, 1365, 1319, 1261, 1209, 1176, 1156, 1053, 960, 871, 746. MS (EI 70 eV, *m/z*, %): 241 (M^+, 8), 211 (2) 194 (50), 169 (4), 146 (100), 136 (11), 131 (43), 117 (4), 105 (5). HRMS for $C_{12}H_{19}NS_2$ calcd 241.0959, found 241.0970. Elemental analysis for $C_{12}H_{19}NS_2$ (241.41) calcd C 59.70, H 7.94, N 5.80, found C 59.78, H 7.80, N 6.05.

4.2.7. {2-[Bis-(methylsulfanyl)methyl]pyridin-3-yl}methylamine (12). Colorless needles. Mp 72–73 °C (from cyclohexane). ^{1}H NMR (400 MHz): δ=2.10 (s, 6H), 2.89 (d, J=5.1 Hz, 3H), 4.56 (br s, 1H), 5.01 (s, 1H), 6.96 (dd, J=8.2, 1.3 Hz, 1H), 7.13 (dd, J=8.3, 4.7 Hz, 1H), 7.94 (dd, J=4.7, 1.3 Hz, 1H). ^{13}C NMR (100 MHz): δ=13.72, 30.15, 56.52, 117.25, 123.50, 136.66, 142.28. IR (KBr) ν: 3398, 2992, 2913, 2816, 1582, 1504, 1460, 1416, 1401, 1322, 1310, 1292, 1247, 1217, 1147, 1093, 964, 788, 762, 719, 652. MS (EI 70 eV, *m/z*, %): 214 (M^+, 9), 167 (100), 151 (98), 132 (10), 119 (63), 92 (32). HRMS for $C_9H_{14}N_2S_2$ calcd 214.0598, found 214.0607. Elemental analysis for $C_9H_{14}N_2S_2$ (214.34) calcd C 50.43, H 6.58, N 13.07, found C 50.24, H 6.56, N 12.97.

4.2.8. {2-[Bis-(phenylsulfanyl)-methyl]pyridin-3-yl}methylamine (13). Colorless plates. Mp 94–95 °C (from cyclohexane). ^{1}H NMR (400 MHz): δ=2.92 (s, 3H), 4.86 (br s, 1H), 5.87 (br s, 1H), 6.96 (d, J=7.8 Hz, 1H), 7.09 (dd, J=7.8, 4.3 Hz, 1H), 7.20–7.26 (m, 6H), 7.39–7.45 (m, 4H), 7.75 (d, J=4.3 Hz, 1H). ^{13}C NMR due to dynamic phenomena no legible spectrum recordable under standard conditions. IR (KBr) ν: 3362, 3339, 3054, 2817, 1583, 1437, 1425, 1321, 1292, 1250, 1162, 1086, 1023, 881, 794, 734, 688. MS (EI 70 eV, *m/z*, %): 338 (M^+, 0.5), 229 (100), 195 (96), 119 (55), 110 (13), 92 (25). HRMS for $C_{19}H_{18}N_2S_2$ calcd 338.0911, found 338.0909. Elemental analysis for $C_{19}H_{18}N_2S_2$ (338.35) calcd C 67.42, H 5.36, N 8.28, found C 67.20, H 5.41, N 8.25.

4.3. Reactions of 1,3-dimethylbenzosultam 19 with dialkyl(aryl) disulfides. General procedure

To a solution of 1,3-dimethylbenzosultam (400 mg, 2 mmol) and dialkyl(aryl) disulfide (2 mmol) in DMSO (5 mL), powdered sodium hydroxide (1.0 g, 25 mmol) was added in one portion. The reaction mixture was stirred under argon for 30 min at room temperature. The reaction mixture was then poured into water (50 mL) and extracted with ethyl acetate (3×25 mL). The combined extracts were washed with water and dried with Na_2SO_4. The products were purified by column chromatography on silica gel (hexane/ethyl acetate 4:1) and crystallized from ethanol. The following compounds were obtained:

4.3.1. 1,3-Dimethyl-3-methylsulfanyl-1,3-dihydro-2,1-benzisothiazole 2,2-dioxide (20). White solid. Mp 63–64 °C. ^{1}H NMR (400 MHz): δ=1.94 (s, 3H), 2.09 (s, 3H), 3.17 (s, 3H), 6.75 (d, J=8.0 Hz, 1H), 7.09 (dt, J=7.5, 1.0 Hz, 1H), 7.30–7.38 (m, 2H). ^{13}C NMR (100 MHz): δ=13.62, 22.53, 27.09, 69.05, 109.48, 122.46, 124.36, 125.17, 130.05, 140.20. IR (KBr) ν: 2929, 1602, 1482, 1467, 1445, 1318, 1306, 1191, 1153, 1121, 1082, 1064, 1037, 847, 753. MS (EI 70 eV, *m/z*, %): 243 (M^+, 8), 197 (1), 196 (1), 179 (10), 164 (100), 149 (6), 132 (11), 130 (11), 117 (13). HRMS for $C_{10}H_{13}NO_2S_2$ calcd 243.0388, found 243.0376. Elemental analysis for $C_{10}H_{13}NO_2S_2$ (243.34) calcd C 49.35, H 5.39, N 5.76, found C 49.58, H 5.27, N 5.75.

4.3.2. 1,3-Dimethyl-3-phenylsulfanyl-1,3-dihydro-2,1-benzisothiazole 2,2-dioxide (21). White solid. Mp 96–97 °C. ^{1}H NMR (400 MHz): δ=1.98 (s, 3H), 2.88 (s, 3H), 6.48 (d, J=8.0 Hz, 1H), 7.05 (dt, J=7.7, 1.1 Hz, 1H),

7.16–7.22 (m, 2H), 7.26 (dt, 7.7, 1.4 Hz, 1H), 7.29–7.34 (m, 2H), 7.37–7.39 (m, 2H). ^{13}C NMR (100 MHz): δ=20.23, 26.92, 71.60, 109.37, 122.19, 124.38, 125.87, 128.31, 128.52, 129.95, 130.02, 137.39, 139.86. IR (KBr) ν: 3072, 1602, 1470, 1437, 1317, 1281, 1185, 1156, 1122, 1084, 1025, 841, 825, 762, 743, 691. MS (EI 70 eV, *m/z*, %): 305 (M^+, 6), 241 (30), 208 (5), 197 (14), 196 (31), 164 (100), 152 (11), 148 (19), 136 (11), 132 (62), 130 (22), 118 (20), 117 (32). HRMS for $C_{15}H_{15}NO_2S_2$ calcd 305.0544, found 305.0545. Elemental analysis for $C_{15}H_{15}NO_2S_2$ (305.41) calcd C 58.99, H 4.95, N 4.59, found C 58.98, H 4.80, N 4.56.

4.3.3. 1,3-Dimethyl-3-(4-chlorophenylsulfanyl)-1,3-dihydro-2,1-benzisothiazole 2,2-dioxide (22). White solid. Mp 93–94 °C. ^{1}H NMR (400 MHz): δ=1.98 (s, 2H), 2.89 (s, 3H), 6.50 (br d, J=7.8 Hz, 1H), 7.07 (dt, J=7.8, 1.0 Hz, 1H), 7.13–7.19 (m, 2H), 7.25–7.35 (m, 4H). ^{13}C NMR (100 Hz): δ=19.99, 26.81, 71.52, 109.41, 122.30, 124.31, 125.50, 126.97, 128.47, 130.24, 136.60, 138.54, 139.85. IR (KBr) ν: 3076, 2981, 2931, 1603, 1570, 1476, 1469, 1321, 1298, 1273, 1183, 1157, 1123, 1079, 1030, 1016, 832, 810, 770. MS (EI 70 eV, m/z, %): 339 (M^+, 7), 275 (22), 196 (74), 164 (100), 148 (36), 132 (95), 130 (34), 117 (43), 109 (10). HRMS for $C_{15}H_{14}NO_2S_2Cl$ calcd 339.0155, found 339.0163. Elemental analysis for $C_{15}H_{14}NO_2S_2Cl$ (339.86) calcd C 53.01, H 4.15, N 4.12, found C 52.97, H 4.19, N 4.09.

4.4. Extrusion of SO_2 from 1,3-dimethyl-3-alkyl(aryl) sulfanylbenzosultam. General procedure

Benzosultam (**20**–**22**, 1 mmol) was heated in chlorobenzene (5 mL) at reflux. The progress of the reaction was monitored by TLC (hexane/ethyl acetate 4:1, 1.5–6 h). The reaction mixture was then subjected to column chromatography. The chlorobenzene was eluted with hexane/ethyl acetate (50:1) and then product with hexane/ethyl acetate 2:1. The following compounds were obtained:

4.4.1. Methyl-[2-(1-methylsulfanylvinyl)phenyl]amine (24). Oil. ^{1}H NMR (400 MHz): δ=2.23 (s, 3H), 2.85 (s, 3H), 5.17 (s, 1H), 5.24 (s, 1H), 6.62 (br d, J=8.2 Hz, 1H), 6.68 (dt, J=7.5, 1.0 Hz, 1H), 7.10 (dd, J=7.5, 1.5 Hz, 1H), 7.23 (ddd, J=8.2, 7.5, 1.5 Hz, 1H). ^{13}C NMR (100 MHz): δ=15.31, 30.62, 109.75, 109.85, 116.23, 124.72, 129.54, 129.65, 144.19, 146.17. IR (KBr) ν: 3420, 2982, 2917, 2814, 1605, 1589, 1578, 1511, 1460, 1425, 1315, 1290, 1259, 1212, 1169, 1077, 1038, 858, 747. MS (EI 70 eV, *m/z*, %): 179 (M^+, 33), 164 (100), 149 (10), 132 (27), 130 (26), 117 (39), 103 (7). HRMS for $C_{10}H_{13}NS$ calcd 179.0769, found 179.0772. Elemental analysis for $C_{10}H_{13}NS$ (179.28) calcd C 66.99, H 7.31, N 7.81, found C 66.96, H 7.35, N 7.61.

4.4.2. Methyl-{2-[1-(phenylsulfanyl)vinyl]phenyl}amine (25). Oil. ^{1}H NMR (200 MHz): δ=2.87 (s, 3H), 4.60 (br s, 1H), 5.16 (s, 1H), 5.29 (s, 1H), 6.50–6.70 (m, 2H), 7.12–7.55 (m, 7H). ^{13}C NMR (100 MHz): δ=23.69, 102.85, 106.82, 109.17, 121.47, 122.07, 122.56, 123.02, 124.96, 127.33, 132.24, 139.25. IR (KBr) ν: 3425, 3059, 2917, 2814, 1721, 1602, 1577, 1512, 1477, 1460, 1439, 1425, 1315, 1260, 1207, 1169, 1075, 1039, 1024, 872, 744, 691. MS (EI 70 eV, *m/z*, %): 241 (M^+, 27), 164 (72), 149 (3), 132 (100), 131 (16), 130 (30), 117 (68), 115 (16), 105 (13), 99 (52). HRMS for $C_{15}H_{15}NS$ calcd 241.0925, found 241.0918.

4.4.3. {2-[1-(4-Chlorophenylsulfanyl)vinyl]phenyl}-methylamine (26). Oil. ^{1}H NMR (400 MHz): δ=2.84 (s, 3H), 4.60 (s, 1H), 5.22 (s, 1H), 5.31 (s, 1H), 6.54–6.75 (m, 2H), 7.05–7.30 (m, 6H). ^{13}C NMR (100 MHz): δ=30.64, 109.88, 114.75, 116.20, 123.60, 129.19, 129.71, 130.06, 131.36, 134.58, 135.27, 143.71, 146.17. IR (KBr) ν: 3424, 2916, 2815, 1629, 1600, 1575, 1513, 1475, 1424, 1388, 1314, 1260, 1170, 1093, 1012, 821, 747. MS (EI 70 eV, *m/z*, %): 275 (M^+, 28), 242 (7), 164 (46), 132 (100), 131 (11), 130 (20), 117 (37). HRMS for $C_{15}H_{14}ClNS$ calcd 275.0536, found 275.0539.

4.5. Synthesis of spiro compounds 27–30. General procedure

Sultam **1a**–**d** (2 mmol), 4-bromobutyl thiocyanate (2.2 mmol), tetrabutylammonium bromide (32 mg, 0.1 mmol) in toluene (5 mL) were stirred vigorously with 50% aqueous NaOH (10 mL) at room temperature under argon. The progress of the reaction was monitored by TLC (30–90 min). Then the reaction mixture was poured into water (50 mL) and the product was extracted with dichloromethane (3×25 mL). The combined extracts were washed with water and dried over Na_2SO_4. After evaporation of solvent the product was purified by column chromatography (hexane/ethyl acetate 2:1) and crystallized from ethanol. The following compounds were obtained:

4.5.1. 1-Methyl-1,3-dihydro-2,1-benzisothiazole-3-spiro-2′-tetrahydrothiopyran 2,2-dioxide (27). White solid. Mp 122–123 °C. ^{1}H NMR (400 MHz): δ=1.82–2.10 (m, 3H), 2.14–2.22 (m, 1H), 2.37 (ddd, J=14.3, 13.2, 3.7 Hz, 1H), 2.55 (ddd, J=14.0, 3.2, 2.9 Hz, 1H), 2.70 (ddd, J=14.3, 3.1, 2.9 Hz, 1H), 3.19 (s, 3H), 3.57 (ddd, J=13.5, 12.7, 2.7 Hz, 1H), 6.74 (br d, J=8.0 Hz, 1H), 7.05 (ddd, J=7.8, 7.7, 1.1 Hz, 1H), 7.28 (dd, J=8.0, 1.4 Hz, 1H), 7.33 (ddd, J=8.0, 7.7, 1.4 Hz, 1H). ^{13}C NMR (100 MHz): δ=22.78, 25.29, 27.57, 28.12, 31.45, 67.68, 109.86, 122.44, 124.37, 125.92, 130.20, 140.06. IR (KBr) ν: 2943, 2922, 1603, 1483, 1470, 1311, 1190, 1152, 1134, 1066, 1029, 839, 752. MS (EI 70 eV, m/z, %): 269 (M^+, 0.5), 205 (53), 176 (49), 162 (12), 144 (38), 130 (100), 117 (17). HRMS for $C_{12}H_{15}NO_2S_2$ calcd 269.0544, found 269.0557.

4.5.2. 1,5-Dimethyl-1,3-dihydro-2,1-benzisothiazole-3-spiro-2′-tetrahydrothiopyran 2,2-dioxide (28). Pale yellow solid. Mp 110–111 °C. ^{1}H NMR (400 MHz): δ= 1.75–2.20 (m, 4H), 2. 34 (s, 3H), 2. 36 (ddd, J=16.9, 13.2, 3.5 Hz, 1H), 2.50–2.57 (m, 1H), 2.69 (dt, J=14.2, 3.2 Hz, 1H), 3.17 (s, 3H), 3.57 (ddd, J=13.4, 12.8, 2.9 Hz, 1H), 6.65 (d, J=8.1 Hz, 1H), 7.09 (dd, J=1.7, 0.7 Hz, 1H), 7.13 (ddd, J=8.1, 1.7, 0.7 Hz, 1H). ^{13}C NMR (100 MHz): δ= 20.97, 22.78, 25.35, 28.09, 28.11, 31.41, 67.75, 110.07, 124.86, 125.93, 130.66, 132.22, 137.87. IR (KBr) ν: 2944, 2919, 2862, 1614, 1492, 1464, 1445, 1428, 1312, 1186, 1162, 1132, 839, 819. MS (EI 70 eV, m/z, %): 283 (M^+, 4), 219 (62), 204 (12), 190 (66), 176 (15), 158 (34), 144 (100), 130 (12), 115 (11). HRMS for $C_{13}H_{17}NO_2S_2$ calcd 283.0701, found 283.0693. Elemental analysis for

$C_{13}H_{17}NO_2S_2$ (283.39) calcd C 55.09, H 6.04, N 4.94, found C 54.97, H 6.38, N 4.98.

4.5.3. 1-Isopropyl-1,3-dihydro-2,1-benzisothiazoline-3-spiro-2′-tetrahydrothiopyran 2,2-dioxide (29). White solid. Mp 108–109 °C. ^{1}H NMR (400 MHz): δ=1.53 (d, J=7.0 Hz, 3H), 1.59 (d, J=6.9 Hz, 3H), 1.75–2.20 (m, 4H), 2.36 (ddd, J=14.1, 13.2, 3,6 Hz, 1H), 2.50 (ddd, J=13.7, 3.3, 3.0 Hz, 1H), 2.68 (ddd, J=14.1, 3.5, 3.2 Hz, 1H), 3.53 (ddd, J=13.4, 12.8, 2.8 Hz, 1H), 4.36 (dq, J=7.0, 6.9 Hz, 1H), 6.89 (br d, J=8.1 Hz, 1H), 7.02 (ddd, J=7.7, 7.6, 1.0 Hz, 1H), 7.25–7.31 (m, 2H). ^{13}C NMR (100 MHz): δ= 19.51, 21.52, 22.85, 25.36, 28.17, 31.46, 47.78, 67.35, 112.01, 122.02, 124.76, 126.56, 129.77, 138.28. IR (KBr) ν: 2979, 2935, 1600, 1476, 1311, 1262, 1166, 1142, 1017, 887, 762, 749. MS (EI 70 eV, m/z, %): 297 (M^+, 5), 233 (79), 218 (71), 204 (22), 190 (100), 184 (11), 162 (66), 158 (38), 157 (25), 156 (26), 148 (22), 144 (37), 130 (31, 115 (17). HRMS for $C_{14}H_{19}NO_2S_2$ calcd 297.0857, found 297.0852. Elemental analysis for $C_{14}H_{19}NO_2S_2$ (297.43) calcd C 56.53, H 6.44, N 4.71, found C 56.23, H 6.40, N 4.56.

4.5.4. 1-Methyl-1,3-dihydroisothiazolo[4,3-b]pyridine-3-spiro-2′-tetrahydrothiopyran 2,2-dioxide (30). White solid. Mp 108–109 °C. ^{1}H NMR (200 MHz): δ=1.80–1.96 (m, 2H), 2.05–2.21 (m, 2H), 2.60–2.78 (m, 3H), 3.21 (s, 3H), 3.54 (ddd, J=15.4, 12.7, 2.8 Hz, 1H), 7.03 (dd, J= 8.1, 1.3 Hz, 1H), 7.27 (dd, J=8.1, 5.0 Hz, 1H), 8.26 (dd, J=5.0, 1.3 Hz, 1H). ^{13}C NMR (100 MHz): δ=22.36, 25.05, 27.20, 27.75, 29.30, 68.49, 116.73, 124.72, 136.56, 143.21, 144.81. IR (KBr) ν: 2933, 1585, 1575, 1472, 1437, 1312, 1244, 1230, 1200, 1175, 1159, 1131, 1032, 839, 798. MS (EI 70 eV, m/z, %): 270 (M^+, 6), 206 (89), 205 (18),191 (37), 177 (57), 173 (54), 163 (30), 159 (60), 146 (40), 145 (100), 131 (36), 119 (21). HRMS for $C_{11}H_{14}N_2O_2S_2$ calcd 270.0497, found 270.0485. Elemental analysis for $C_{11}H_{14}N_2O_2S_2$ (270.38) calcd C 48.86, H 5.22, N 10.36, found C 48.96, H 5.47, N 10.19.

4.6. Synthesis of 2-aryl-5,6-dihydro-4*H*-thiopyrans 32–35. General procedure

Spiro compound 27–30 (1 mmol) was heated at reflux in 1, 2-dichlorobenzene (3 mL) for 30–60 min (TLC control). The reaction mixture was then subjected to column chromatography. The dichlorobenzene was eluted with hexane/ethyl acetate (50:1) and then product with hexane/ethyl acetate 2:1. The following compounds were obtained:

4.6.1. [2-(5,6-Dihydro-4*H*-thiopyran-2-yl)phenyl]methylamine (32). Oil. ^{1}H NMR (400 MHz): δ=2.02–2.09 (m, 2H), 2.28–2.32 (m, 2H), 2.84 (s, 3H), 2.99–3.01 (m, 2H), 4.50 (br s, 1H), 5.74 (t, J=4.4 Hz, 1H), 6.60 (dd, J=8.1, 1.0 Hz, 1H), 6.66 (dt, J=7.4, 1.0 Hz, 1H), 7.08 (dd, J=7.4, 1.6 Hz, 1H), 7.20 (ddd, J=8.1, 7.5, 1.6 Hz, 1H). ^{13}C NMR (100 MHz): δ=21.71, 24.50, 27.71, 30.69, 109.82, 116.38, 121.41, 126.03, 129.14, 129.75, 130.40, 146.52. IR (KBr) ν: 3419, 2918, 2833, 2813, 1598, 1579, 1514, 1460, 1427, 1316, 1267, 1168, 1070, 973, 960, 871, 853, 747. MS (EI 70 eV, *m/z*, %): 205 (M^+, 62), 190 (7), 176 (50), 162 (13), 144 (41), 130 (100), 117 (18), 103 (5), 91 (7). HRMS for $C_{12}H_{15}NS$ calcd 205.0925, found 205.0929. Elemental analysis for $C_{12}H_{15}NS$ (205.31) calcd C 70.19, H 7.36, N 6.82, found C 70.28, H 7.30, N 6.68.

4.6.2. [2-(5,6-Dihydro-4*H*-thiopyran-2-yl)-4-methylphenyl]methylamine (33). White solid. Mp 63–65 °C. ^{1}H NMR (400 MHz): δ=2.02–2.09 (m, 2H), 2.23 (s, 3H), 2.29–2.34 (m, 2H), 2.83 (s, 1H), 2.97–3.02 (m, 2H), 4.30 (br s, 1H), 5.73 (t, J=4.4 Hz, 2H), 6.53 (d, J=8.2 Hz, 1H), 6.92 (d, J=2.2 Hz, 1H), 7.01 (dd, J=8.2, 2.2 Hz, 1H). ^{13}C NMR (100 MHz): δ=20.20, 21.76, 24.52, 27.76, 31.07, 110.19, 121.30, 125.62, 126.17, 129.61, 130.39, 130.48, 144.35. IR (KBr) ν: 3412, 2908, 1632, 1612, 1579, 1515, 1473, 1443, 1431, 1401, 1313, 1276, 1265, 1168, 1128, 1070, 973, 848, 800. MS (EI 70 eV, *m/z*, %): 219 (M^+, 81), 204 (11), 190 (49), 176 (13), 158 (42), 144 (100), 130 (12), 115 (12). HRMS for $C_{13}H_{17}NS$ calcd 219.1082, found 219.1091. Elemental analysis for $C_{13}H_{17}NS$ (219.33) calcd C 71.18, H 7.81, N 6.39, found C 71.04, H 7.92, N 6.36.

4.6.3. [2-(5,6-Dihydro-4*H*-thiopyran-2-yl)phenyl]isopropylamine (34). Oil. ^{1}H NMR (400 MHz): δ=1.23 (d, J=6.3 Hz, 6H), 2.02–2.09 (m, 2H), 2.32–2.37 (m, 2H), 2.99–3.00 (m, 2H), 3.64 (sept, J=6.3 Hz, 1H), 4.50 (br s, 1H), 5.74 (dd, J=4.4, 4.3 Hz, 1H), 6.60–6.74 (m, 2H), 7.05–7.20 (m, 2H). ^{13}C NMR (100 MHz): δ=21.44, 21.82, 22.79, 24.56, 27.72, 111.95, 116.43, 121.71, 128.32, 129.01, 130.13, 130.45, 144.44. IR (KBr) ν: 3398, 2963, 2932, 1598, 1579, 1508, 1462, 1453, 1382, 1364, 1317, 1266, 1176, 1124, 1048, 974, 875, 745. MS (EI 70 eV, m/z, %): 233 (M^+, 91), 218 (67), 200 (11), 190 (100), 184 (16), 170 (11), 162 (57), 158 (50), 157 (38), 156 (35), 144 (52), 130 (39), 115 (22), 87 (80). HRMS for $C_{14}H_{19}NS$ calcd 233.1238, found 233.1232. Elemental analysis for $C_{14}H_{19}NS$ (233.37) calcd C 72.05, H 8.21, N 6.00, found C 72.01, H 8.28, N 5.80.

4.6.4. [2-(5,6-Dihydro-4*H*-thiopyran-2-yl)pyridin-3-yl]methylamine (35). Yellow needles. Mp 134–135 °C. ^{1}H NMR (200 MHz): δ=2.06–2.14 (m, 2H), 2.34–2.40 (m, 2H), 2.84 (s, 3H), 3.02–3.07 (m, 2H), 4.63 (br s, 1H), 6.01 (t, J=4.4 Hz, 1H), 6.90 (dd, J=8.2, 1.3 Hz, 1H), 7.11 (dd, J=8.2, 4.7 Hz, 1H), 7.95 (dd, J=4.7, 1.3 Hz, 1H). ^{13}C NMR (50 MHz): δ=21.64, 24.32, 27.41, 30.21, 116.87, 122.96, 123.66, 130.21, 136.63, 142.79, 143.34. IR (KBr) ν: 3355, 2919, 2811, 1579, 1494, 1424, 1317, 1258, 1162, 969, 795, 755. MS (EI 70 eV, *m/z*, %): 206 (M^+, 77), 191 (23), 177 (39), 173 (46), 163 (28), 159 (57), 146 (9100), 131 (37), 119 (21). HRMS for $C_{11}H_{14}N_2S$ calcd 206.0878, found 207.0886. Elemental analysis for $C_{11}H_{14}N_2S$ (206.30) calcd C 64.04, H 6.84, N 13.58, found C 63.84, H 7.08, N 13.39.

4.6.5. [2-(4,5-Dihydrothiophen-2-yl)pyridin-3-yl]methylamine (37). Pyridosultam **1d** (200 mg, 1 mmol) 3-chloropropyl thiocyanate (145 mg, 1.1 mmol), tetrabutylammonium bromide (32 mg, 0.1 mmol) in toluene (5 mL) were stirred vigorously with 50% aqueous NaOH (10 mL) at room temperature under argon. After 1 h the reaction mixture was poured into water (50 mL) and the product was extracted with dichloromethane (3×25 mL). The combined extracts were washed with water and dried over Na_2SO_4. After evaporation of solvent the product was purified by column chromatography (hexane/ethyl acetate 2:1) and crystallized from ethanol. Yield 106 mg (55%). Orange

solid. Mp 119–120 °C. ^{1}H NMR (400 MHz): δ=2.86 (d, J=5.0 Hz, 3H), 3.01–3.07 (m, 2H), 3.31–3.36 (m, 2H), 4.56 (br s, 1H), 6.07 (t, J=3.0 Hz, 1H), 6.92 (dd, J=8.3, 1.3 Hz, 1H), 7.09 (dd, J=8.3, 4.7 Hz, 1H), 7.97 (dd, J=4.7, 1.3 Hz, 1H). ^{13}C NMR (100 MHz): δ=30.32, 31.94, 37.09, 116.72, 121.92, 123.45, 137.18, 138.82, 141.34, 143.07. IR (KBr) ν: 3319, 2924, 2886, 2860, 2811, 1580, 1496, 1451, 1422, 1406, 1316, 1263, 1225, 1161, 1124, 1017, 946, 793, 753. MS (EI 70 eV, *m*/*z*, %): 192 (100, M^{+}), 177 (16), 175 (8), 164 (57), 159 (18), 149 (28), 145 (54), 133 (53), 132 (78), 131 (44), 118 (15), 104 (7). Elemental analysis for $C_{10}H_{12}N_2S$ (192.28) calcd C 62.47, H 6.29, N 14.57, found C 62.26, H 6.03, N 14.44.

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Tetrahedron 61 (2005) 8855–8859

A formal total synthesis of (±)-9-isocyanoneopupukeanane

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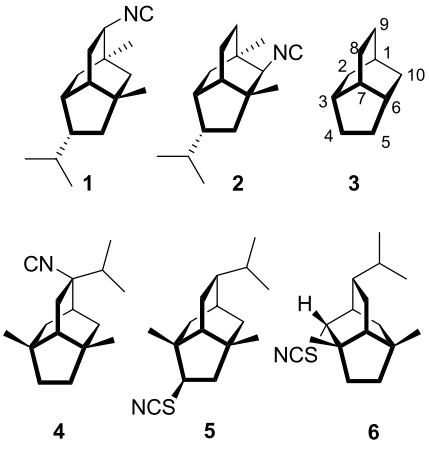
Received 30 April 2005; revised 27 June 2005; accepted 7 July 2005

Available online 28 July 2005

Abstract—A formal total synthesis of the marine sesquiterpene (±)-9-isocyanoneopupukeanane starting from the readily available monoterpene carvone has been accomplished employing a combination of intermolecular Michael addition–intramolecular Michael addition reaction and an intramolecular rhodium carbenoid C–H insertion reaction as key steps, and identifying the isopropenyl group as a masked hydroxy group.

1. Introduction

In a variety of marine organisms, chemical defence via secretion of toxic and/or strong smelling organic compounds from skin glands is a common phenomenon as part of the self-defence mechanism to protect themselves from higher animals. Based on the observation that the nudibranch *Phyllidia varicosa* Lamarck secretes a toxic substance lethal to fish and crustaceans, investigations on the chemical constituents of the skin extracts of *P. varicosa* and also from its prey, a sponge, *Hymeniacidon* sp. by the research group of Scheuer led to the isolation[1] of two novel sesquiterpenes, 9-isocyano- and 2-isocyanopupukeananes **1** and **2**. The pupukeananes **1** and **2** contain a tricyclo[4.3.1.0^{3,7}]decane (isotwistane **3**) carbon framework, which was observed for the first time among the natural products. Subsequently, during their biosynthetic experiments directed towards discovering the origin of the isocyano group in marine sponges, Scheuer and co-workers[2] isolated a new sesquiterpene **4** from the sponge *Ciocalypta* sp. containing the isocyano functionality and a new carbon framework named as neopupukeanane. Later, the research groups of Scheuer, Higa and Faulkner reported[3] the isolation of two more sesquiterpenes belonging to this group, 4- and 2-thiocyanatoneopupukeananes **5** and **6**, from the sponge *Phycopsis terpnis* (from Okinawa) and from an unidentified species from Pohnpei.



The presence of the tricyclo[4.3.1.0^{3,7}]decane carbon framework (isotwistane) incorporating two quaternary carbon centres besides the thiocyanate and isonitrile functionalities made neopupukeananes attractive and challenging synthetic targets. In 1999, Ho and Jana reported[4,5] the first total synthesis of (±)-9-isocyanoneopupukeanane **4** via the symmetric isotwistanone **7**. Herein we report a formal total synthesis of (±)-**4** starting from readily available monoterpene carvone **8**.

2. Results and discussion

It was anticipated (Scheme 1) that the rhodium carbenoid C–H insertion[6] of the diazoketone **10**, derived from bicyclo[2.2.2]octanecarboxylate **11**, would generate the isotwistanedione **9** in a regioselective manner via preferential formation of a five-membered ring by the insertion of the rhodium carbenoid into the only available γ C–H bond,

Keywords: Marine sesquiterpenes; Neopupukeanane; Double Michael reaction; Rhodium CH insertion reaction.
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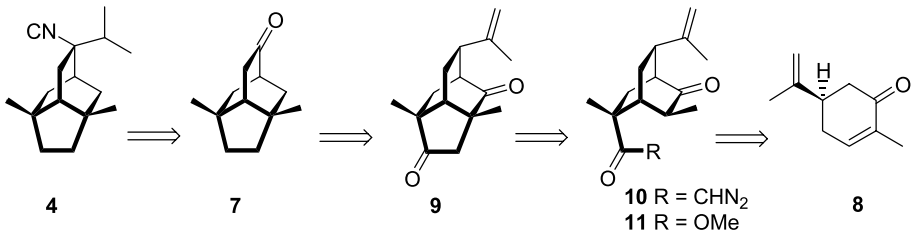
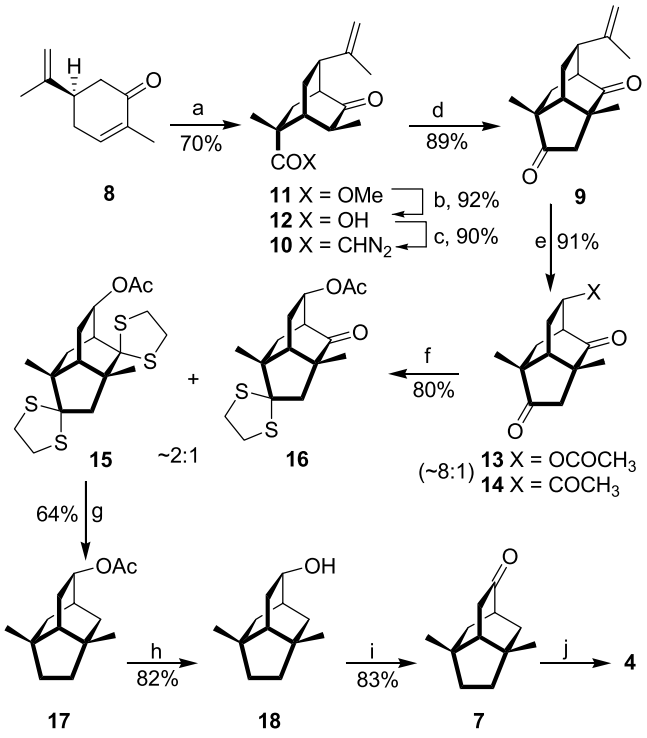
doi:10.1016/j.tet.2005.07.016



Scheme 1.

which could be further elaborated to symmetric isotwistanone **7** via reductive deoxygenation of the two ketones and degradation of the isopropenyl group. An intermolecular Michael addition followed by an intramolecular Michael reaction of carvone **8** with methyl methacrylate was exploited for generating the bicyclo[2.2.2]octanone carboxylate **11**.[7] Thus, reaction of carvone **8** with lithium hexamethyldisilazide (LiHMDS) in hexane at −70 °C followed by reaction of the resultant kinetic dienolate with one equivalent of methyl methacrylate furnished the bicyclic keto ester **11** via the Michael–Michael reaction in 70% yield in a highly regio- and stereoselective manner (Scheme 2). Base catalysed hydrolysis of the keto ester **11** furnished the keto acid **12**, which was converted into the diazoketone **10** via the corresponding acid chloride. Treatment of the diazoketone **10** with a catalytic amount of rhodium acetate in dichloromethane at reflux furnished the isotwistane dione **9** in 89% yield, containing the complete carbon framework of neopupukeananes,[8] via regiospecific C–H insertion of the intermediate rhodium carbenoid. As the olefin moiety in the isopropenyl group was found to isomerise under acidic conditions, it was contemplated to degrade the isopropenyl group prior to the reductive deoxygenation of the ketones. Since the ozonolysis followed by Baeyer–Villiger rearrangement would lead to regiochemical problems, a one pot ozonolysis-Criegee rearrangement[9] was chosen for the conversion of the isopropenyl moiety into an acetoxy group. Thus, ozonolysis of the isotwistanedione **9** in methanol–methylene chloride medium followed by treatment of the resultant methoxyhydroperoxide with acetic anhydride, triethylamine and a catalytic amount of 4-*N*,*N*-dimethylaminopyridine (DMAP) in benzene at reflux furnished the diketoacetate **13** in 81% yield along with the normal ozonolysis product triketone **14** (11%). For the reductive deoxygenation of the two ketones, a two step protocol was employed via the corresponding bis-dithioketal. Accordingly, treatment of the diketoacetate with ethanedithiol in the presence of a catalytic amount of either iodine[10] or boron trifluoride diethyl etherate furnished a mixture of bis and mono dithioketals **15** and **16** in 52 and 28% yields, respectively. Raney nickel mediated desulfurisation of the bis-dithioketal **15** furnished the 9-isotwistanyl acetate **17**. Hydrolysis of the acetate group in **17** with potassium carbonate in methanol followed by oxidation of the resultant isotwistanol **18** with pyridinium chlorochromate (PCC) and silica gel in methylene chloride furnished the isotwistanone **7**. Both the alcohol **18** and the ketone **7** exhibited spectral data, in particular ^{1}H and ^{13}C NMR, identical to those reported by Ho and Jana. Since the conversion of isotwistanone **7** into 9-isocyanoneopupukeanane (±)-**4** has already been accomplished by Ho and Jana,[4] the present sequence constitute a formal total synthesis of the marine sesquiterpene **4**.



Scheme 2. (a) $LiN(TMS)_2$, $CH_2{=}C(Me)COOMe$, hexane; (b) NaOH, MeOH–H_2O, reflux; (c) (i) $(COCl)_2$, C_6H_6; (ii) CH_2N_2, Et_2O; (d) $Rh_2(OAc)_4$, CH_2Cl_2; (e) O_3/O_2, MeOH–CH_2Cl_2, −70 °C; Ac_2O, NEt_3, DMAP, C_6H_6, reflux; (f) $(CH_2SH)_2$, I_2, $CHCl_3$; (g) Raney Ni, EtOH, reflux; (h) K_2CO_3, MeOH; (i) PCC, silica gel, CH_2Cl_2; (j) Ref. 4.

In summary, we have developed a convenient approach to 9-isocyanoneopupukeanane starting from readily available monoterpene carvone. The isopropenyl group of carvone has been identified as a masked hydroxy group. A combination of double Michael reaction and a regiospecific intramolecular rhodium carbenoid CH insertion reaction have been employed as key steps for the regio- and stereospecific efficient construction of the isotwistane skeleton.

3. Experimental

3.1. General

Melting points are recorded using Tempo and Mettler FP1 melting point apparatus in capillary tubes and are uncorrected. IR spectra were recorded on a Jasco FTIR

410 spectrophotometer. ^{1}H (300 MHz) and ^{13}C (75, 22.5 MHz) spectra were recorded on JNM λ-300 or JEOL FX-90Q spectrometers. The chemical shifts (δ ppm) and coupling constants (Hz) are reported in the standard fashion with reference to either internal tetramethylsilane (for ^{1}H) or the central line (77.1 ppm) of $CDCl_3$ (for ^{13}C). In the ^{13}C NMR spectra, the nature of the carbons (C, CH, CH_2 or CH_3) were determined either by recording the DEPT-135 or off-resonance decoupling, and are given in parentheses. Low-resolution mass spectra were recorded using a Shimadzu QP-5050A GCMS instrument using direct inlet (EI) mode. Relative intensities are given in parentheses. High resolution mass spectra were recorded on a Micromass Q-TOF micro mass spectrometer using electron spray ionization mode. Optical rotations were measured using a Jasco DIP-370 digital polarimeter and $[\alpha]_D$ values are given in units of 10^{-1} deg cm^2 g^{-1}. Ozonolysis experiments were carried out using Fischer 502 ozone generator by passing pre-cooled (−70 °C) oxygen (for generating dry ozone). Acme's silica gel (100–200 mesh) was used for column chromatography (approximately 15–20 g per 1 g of the crude product). Dry THF was obtained by distillation over sodium-benzophenone ketyl. Dry ether was obtained by distillation over sodium and stored over sodium wire. Dry dichloromethane was prepared by distilling over calcium hydride. All the commercial reagents were used as such without further purification.

3.1.1. Methyl (1*R*,2*R*,4*S*,6*S*,8*R*)-8-isopropenyl-2,6-dimethyl-5-oxobicyclo[2.2.2]octane-2-carboxylate (11). To a cold (−78 °C), magnetically stirred solution of hexamethyldisilazane (2.02 ml, 9.33 mmol) in dry hexane (20 ml) was slowly added by a syringe a solution of *n*-BuLi (4.56 ml, 1.9 M in hexane, 20 mmol) and the mixture stirred for 15 min. To the LiHMDS thus, formed was added drop wise a solution of (*R*)-carvone **8** (1 g, 6.66 mmol) in dry hexane (5 ml) and continued stirring for 45 min at the same temperature. The enolate was treated with methyl methacrylate (0.78 ml, 7.33 mmol) and stirred for 3 h at rt. The reaction mixture was then filtered through a small silica gel column. Evaporation of the solvent and purification of the residue on a silica gel column using ethyl acetate/hexane (1:20) as eluent furnished the bicyclic adduct **11** (1.16 g, 70%) as a white solid,[7] which was recrystallized from hexanes, mp 59–61 °C (lit.[7] 60–61.5 °C). $[\alpha]_D^{22}$ −43.5 (*c* 3.77, MeOH) [lit.[7] $[\alpha]_D^{25}$ −42.6 (*c* 1.00, MeOH)]. ν_{max} (neat) 1720, 1645, 895 cm^{-1}. δ_H (300 MHz, $CDCl_3$) 4.75 (1H, s), 4.72 (1H, s), 3.71 (3H, s), 2.70 (1H, dd, J=14.5, 2.5 Hz), 2.60–2.40 (2H, m), 2.25–2.00 (3H, m), 1.71 (3H, s), 1.70–1.55 (2H, m), 1.46 (3H, s), 1.10 (3H, d, J=6.9 Hz). δ_C (22.5 MHz, $CDCl_3$) 216.7 (s), 177.5 (s), 146.7 (s), 109.7 (t), 52.0 (q), 47.2 (d), 44.5 (d), 44.2 (s), 42.1 (d), 41.5 (d), 33.9 (t), 26.4 (q), 21.7 (2C, q and t), 12.3 (q).

3.1.2. (1*R*,2*R*,4*S*,6*S*,8*R*)-8-Isopropenyl-2,6-dimethyl-5-oxobicyclo[2.2.2]octane-2-carboxylic acid (12). To a solution of the keto ester **11** (1.0 g, 4 mmol) in 5 ml methanol was added 10% aq NaOH solution (5 ml), and the reaction heated at reflux for 8 h. It was then cooled to rt and washed with CH_2Cl_2 (10 ml). The aqueous layer was acidified with 3 M aq HCl and extracted with CH_2Cl_2 (3×15 ml). The CH_2Cl_2 extract was washed with brine, dried (Na_2SO_4) and the solvent evaporated in vacuo to furnish the acid **12** (870 mg, 92%) as a sticky solid, which was recrystallized from a mixture of hexane and CH_2Cl_2,[8] mp 119–120 °C. $[\alpha]_D^{26}$ −47.7 (*c* 1.30, $CHCl_3$). ν_{max} (neat) 1720, 1700, 890 cm^{-1}. δ_H (300 MHz, 1:1 $CDCl_3$+CCl_4) 4.74 (1H, s), 4.72 (1H, s), 2.68 (1H, dd, J=14.7, 2.4 Hz), 2.55–2.45 (2H, m), 2.30–2.10 (3H, m), 1.71 (3H, s), 1.75–1.50 (2H, m), 1.59 (1H, dd, J=14.7, 3.3 Hz), 1.52 (3H, s), 1.12 (3H, d, J=6.9 Hz). δ_C (22.5 MHz, $CDCl_3$) 217.7 (s), 183.4 (s), 146.7 (s), 110.0 (t), 47.3 (d), 44.7 (d), 44.1 (s), 42.1 (d), 41.5 (d), 33.7 (t), 26.5 (q), 21.7 (2C, q and t), 12.5 (q).

3.1.3. (1*S*,3*R*,6*R*,7*S*,9*R*)-9-Isopropenyl-3,6-dimethyltricyclo[4.3.1.0^{3,7}]decane-2,5-dione (9). To a magnetically stirred solution of the acid **12** (500 mg, 2.12 mmol) in dry benzene (3 ml) was added oxalyl chloride (0.93 ml, 10.6 mmol) and the reaction mixture was stirred for 2 h at rt. Evaporation of benzene and the excess oxalyl chloride in vacuo furnished the acid chloride, which was taken in dry ether (5 ml) and added to a cold (0 °C) magnetically stirred solution of diazomethane (25 ml, prepared from 3 g of *N*-nitroso-*N*-methylurea and 30 ml of 60% aq KOH solution). The reaction mixture was slowly warmed up to rt, stirred for 2 h and the excess diazomethane and ether were carefully evaporated on a water bath. Rapid purification by filtration of the crude product through a neutral alumina column using CH_2Cl_2 as eluent furnished the diazoketone **10** (495 mg, 90%) as yellow oil. IR (neat). ν_{max}/cm^{-1} 2100, 1710, 1620, 890 cm^{-1}.

To a magnetically stirred, refluxing solution of rhodium acetate (4 mg) in dry CH_2Cl_2 (30 ml) was added drop wise a solution of the diazoketone **71** (495 mg, 1.91 mmol) in CH_2Cl_2 (10 ml) and the reaction mixture was refluxed for 4 h. Evaporation of the solvent and purification of the residue on a silica gel column using ethyl acetate/hexane (1:10) as eluent furnished the dione **9** (392 mg, 89%) as a white solid, which was recrystallized from a mixture of ethyl acetate/hexane,[8] mp 111–113 °C. $[\alpha]_D^{26}$ −45.5 (*c* 1.32, $CHCl_3$). ν_{max} (neat) 1740, 1710, 890 cm^{-1}. δ_H (300 MHz, $CDCl_3$) 4.83 (1H, s), 4.79 (1H, s), 2.55–2.50 (2H, m), 2.52 (1H, d, J=18.9 Hz), 2.21 (1H, ddd, J=14.4, 10.5, 3.3 Hz), 2.10 (1H, d, J=18.9 Hz), 1.94 (1H, ddd, J=14.7, 6.3, 2.7 Hz), 1.90 (1H, br s), 1.80 (1H, dd, J=14.7, 4.2 Hz), 1.76 (3H, s), 1.59 (1H, d, J=14.7 Hz), 1.25 (3H, s), 1.24 (3H, s). δ_C (75 MHz, 1:1 $CDCl_3$+CCl_4) 218.6 (C), 217.6 (C), 146.8 (C), 110.4 (CH_2), 50.9 (C), 49.0 (CH), 48.5 (C), 48.1 (CH_2), 46.3 (CH), 45.1 (CH), 35.2 (CH_2), 22.0 (CH_3), 20.7 (CH_2), 19.5 (CH_3), 18.1 (CH_3).

3.1.4. (1*S*,3*R*,6*R*,7*S*,9*R*)-9-Acetoxy-3,6-dimethyltricyclo[4.3.1.0^{3,7}]decane-2,5-dione (13). Dry ozone in oxygen gas was passed through a cold (−70 °C) solution of the diketone **9** (215 mg, 0.92 mmol) and $NaHCO_3$ (100 mg) in 1:9 MeOH/CH_2Cl_2 (10 ml) until (ca. 4.5 min) pale blue colour appeared. Excess ozone was flushed off with oxygen. The solvent was evaporated in vacuo and the residue was dissolved in dry benzene (30 ml). Acetic anhydride (0.61 ml, 6.49 mmol), triethylamine (1.29 ml, 9.26 mmol) and a catalytic amount of DMAP (10 mg) were added to the reaction mixture and heated at reflux for 10 h. It was then cooled, diluted with water (10 ml) and extracted with ether (3×5 ml). The ether extract was washed with

3 M aqueous HCl, water and brine, and dried (Na_2SO_4). Evaporation of the solvent and purification of the residue over a silica gel column using ethyl acetate/hexane (1:5) as eluent furnished the diketoacetate **13** (187 mg, 81%) as colourless solid, which was recrystallised from a mixture of CH_2Cl_2/hexane, mp 73–75 °C. $[\alpha]_D^{24}-5.8$ (*c* 5.5, $CHCl_3$). ν_{max} (neat) 1740, 1729 cm^{-1}. δ_H (300 MHz, 1:1 $CDCl_3+CCl_4$) 5.09 (1H, ddd, *J*=9.0, 4.2, 2.1 Hz, C*H*OAc), 2.56 (1H, t, *J*=4.2 Hz), 2.44 (1H, d, *J*=18.9 Hz), 2.37 (1H, ddd, *J*=16.5, 9.3, 3.0 Hz), 2.10 (1H, d, *J*=18.9 Hz), 2.08 (1H, d, *J*=4.8 Hz), 2.03 (3H, s), 2.00–1.85 (1H, m), 1.76 (1H, dd, *J*=14.4, 4.8 Hz), 1.46 (1H, d, *J*=14.4 Hz), 1.33 (3H, s), 1.16 (3H, s). δ_C (75 MHz, 1:1 $CDCl_3+CCl_4$) 216.6 (C), 214.5 (C), 169.4 (C), 71.6 (CH), 50.9 (C), 48.8 (CH), 48.0 (CH_2), 47.7 (C), 46.4 (CH), 30.5 (CH_2), 24.0 (CH_2), 20.9 (CH_3), 19.6 (CH_3), 17.9 (CH_3). *m/z* 250 (M^+, 22), 234 (12), 208 (14), 191 (20), 190 (100), 175 (14), 162 (32), 147 (68), 134 (32), 121 (43), 119 (43), 105 (22), 93 (82%). HRMS: M^++Na, found 273.1095. $C_{14}H_{18}O_4Na$ requires 273.1103.

Further elution of the column with ethyl acetate/hexane (1:3) furnished the triketone **14** (21 mg, 10%) as a colourless solid, which was recrystallized from a mixture of CH_2Cl_2/hexane, mp 68–70 °C. $[\alpha]_D^{27}-70.0$ (*c* 2.8, $CHCl_3$). ν_{max} (neat) 1742, 1713 cm^{-1}. δ_H (300 MHz, 1:1 $CDCl_3+CCl_4$) 3.40–3.25 (1H, m), 3.00–2.88 (1H, m), 2.70–2.52 (2H, m), 2.42 (1H, d, *J*=19.2 Hz), 2.18 (3H, s), 2.07 (1H, d, *J*=19.2 Hz), 1.95–1.75 (2H, m), 1.59 (1H, d, *J*=14.7 Hz), 1.27 (3H, s), 1.20 (3H, s). δ_C (75 MHz, 1:1 $CDCl_3+CCl_4$) 216.6 (C), 215.4 (C), 206.1 (C), 51.6 (CH), 51.2 (C), 48.8 (CH), 48.3 (C), 47.9 (CH_2), 44.9 (CH), 34.0 (CH_2), 28.0 (CH_3), 19.6 (CH_3), 18.0 (CH_3), 15.5 (CH_2). *m/z* 234 (M^+, 46), 219 (16), 206 (36), 191 (29), 164 (14), 163 (13), 149 (26), 135 (20), 123 (62), 121 (44), 110 (72), 109 (100), 93 (73%). HRMS: M+Na, found 257.1148. $C_{14}H_{18}O_3Na$ requires 257.1154.

3.1.5. (1*S*,3*R*,6*R*,7*S*,9*R*)-9-Acetoxy-3,6-dimethyltricyclo[4.3.1.0^{3,7}]decane-2,5-dione bis-dithioketal (15). To a cold (0 °C) magnetically stirred solution of the diketone **13** (50 mg, 0.20 mmol) and ethanedithiol (0.33 ml, 4.0 mmol) in dry $CHCl_3$ (5 ml) was added a catalytic amount of iodine (10 mg) and stirring continued for 16 h at rt. It was then diluted with ether (10 ml), washed with 5% aqueous NaOH solution and brine, and dried (Na_2SO_4). Evaporation of the solvent and purification of the residue over a silica gel column using ethyl acetate/hexane (1:40) as eluent first furnished the bis-thioketal **15** (42 mg, 52%) as oil. $[\alpha]_D^{23}-45.0$ (*c* 5.8, $CHCl_3$). ν_{max} (neat) 1731 cm^{-1}. δ_H (300 MHz, 1:1 $CDCl_3+CCl_4$) 4.76 (1H, t, *J*=9.3 Hz), 3.43 (1H, d, *J*=16.5 Hz), 3.35–3.10 (6H, m), 3.12–2.90 (2H, m), 2.40 (1H, d, *J*=16.5 Hz), 2.28 (1H, s), 2.20–1.80 (2H, m), 2.04 (3H, s), 1.89 (1H, ddd, *J*=14.4, 9.0, 1.2 Hz), 1.75–1.50 (2H, m), 1.31 (3H, s), 1.21 (3H, s). δ_C (75 MHz, 1:1 $CDCl_3+CCl_4$) 170.2 (C), 79.1 (C), 78.0 (C), 72.4 (CH), 60.8 (CH_2), 50.6 (CH), 49.3 (C), 48.3 (CH), 47.7 (C), 40.6 (CH_2), 40.5 (CH_2), 40.4 (CH_2), 39.4 (CH_2), 37.0 (CH_2), 24.5 (CH_3), 22.9 (CH_2), 21.7 (2C, CH_3). *m/z* 402 (M^+, 34), 374 (29), 158 (13), 145 (36), 119 (100), 105 (22%). HRMS: M+Na, found 425.0694. $C_{18}H_{26}O_2S_4Na$ requires 425.0713.

Further elution of the column using ethyl acetate/hexane (1:20) as eluent furnished monodithioketal **16** (18 mg, 28%) as a colourless solid, which was recrystallized from a mixture of CH_2Cl_2/hexane, mp 123–125 °C. $[\alpha]_D^{27}-103.2$ (*c* 5.8, $CHCl_3$). ν_{max} (neat) 1736, 1716 cm^{-1}. δ_H (300 MHz, 1:1 $CDCl_3+CCl_4$) 5.00–4.90 (1H, m), 3.40–3.10 (4H, m), 2.64 and 2.47 (2H, 2×d, *J*=15.6 Hz), 2.42–2.30 (2H, m), 2.13–2.00 (1H, m), 2.10–1.95 (1H, m), 2.02 (3H, s), 1.70–1.50 (2H, m), 1.32 (3H, s), 1.19 (3H, s). δ_C (75 MHz, 1:1 $CDCl_3+CCl_4$) 214.9 (C), 169.8 (C), 79.1 (C), 72.4 (CH), 56.0 (CH_2), 52.2 (C), 50.7 (CH), 48.8 (C), 46.6 (CH), 40.7 (CH_2), 39.7 (CH_2), 34.1 (CH_2), 25.0 (CH_2), 22.2 (CH_3), 21.1 (CH_3), 17.8 (CH_3). *m/z* 328 (M^++2, 9), 326 (82), 298 (34), 270 (46), 239 (27), 238 (31), 237 (30), 210 (37), 199 (45), 145 (100), 105 (41), 91 (26%). HRMS: M+Na, found 349.0912. $C_{16}H_{22}O_3S_2Na$ requires 349.0908.

3.1.6. (*R*)-3,6-Dimethyltricyclo[4.3.1.0^{3,7}]decan-9-yl acetate (17). To a magnetically stirred solution of the bis-dithioketal **15** (34 mg, 0.08 mmol) in dry ethanol (3 ml) was added Raney nickel (150 mg, excess) and the reaction heated at reflux for 5 h. The reaction mixture was then cooled and filtered through a short silica gel column to remove the catalyst. Evaporation of the solvent and purification of the residue over a silica gel column using ethyl acetate/hexane (1:30) as eluent furnished the acetate **17** (12 mg, 64%) as oil. $[\alpha]_D^{27}-13.0$ (*c* 3.0, $CHCl_3$). ν_{max} (neat) 1737 cm^{-1}. δ_H (300 MHz, 1:1 $CDCl_3+CCl_4$) 4.70–4.55 (1H, m), 2.14–1.90 (2H, m), 2.03 (3H, s), 1.75–1.22 (10H, m), 1.05 (3H, s), 1.01 (3H, s). δ_C (75 MHz, 1:1 $CDCl_3+CCl_4$) 170.4 (C), 71.9 (CH), 48.3 (CH), 41.7 (CH_2), 40.9 (CH_2), 40.4 (CH_2), 39.8 (C), 39.4 (C), 36.4 (CH_2), 30.4 (CH), 27.1 (CH_3), 26.6 (CH_3), 24.2 (CH_2), 21.4 (CH_3). *m/z* 162 (M^+−AcOH, 91), 147 (21), 106 (80), 95 (100), 93 (47%). HRMS: M+Na found 245.1515. $C_{14}H_{22}O_2Na$ requires 245.1517.

3.1.7. (*R*)-3,7-Dimethyltricyclo[4.3.1.0^{3,7}]decan-9-ol (18). To a magnetically stirred solution of the acetate **17** (12 mg, 0.05 mmol) in methanol (1 ml) was added K_2CO_3 (50 mg) and the reaction stirred for 10 h at rt. The reaction mixture was then filtered through a short silica gel column using ethyl acetate/hexane (1:5). Evaporation of the solvent and purification of the residue over a silica gel column using ethyl acetate/hexane (1:10) as eluent furnished the alcohol **18** (8 mg, 82%) as oil.[4] $[\alpha]_D^{23}-15.8$ (*c* 1.2, $CHCl_3$). ν_{max} (neat) 3276 cm^{-1}. δ_H (300 MHz, 1:1 $CDCl_3+CCl_4$) 3.75–3.65 (1H, m), 1.98 (1H, ddd, *J*=14.7, 9.6, 3.3 Hz), 1.76 (1H, d, *J*=13.5 Hz), 1.60–1.10 (11H, m), 1.06 (3H, s), 0.98 (3H, s). δ_C (75 MHz, 1:1 $CDCl_3+CCl_4$) 68.7 (CH, CHOH), 48.6 (CH), 42.0 (CH_2), 40.8 (CH_2), 40.4 (CH_2), 39.9 (C), 39.2 (C), 35.6 (CH_2), 33.9 (CH), 27.0 (CH_3), 26.9 (CH_2), 26.6 (CH_3). *m/z* 162 (M^+-H_2O, 62), 149 (8), 107 (100), 106 (68), 105 (37), 95 (52), 93 (50), 91 (40%).

3.1.8. 3,7-Dimethyltricyclo[4.3.1.0^{3,7}]decan-9-one (7). To a magnetically stirred solution of the alcohol **18** (6 mg, 0.03 mmol) in dry CH_2Cl_2 (1 ml) was added a homogeneous mixture of PCC (71 mg, 0.33 mmol) and silica gel (71 mg) and the reaction stirred for 90 min at rt. The reaction mixture was then filtered through a short silica gel column and eluted with excess CH_2Cl_2. Evaporation of the solvent and purification of the residue over a silica gel column using ethyl acetate/hexane (1:30) as eluent furnished the ketone **7**

(5 mg, 83%) as oil.[4] ν_{max} (neat) 1732 cm^{-1}. δ_H (300 MHz, 1:1 $CDCl_3+CCl_4$) 2.26 (2H, d, $J=3.3$ Hz), 2.20–2.14 (1H, m), 1.73–1.55 (8H, m), 1.31 (1H, t, $J=3.3$ Hz), 1.08 (6H, s). δ_C (75 MHz, 1:1 $CDCl_3+CCl_4$) 217.0 (C), 50.8 (CH), 43.6 (CH), 40.5 (2C, CH_2), 40.1 (2C, CH_2), 39.8 (2C, C), 33.1 (CH_2), 26.7 (2C, CH_3). *m/z* 178 (M^+, 18%), 149 (100), 135 (7), 121 (10), 107 (21), 105 (73). HRMS: M+Na, found 201.1263. $C_{12}H_{18}ONa$ requires 201.1255.

Acknowledgements

We thank the Council of Scientific and Industrial Research, New Delhi for the award of a research fellowship to GS.

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Tetrahedron 61 (2005) 8860–8867
Tetrahedron

Manganese(III) acetate based oxidative cyclizations of 3-oxopropanenitriles with conjugated alkenes and synthesis of 4,5-dihydrofuran-3-carbonitriles containing heterocycles

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Received 29 April 2005; revised 20 June 2005; accepted 7 July 2005

Available online 26 July 2005

Abstract—4,5-Dihydrofuran-3-carbonitriles **3a–i** were obtained through oxidative cyclizations of 3-oxo-3-phenylpropanenitrile **1a**, 3-oxo-3-thien-2-ylpropanenitrile **1b**, 3-(2-furyl)-3-oxopropanenitirle **1c**, 3-(1-benzofuran-2-yl)-3-oxopropanenitrile **1d**, and 4,4-dimethyl-3-oxopropanenitrile **1e** mediated manganese(III) acetate with 1,1-diphenyl-1-butene **2a** and 1,2-diphenyl-1-pentene **2b**. The treatments of these 3-oxopropanenitriles with 2-thienyl substituted alkenes such as 2-[(*E*)-2-phenylvinyl]thiophene **2c**, 2-[(*E*)-1-methyl-2-phenylvinyl]thiophene **2d**, and 2-(1-phenylvinyl)thiophene **2e** formed 5-(2-thienyl)-4,5-dihydrofuran-3-carbonitriles **3j–r** in good yields (45–93%). As a result, 2-thienyl substituted alkenes formed products in higher yields than phenyl substituted derivatives.

1. Introduction

Oxidative cyclization reactions have become increasingly important in the synthesis of useful and complex molecules in the past two decades.[1] The oxidative addition of carbon-centered radicals to alkenes mediated by transition metal salts (Mn^{+3}, Ce^{+4}, Co^{+3}, Cu^{+2}, etc.) has received considerable attention in the organic synthesis for the construction of carbon–carbon bonds. Among these, manganese(III) acetate[1c–e,2] and cerium(IV) ammonium nitrate[2h,3] encompasses a prominent place and have been used efficiently. Thus, they provide the formation of highly functionalized products by inter- and intramolecular cyclization, such as, furans,[1d,2i,k,4] γ-lactones,[5] β-lactams,[6] biologically active compounds and natural products.[1d,e,7] The oxidative cyclizations of active methylene compounds, which can be enolized such as 1,3-dicarbonyls, β-ketoesters and β-ketoamides using manganese(III) acetate with alkenes were studied in detail and various 4,5-dihydrofurans were obtained.[2c–k]

Recently, we have reported the formation of 4,5-dihydrofuran and furan derivatives as a result of oxidative cyclizations of 1,3-dicarbonyl compounds with alkenes and alkynes[2k] by using $Mn(OAc)_3$. Additionally, we have reported the synthesis of carbamoyl-4,5-dihydrofurans and tetralones due to the reaction of 1,3-dicarbonyls with α,β-unsaturated amides.[2j] Previously, we have described the synthesis of 3-trifluoroacetyl-4,5-dihydrofurans and 3-(dihydrofuran-2(3*H*)-ylidene)-1,1,1-trifluoroacetones by the treatment of trifluoromethyl-1,3-dicarbonyl compounds with conjugated alkenes.[8]

The oxidative cyclizations of 3-oxopropanenitriles with 1,1-diarylethylenes and their reaction mechanism have been reported by Nishino et al.[9] Here we studied oxidative cyclization of various 3-oxopropanenitriles **1a–g** mediated $Mn(OAc)_3$ with 1,1-diphenyl **2a** and 1,2-diphenyl **2b** trisubstituted sterically hindered alkenes, and 2-thienyl substitute alkenes **2c–e** comparatively. As a result of these reactions we have obtained 4,5-dihydrofuran-3-carbonitriles containing heterocycles.

2. Results and discussion

Manganese(III) acetate dihydrate used as a radical oxidant has been obtained from the bipolar packed-bed reactor by electrochemical method in literature.[10] Conjugated alkenes **2b–d** were synthesized using benzyltriphenylphosphonium bromide and suitable carbonyl compounds.[11] **2a** and **2e** were prepared by removing water from the carbinols, which formed the reaction of phenylmagnesium bromide and suitable carbonyl compounds.[12] All 3-oxopropanenitriles **1a–g** were prepared according to literature.[13]

Keywords: Manganese(III) acetate; Oxidative cyclization; Heterocycles; 4,5-Dihydrofuran-3-carbonitrile; 3-Oxopropanenitrile; Conjugated alkene.
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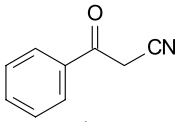
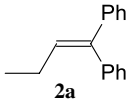
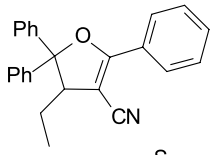
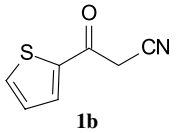
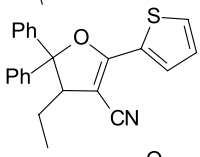
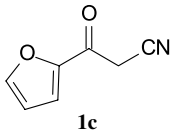
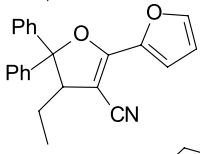
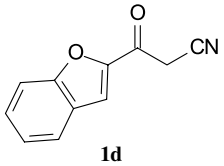
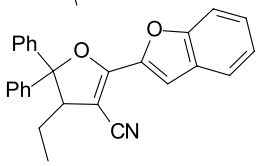
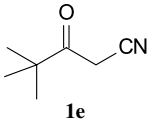
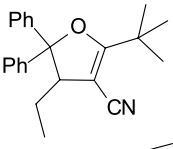
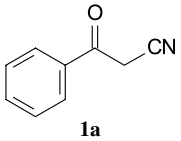
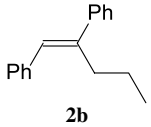
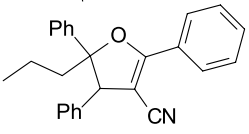
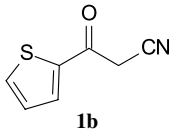
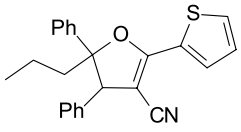
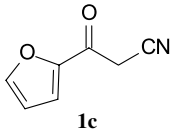
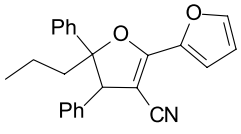
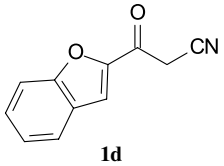
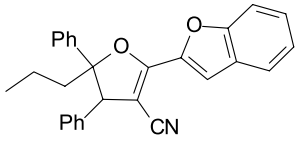
doi:10.1016/j.tet.2005.07.019

Oxidative cyclizations have been carried out at 2:1:3 mol ratio (**1**–**2**–$Mn(OAc)_3$) under N_2 atmosphere in AcOH. The reactions of **2a** and **2b** with 3-oxopropanenitriles at 80 °C and the reactions of 2-thienyl substituted alkenes **2c**–**e** with 3-oxopropanenitriles at 60 °C produced 4,5-dihydrofuran-3-carbonitriles in good yields. All compounds purified through column chromatography or preparative TLC were characterized by IR, ^{1}H NMR, ^{13}C NMR, MS and microanalysis.

The results of the reactions of **1a**–**e** with **2a** and **2b** are given in Table 1. The treatment of **1a** with **2a** formed **3a** in good yield (63%, entry 1), however we have obtained 4,5-dihydrofuran-3-carbonitrile **3f** in low yield (40%, entry 6) as a result of the treatment of **1a** with **2b**. Also, the reactions of 3-oxopropanenitriles **1b**–**d** containing heterocycles with **2a** gave **3b**–**d** in moderate yields (entries 2–4). On the other hand, oxidative cyclizations of **1b**–**d** with **2b** gave 4,5-dihydrofuran-3-carbonitriles **3g**–**i** in low yields (entries 7–9).

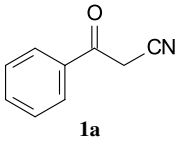
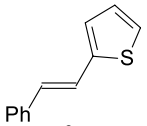
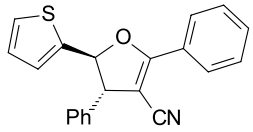
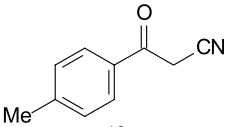
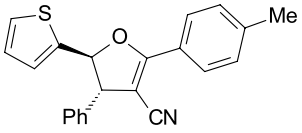
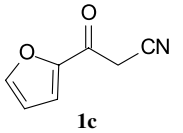
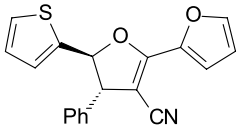
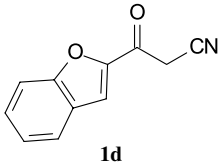
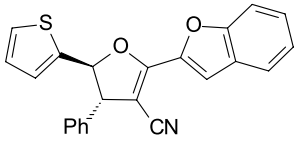
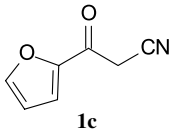
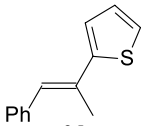
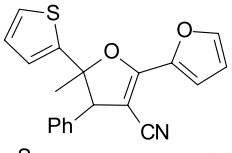
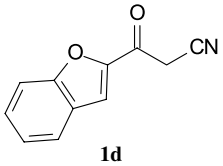
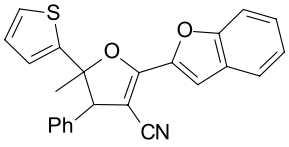
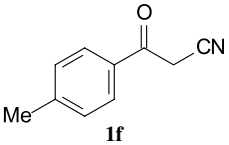
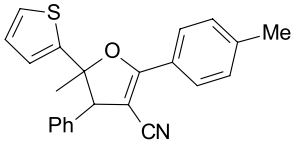
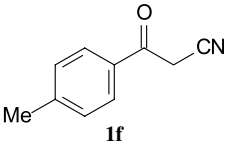
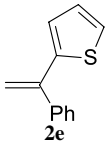
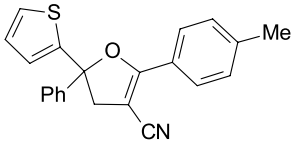
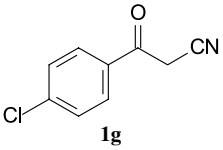
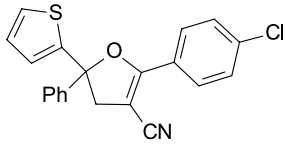
From these results we can conclude that 1,1-diphenyl substituted alkene **2a** is more reactive than 1,2-diphenyl substituted alkene **2b**. This high reactivity can be explained by the stability of the intermediate products formed with the addition of an α-carbon radical, which was obtained by the

Table 1. Oxidative cyclization of 3-oxopropanenitriles **1a**–**e** with **2a** and **2b**

Entry	3-Oxopropanenitrile	Alkene	4,5-Dihydrofuran-3-carbonitrile	Yield (%)[a]
1	 **1a**	 **2a**		**3a** (63)
2	 **1b**	**2a**		**3b** (44)
3	 **1c**	**2a**		**3c** (42)
4	 **1d**	**2a**		**3d** (55)
5	 **1e**	**2a**		**3e** (48)
6	 **1a**	 **2b**		**3f** (40)
7	 **1b**	**2b**		**3g** (26)
8	 **1c**	**2b**		**3h** (23)
9	 **1d**	**2b**		**3i** (30)

[a] Yield of isolated product based on the alkene.

Table 2. Synthesis of heterocycles containing 4,5-dihydrofuran-3-carbonitriles

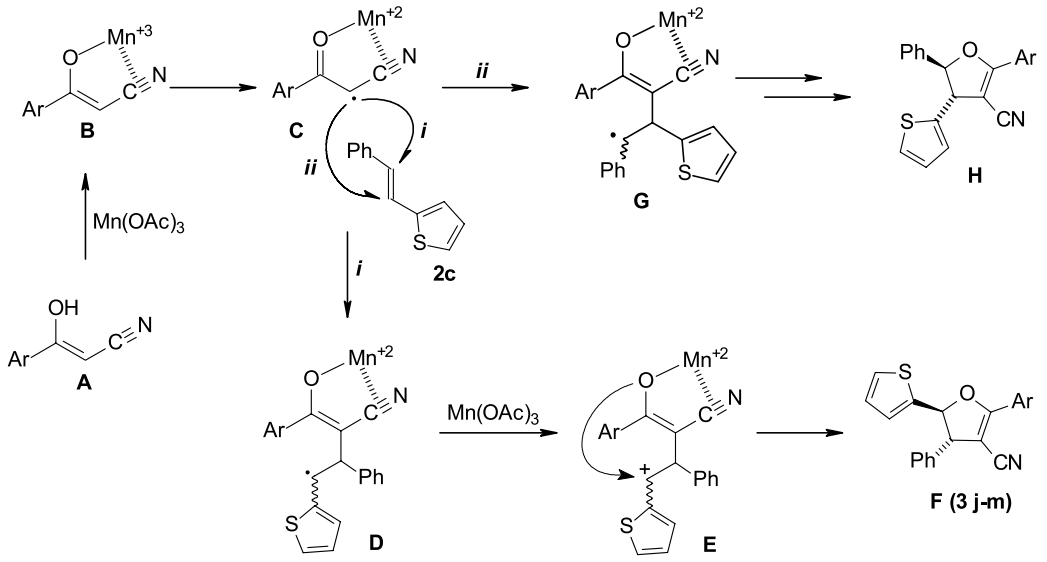
Entry	3-Oxopropanenitrile	Alkene	4,5-Dihydrofuran-3-carbonitrile	Yield (%)[a]
1	 **1a**	 **2c**		**3j** (56)
2	 **1f**	**2c**		**3k** (60)
3	 **1c**	**2c**		**3l** (58)
4	 **1d**	**2c**		**3m** (52)
5	 **1c**	 **2d**		**3n** (67)
6	 **1d**	**2d**		**3o** (60)
7	 **1f**	**2d**		**3p** (71)
8	 **1f**	 **2e**		**3r** (83)
9	 **1g**	**2e**		**3s** (93)

[a] Yield of isolated product based on the alkene.

treatment of $Mn(OAc)_3$ and 3-oxopropanenitrile, to **2a**. Both of the intermediate products are tertiary carbon radicals but the stability of tertiary radical formed on **2a** is higher than that of the tertiary radical formed on **2b** since the tertiary radical of **2a** is conjugated with phenyl groups, and the cyclization of more stable intermediate product forms 4,5-dihydrofuran-3-carbonitrile in a higher yield.

Oxidative cyclizations of 2-thienyl substituted alkenes mediated manganese(III) acetate with 3-oxopropanenitriles and the other active methylene compounds have not been studied so far. Herein, we first reported the reactions of these alkenes **2c**–**e** with 3-oxopropanenitriles and the synthesis of 4,5-dihydrofuran-3-carbonitrile containing heterocycles (Table 2).

There are two possible ways in oxidative cyclization of 3-oxopropanenitriles with **2c**. In one of them 4-(2-thienyl) substituted 4,5-dihydrofurans can be formed, in the other one 5-(2-thienyl) substituted 4,5-dihydrofurans can be formed. The mechanism of these possible cyclizations is explained in Scheme 1. According to this mechanism, $Mn(OAc)_3$ with the enol form of 3-oxo-propanenitrile **A** gives manganese(III)-enolate complex **B**. In complex **B**, an



Scheme 1.

α-carbon radical **C** is formed on 3-oxopropanenitrile while Mn^{+3} is reduced to Mn^{+2}. This α-carbon radical attacks to **2c**, and compound **D** can be formed with the following pathway i, or compound **G** can be formed with the following pathway ii. Structure **D** is oxidized to carbocation **E** with $Mn(OAc)_3$ and intermolecular cyclization of **E** forms 5-(2-thienyl) substituted 4,5-dihydrofuran **F**. Structure **G** following the same two steps forms 4-(2-thienyl) substituted 4,5-dihydrofuran **H**. In literature the chemical shifts of H4 and H5 have been reported as 4.4 and 5.6 ppm according to its ^{1}H NMR spectrum.[9] However, it has been observed that in the ^{1}H NMR spectra of the compounds **3j–m** that we have synthesized, H4 and H5 gave the chemical shifts of 4.58–4.88 and 6.02–6.11 ppm, respectively. According to these results, it has been concluded that 5-(2-thienyl) substituted 4,5-dihydrofurans **F** are produced from path i.

Nishino et al. obtained 2,4,5,-triphenyl-4,5-dihydrofuran-3-carbonitrile in 34% yield by the treatment of *trans*-stilbene with **1a** at reflux temperature.[9] However, we have obtained 2,4-diphenyl-5-thien-2-yl-4,5-dihydrofuran-3-carbonitrile **3j** in 56% yield by the treatment of **1a** with 2-[(*E*)-2-phenylvinyl]thiophene **2c**. Oxidative cyclizations of 3-oxopropanenitriles containing 2-furyl **1c** and 2-benzofuryl **1d** with **2c** gave **3l** (58%) and **3m** (52%) 4,5-dihydrofuran-3-carbonitriles, respectively (entries 3 and 4). In NMR spectra, the coupling constants (J=7.3–7.7 Hz) of H4 and H5 protons of **3j–m** show that 2-thienyl and phenyl groups are in trans position.

It has been mentioned that oxidative cyclization of 3-oxopropanenitriles with **2b** produces 4,5-dihydrofuran-3-carbonitriles in low yields in Table 1. Yet, the treatments of 2-[(*E*)-1-methyl-2-phenylvinyl]thiophene **2d** with **1c** and **1d** gave **3n** and **3o** in good yields, respectively (entries 5 and 6).

Additionally, the reaction of **1f** with **2d** gave 4,5-dihydrofuran-3-carbonitrile **3p** in 71% yields. In literature, the reactions of **1f** and **1g** with 1,1-diphenylethylene at reflux temperature gave 4,5-dihidrofurans in 67 and 74% yields, respectively.[9] However, we obtained 5-(2-thienyl) substituted 4,5-dihydrofuran-3-carbonitriles **3r** in 83% and **3s** in 93% yields, respectively, by the cyclization of 2-(1-phenylvinyl)thiophene **2e** with **1f** and **1g** (entries 8 and 9).

Consequently, oxidative cyclizations of 3-oxopropanenitriles mediated manganese(III) acetate with 1,1 and 1,2-diphenyl substituted alkenes and 2-thienyl substituted derivatives have comparatively been studied in this work. As a result, 2-thienyl substituted alkenes formed products in higher yields than phenyl substituted derivatives.

3. Experimental

Melting points were determined on a Gallencamp capillary melting point. IR spectra (KBr disc) were obtained with a Matson 1000 FT-IR in the 400–4000 cm^{-1} range with 4 cm^{-1} resolution. ^{1}H NMR (400 MHz), and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker Avance DPX- 400 MHz high performance digital FT NMR in $CDCl_3$ solution. The electron impact mass spectra were measured on a Agilent 1100 MSD LC/MS (APCI, 100–150 eV), and a Shimadzu GC-17A/GC-MS-QP5000 (EIMS, 70 eV) spectrophotometers. Elemental analyses were performed on a Leco 932 CHNS-O instrument.

Thin layer chromatography (TLC) was performed on Merck aluminum-packed silica gel plates. Purification of products was performed by column chromatography on silica gel (Merck silica gel 60, 40–63 μm) or preparative TLC on silica gel of Merck ($PF_{254-366\ nm}$). All solvents purchased from DOP.

3.1. General procedure for synthesis of 4,5-dihydrofuran-3-carbonitriles

A solution of manganese(III) acetate dihydrate (6 mmol, 1.64 g) in 30 mL in glacial acetic acid was heated under nitrogen atmosphere at 80 °C until it dissolved. After

$Mn(OAc)_3$ dissolved completely, the solution was cooled down to 50 °C. A solution of **1a–f** (4 mmol) and alkene (2 mmol) in 5 mL acetic acid was added to this mixture and the temperature was raised up to 80 °C (for **2c–e** at 60 °C). The reaction was completed when the dark brown colour of the solution disappeared (in 1–15 min). Acetic acid was evaporated under reduced pressure. Water was added to the residue and extracted with EtOAc (3×20 mL). The combined organic phases were neutralized with satd $NaHCO_3$ solution, and dried over anhydrous Na_2SO_4. Crude products were purified by column chromatography on silica gel or preparative TLC (20×20 cm plates, 2 mm thickness) using *n*-hexane/ EtOAc (5:1) as eluent.

3.1.1. 4-Ethyl-2,5,5-triphenyl-4,5-dihydrofuran-3-carbonitrile (3a). Yield 63% (442 mg) as a colourless solid, mp 143–144 °C (hexane/EtOAc). [Found: C, 85.7; H, 5.8; N, 4.1. $C_{25}H_{21}NO$ requires C, 85.4; H, 6.0; N, 4.0%]; ν_{max} (KBr disc) 2201 (CN), 1627 (C=C), 1234 (C–O–C), 756, 696; δ_H (400 MHz, $CDCl_3$) 8.26 (2H, dd, J=7.4, 1.7 Hz, arom.), 7.76 (2H, dd, J=7.5, 1.5 Hz, arom.), 7.67 (3H, m, arom.), 7.56 (2H, tt, J=7.0, 1.6 Hz, arom.), 7.52–7.44 (6H, m, arom.), 4.04 (1H, dd, J=7.7, 5.9 Hz, H4), 1.56 (2H, m, CHCH_2Me), 1.14 (3H, t, J=7.4 Hz, *Me*); δ_C (100 MHz, $CDCl_3$) 165.3 (C2), 144.1, 140.5, 131.8, 129.1, 128.7, 128.5, 128.4, 128.3, 127.9, 127.6, 126.9, 126.4, 118.5 (CN), 96.3 (C3), 85.8 (C5), 53.3 (C4), 26.4 (CH_2Me), 11.8 (Me); *m/z* (APCI, 150 eV) 352 (MH^+, 4.4), 351 (M^+, 16.2), 336 (M^+-CH_3, 4.7), 322 ($M^+-C_2H_5$, 7.1), 306 ($M^+-C_3H_8$, 4.6), 246 (M^+−PhCO, 3.1), 167 ($C_{13}H_{11}^+$, 4.1), 105 ($PhCO^+$, 100.00), 91 ($PhCH_2^+$, 5.01), 77 ($C_6H_5^+$, 48.6).

3.1.2. 4-Ethyl-5,5-diphenyl-2-thien-2-yl-4,5-dihydrofuran-3-carbonitrile (3b). Yield 44% (314 mg) as a colourless oil. [Found: C, 77.1; H, 5.6; N, 4.1; S, 8.8. $C_{23}H_{19}NOS$ requires C, 77.3; H, 5.4; N, 3.9; S, 9.0%]; ν_{max} (KBr disc, $CHCl_3$) 2198 (CN), 1616 (C=C), 1211 (C–O–C), 731, 696; δ_H (400 MHz, $CDCl_3$) 7.88 (1H, d, J=3.8 Hz, arom.), 7.51 (1H, d, J=7.2 Hz, arom.), 7.48 (1H, d, J=5.0 Hz, arom.), 7.32 (1H, t, J=7.8 Hz, arom.), 7.26–7.19 (7H, m, arom.), 7.09 (1H, t, J=4.8 Hz, arom.), 3.76 (1H, t, J=6.9 Hz, H4), 1.41 (2H, m, CHCH_2Me), 0.89 (3H, t, J=7.4 Hz, *Me*); *m/z* (APCI, 150 eV) 358 (MH^+, 1.6), 357 (M^+, 6.5), 342 (M^+-CH_3, 2.6), 328 ($M^+-C_2H_5$, 4.2), 312 ($M^+-C_3H_8$, 3.2), 274 ($M^+-C_4H_3S$, 4.7), 246 ($M^+-C_5H_3SO$, 9.9), 231 ($M^+-C_5H_3SO-CH_3$, 6.3), 165 ($C_{13}H_9^+$, 6.7), 111 ($C_5H_3SO^+$, 100.00), 105 ($PhCO^+$, 3.9), 91 ($PhCH_2^+$, 4.0), 83 ($C_4H_3S^+$, 5.01).

3.1.3. 4-Ethyl-5,5-diphenyl-4,5-dihydro-2,2′-bifuran-3-carbonitrile (3c). Yield 42% (86 mg) as a colourless solid, mp 133–134 °C (hexane/EtOAc). [Found: C, 80.7; H, 5.6; N, 3.8. $C_{23}H_{19}NO_2$ requires C, 80.9; H, 5.6; N, 4.1%]; ν_{max} (KBr disc) 2970, 2876, 2201 (CN), 1635 (C=C), 1180 (C–O–C), 764, 702; δ_H (400 MHz, $CDCl_3$) 7.85 (1H, d, J=1.4 Hz, arom.), 7.79 (2H, d, J=7.4 Hz, arom.), 7.62 (1H, t, J=6.9 Hz, arom.), 7.58–7.50 (7H, m, arom.), 7.34 (1H, d, J=3.5 Hz, arom.), 6.79 (1H, dd, J=3.5, 1.7 Hz, arom.), 4.05 (1H, t, J=6.8 Hz, H4), 1.58 (2H, m, CHCH_2Me), 1.28 (3H, t, J=7.4 Hz, *Me*); δ_C (100 MHz, $CDCl_3$) 156.8 (C2), 145.7, 144.1, 143.7, 140.1, 128.7, 128.6, 128.3, 127.9, 126.8, 126.3, 117.5 (CN), 114.5, 112.4, 97.2 (C3), 84.5 (C5), 52.8 (C4), 26.3 (CH_2Me), 11.7 (Me); *m/z* (APCI, 150 eV) 342 (MH^+, 5.8), 341 (M^+, 21.1), 326 (M^+-CH_3, 7.7), 312 ($M^+-C_2H_5$, 10.5), 295 ($M^+-C_3H_8$, 9.01), 274 ($M^+-C_4H_3O$, 12.5), 246 ($M^+-C_5H_3O_2$, 83.6), 232 ($M^+-C_5H_3O_2-CH_3$, 52.3), 217 ($M^+-C_5H_3O_2-C_2H_5$, 13.3), 207 ($M^+-C_{10}H_{14}$, 3.8), 167 ($C_{13}H_{11}^+$, 22.8), 152 ($C_{12}H_{10}^+$, 23.1), 105 ($PhCO^+$, 6.5), 217 ($C_5H_3O_2^+$, 100.00), 91 ($PhCH_2^+$, 12.10), 77 ($C_6H_5^+$, 1.2).

3.1.4. 2-(1-Benzofuran-2-yl)-4-ethyl-5,5-diphenyl-4,5-dihydrofuran-3-carbonitrile (3d). Yield 55% (430 mg) as a colourless solid, mp 186 °C (hexane/ EtOAc). [Found: C, 82.6; H, 5.1; N, 3.5. $C_{27}H_{21}NO_2$ requires C, 82.8; H, 5.4; N, 3.6%]; ν_{max} (KBr disc) 2201 (CN), 1639 (C=C), 1180 (C–O–C), 754, 698; δ_H (400 MHz, $CDCl_3$) 7.58 (1H, d, J=7.9 Hz, arom.), 7.53 (2H, m, arom.), 7.38 (1H, s, arom.), 7.32 (1H, t, J=7.9 Hz, arom.), 7.28–7.19 (10H, m, arom.), 3.80 (1H, dd, J=7.5, 6.2 Hz, H4), 1.41 (2H, m, CHCH_2Me), 1.03 (3H, t, J=7.5 Hz, *Me*); *m/z* (APCI, 150 eV) 392 (MH^+, 6.8), 391 (M^+, 23.4), 376 (M^+-CH_3, 10.3), 362 ($M^+-C_2H_5$, 4.8), 346 ($M^+-C_3H_8$, 7.3), 274 ($M^+-C_8H_5O$, 12.8), 246 ($M^+-C_9H_5O_2$, 15.7), 167 ($C_{13}H_{11}^+$, 5.7), 145 ($C_9H_5O_2^+$, 92.6), 89 ($C_7H_5^+$, 100.0).

3.1.5. 2-*tert*-Butyl-4-ethyl-5,5-diphenyl-4,5-dihydrofuran-3-carbonitrile (3e). Yield 48% (318 mg) as a colourless solid, mp 99 °C (hexane/EtOAc). [Found: C, 83.2; H, 7.7; N, 4.0. $C_{23}H_{25}NO$ requires C, 83.3; H, 7.6; N, 4.2%]; ν_{max} (KBr disc) 2970, 2876, 2201 (CN), 1635 (C=C), 1180 (C–O–C), 765, 702; δ_H (400 MHz, $CDCl_3$) 7.55 (2H, m, arom.), 7.38–7.21 (8H, m, arom.), 3.67 (1H, dd, J=7.3, 5.8 Hz, H4), 1.42 (9H, s, 3×*Me*), 1.32 (2H, m, CHCH_2Me), 0.84 (3H, t, J=7.4 Hz, *Me*); *m/z* (APCI, 150 eV) 332 (MH^+, 5.6), 331 (M^+, 22.01), 316 (M^+-CH_3, 3.4), 248 ($M^+-C_6H_{11}$, 22.2), 246 ($M^+-C_5H_9O$, 11.3), 219 ($M^+-CN-C_5H_9O$, 100.00), 196 (M^+−PhCO−C_2H_4, 3.9), 167 ($C_{13}H_{11}^+$, 10.1), 152 ($C_{12}H_{10}^+$, 5.4), 105 ($PhCO^+$, 8.6), 91 ($PhCH_2^+$, 9.2), 57 ($C_4H_9^+$, 29.2).

3.1.6. 2,4,5-Triphenyl-5-propyl-4,5-dihydrofuran-3-carbonitrile (3f). Yield 40% (292 mg) as a pale yellow oil. [Found: C, 85.8; H, 6.1; N, 4.1. $C_{26}H_{23}NO$ requires C, 85.5; H, 6.3; N, 3.8%]; ν_{max} (KBr disc, $CHCl_3$) 2957, 2874, 2203 (CN), 1623 (C=C), 760, 649; δ_H (400 MHz, $CDCl_3$) 8.00 (2H, dd, J=7.9, 1.5 Hz, arom.), 7.34 (3H, t, J=6.5 Hz, arom.), 7.20 (7H, m, arom.), 7.11 (3H, td, J=7.5, 1.4 Hz, arom.), 4.07 (1H, s, H4), 1.32 (1H, m), 1.22 (1H, m), 1.12 (1H, m), 0.55 (1H, m), 0.48 (3H, t, J=7.3 Hz, *Me*); *m/z* (APCI, 150 eV) 366 (MH^+, 18.9), 365 (M^+, 63.1), 350 (M^+-CH_3, 2.6), 322 ($M^+-C_3H_7$, 3.6), 288 ($M^+-C_6H_5$, 4.1), 260 ($M^+-C_7H_5O$, 1.3), 246 ($M^+-C_6H_5-C_3H_8$, 4.9), 179 ($M^+-C_{14}H_{11}$, 4.1), 154 ($C_{12}H_{10}^+$, 5.5), 143 ($C_{11}H_{11}^+$, 26.7), 128 ($C_9H_6N^+$, 36.4), 105 ($C_7H_5O^+$, 77.6), 91 ($PhCH_2^+$, 100.00), 77 ($C_6H_5^+$, 11.6).

3.1.7. 4,5-Diphenyl-5-propyl-2-thien-2-yl-4,5-dihydrofuran-3-carbonitrile (3g). Yield 26% (193 mg) as a pale yellow oil. [Found: C, 77.5; H, 5.5; N, 4.0; S, 8.5. $C_{24}H_{21}NOS$ requires C, 77.6; H, 5.7; N, 3.8; S, 8.6%]; ν_{max} (KBr disc, $CHCl_3$) 2955, 2876, 2203 (CN), 1617 (C=C), 756, 701; δ_H (400 MHz, $CDCl_3$) 7.83 (1H, d, J=3.8 Hz), 7.40 (1H, d, J=4.9 Hz, arom.), 7.23–7.08 (10H, m, arom.), 7.04 (1H, t, J=4.8 Hz, arom.), 4.22 (1H, s, H4), 1.31 (1H, td, J=11.0, 4.7 Hz, CH_aH$_b$CH$_2$Me), 1.20 (1H, td,

$J=11.3$, 4.5 Hz, $CH_aH_bCH_2Me$), 1.16 (1H, m), 0.66 (1H, m), 0.45 (3H, t, $J=7.0$ Hz, *Me*); *m/z* (APCI, 150 eV) 372 (MH^+, 11.6), 371 (M^+, 42.4), 356 (M^+-CH_3, 1.6), 328 ($M^+-C_3H_7$, 2.6), 294 ($M^+-C_6H_5$, 2.8), 288 ($M^+-C_4H_3S$, 18.7), 260 ($M^+-C_5H_3SO$, 2.6), 245 ($M^+-C_4H_3S-C_3H_7$, 7.9), 223 ($C_{10}H_{12}O^+$, 7.9), 143 ($C_{11}H_{11}^+$, 27.7), 128 ($C_9H_6N^+$, 27.3), 111 ($C_5H_3SO^+$, 30.6), 105 ($C_7H_5O^+$, 25.7), 91 ($C_7H_7^+$, 100.00), 83 ($C_4H_3S^+$, 2.5).

3.1.8. 4,5-Diphenyl-5-propyl-4,5-dihydro-2,2′-bifuran-3-carbonitrile (3h). Yield 23% (163 mg) as a light yellow oil. [Found: C, 80.8; H, 6.2; N, 4.1. $C_{24}H_{21}NO_2$ requires C, 81.1; H, 6.0; N, 4.0%]; ν_{max} (KBr disc, $CHCl_3$) 2961, 2874, 2207 (CN), 1650 (C=C), 758, 702; δ_H (400 MHz, $CDCl_3$) 7.62 (1H, d, $J=0.9$ Hz, arom.), 7.38–7.25 (10H, m, arom.), 7.23 (1H, t, $J=7.0$ Hz, arom.), 7.13 (1H, d, $J=3.7$ Hz, arom.), 6.56 (1H, dd, $J=3.4$, 1.7 Hz, arom.), 4.41 (1H, s, H4), 1.48 (1H, td, $J=11.3$, 4.7 Hz, $CH_aH_bCH_2Me$), 1.35 (1H, td, $J=11.5$, 4.6 Hz, $CH_aH_bCH_2Me$), 1.20 (1H, m), 0.81 (1H, m), 0.52 (3H, t, $J=7.3$ Hz, *Me*); *m/z* (APCI, 150 eV) 356 (MH^+, 19.7), 355 (M^+, 71.3), 340 (M^+-CH_3, 3.2), 313 ($MH^+-C_3H_7$, 3.6), 288 ($M^+-C_4H_3O$, 10.9), 278 ($M^+-C_6H_5$, 2.9), 260 ($M^+-C_5H_3O_2$, 12.9), 246 ($M^+-C_4H_3O-C_3H_9$, 17.1), 232 ($M^+-C_7H_7O_2$, 18.4), 223 ($M^+-C_{10}H_{12}$, 24.4), 210 ($M^+-C_6H_5-C_4H_3O$, 4.5), 178 ($C_{14}H_{10}^+$, 5.4), 143 ($C_{11}H_{11}^+$, 27.8), 128 ($C_9H_6N^+$, 32.6), 105 ($C_7H_5O^+$, 33.8), 95 ($C_5H_3O_2^+$, 31.6), 91 ($C_7H_7^+$, 100.00), 77 ($C_6H_5^+$, 2.1).

3.1.9. 2-(1-Benzofuran-2-yl)-5-propyl-4,5-diphenyl-4,5-dihydrofuran-3-carbonitrile (3i). Yield 30% (243 mg) as a yellow oil. [Found: C, 83.1; H, 5.6; N, 3.3. $C_{28}H_{23}NO_2$ requires C, 82.9; H, 5.7; N, 3.5%]; ν_{max} (KBr disc, $CHCl_3$) 2209 (CN), 1648 (C=C), 1180 (C–O–C), 758, 702; δ_H (400 MHz, $CDCl_3$) 7.72 (1H, d, $J=7.7$ Hz, arom.), 7.56 (1H, d, $J=8.4$ Hz, arom.), 7.48 (1H, s, arom.), 7.48–7.35 (12H, m, arom.), 4.63 (1H, s, H4), 1.86 (1H, td, $J=12.0$, 6.1 Hz, $CH_aH_bCH_2Me$), 1.65 (1H, m), 1.52 (1H, td, $J=11.6$, 4.7 Hz, $CH_aH_bCH_2Me$), 0.58 (1H, m), 0.54 (3H, t, $J=7.3$ Hz, *Me*); *m/z* (APCI, 150 eV) 406 (MH^+, 29.9), 405 (M^+, 100.00), 390 (M^+-CH_3, 3.2), 362 ($M^+-C_3H_6$, 2.3), 228 ($M^+-C_6H_5$, 3.5), 288 ($M^+-C_8H_5O$, 37.0), 260 ($M^+-C_9H_5O_2$, 5.5), 246 ($MH^+-C_8H_5O-C_3H_7$, 15.2), 145 ($C_9H_5O_2^+$, 38.1), 105 ($C_7H_5O^+$, 16.7), 91 ($C_7H_7^+$, 48.2).

3.1.10. 2,4-Diphenyl-5-thien-2-yl-4,5-dihydrofuran-3-carbonitrile (3j). Yield 56% (369 mg) as a yellow oil. [Found: C, 76.3; H, 4.8; N, 4.2; S, 9.5. $C_{21}H_{15}NOS$ requires C, 76.6; H, 4.6; N, 4.3; S, 9.7%]; ν_{max} (KBr disc, $CHCl_3$) 2203 (CN), 1623 (C=C), 1124 (C–O–C), 754, 700; δ_H (400 MHz, $CDCl_3$) 8.32 (2H, dt, $J=6.7$, 1.7 Hz, arom.), 7.79–7.73 (3H, m, arom.), 7.67–7.56 (6H, m, arom.), 7.35 (1H, dt, $J=3.0$, 0.5 Hz, arom.), 7.28 (1H, dd, $J=3.6$, 5.1 Hz, arom.), 6.07 (1H, d, $J=7.3$ Hz, H5), 4.84 (1H, d, $J=7.3$ Hz, H4); δ_C (100 MHz, $CDCl_3$) 166.6 (C-2), 141.9, 139.3, 132.1, 129.7, 129.1, 128.6, 127.8, 127.7, 127.4, 126.8, 126.4, 117.4 (CN), 88.4 (C3), 84.7 (C5), 59.1 (C4); *m/z* (EI, 70 eV) 330 (MH^+, 19.57), 329 (M^+, 81.05), 328 (M^+-H, 25.51), 252 ($M^+-C_6H_5$, 4.33), 224 ($M^+-C_6H_5CO$, 5.88), 105 ($C_6H_5CO^+$, 100.00), 83 ($C_4H_3S^+$, 2.33), 77 ($C_6H_5^+$, 47.02).

3.1.11. 2-(4-Methylphenyl)-4-phenyl-5-thien-2-yl-4,5-dihydofuran-3-carbonitrile (3k). Yield 60% (412 mg) as a yellow oil. [Found: C, 77.1; H, 5.2; N, 4.2; S, 9.5. $C_{22}H_{17}NOS$ requires C, 76.9; H, 5.0; N, 4.1; S, 9.3%]; ν_{max} (KBr disc, $CHCl_3$) 2203 (CN), 1617 (C=C), 1114 (C–O–C), 708; δ_H (400 MHz, $CDCl_3$) 8.01 (2H, d, $J=8.3$ Hz, arom.), 7.44–7.28 (8H, m, arom.), 7.12 (1H, d, $J=3.5$ Hz, arom.), 7.05 (1H, dd, $J=5.0$, 3.6 Hz, arom.), 6.02 (1H, d, $J=7.3$ Hz, H5), 4.58 (1H, d, $J=7.3$ Hz, H4), 2.45 (3H, s, *Me*); *m/z* (EI, 70 eV) 344 (MH^+, 7.63), 343 (M^+, 29.60), 328 (M^+-CH_3, 6.76), 251 ($M^+-C_7H_8$, 2.33), 223 ($M^+-C_8H_8O$, 5.27), 119 ($C_8H_6O^+$, 100.00), 91 ($PhCH_2^+$, 63.68), 77 ($C_6H_5^+$, 7.53).

3.1.12. 4-Phenyl-5-thien-2-yl-4,5-dihydro-2,2′bifuran-3-carbonitrile (3l). Yield 58% (370 mg) as a yellow oil. [Found: C, 71.6; H, 3.8; N, 4.1; S, 10.2. $C_{19}H_{13}NO_2S$ requires C, 71.5; H, 4.1; N, 4.4; S, 10.0%]; ν_{max} (KBr disc, $CHCl_3$) 2207 (CN), 1646 (C=C), 1174 (C–O–C), 758, 706; δ_H (400 MHz, $CDCl_3$) 7.89 (1H, d, $J=1.2$ Hz, arom.), 7.66–7.50 (8H, m, arom.), 7.27 (1H, dd, $J=5.1$, 3.6 Hz, arom.), 6.81 (1H, dd, $J=3.6$, 1.7 Hz, arom.), 6.04 (1H, d, $J=7.7$ Hz, H5), 4.82 (1H, d, $J=7.7$ Hz, H4); *m/z* (EI, 70 eV) 320 (MH^+, 19.14), 319 (M^+, 100.00), 236 ($M^+-C_4H_3S$, 3.85), 224 ($M^+-C_5H_3O_2$, 13.39), 178 ($M^+-C_{10}H_7N$, 15.11), 163 ($C_9H_7OS^+$, 10.96), 140 ($C_{10}H_6N^+$, 51.87), 95 ($C_5H_4O_2^+$, 96.79), 83 ($C_4H_3S^+$, 1.67), 77 ($C_6H_5^+$, 6.39).

3.1.13. 2-(1-Benzofuran-2-yl)-4-phenyl-5-thien-2-yl-4,5-dihydrofuran-3-carbonitrile (3m). Yield 52% (384 mg) as a colourless solid, mp 107–108 °C (hexane/EtOAc). [Found: C, 75.0; H, 4.1; N, 3.7; S, 8.6. $C_{23}H_{15}NO_2S$ requires C, 74.8; H, 4.1; N, 3.8; S, 8.7%]; ν_{max} (KBr disc) 2200 (CN), 1643 (C=C), 1172 (C–O–C), 754, 700; δ_H (400 MHz, $CDCl_3$) 7.92 (1H, d, $J=7.8$ Hz, arom.), 7.85 (1H, d, $J=8.4$ Hz, arom.), 7.69 (1H, s, arom.), 7.60–7.54 (8H, m, arom.), 7.37 (1H, d, $J=3.4$ Hz, arom.), 7.28 (1H, dd, $J=5.0$, 3.6 Hz, arom.), 6.11 (1H, d, $J=7.7$ Hz, H5), 4.88 (1H, d, $J=7.7$ Hz, H4); *m/z* (EI, 70 eV) 370 (MH^+, 22.68), 369 (M^+, 100.00), 285 ($M^+-C_4H_4S$, 2.44), 227 ($MH^+-C_8H_5O-CN$, 7.16), 145 ($C_9H_5O_2^+$, 75.44), 83 ($C_4H_3S^+$, 1.28), 89 ($C_7H_5^+$, 58.15), 77 ($C_6H_5^+$, 6.4).

3.1.14. 5-Methyl-4-phenyl-5-thien-2-yl-4,5-dihydo-2,2′bifuran-3-carbonitrile (3n). Yield 67% (446 mg) as a yellow oil. [Found: C, 72.2; H, 4.3; N, 4.0; S, 9.7. $C_{20}H_{15}NO_2S$ requires C, 72.1; H, 4.5; N, 4.2; S, 9.6%]; ν_{max} (KBr disc, $CHCl_3$) 2205 (CN), 1629 (C=C), 1018 (C–O–C), 754, 702; δ_H (400 MHz, $CDCl_3$) 8.08 (2H, dt, $J=8.6$, 1.9 Hz, arom.), 7.50 (2H, dt, $J=8.6$, 1.9 Hz, arom.), 7.45–7.35 (3H, m, arom.), 7.32 (1H, dd, $J=5.0$, 1.2 Hz, arom.), 7.27 (1H, m, arom.), 7.10 (1H, dd, $J=3.7$, 1.2 Hz, arom.), 7.05 (1H, dd, $J=5.0$, 3.6 Hz, arom.), 4.79 (1H, s, H4), 1.47 (3H, s, *Me*); δ_C (100 MHz, $CDCl_3$) 165.1 (C2), 149.7, 137.9, 135.6, 129.2, 128.9, 128.85, 128.8, 128.5, 127.1, 126.3, 124.9, 123.1, 117.1 (CN), 91.5 (C3), 84.3 (C5), 61.8 (C4), 25.3 (Me); *m/z* (APCI, 100 eV) 334 (MH^+, 21.52), 333 (M^+, 89.45), 316 (MH^+-H_2O, 17.42), 266 ($M^+-C_4H_3O$, 28.67), 239 ($MH^+-C_5H_3O_2$, 42.94), 224 ($MH^+-C_5H_4OS$, 13.81), 199 ($MH^+-C_5H_3O_2-C_2H_2N$, 100.00), 95 ($C_5H_3O_2^+$, 75.18).

3.1.15. 2-(1-Benzofuran-2-yl)-5-methyl-4-phenyl-5-thien-2-yl-4,5-dihydofuran-3-carbonitrile (3o). Yield 60% (460 mg) as a colourless solid, mp 106–107 °C (hexane/EtOAc). [Found: C, 75.1; H, 4.4; N, 3.7; S, 8.4. $C_{24}H_{17}NO_2S$ requires C, 75.2; H, 4.5; N, 3.6; S, 8.3%]; ν_{max} (KBr disc) 2204 (CN), 1635 (C=C), 1132 (C–O–C), 751, 694; δ_H (400 MHz, $CDCl_3$) 7.65 (1H, d, J=1.2 Hz, arom.), 7.41–7.34 (5H, m, arom.), 7.28 (1H, dd, J=5.2, 1.2 Hz, arom.), 7.25 (2H, dd, J=6.8, 1.6 Hz, arom.), 7.13 (1H, d, J=4.1 Hz, arom.), 7.07 (1H, dd, J=3.6, 1.2 Hz, arom.), 7.01 (1H, dd, J=5.2, 3.2 Hz, arom.), 6.58 (1H, dd, J=3.6, 2.1 Hz, arom.), 4.70 (1H, s, H4), 1.38 (3H, s, *Me*); δ_C (100 MHz, $CDCl_3$) 157.7 (C2), 149.5, 145.9, 143.6, 135.9, 129.1, 129.0, 128.6, 127.2, 125.3, 123.65, 116.5 (CN), 115.1, 112.3, 92.6 (C3), 82.7 (C5), 61.45 (C4), 60.65, 25.38, 14.4 (Me); *m/z* (EI, 70 eV) 384 (MH^+, 15.17), 383 (M^+, 58.51), 306 ($M^+-C_6H_5$, 2.43), 257 ($M^+-C_6H_6OS$, 47.49), 238 ($M^+-C_9H_5O_2$, 15.51), 155 ($M^+-C_9H_5O_2-C_4H_4S$, 40.95), 145 ($C_9H_5O_2^+$, 100.00), 126 ($C_6H_6OS^+$, 15.55), 111 ($C_5H_3OS^+$, 35.71), 83 ($C_4H_3S^+$, 9.45), 77 ($C_6H_5^+$, 21.81).

3.1.16. 5-Methyl-2-(4-methylphenyl)-4-phenyl-5-thien-2-yl-4,5-dihydofuran-3-carbonitrile (3p). Yield 71% (507 mg) as a colourless solid, mp 152 °C (hexane/EtOAc). [Found: C, 77.1; H, 5.4; N, 4.2; S, 9.2. $C_{23}H_{19}NOS$ requires C, 77.3; H, 5.3; N, 3.9; S, 9.0%]; ν_{max} (KBr disc) 2205 (CN), 1617 (C=C), 1238 (C–O–C), 1078, 835, 696; δ_H (400 MHz, $CDCl_3$) 8.04 (2H, d, J=8.3 Hz, arom.), 7.44–7.27 (8H, m, arom.), 7.09 (1H, dd, J=3.6, 1.2 Hz, arom.), 7.04 (1H, dd, J=5.0, 3.6 Hz, arom.), 4.71 (1H, s, H4), 2.46 (3H, s, *Me*), 1.41 (3H, s, *Me*); δ_C (100 MHz, $CDCl_3$) 166.5 (C2), 150.1, 142.5, 136.1, 129.5, 128.83, 128.8, 128.3, 127.4, 126.9, 125.1, 124.8, 123.1, 117.7 (CN), 91.1 (C3), 82.9 (C5), 61.8 (C4), 25.3 (Me), 21.7 (Me); *m/z* (APCI, 100 eV) 358 (MH^+, 11.82), 357 (M^+, 74.01), 343 (MH^+-CH_3, 13.81), 238 ($M^+-C_8H_7O$, 35.24), 119 ($C_8H_7O^+$, 100.0), 91 ($C_7H_7^+$, 19.12), 77 ($C_6H_5^+$, 4.15).

3.1.17. 2-(4-Methylphenyl)-5-phenyl-5-thien-2-yl-4,5-dihydrofuran-3-carbonitrile (3r). Yield 83% (569 mg) as a yellow oil. [Found: C, 77.1; H, 5.3; N, 3.9; S, 9.5. $C_{22}H_{17}NOS$ requires C, 76.9; H, 5.0; N, 4.1; S, 9.3%]; ν_{max} (KBr disc, $CHCl_3$) 2198 (CN), 1608 (C=C), 1255 (C–O–C), 1182, 835, 704; δ_H (400 MHz, $CDCl_3$) 8.0 (2H, d, J=8.3 Hz, arom.), 7.50 (2H, dt, J=6.9, 1.7 Hz, arom.), 7.38–7.44 (3H, m, arom.), 7.33 (3H, m, arom.), 7.0 (2H, m, arom.), 3.89 (1H, d, J=14.8 Hz, H_a4), 3.71 (1H, d, J=14.8 Hz, H_b4), 2.45 (3H, s, *Me*); δ_C (100 MHz, $CDCl_3$) 166.4 (C2), 148.9, 144.5, 143.1, 130.3, 129.4, 129.2, 128.1, 127.5, 127.1, 126.6, 126.0, 125.9, 118.1 (CN), 91.1 (C3), 78.7 (C5), 47.3 (C4), 21.8 (Me); *m/z* (APCI, 150 eV) 344 (MH^+, 16.8), 343 (M^+, 29.8), 328 (M^+-CH_3, 28.79), 276 ($M^+-C_4H_3S$, 43.23), 224 ($M^+-C_8H_7O$, 25.70), 155 ($MH^+-C_{11}H_8OS$, 34.45), 119 ($C_8H_7O^+$, 100.00), 83 ($C_4H_3S^+$, 16.20), 77 ($C_6H_5^+$, 12.03).

3.1.18. 2-(4-Chlorophenyl)-5-phenyl-5-thien-2-yl-4,5-dihydrofuran-3-carbonitrile (3s). Yield 93% (675 mg) as a colourless solid, mp 122 °C (hexane/ EtOAc). [Found: C, 69.5; H, 3.8; N, 3.9; S, 8.9. $C_{21}H_{14}ClNOS$ requires C, 69.3; H, 3.9; N, 3.9; S, 8.8%]; ν_{max} (KBr disc) 2200 (CN), 1623 (C=C), 829, 702; δ_H (400 MHz, $CDCl_3$) 8.05 (2H, dt, J=8.7, 1.9 Hz, arom.), 7.50–7.38 (7H, m, arom.), 7.35 (1H, dd, J=3.6, 2.8 Hz, arom.), 7.0 (2H, dd, J=2.7, 0.9 Hz, arom.), 3.91 (1H, d, J=15.0 Hz, H_a4), 3.73 (1H, d, J=15.0 Hz, H_b4); δ_C (100 MHz, $CDCl_3$) 164.2 (C2), 147.8, 143.5, 137.9, 129.4, 128.9, 128.8, 128.7, 127.1, 126.7, 126.5, 126.3, 125.5, 117.1 (CN), 91.1 (C3), 80.0 (C5), 47.2 (C4); *m/z* (EI, 70 eV) 364 (MH^+, 11.35), 363 (M^+, 19.80), 362 (M^+-H, 18.54), 328 (M^+-Cl, 6.21), 252 ($M^+-C_6H_5Cl$, 2.20), 224 ($M^+-C_7H_5ClO$, 7.17), 139 ($C_7H_5ClO^+$, 100.00), 111 ($C_6H_5Cl^+$, 43.70), 77 ($C_6H_5^+$, 7.66).

Acknowledgements

The authors thanks to Ankara University Scientific Research Projects (BAP 20010705065), and the Scientific and Technical Research Council of Turkey (TBAG-104T348) for financial supports. EIMS analyses were performed at Scientific Research Center of Ankara University (BITAUM).

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A convenient and new approach to the synthesis of ω-heterocyclic amino acids from carboxy lactams through ring-chain-transformation. Part 2: Synthesis of (2*R*)-/(2*S*)-2-aminomethyl-3-(1-aryl-/1,5-diaryl-1*H*-pyrazol-3-yl)-propionic acid

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Received 27 April 2005; revised 24 June 2005; accepted 7 July 2005

Available online 28 July 2005

Abstract—Making use of amide activation, a convenient and short path synthesis of optically pure ω-heterocyclic-β-amino acids has been achieved from (1*R*,3*R*)- and (1*R*,3*S*)-5-oxo-1-(1-phenyl-ethyl)-pyrrolidine-3-carboxylic acid methyl ester. The key step of the synthesis involves a regiospecific ring-chain-transformation of the enaminones when subjected to 1,2-binucleophilic attacks. The method is illustrated by the synthesis of (2*R*)-/(2*S*)-2-aminomethyl-3-(1-aryl-/1,5-diaryl-1*H*-pyrazol-3-yl)-propionic acid.

1. Introduction

Unnatural amino acids[1–3] have stimulated much recent interest. Among unnatural amino acids, β-amino acids[4] has attracted much attention. Oligomeric β-amino acids form novel folded structures, which have shown to exhibit novel physicochemical and biological activities. They are being used as peptidomimetics and have emerged as an important class of building blocks in combinatorial library building and drug discovery. Moreover many of the developmental pharmaceuticals contain β-amino acids as critical components.[5]

We had earlier demonstrated the convenient synthesis of optically active ω-heterocyclic-α-amino acids.[6] The present study is aimed as an extension of this efficient methodology to the synthesis of optically active ω-heterocyclic-β-amino acid from activated lactams. This paper describes chiral syntheses of 2-aminomethyl-3-[1-(un)substituted-5-aryl-1*H*-pyrazol-3-yl]-propionic acids from optically active lactams derived from itaconic acid.

2. Results and discussion

With a view to establish the feasibility of this approach racemic 2-methylaminomethyl-3-[1-(un)substituted-5-aryl-1*H*-pyrazol-3-yl/5-aryl-isoxazol-3-yl]-propionic acid methyl esters **4** and **5** were first synthesized as model compounds. The compounds **4** and **5** were prepared as described in Scheme 1, starting from 1-methyl-5-oxopyrrolidine-3-carboxylic acid methyl ester (**1**), which in turn was prepared from itaconic acid according to the procedure described in literature.[7] The compound **1** was then treated with Lawesson's reagent to give the corresponding thiolactam **2** in quantitative yield. The sulfur extrusion reaction[6] on thiolactam **2** with various phenacyl bromide afforded 1-methyl-5-(2-aryl-2-oxo-ethylidene)-pyrrolidine-

Keywords: Unnatural β-amino acids; 2-Aminomethyl-3-(substituted-1*H*-pyrazol-3-yl)-propionic acid; Chirality; Ring-chain-transformation reaction; Sulfur extrusion reaction; Hydrogenation.
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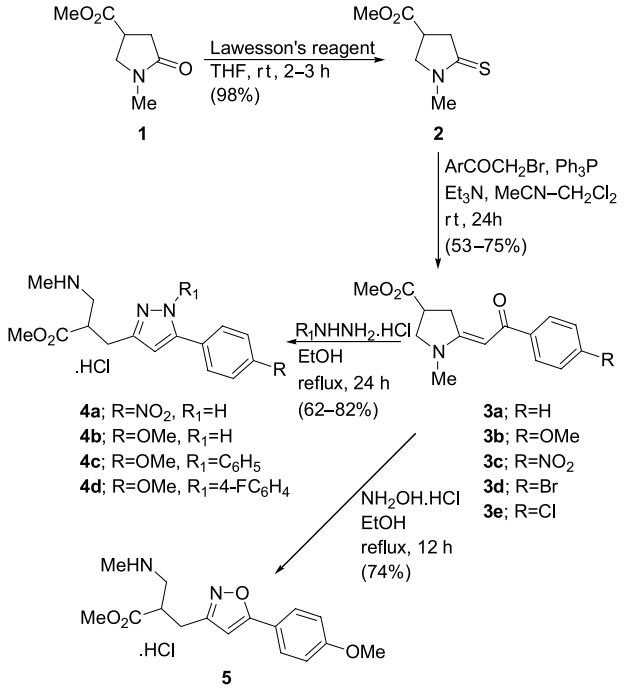
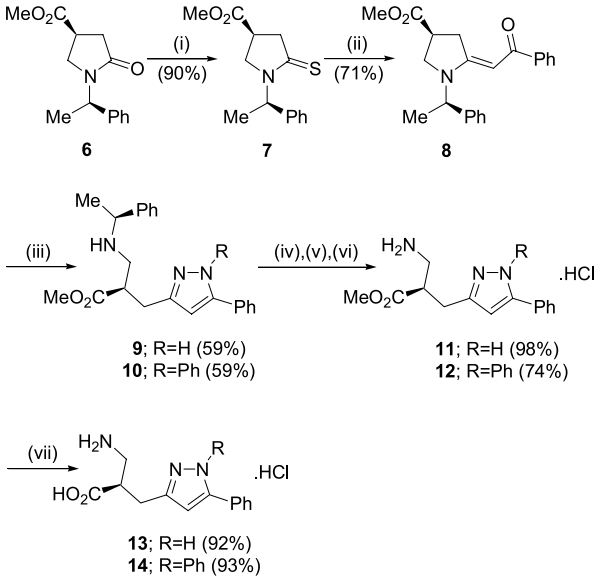
doi:10.1016/j.tet.2005.07.018



Scheme 1.



Scheme 2. Reagents and conditions: (i) Lawesson's reagent, THF, rt, 3–4 h; (ii) $PhCOCH_2Br$, Ph_3P, Et_3N, $MeCN–CH_2Cl_2$, rt, 24 h; (iii) $RNHNH_2 \cdot HCl$, MeOH or EtOH, reflux, 18–32 h, $NH_3–CHCl_3$; (iv) HCOOH or cyclohexene, 10% Pd–C, reflux, 6–18 h; (v) $(Boc)_2O$, Et_3N, CH_2Cl_2, 0 °C, 2–3 h; (vi) MeOH–HCl, 0→25 °C, 12 h; (vii) 6 N HCl, reflux 2–4 h.

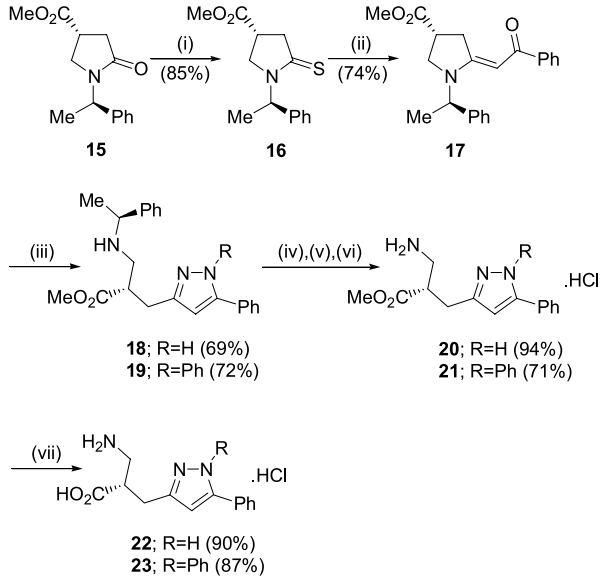
3-carboxylic acid methyl esters (**3**) in good yields. These enaminones **3** on condensation with hydroxyl amine/(un)substituted hydrazines hydrochloride in EtOH under reflux temperature underwent facile ring-chain-transformation reaction[8] to give 2-methylamino-methyl-3-[1-(un)substituted-5-aryl-1*H*-pyrazol-3-yl/5-aryl-isoxazol-3-yl]-propionic acid methyl ester hydrochloride (**4** and **5**) in 62–82% yields (Scheme 1).

Attempted reaction of these enaminones with 1,3-dinucleophiles such as guanidine, benzamidine, acetamidine, urea, and thiourea were unsuccessful. Compounds of type **3**, **4** and **5** are hitherto unknown in the literature and their structures were confirmed on the basis of elemental and spectroscopic analysis.

Having established the route to racemic ω-heterocyclic-β-amino acid esters, attention was turned to the synthesis of optically pure ω-heterocyclic-β-amino acids. (*R*)-(+)-1-Phenyl-ethylamine was used as chiral auxiliary to function both as resolving agent and protecting group during synthesis. The required (1*R*,3*R*)-5-oxo-1-(1-phenyl-ethyl)-pyrrolidine-3-carboxylic acid methyl ester **6** and (1*R*,3*S*)-5-oxo-1-(1-phenyl-ethyl)-pyrrolidine-3-carboxylic acid methyl ester **15** were prepared from itaconic acid and (*R*)-(+)-1-phenyl-ethylamine as described in literature.[9] The purity and stereochemical homogeneity of **6** and **15** were established by TLC analysis, ^{1}H NMR and HPLC analysis and each isomer was found to be free from any contamination from other isomer. The optically pure methyl ester **6** was converted to corresponding thiolactam **7** in 90% yields following the procedure described in literature.[6] Sulfur extrusion reaction on thiolactam **7** with phenacyl bromide yielded the enaminone **8** in 71% yield (Scheme 2). The enaminone **8** was next subjected to ring-chain-transformation with hydrazine hydrochloride and phenyl-hydrazine hydrochloride to afford (1*R*,2*R*)-2-[(1-phenyl-ethylamino)-methyl]-3-(5-phenyl-1*H*-pyrazol-3-yl)-propionic acid methyl ester **9** and (1*R*,2*R*)-3-(1,5-diphenyl-1*H*-pyrazol-3-yl)-2-[(1-phenyl-ethylamino)-methyl]-propionic acid methyl ester **10**, respectively, in 59% yields (Scheme 2). The *N*-debenzylation of compounds **9** and **10** using catalytic transfer hydrogenation conditions[6] gave crude (2*R*)-2-aminomethyl-3-(5-phenyl-1*H*-pyrazol-3-yl)-propionic acid methyl ester **11** and (2*R*)-2-aminomethyl-3-(1,5-diphenyl-1*H*-pyrazol-3-yl)-propionic acid methyl ester **12**, respectively. These crude amino acid ester **11** and **12** were converted to corresponding *N*-Boc derivatives and then purified by column chromatography over silica gel to afford pure *N*-Boc analog, which on treatment with methanolic-HCl at low temperature to afford pure amino acid ester **11** and **12**, which were isolated as hydrochloride salts. Finally the acidic hydrolysis of the methyl esters **11** and **12** afforded the desired (2*R*)-2-aminomethyl-3-(5-phenyl-1*H*-pyrazol-3-yl)-propionic acid **13** and (2*R*)-2-aminomethyl-3-(1,5-diphenyl-1*H*-pyrazol-3-yl)-propionic acid **14**, respectively, in quantitative yields, which were isolated as hydrochloride salts (Scheme 2).

Following a similar sequence of reactions, (1*R*,3*S*)-5-oxo-1-(1-phenyl-ethyl)-pyrrolidine-3-carboxylic acid methyl ester **15** was converted to (2*S*)-2-aminomethyl-3-(5-phenyl-1*H*-pyrazol-3-yl)-propionic acid **22** and (2*S*)-2-aminomethyl-3-(1,5-diphenyl-1*H*-pyrazol-3-yl)-propionic acid **23** (Scheme 3).

All new compounds reported here, were fully characterized on the basis of complementary spectroscopic (IR, NMR and MS) and analytical data. Specific optical rotations of the antipodes were found to be opposite and identical.



Scheme 3. Reagents and conditions: (i) Lawesson's reagent, THF, rt, 3–4 h; (ii) $PhCOCH_2Br$, Ph_3P, Et_3N, MeCN–CH_2Cl_2, rt, 24 h; (iii) $RNHNH_2 \cdot HCl$, MeOH or EtOH, reflux, 18–32 h, NH_3–$CHCl_3$; (iv) HCOOH or cyclohexene, 10% Pd–C, reflux, 6–18 h; (v) $(Boc)_2O$, Et_3N, CH_2Cl_2, 0 °C, 2–3 h; (vi) MeOH–HCl, 0→25 °C, 12 h; (vii) 6 N HCl, reflux 2–4 h.

3. Conclusion

In conclusion, we have developed a convenient and new approach for the synthesis of ω-heterocyclic-β-amino acids from activated lactams derived from itaconic acid. This methodology can be used to afford the higher analogs, simply by extending either the lactam ring size or changing the position of carboxyl group on a given lactam. Further, investigation on this type of reaction is currently in progress in our laboratories.

4. Experimental

4.1. General

Melting points were recorded on a Büchi B-540 melting point apparatus. Compounds were routinely checked for their purity on silica gel 60 F_{254} TLC plates and their spots were visualized by exposing them to iodine vapor, UV lamp or by spraying the plates with Dragendorff's or ninhydrine or $KMnO_4$ reagents. IR spectra (λ_{max} in cm^{-1}) were recorded on Perkin Elmer Paragon-1000 PC instrument and NMR (300 MHz) spectra were recorded on Bruker 300-DRX instrument as solutions using TMS as internal standard, and chemical shifts are expressed in δ units. Mass spectra were recorded on PE-SCIEX LC-MS/MS instrument. Optical rotations were taken on Autopol-III instrument. Elemental analyses were carried out with a Perkin Elmer 2400 analyzer and values found were within $\pm 0.4\%$ of theoretical values.

4.2. 1-Methyl-5-thioxo-pyrrolidine-3-carboxylic acid methyl ester (2)

To a soln of lactam **1**[7] (15.07 g, 96 mmol) in dry THF (75 mL) was added Lawesson's reagent (19.4 g, 48 mmol) portion-wise under stirring at 25–30 °C and resulting reaction mixture stirred for 2–3 h at same temperature. THF was removed under reduced pressure to obtain a viscous residue, which was dissolved in EtOAc (200 mL), washed with 10% $NaHCO_3$ (5×50 mL), brine (50 mL), dried (Na_2SO_4) and filtered. The filtrate was concentrated under reduced pressure to give the thiolactam **2** as viscous oil, yield 16.2 g (98%); ν_{max} (CH_2Cl_2) 1736, 1210 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 3.26 (s, 3H, NC*H*$_3$), 3.31–3.35 (m, 3H, 3-C*H*$_2$, 4-C*H*), 3.74 (s, 3H, CO_2CH_3), 3.90–3.97 (m, 1H, 5-C*H*$_a$), 4.04–4.09 (m, 1H, 5-C*H*$_b$); *m*/*z* 174 (M+1).

4.3. Sulfur extrusion reaction on thiolactam with phenacyl bromides

General procedure. To a soln of thiolactam **2** (5.0 mmol) in dry MeCN (2 mL), phenacyl bromide (6.25 mmol, 1.25 equiv) was added and the reaction mixture stirred for 10 h at 25–30 °C. The solid, which separated out, was dissolved by addition of dry CH_2Cl_2 (25 mL) and stirred for 10 min at the same temperature. To this Ph_3P (7.5 mmol) and Et_3N (15.0 mmol) were added and the reaction mixture stirred for another 14 h at same temperature. When the reaction was completed, solvents were removed under reduced pressure and the residue was dissolved in EtOAc (50 mL), washed with H_2O (3×10 mL), brine (10 mL), dried (Na_2SO_4) and filtered. The filtrate was concentrated under reduced pressure to give a crude product, which was purified by column chromatography over silica gel (230–400 mesh) using hexanes–EtOAc gradient as eluent to afford enaminones **3a**–**3e**.

4.3.1. 1-Methyl-5-(2-oxo-2-phenyl-ethylidene)-pyrrolidine-3-carboxylic acid methyl ester (3a). This was obtained as colorless thick oil by condensing phenacyl bromide with thiolactam **2**, 67% yield; ν_{max} (CH_2Cl_2) 1735, 1626, 1579, 1546, 1217 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 2.96 (s, 3H, NC*H*$_3$), 3.20–3.25 (m, 1H, 4-C*H*), 3.61–3.67 (m, 2H, 3-C*H*$_2$), 3.73 (s, 3H, CO_2CH_3), 3.76–3.83 (m, 2H, 5-C*H*$_2$), 5.70 (s, 1H, COC*H*), 7.37–7.43 (m, 3H, Ar*H*), 7.86–7.80 (m, 2H, Ar*H*); δ_C (75 MHz, $CDCl_3$) 187.0, 174.5, 169.0, 136.7, 134.3, 129.7, 129.0, 93.5, 58.1, 50.7, 46.9, 36.2, 35.1; *m*/*z* 260 (M+1). Anal. Calcd for $C_{15}H_{17}NO_3$ (259.30): C, 69.48; H, 6.61; N, 5.40. Found: C, 69.22; H, 6.53; N, 5.29%.

4.3.2. 1-Methyl-5-[2-(4-methoxyphenyl)-2-oxo-ethylidene]-pyrrolidine-3-carboxylic acid methyl ester (3b). This was obtained in 83% yield as white needles by condensing 4-methoxyphenacyl bromide with thiolactam **2**, mp 96–97 °C; ν_{max} (KBr) 1745, 1618, 1219 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 2.96 (s, 3H, NC*H*$_3$), 3.20–3.30 (m, 1H, 4-C*H*), 3.59–3.67 (m, 2H, 3-C*H*$_2$), 3.72 (s, 3H, CO_2CH_3), 3.75–3.81 (m, 2H, 5-C*H*$_2$), 3.84 (s, 3H, OC*H*$_3$), 5.68 (s, 1H, COC*H*), 6.89 (d, *J*=9.0 Hz, 2H, Ar*H*), 7.88 (d, *J*=9.0 Hz, 2H, Ar*H*); *m*/*z* 290 (M+1). Anal. Calcd for $C_{16}H_{19}NO_4$ (289.33): C, 66.42; H, 6.62; N, 4.84. Found: C, 66.54; H, 6.47; N, 4.44%.

4.3.3. 1-Methyl-5-[2-(4-nitrophenyl)-2-oxo-ethylidene]-pyrrolidine-3-carboxylic acid methyl ester (3c). This was obtained in 75% yield as yellow solid by condensing 4-nitrophenacyl bromide with thiolactam **2**, mp 102–103 °C; ν_{max} (KBr) 1741, 1625, 1598, 1542, 1344 cm^{-1};

δ_H (300 MHz, $CDCl_3$) 3.01 (s, 3H, NCH_3), 3.03–3.33 (m, 1H, 4-C*H*), 3.69–3.75 (m, 6H, CO_2CH_3, 3-CH_2, 5-CH_a), 3.84–3.89 (m, 1H, 5-CH_b), 5.64 (s, 1H, COC*H*), 7.99 (d, J=9.0 Hz, 2H, Ar*H*), 8.23 (d, J=9.0 Hz, 2H, Ar*H*); *m*/*z* 305 (M+1). Anal. Calcd for $C_{15}H_{16}N_2O_5$ (304.30): C, 59.21; H, 5.30; N, 9.21. Found: C, 58.90; H, 5.64; N, 9.29%.

4.3.4. 1-Methyl-5-[2-(4-bromophenyl)-2-oxo-ethylidene]-pyrrolidine-3-carboxylic acid methyl ester (3d). This was obtained in 53% yield as off white solid by condensing 4-bromophenacyl bromide with thiolactam **2**, mp 126–128 °C; ν_{max} (KBr) 1736, 1624, 1584, 1214, 976, 767 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 2.97 (s, 3H, NCH_3), 3.27–3.30 (m, 1H, 4-C*H*), 3.62–3.71 (m, 6H, CO_2CH_3, 3-CH_2, 5-CH_a), 3.78–3.84 (m, 1H, 5-CH_b), 5.62 (s, 1H, COC*H*), 7.51 (d, J=9.0 Hz, 2H, Ar*H*), 7.74 (d, J=9.0 Hz, 2H, Ar*H*); *m*/*z* 339 (M+1). Anal. Calcd for $C_{15}H_{16}BrNO_3$ (338.20): C, 53.27; H, 4.77; N, 4.14. Found: C, 53.11; H, 4.83; N, 3.92%.

4.3.5. 1-Methyl-5-[2-(4-chlorophenyl)-2-oxo-ethylidene]-pyrrolidine-3-carboxylic acid methyl ester (3e). This was obtained in 58% yield as thick oil by condensing 4-chlorophenacyl bromide with thiolactam **2**; ν_{max} ($CHCl_3$) 1735, 1573, 1542, 1216, 768 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 2.97 (s, 3H, NCH_3), 3.25–3.30 (m, 1H, 4-C*H*), 3.62–3.68 (m, 2H, 3-CH_2), 3.71 (s, 3H, CO_2CH_3), 3.73–3.84 (m, 2H, 5-CH_2), 5.63 (s, 1H, COC*H*), 7.35 (d, J=9.0 Hz, 2H, Ar*H*), 7.81 (d, J=9.0 Hz, 2H, Ar*H*); *m*/*z* 294 (M+1). Anal. Calcd for $C_{15}H_{16}ClNO_3$ (293.75): C, 61.33; H, 4.49; N, 4.77. Found: C, 61.49; H, 4.21; N, 4.70%.

4.4. Ring-chain-transformation reaction of enaminones with NH_2XH

General procedure. $NH_2XH\cdot HCl$ (2.99 mmol, 1.0 equiv) was added to a soln of enaminone **3** (2.99 mmol) in MeOH (10 mL) at 25–30 °C with stirring and the resulting reaction mixture was refluxed for 28 h. Solvent was removed under reduced pressure; the solid so obtained was stirred with Et_2O (20 mL) and filtered, dried under reduced pressure to afford **4a**–**4d** and **5**.

4.4.1. 2-Methylaminomethyl-3-[5-(4-nitrophenyl)-1*H*-pyrazol-3-yl]-propionic acid methyl ester hydrochloride (4a). This was obtained in 82% yield as white solid by condensing hydrazine dihydrochloride with enaminone **3c**, mp 190–191 °C; ν_{max} (KBr) 1739, 1601, 1524, 1342 cm^{-1}; δ_H (300 MHz, CD_3OD) 2.76 (s, 3H, NCH_3), 3.04–3.16 (m, 4H, 2×CH_2), 3.32–3.33 (m, 1H, 4-C*H*), 3.71 (s, 3H, CO_2CH_3), 6.68 (s, 1H, Ar*H*), 7.90 (d, J=9.0 Hz, 2H, Ar*H*), 8.22 (d, J=9.0 Hz, 2H, Ar*H*); *m*/*z* 319 (M+1). Anal. Calcd for $C_{15}H_{18}N_4O_4\cdot 2HCl$ (391.25): C, 46.05; H, 5.15; N, 14.32. Found: C, 46.19; H, 4.96; N, 13.97%.

4.4.2. 3-[5-(4-Methoxyphenyl)-1*H*-pyrazol-3-yl]-2-methylaminomethyl-propionic acid methyl ester hydrochloride (4b). This was obtained as white powder by condensing hydrazine dihydrochloride with enaminone **3b**, which was recrystallized from MeOH–Et_2O to give colorless long needles (62% yield), mp 200–201 °C; ν_{max} (KBr) 1734, 1617 cm^{-1}; δ_H (300 MHz, TFA-*d*) 3.05 (s, 3H, NCH_3), 3.36–3.66 (m, 4H, 2×CH_2), 3.85 (br s, 1H, 4-C*H*), 3.91 (s, 3H, CO_2CH_3), 4.04 (s, 3H, OCH_3), 6.99 (s, 1H, Ar*H*), 7.21 (d, J=9.0 Hz, 2H, Ar*H*), 7.80 (d, J=9.0 Hz, 2H, Ar*H*); *m*/*z* 304 (M+1). Anal. Calcd for $C_{16}H_{21}N_3O_3\cdot 2HCl$ (376.28): C, 51.07; H, 6.16; N, 11.17. Found: C, 50.85; H, 6.13; N, 10.95%.

4.4.3. 3-[5-(4-Methoxyphenyl)-1-phenyl-1*H*-pyrazol-3-yl]-2-methylaminomethyl-propionic acid methyl ester hydrochloride (4c). This was obtained as off white solid by condensing phenyl hydrazine dihydrochloride with enaminone **3b**, which was recrystallized from MeOH–Et_2O to give off white solid (78% yield), mp 180–181 °C; ν_{max} (KBr) 1733, 1611, 1506, 1250 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 2.72 (s, 3H, NCH_3), 3.15–3.45 (m, 4H, 2×CH_2), 3.61–3.64 (m, 1H, 4-C*H*), 3.80 (s, 3H, CO_2CH_3), 3.82 (s, 3H, OCH_3), 6.32 (s, 1H, Ar*H*), 6.81 (d, J=9.0 Hz, 2H, Ar*H*), 7.12 (d, J=9.0 Hz, 2H, Ar*H*), 7.24–7.37 (m, 5H, Ar*H*); *m*/*z* 380 (M+1). Anal. Calcd for $C_{22}H_{25}N_3O_3\cdot HCl$ (415.91): C, 63.53; H, 6.30; N, 10.10. Found: C, 63.39; H, 6.33; N, 10.08%.

4.4.4. 3-[1-(4-Fluorophenyl)-5-(4-methoxyphenyl)-1*H*-pyrazol-3-yl]-2-methylaminomethyl-propionic acid methyl ester hydrochloride (4d). This was obtained as off white solid by condensing 4-fluorophenyl hydrazine dihydrochloride with enaminone **3b**, which was recrystallized from MeOH–Et_2O to give white solid (81% yield), mp 162–163 °C; ν_{max} (KBr) 2944, 1733, 1613, 1512 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 2.69 (s, 3H, NCH_3), 2.93–3.37 (m, 4H, 2×CH_2), 3.55 (br s, 1H, 4-C*H*), 3.80 (br s, 6H, CO_2CH_3, OCH_3), 6.30 (s, 1H, Ar*H*), 6.81 (d, J=9.0 Hz, 2H, Ar*H*), 7.03 (d, J=9.0 Hz, 2H, Ar*H*), 7.06 (d, J=12.0 Hz, 2H, Ar*H*), 7.15 (d, J=9.0 Hz, 2H, Ar*H*); *m*/*z* 398 (M+1). Anal. Calcd for $C_{22}H_{24}FN_3O_3\cdot HCl$ (433.90): C, 60.90; H, 5.81; N, 9.68. Found: C, 61.13; H, 5.67; N, 9.80%.

4.4.5. 3-[5-(4-Methoxyphenyl)-isoxazol-3-yl]-2-methyl-aminomethyl-propionic acid methyl ester hydrochloride (5). This was obtained as gummy solid by condensing hydroxylamine hydrochloride with enaminone **3b**, which was recrystallized from MeOH–Et_2O to give white crystalline solid (74% yield), mp 139–140 °C; ν_{max} (KBr) 1731, 1618, 1444, 1255 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 2.79 (s, 3H, NCH_3), 3.25–3.49 (m, 4H, 2×CH_2), 3.70 (br s, 1H, 4-C*H*), 3.84 (s, 3H, CO_2CH_3), 3.87 (s, 3H, OCH_3), 6.45 (s, 1H, Ar*H*), 6.97 (d, J=9.0 Hz, 2H, Ar*H*), 7.70 (d, J=9.0 Hz, 2H, Ar*H*); *m*/*z* 305 (M+1). Anal. Calcd for $C_{16}H_{20}N_2O_4\cdot HCl$ (340.80): C, 56.39; H, 6.21; N, 8.22. Found: C, 56.34; H, 6.37; N, 8.15%.

4.5. (1*R*,3*R*)-5-Oxo-1-(1-phenyl-ethyl)-pyrrolidine-3-carboxylic acid methyl ester (6) and (1*R*,3*S*)-5-oxo-1-(1-phenyl-ethyl)-pyrrolidine-3-carboxylic acid methyl ester (15)

The compounds **6** and **15** were prepared according to the literature method.[9]

Compound **6** was isolated as oil in 41% yield; ν_{max} (CH_2Cl_2) 1732, 1680, 1492, 1430 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 1.53 (d, J=7.0 Hz, 3H, CHCH_3), 2.63–2.80 (m, 2H, 3-CH_2), 3.07–3.22 (m, 2H, 5-CH_2), 3.51–3.62 (m, 1H, 4-C*H*), 3.72 (s, 3H, CO_2CH_3), 5.50 (q, J=7.0 Hz, 1H, C*H*CH_3),

7.26–7.31 (m, 5H, Ar*H*); *m/z* 248 (M+1); $[\alpha]_D^{24}$ +52.9 (*c* 1, MeOH) {lit.,[9] $[\alpha]_D^{24}$ +51.3 (*c* 2.1, EtOAc)}. Anal. Calcd for $C_{14}H_{17}NO_3$ (247.29): C, 68.00; H, 6.93; N, 5.66. Found: C, 67.81; H, 6.66; N, 5.95%.

Compound **15** was isolated in 47% yield as long needles, mp 68–69 °C (lit.,[9] mp 70 °C); ν_{max} (KBr) 1736, 1682, 1493, 1434 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 1.56 (d, *J*=7.0 Hz, 3H, CHC*H*$_3$), 2.69–2.79 (m, 2H, 3-C*H*$_2$), 3.16–3.25 (m, 2H, 5-C*H*$_2$), 3.52–3.60 (m, 1H, 4-C*H*), 3.67 (s, 3H, CO_2CH_3), 5.52 (q, *J*=7.0 Hz, 1H, C*H*CH$_3$), 7.30–7.40 (m, 5H, Ar*H*); *m/z* 248 (M+1); $[\alpha]_D^{24}$ +107.2 (*c* 1, MeOH) {lit.,[9] $[\alpha]_D^{24}$ +109.6 (*c* 2.6, EtOAc)}. Anal. Calcd for $C_{14}H_{17}NO_3$ (247.29): C, 68.00; H, 6.93; N, 5.66. Found: C, 68.05; H, 7.13; N, 5.49%.

4.6. (1*R*,3*R*)-1-(1-Phenyl-ethyl)-5-thioxo-pyrrolidine-3-carboxylic acid methyl ester (7)

This was obtained from **6** as thick oil in 90% yield according to the procedure described for compound **2**; ν_{max} (CH_2Cl_2) 1737, 1495, 1443 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 1.61 (d, *J*=7.2 Hz, 3H, CHC*H*$_3$), 3.13 (dd, *J*=7.2, 8.7 Hz, 1H, 3-C*H*$_a$), 3.31–3.43 (m, 3H, 3-C*H*$_b$ and 5-C*H*$_2$), 3.72 (s, 3H, CO_2CH_3), 3.86 (dd, *J*=6.3, 6.1 Hz, 1H, 4-C*H*), 6.34 (q, *J*=7.2 Hz, 1H, C*H*CH$_3$), 7.31–7.35 (m, 5H, Ar*H*); *m/z* 264 (M+1); $[\alpha]_D^{24}$ +70.9 (*c* 1, MeOH). Anal. Calcd for $C_{14}H_{17}NO_2S$ (263.36): C, 63.85; H, 6.51; N, 5.32. Found: C, 63.78; H, 6.42; N, 5.28%.

4.7. (1*R*,3*S*)-1-(1-Phenyl-ethyl)-5-thioxo-pyrrolidine-3-carboxylic acid methyl ester (16)

This was obtained from **15** as thick oil in 85% yield according to the procedure described for compound **2**; ν_{max} (CH_2Cl_2) 1737, 1495, 1451 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 1.61 (d, *J*=7.2 Hz, 3H, CHC*H*$_3$), 3.20–3.60 (m, 4H, 3-C*H*$_2$ and 5-C*H*$_2$), 3.64 (s, 3H, CO_2CH_3), 3.74–3.81 (m, 1H, 4-C*H*), 6.36 (q, *J*=7.2 Hz, 1H, C*H*CH$_3$), 7.30–7.40 (m, 5H, Ar*H*); *m/z* 264 (M+1); $[\alpha]_D^{24}$ +133 (*c* 1, MeOH). Anal. Calcd for $C_{14}H_{17}NO_2S$ (263.36): C, 63.85; H, 6.51; N, 5.32. Found: C, 63.90; H, 6.67; N, 5.21%.

4.8. (1*R*,3*R*)-5-(2-Oxo-2-phenyl-ethylidene)-1-(1-phenyl-ethyl)-pyrrolidine-3-carboxylic acid methyl ester (8)

This was obtained in 71% yield as light yellow solid by condensing phenacyl bromide with thiolactam **7** according to the procedure described for compound **3**, mp 89–90 °C; ν_{max} (KBr) 1733, 1625, 1532, 1374 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 1.66 (d, *J*=6.9 Hz, 3H, CHC*H*$_3$), 3.13–3.37 (m, 2H, 3-C*H*$_2$), 3.61–3.69 (m, 2H, 5-C*H*$_2$), 3.72 (s, 3H, CO_2CH_3), 3.87 (dd, *J*=9.3, 9.6 Hz, 1H, 4-C*H*), 5.09 (q, *J*=6.9 Hz, 1H, C*H*CH$_3$), 5.93 (s, 1H, COC*H*), 7.28–7.44 (m, 8H, Ar*H*), 7.80–7.83 (m, 2H, Ar*H*); δ_C (75 MHz, $CDCl_3$) 187.0, 174.5, 169.0, 137.2, 136.7, 134.3, 129.7, 129.0, 128.3, 128.1, 126.8, 93.5, 53.7, 53.0, 50.7, 47.5, 35.7, 22.3; *m/z* 350 (M+1); $[\alpha]_D^{24}$ +341.1 (*c* 0.7, MeOH). Anal. Calcd for $C_{22}H_{23}NO_3$ (349.42): C, 75.62; H, 6.63; N, 4.01. Found: C, 75.63; H, 6.77; N, 4.15%.

4.9. (1*R*,3*S*)-5-(2-Oxo-2-phenyl-ethylidene)-1-(1-phenyl-ethyl)-pyrrolidine-3-carboxylic acid methyl ester (17)

This was obtained in 74% yield as light yellow thick oil by condensing phenacyl bromide with thiolactam **16** according to the procedure described for compound **3**; ν_{max} (CH_2Cl_2) 1735, 1625, 1576, 1539 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 1.65 (d, *J*=6.9 Hz, 3H, CHC*H*$_3$), 3.20–3.25 (m, 2H, 3-C*H*$_2$), 3.54–3.64 (m, 5H, 5-C*H*$_2$, CO_2CH_3), 3.75–3.78 (m, 1H, 4-C*H*), 5.05 (q, *J*=7.0 Hz, 1H, C*H*CH$_3$), 5.86 (s, 1H, COC*H*), 7.29–7.41 (m, 8H, Ar*H*), 7.67–7.79 (m, 2H, Ar*H*); *m/z* 350 (M+1); $[\alpha]_D^{24}$ +168.6 (*c* 0.7, MeOH). Anal. Calcd for $C_{22}H_{23}NO_3$ (349.42): C, 75.62; H, 6.63; N, 4.01. Found: C, 75.81; H, 6.59; N, 3.95%.

4.10. (1*R*,2*R*)-2-[(1-Phenyl-ethylamino)-methyl]-3-(5-phenyl-1*H*-pyrazol-3-yl)-propionic acid methyl ester (9)

This was obtained in 59% yield as oil by condensing hydrazine dihydrochloride with enaminone **8** according to the procedure described for compound **4**; ν_{max} (CH_2Cl_2) 3208, 2905, 1732, 1568, 1436 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 1.35 (d, *J*=6.8 Hz, 3H, CHC*H*$_3$), 2.69–2.79 (m, 2H, NC*H*$_2$), 2.86–2.94 (m, 2H, CHC*H*$_2$), 2.99–3.05 (m, 1H, C*H*CO$_2$CH$_3$), 3.68 (s, 3H, CO_2CH_3), 3.75 (q, *J*=6.0 Hz, 1H, C*H*CH$_3$), 6.28 (s, 1H, Ar*H*), 7.21–7.41 (m, 8H, Ar*H*), 7.67–7.70 (m, 2H, Ar*H*); *m/z* 364 (M+1); $[\alpha]_D^{24}$ +19.2 (*c* 0.5, MeOH). Anal. Calcd for $C_{22}H_{25}N_3O_2$ (363.45): C, 72.70; H, 6.93; N, 11.56. Found: C, 72.99; H, 6.90; N, 11.39%.

4.11. (1*R*,2*S*)-2-[(1-Phenyl-ethylamino)-methyl]-3-(5-phenyl-1*H*-pyrazol-3-yl)-propionic acid methyl ester (18)

This was obtained in 69% yield as thick oil by condensing hydrazine dihydrochloride with enaminone **17** according to the procedure described for compound **4**; ν_{max} (CH_2Cl_2) 3344, 2923, 1732, 1493, 1463 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 1.30 (d, *J*=6.8 Hz, 3H, CHC*H*$_3$), 2.60–2.85 (m, 2H, NC*H*$_2$), 2.90–3.06 (m, 3H, CHC*H*$_2$, C*H*CO$_2$CH$_3$), 3.65 (s, 3H, CO_2CH_3), 3.78 (q, *J*=7.0 Hz, 1H, C*H*CH$_3$), 6.30 (s, 1H, Ar*H*), 7.22–7.41 (m, 8H, Ar*H*), 7.68–7.70 (m, 2H, Ar*H*); *m/z* 364 (M+1); $[\alpha]_D^{24}$ +15.9 (*c* 0.51, MeOH). Anal. Calcd for $C_{22}H_{25}N_3O_2$ (363.45): C, 72.70; H, 6.93; N, 11.56. Found: C, 72.57; H, 7.03; N, 11.60%.

4.12. (1*R*,2*R*)-3-(1,5-Diphenyl-1*H*-pyrazol-3-yl)-2-[(1-phenyl-ethylamino)-methyl]-propionic acid methyl ester (10)

This was obtained in 59% yield as thick oil by condensing phenylhydrazine with enaminone **8** according to the procedure described for compound **4**; ν_{max} (CH_2Cl_2) 1739, 1696, 1610, 1585 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 1.28 (d, *J*=6.6 Hz, 3H, CHC*H*$_3$), 2.70–2.74 (m, 2H, NC*H*$_2$), 2.79–2.88 (m, 1H, C*H*CO$_2$CH$_3$), 3.01–3.06 (m, 2H, CHC*H*$_2$), 3.67 (s, 3H, CO_2CH_3), 3.74 (q, *J*=6.8 Hz, 1H, C*H*CH$_3$), 6.16 (s, 1H, Ar*H*), 7.11–7.26 (m, 15H, Ar*H*); *m/z* 440 (M+1); $[\alpha]_D^{24}$ +20.0 (*c* 0.25, MeOH). Anal. Calcd for $C_{28}H_{29}N_3O_2$ (439.55): C, 76.51; H, 6.65; N, 9.56. Found: C, 76.29; H, 6.49; N, 9.77%.

4.13. (1*R*,2*S*)-3-(1,5-Diphenyl-1*H*-pyrazol-3-yl)-2-[(1-phenyl-ethylamino)-methyl]-propionic acid methyl ester (19)

This was obtained in 72% yield as thick oil by condensing phenylhydrazine with enaminone **17** according to the procedure described for compound **4**; ν_{max} (CH_2Cl_2) 1743, 1695, 1619, 1595 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 1.31 (d, J= 6.6 Hz, 3H, CHCH_3), 2.04 (br s, 1H, N*H*), 2.67 (dd, J=7.2, 5.7 Hz, 2H, NCH_2), 2.83–3.07 (m, 3H, CHCH_2, C*H*CO_2-CH_3), 3.70 (s, 3H, CO_2CH_3), 3.73 (q, J=6.9 Hz, 1H, C*H*CH_3), 6.23 (s, 1H, Ar*H*), 7.15–7.36 (m, 15H, Ar*H*); *m*/*z* 440 (M+1); $[\alpha]_D^{24}$ +29.0 (*c* 0.41, MeOH). Anal. Calcd for $C_{28}H_{29}N_3O_2$ (439.55): C, 76.51; H, 6.65; N, 9.56. Found: C, 76.31; H, 6.58; N, 9.60%.

4.14. General method of *N*-debenzylation

Method 1. A mixture of *N*-(phenyl-ethyl)-amino acid esters (1.0 g), 10% Pd–C (1.0 g), formic acid (50 mL) was refluxed with stirring for 4–12 h. After completion of reaction, reaction mixture was cooled to rt and filtered through Celite bed, washed with EtOH (2×5 mL) and the combined filtrate was concentrated under reduced pressure to afford crude amino acid esters. These crude amino acid esters were purified by treating it with ethereal-HCl or else its *N*-Boc derivative was prepared.

Purification of amino acid esters. General method for N-Boc protection. To a stirred solution of crude amino acid ester (1 equiv) in CH_2Cl_2 (20 mL) was added Boc anhydride (1.1 equiv) and Et_3N (1.1 equiv) at 0 °C. The resulting reaction mixture was stirred for 1–3 h at the same temperature. After completion of reaction, the reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed with chilled 10% $NaHCO_3$ (3×50 mL), brine (50 mL), dried (Na_2SO_4) and filtered. The filtrate was concentrated under reduced pressure to give a crude product, which was purified by column chromatography over silica gel (100–200 mesh) using 0.5% MeOH–$CHCl_3$ as eluent to afford pure *N*-Boc derivative of amino acid ester.

General method for N-Boc deprotection. To a stirred solution of pure *N*-Boc amino acid ester in MeOH (5 mL) was added 1 M MeOH–HCl (5 equiv) 0 °C and resulting reaction mixture stirred at the same temperature till TLC indicated complete disappearance of starting material (10–12 h). Solvent was removed under reduced pressure to afford solid material, which was dried under reduced pressure to afford corresponding amino acid esters as HCl salts.

Method 2. A mixture of *N*-(phenyl-ethyl)-amino acid esters (1.0 g), 10% Pd–C (0.5 g), cyclohexene (150 mL) was refluxed with stirring for 6–18 h. After completion of reaction, reaction mixture was cooled to rt and filtered through a Celite bed, washed with EtOH (2×5 mL) and the combined filtrate was concentrated under reduced pressure to afford crude amino acid esters, which was purified as described above in Method 1.

Using the above described methods were prepared:

4.14.1. (2*R*)-2-Aminomethyl-3-(5-phenyl-1*H*-pyrazol-3-yl)-propionic acid methyl ester hydrochloride (11).

Method 1. This was isolated in 98% yield as white hydrochloride salt from compound **9**, mp 183–184 °C; ν_{max} (KBr) 3420, 1750, 1623, 1593 cm^{-1}; δ_H (300 MHz, D_2O) 3.17–3.67 (m, 5H, NCH_2, C*H*CH_2), 3.75 (s, 3H, CO_2CH_3), 6.77 (s, 1H, Ar*H*), 7.48–7.60 (m, 3H, Ar*H*), 7.68–7.75 (m, 2H, Ar*H*); *m*/*z* 260 (M+1); $[\alpha]_D^{24}$ +18.3 (*c* 0.7, MeOH). Anal. Calcd for $C_{14}H_{17}N_3O_2 \cdot 2HCl$ (332.23): C, 50.61; H, 5.76; N, 12.65. Found: C, 50.63; H, 5.88; N, 12.96%.

4.14.2. (2*R*)-2-Aminomethyl-3-(1,5-diphenyl-1*H*-pyrazol-3-yl)-propionic acid methyl ester hydrochloride (12).

Method 2. This was isolated in 74% yield as off white hydrochloride salt from compound **10**, mp 59–60 °C; ν_{max} (KBr) 3473, 2944, 1740, 1618, 1603, 1492 cm^{-1}; δ_H (300 MHz, D_2O) 2.98–3.00 (d, J=4.7 Hz, 2H, NCH_2), 3.12–3.28 (m, 3H, C*H*CH_2), 3.63 (s, 3H, CO_2CH_3), 6.38 (s, 1H, Ar*H*), 7.09–7.53 (m, 10H, Ar*H*); *m*/*z* 336 (M+1); $[\alpha]_D^{24}$ +5.6 (*c* 0.7, MeOH). Anal. Calcd for $C_{20}H_{21}N_3O_2 \cdot 2HCl$ (408.32): C, 58.83; H, 5.68; N, 10.29. Found: C, 59.01; H, 5.80; N, 10.26%.

4.14.3. (2*S*)-2-Aminomethyl-3-(5-phenyl-1*H*-pyrazol-3-yl)-propionic acid methyl ester hydrochloride (20).

Method 1. This was isolated in 94% yield as off white hydrochloride salt from compound **18**, mp 199–200 °C; ν_{max} (KBr) 3427, 1738, 1623, 1595, 1585 cm^{-1}; δ_H (300 MHz, D_2O) 3.17–3.65 (m, 5H, NCH_2, C*H*CH_2), 3.77 (s, 3H, CO_2CH_3), 6.77 (s, 1H, Ar*H*), 7.53–7.55 (m, 3H, Ar*H*), 7.69–7.75 (m, 2H, Ar*H*); *m*/*z* 260 (M+1); $[\alpha]_D^{24}$ −18.0 (*c* 0.66, MeOH). Anal. Calcd for $C_{14}H_{17}N_3O_2 \cdot 2HCl$ (332.23): C, 50.61; H, 5.76; N, 12.65. Found: C, 50.59; H, 5.77; N, 12.51%.

4.14.4. (2*S*)-2-Aminomethyl-3-(1,5-diphenyl-1*H*-pyrazol-3-yl)-propionic acid methyl ester hydrochloride (21).

Method 2. This was isolated in 71% yield as white hydrochloride salt from compound **19**, mp 62–63 °C; ν_{max} (KBr) 3469, 2939, 1753, 1620, 1595, 1499 cm^{-1}; δ_H (300 MHz, D_2O) 3.22 (d, J=4.8 Hz, 2H, NCH_2), 3.33–3.48 (m, 3H, C*H*CH_2), 3.84 (s, 3H, CO_2CH_3), 6.62 (s, 1H, Ar*H*), 7.36–7.52 (m, 10H, Ar*H*); *m*/*z* 336 (M+1); $[\alpha]_D^{24}$ −5.7 (*c* 0.79, MeOH). Anal. Calcd for $C_{20}H_{21}N_3O_2 \cdot 2HCl$ (408.32): C, 58.83; H, 5.68; N, 10.29. Found: C, 58.79; H, 5.58; N, 10.20%.

4.15. General method for ester hydrolysis

A soln of amino acid ester hydrochloride (1.0 g) in 6 N HCl soln (5 mL) was refluxed under stirring for 4–6 h after cooled to rt this was washed with Et_2O (3×15 mL). The aq layer was concentrated under reduced pressure to afford corresponding amino acid hydrochloride. Using the above described methods were prepared:

4.15.1. (2*R*)-2-Aminomethyl-3-(5-phenyl-1*H*-pyrazol-3-yl)-propionic acid hydrochloride (13).

This was isolated in 92% yield as off white hydrochloride salt from compound **11**, mp 133–134 °C; ν_{max} (KBr) 3420, 1725, 1620, 1489 cm^{-1}; δ_H (300 MHz, D_2O) 3.17–3.35 (m, 5H, NCH_2, C*H*CH_2), 6.78 (s, 1H, Ar*H*), 7.53–7.55 (m, 3H,

Ar*H*), 7.71–7.72 (m, 2H, Ar*H*); δ_C (75 MHz, D_2O) 179.5, 152.9, 145.5, 136.5, 129.0, 128.5, 127.0, 104.3, 50.6, 41.4, 26.7; *m*/*z* 246 (M+1); $[\alpha]_D^{24}$ +8.2 (*c* 0.72, H_2O). Anal. Calcd for $C_{13}H_{15}N_3O_2 \cdot 2HCl$ (318.20): C, 49.07; H, 5.38; N, 13.21. Found: C, 48.87; H, 5.15; N, 13.10%.

4.15.2. (2*R*)-2-Aminomethyl-3-(1,5-diphenyl-1*H*-pyrazol-3-yl)-propionic acid hydrochloride (14). This was isolated in 93% yield as off white hydrochloride salt from compound **12**, mp 166–167 °C; ν_{max} (KBr) 3434, 3055, 2943, 1717, 1597, 1503 cm^{-1}; δ_H (300 MHz, D_2O) 3.02 (br s, 2H, NCH_2), 3.20–3.28 (m, 3H, CHCH_2), 6.38 (s, 1H, Ar*H*), 6.65–7.00 (m, 7H, Ar*H*), 7.10–7.20 (m, 3H, Ar*H*); δ_C (75 MHz, D_2O) 179.5, 152.9, 145.8, 139.7, 136.5, 129.1, 129.0, 128.5, 127.0, 126.0, 118.8, 106.9, 50.6, 41.4, 27.0; *m*/*z* 322 (M+1); $[\alpha]_D^{24}$ +1.9 (*c* 1.05, H_2O). Anal. Calcd for $C_{19}H_{19}N_3O_2 \cdot 2HCl$ (394.29): C, 57.88; H, 5.37; N, 10.66. Found: C, 58.10; H, 5.31; N, 10.39%.

4.15.3. (2*S*)-2-Aminomethyl-3-(5-phenyl-1*H*-pyrazol-3-yl)-propionic acid hydrochloride (22). This was isolated in 90% yield as off white hydrochloride salt from compound **20**, mp 145–146 °C; ν_{max} (KBr) 3417, 1726, 1620, 1468 cm^{-1}; δ_H (300 MHz, D_2O) 3.18–3.35 (m, 5H, NCH_2, CHCH_2), 6.83 (s, 1H, Ar*H*), 7.50–7.60 (m, 3H, Ar*H*), 7.69–7.74 (m, 2H, Ar*H*); *m*/*z* 246 (M+1); $[\alpha]_D^{24}$ −8.1 (*c* 0.7, H_2O). Anal. Calcd for $C_{13}H_{15}N_3O_2 \cdot 2HCl$ (318.20): C, 49.07; H, 5.38; N, 13.21. Found: C, 49.08; H, 5.21; N, 13.33%.

4.15.4. (2*S*)-2-Aminomethyl-3-(1,5-diphenyl-1*H*-pyrazol-3-yl)-propionic acid hydrochloride (23). This was isolated in 87% yield as white hydrochloride salt from compound **21**, mp 194–195 °C; ν_{max} (KBr) 3448, 3047, 2939, 1717, 1597, 1503 cm^{-1}; δ_H (300 MHz, D_2O) 3.02 (br s, 2H, NCH_2), 3.11–3.28 (m, 3H, CHCH_2), 6.45 (s, 1H, Ar*H*), 7.10–7.36 (m, 10H, Ar*H*); *m*/*z* 322 (M+1); $[\alpha]_D^{24}$ −1.8 (*c* 1, H_2O). Anal. Calcd for $C_{19}H_{19}N_3O_2 \cdot 2HCl$ (394.29): C, 57.88; H, 5.37; N, 10.66. Found: C, 57.81; H, 5.33; N, 10.59%.

Acknowledgements

We thank CSIR New Delhi, for Research Fellowship to R.K.S. and financial support for this work. We are also grateful to Analytical Chemistry Division for IR, NMR, mass spectroscopy, elemental analyses, and optical rotation analyses of compounds synthesized.

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Tetrahedron
Tetrahedron 61 (2005) 8875–8887

Lipophilic peptide nucleic acids containing a 1,3-diyne function: synthesis, characterization and production of derived polydiacetylene liposomes

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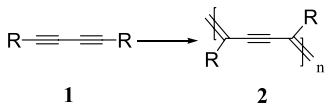
Received 15 April 2005; revised 22 June 2005; accepted 7 July 2005

Available online 28 July 2005

Abstract—Adenine-, cytosine- and thymine-containing peptide nucleic acid (PNA) monomers have been synthesized in which either diacetylenic or stearoyl moieties are attached to the *N*-or *C*-terminus; the diacetylenic group is embedded within a long hydrocarbon chain. A range of analogous lipophilic functionalized PNA oligomers have been prepared using either solid phase synthesis or a post-synthetic solution phase procedure following cleavage of the PNA oligomer from the solid support. Selected functionalized PNA monomers and oligomers have been incorporated into liposomal polydiacetylenes and characterized by UV–vis absorption spectroscopy. Preliminary investigations show that blue PDA-liposomes containing thymine-based PNAs can be formed and that production of liposomes with other PNA systems are viable.

1. Introduction

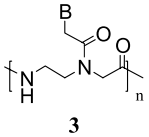
Polydiacetylenes (PDAs) [**2**, Scheme 1] can be prepared from conjugated diynes **1** by solid state polymerization through thermal treatment or by UV- and γ-irradiation.[1] Many PDAs exhibit chromic effects that can be induced by heat,[2] solvent effects[3] and pH changes;[4,5] such effects have also been observed through binding processes designed to mimic molecular recognition events at cell surfaces. For example, Charych et al. prepared a thin film PDA-assembly incorporating a sialic acid head group, which afforded a quantifiable colorimetric response on exposure to influenza virus.[6] Later, they prepared a PDA virus biosensor more efficiently through preliminary incorporation of the sialic acid-labelled diacetylene into liposomes,[7] and related assemblies have been used to detect cholera toxin.[8] More recently, selectively labelled PDA liposomes have been employed to detect the enzymic activities of phospholipases,[9] the interaction of peptides with cell membranes,[10] and antibody–epitope interactions.[11] It is notable from the work cited above[6–11] that the recognition element in these sensors can be either covalently linked to the reporter (PDA) fragment through copolymerization[7] or embedded within the lipid bilayer by physical interactions.[9]



1 **2**

Scheme 1.

We are using this type of approach to design nucleic acid biosensors in which the PDA head group or the embedded, labelled lipid possesses DNA-intercalating or related binding properties. In earlier work, we synthesized lipophilic acridine-labelled diacetylenes, thence PDAs,[12] and more recently described, in preliminary communication, the preparation and polymerization of lipophilic peptide nucleic acids (PNAs; cf. **3** Scheme 2) derived form stearic acid and pentacosa-10,12-diynoic acid.[13] A notable feature of PNAs **3**[14] is that they bind with higher affinity to single-stranded DNA (ssDNA) and RNA than their natural oligonucleotide counterparts.[15]



3

Scheme 2. B = thyminyl, cytosinyl, adeninyl, guaninyl.

Keywords: Nucleic acid biosensors; PNA; Polydiacetylenes; Liposomes.
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doi:10.1016/j.tet.2005.07.021

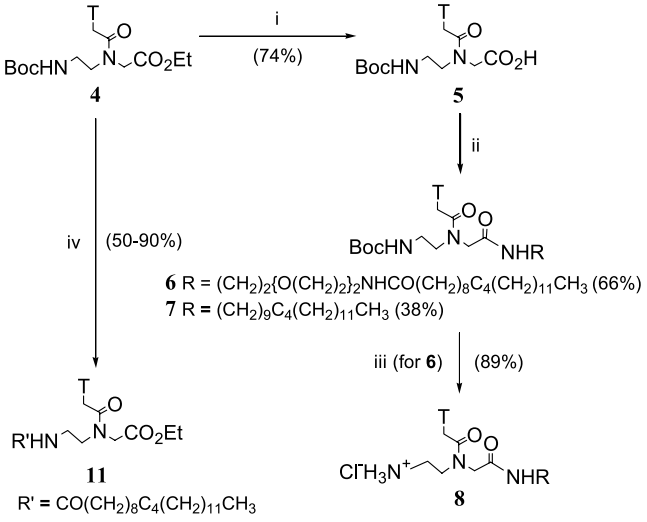
In this paper, we provide full experimental details of our earlier[13] and subsequent investigations in this area.

The objectives of this study were as follows: (1) synthesis of PNA 'monomer' model compounds with diacetylenic or stearoyl moieties at the *N*- or *C*-terminus; (2) solid-phase synthesis of PNA oligomers incorporating diacetylenic and (separately) stearoyl groups; (3) production of novel PNA-containing PDA liposomes.

2. Results and discussion

2.1. *C*-Terminal diacetylenic derivatives

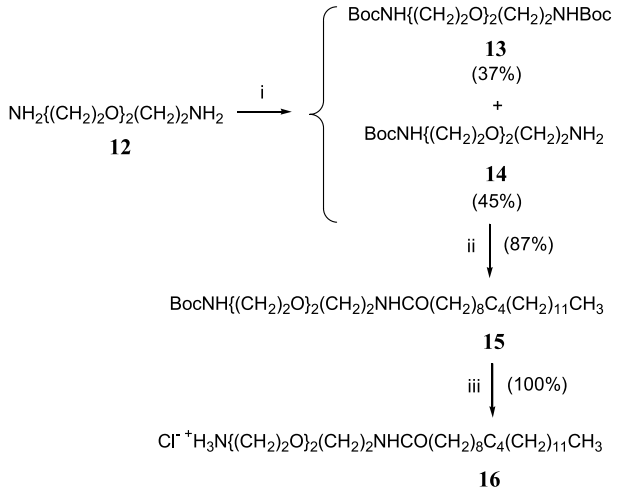
Whilst the primary objective was the synthesis of PNA oligomers attached to diacetylenic units, it was important to prepare model PNA 'monomers' to evaluate their susceptibility to topochemical polymerization. *C*-Terminal diacetylenic derivatives were prepared by two routes: the protected thymine 'monomer' **4**[16,17] was hydrolyzed[18] to afford **5** in 74% yield (Scheme 3).



Scheme 3. Boc = $(CH_3)_3COCO$; T = thyminyl. Reagents and conditions: (i) 1 M aq LiOH, THF, rt; (ii) (a) 1.1 equiv EDC, 1.1 equiv HOBt, DMF, 0 °C-rt (b) 0.94 equiv $CH_3(CH_2)_{11}C_4(CH_2)_8CONH(CH_2CH_2O)_2CH_2CH_2NH_2$[12] **9** or 0.67 equiv $CH_3(CH_2)_{11}C_4(CH_2)_9NH_2$ **10**, 1 equiv DIPEA, DMF, 0 °C-rt; (iii) 6 M aq HCl, DCM, rt; (iv) 1 equiv $CH_3(CH_2)_{11}C_4(CH_2)_8CO_2N(COCH_2)_2$,[12] DMF, rt, or 1 equiv $CH_3(CH_2)_{11}C_4(CH_2)_8COF$,[13] 2:1 DMF:DCM, rt, 3 equiv DIPEA.

Compound **5** was then coupled using *N*-hydroxybenzotriazole (HOBt) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) to (separately) *N*-(8′-amino-3′,6′-dioxaoctyl)-10,12-pentacosadiynamide **9**[12] and 1-amino-10,12-pentacosadiyne **10**; compounds **6** and **7** (Scheme 3) were isolated in 66 and 38% yields, respectively, after purification, with deprotection (6 M HCl) of the former affording the hydrochloride salt **8** in high yield.

An inefficient step in the sequence described above is the low yield (38%) obtained in converting the relatively expensive *N*-succinimidyl-10,12-pentacosadiynoate into **9**.[12] Therefore, an alternative route was also used in this work (**12**→**14**→**15**→**16**; Scheme 4), which provided a less



Scheme 4. Reagents and conditions: (i) $[(CH_3)_3COCO]_2O$, DCM, Et_2O, rt; (ii) $CH_3(CH_2)_{11}C_4(CH_2)_8CO_2N(COCH_2)_2$,[12] DCM, rt; (iii) 6 M aq HCl, DCM, rt.

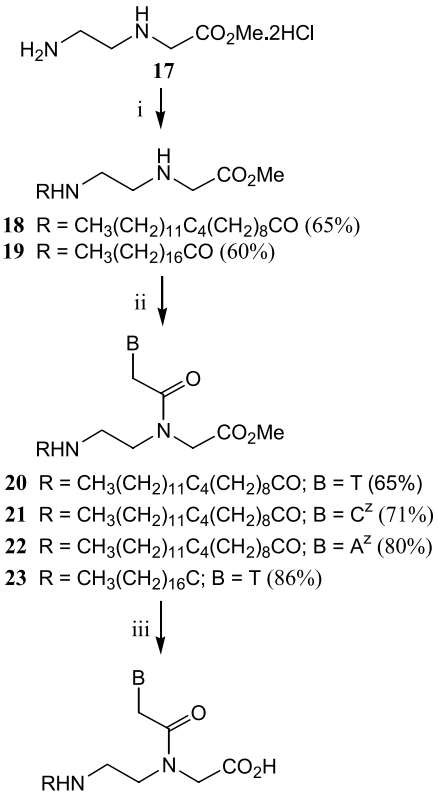
wasteful use of the diacetylenic reagent (87% yield for **14**→**15**) but an unavoidable byproduct **13** is formed in the first step (**12**→**13**+**14**).

2.2. *N*-Terminal diacetylenic and stearoyl derivatives

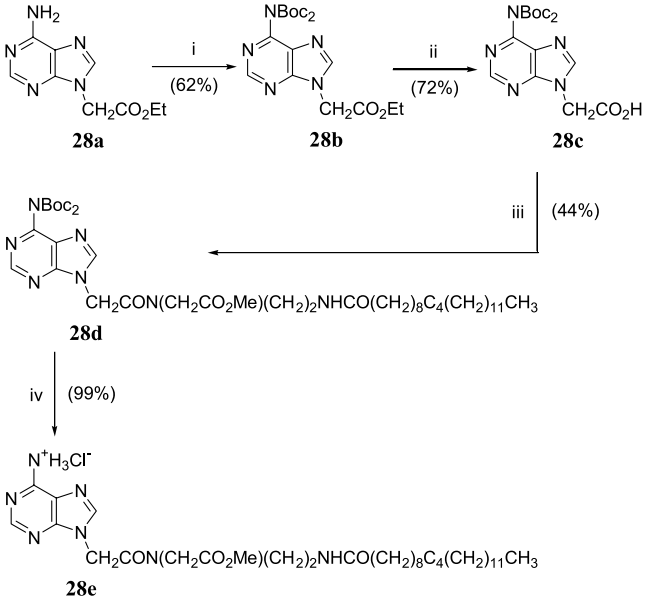
PNA model compounds in this category were prepared by two routes. The diyne **11** (Scheme 3) was prepared in 50% yield from **4**[14,15] by first removing the Boc protecting group with hydrochloric acid, followed by treatment with *N*-succinimidyl-10,12-pentacosadiynoate.[12] We subsequently achieved a higher yield in the coupling step (90%) through use of 10,12-pentacosadiynoyl fluoride.[13]

In the alternative approach, the *N*-terminal diacetylenic derivatives **24**–**26** and *N*-terminal stearoyl derivative **27** were prepared from *N*-(2-aminoethyl)glycine methyl ester dihydrochloride **17** (Scheme 5). Treatment of **17** with either *N*-succinimidyl-10,12-pentacosadiynoate[12] or *N*-succinimidyl stearoate in the presence of *N*,*N*-diisopropylethylamine (DIPEA) afforded **18** and **19**, respectively, in 65 and 60% yield after purification. Compound **18** was then coupled to thyminylacetic acid,[17] N^4-benzyloxycarbonylcytosinylacetic acid[18] and N^6-benzyloxycarbonyladeninylacetic acid using either 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluoro-phosphate (HBTU) or 2-(7-azabenzotriazol-1-yl)-1,1,3,3,-tetramethyluronium hexafluorophosphate (HATU) as the activating agent in the presence of DIPEA. After work-up and purification by flash chromatography, **20**, **21** and **22** were isolated in 63, 71 and 80% yields, respectively. Similar conditions were applied to the coupling of **19** to thyminyl acetic acid to obtain **23** (86% yield). Finally, ester hydrolysis using lithium hydroxide afforded the desired compounds **24**–**27** in yields ranging from 84–97%.

Surprisingly, attempts to deprotect compounds **25** and **26** were unsuccessful using standard procedures[19] [e.g., 6 M aq HCl or 10% aq KOH] and an alternative route[20] was used to produce a fully deprotected adenine-containing PNA-monomer (Scheme 6). Thus, ethyl adenin-9-yl acetate[18] was di-Boc protected (**28a**→**28b**) and the product was hydrolyzed (**28b**→**28c**); a standard coupling procedure



Scheme 5. $A^Z = N^6$-benzyloxycarbonyladeninyl; $C^Z = N^4$-benzyloxycarbonylcytosinyl; T = thyminyl. Reagents and conditions: (i) 1 equiv $CH_3(CH_2)_{11}C_4(CH_2)_8CO_2N(COCH_2)_2$[12] or 1 equiv $CH_3(CH_2)_{16}CO_2N(COCH_2)_2$, 3 equiv DIPEA, DMF, rt; (ii) 1.2 equiv BCH_2CO_2H,[17,18] 1.2 equiv HBTU or HATU, 3 equiv DIPEA, DMF, rt; (iii) 1 M aq LiOH, THF, rt.



Scheme 6. Reagents and conditions: (i) $[(CH_3)_3COCO]_2O$, DMAP, THF, rt; (ii) 1 M aq LiOH, THF, rt; (iii) **18**, DIPEA, HBTU, DMF, rt; (iv) 6 M aq HCl, DCM, 40 °C.

(**28c**→**28d**) afforded the diacetylenic derivative, which was hydrolyzed (6 M aq HCl) in almost quantitative yield to give the desired, deprotected PNA monomer **28e**. A characteristic feature of the *N*-terminal diacetylenic derived PNA monomers (**24–26**, **28e**) is their gradual change on exposure to light from colourless to pale blue in the solid state as a result of topochemical polymerization.

2.3. Synthesis of *N*-stearoyl and diacetylenic PNA oligomers

All PNA oligomers were assembled manually in a stepwise fashion on a suitably derivatized solid support employing the solid-phase protocol described by Christensen et al.[21] and successfully utilized previously.[22] Oligomers **29** and **31–36** (Scheme 7) were constructed on an N^1-Boc-N^5-(2-chloro-*Z*)-L-lysine-derivatized 4-methyl-benzhydrylamine (MBHA) resin whereas, for oligomers **30** and **37–40** (Scheme 7), an *N*-Boc-L-aspartic acid 4-benzyl ester MBHA-derivatized resin was used. The Boc-protected thymine monomer **5** (Scheme 3) was coupled using HBTU as the activating agent in the presence of a slight excess of DIPEA. All the couplings were monitored using the Kaiser test[23] and in all cases only single couplings were required. At the end of the syntheses, the oligomers were cleaved from the solid support using trifluoromethanesulfonic acid (TFMSA) under identical conditions to those employed by Christensen et al.[21] The crude oligomers were purified using RP-HPLC and their identities were verified by MALDI-TOF mass spectrometry.

29 $CH_3(CH_2)_{16}CO$-$(T')_{10}$-$LysNH_2$
30 $CH_3(CH_2)_{16}CO$-$(T')_{10}$-$AspNH_2$
31 Ac-$(T')_{10}$-$LysNH_2$
32 Ac-$(T')_{10}$-Lys{$CO(CH_2)_{16}CH_3$}NH_2
33 Ac-$(T')_{10}$-Lys{$CO(CH_2)_8C_4(CH_2)_{11}CH_3$}$NH_2$
34 AcAsp-$(T')_{10}$-$LysNH_2$
35 AcAsp-$(T')_{10}$-Lys{$CO(CH_2)_{16}CH_3$}NH_2
36 AcAsp-$(T')_{10}$-Lys{$CO(CH_2)_8C_4(CH_2)_{11}CH_3$}$NH_2$
37 H-$(T')_{10}$-$AspNH_2$
38 $CH_3(CH_2)_{11}C_4(CH_2)_8CO$-$(T')_{10}$-$AspNH_2$
39 H-PEG-$(T')_{10}$-$AspNH_2$
40 $CH_3(CH_2)_{11}C_4(CH_2)_8CO$-PEG-$(T')_{10}$-$AspNH_2$
41 $CH_3(CH_2)_{11}C_4(CH_2)_8CO_2H$

Scheme 7. T′ = thyminyl-PNA monomer unit; Lys and Asp = lysine and aspartic acid units, respectively, $CH_3(CH_2)_{16}CO$, Ac, $CH_3(CH_2)_{11}C_4(CH_2)_8CO$, H-PEG and $CH_3(CH_2)_{11}C_4(CH_2)_8CO$-PEG = acyl group attached either to the *N*-terminal amino group of the PNA oligomer or to the side-chain amino group of *C*-terminal lysine unit; H-PEG = H-$NH(CH_2CH_2O)_2CH_2CO$; NH_2 = *C*-terminal amide.

The stearoyl group was incorporated at the *N*-terminus of oligomers **29** and **30** (Scheme 7) in the final coupling step once the PNA T'_{10}-mer had been assembled on the solid support. Initially, for the preparation of **29**, attempts to couple stearic acid to the PNA oligomer involved using HBTU in the presence of DIPEA. However, in this case, it was found necessary to use a 1:1 (v/v) mixture of DCM:DMF as the coupling solvent for solubility reasons. This coupling step was repeated twice in an attempt to ensure complete reaction but, unfortunately, subsequent analysis by RP-HPLC of the crude product obtained, after cleavage and precipitation, showed the presence of almost

equal amounts of the acetyl-capped failure sequence **31** (Scheme 7) [R.T.=17 min] and the desired oligomer **29** [R.T.=29 min]. Therefore, alternative strategies for incorporating the stearoyl moiety were sought. Our earlier success in the use of 10,12-pentacosadiynoyl fluoride for the preparation of model *N*-terminal PNA compounds led us next to investigate the use of stearoyl fluoride in the solid phase synthesis of **29**.[13] Thus, following *N*-terminal deprotection of the PNA T'_{10}-mer, the solid-supported oligomer was treated with a solution of stearoyl fluoride and DIPEA in 1:1 (v/v) DCM:DMF. Once again, this coupling procedure was repeated twice more before the oligomer was cleaved from the support. This time subsequent analysis by RP-HPLC of the crude product revealed the desired oligomer **29** to be the principal component, with the capped failure sequence **31** accounting for $<12\%$ of the material. This strategy for addition of the stearoyl group to the *N*-terminus of a PNA-oligomer was also successfully utilized for preparation of the analogous oligomer **30**.

Preliminary investigations into the preparation of the related *N*-terminal diacetylene-functionalized PNA oligomers employing a similar approach to that described above, using 10,12-pentacosadiynoyl fluoride in the final coupling step, proved to be unsuccessful.[13] The reasons for this are as yet unclear but it appears that the diacetylene function may polymerize under the conditions required to cleave the oligomer from the solid support. Therefore, in order to overcome this limitation, a post-synthetic solution-phase modification method was developed. Thus, following construction of the appropriate PNA oligomer on the solid support, the *N*-terminus was acetyl capped and the oligomer cleaved from the resin to afford **31** and **34**, respectively (Scheme 7). The lysine amino side-chains of the oligomers **31** and **34** were subsequently acylated by treatment of a solution of the appropriate oligomer in 1:1 (v/v) DMF: pyridine with the required acyl fluoride in the presence of DIPEA to give **32**, **33**, **35** or **36**, respectively.

This post-synthetic solution-phase modification approach was also used successfully for the preparation of two *N*-terminal diacetylenic-functionalized PNA oligomers [**38** and **40** (Scheme 7)] needed for completion of our liposome studies. Thus, following construction of the parent PNA-oligomers **37** and **39** (Scheme 7), respectively, the oligomers were cleaved from the solid support and treated with 10,12-pentacosadiynoyl fluoride[13] in the presence of DIPEA. Although, in both cases, these couplings failed to go to completion, analysis of the crude products by RP-HPLC showed that both **38** and **40** had been formed in ca. 50% yield. Furthermore, since there was a large difference in retention times between the diacetylene-functionalized PNA oligomers **38** and **40** and the parent PNA oligomers **37** and **39**, respectively, it was relatively easy to separate out the desired products.

2.4. Formation of liposomes and polymerization of diacetylenes

Liposomes (or, in more general terminology, vesicles) comprise discrete aggregates of amphiphilic molecular bilayers, formed by naturally occurring phospholipids but also by self-assembly of synthetic surfactants and related molecules.[24] Amphiphilic diacetylenes with polar head-groups and extended hydrocarbon chains form liposomes in aqueous solutions with one set of head-groups exposed at the surface and the other enclosing an interior hydration sphere;[25] in many cases the ordered structure permits topochemical polymerization to produce stable PDA-liposomes. Such PDA-liposomes have been described for diacetylene lipids with, inter alia, carboxylic acid,[7,25,26] ester,[25] alcohol,[25] amine,[25] hydrazyl[27] and carbohydrate terminal groups[6,25] and vary in colour from deep blue to red, corresponding to decreasing effective π-conjugation lengths of the ene–yne backbone. Moreover, the incorporation of suitable receptor elements at the surface of such PDA-liposomes, either by covalent linking to the PDA or by physical embedding in the matrix, can produce sensors in which interactions with specific substrates induce changes in polymer conformation with concomitant colour changes.[7–11,25,26,28,29]

In this work, PDA-liposomes were prepared by a procedure similar to that described by Charych and co-workers,[9,28,29] involving sonication of an aqueous suspension of the amphiphilic molecules, filtration, refrigeration and subsequent photo-polymerization of the diacetylene arrays. As previously described,[7,25] 10,12-pentacosadiynoic acid **41** (Scheme 7) formed deep blue solutions of PDA-liposome and this compound can be employed as a matrix for liposomes of mixed composition.[9] The model, mono-thymine diacetylene **24** with an acid head-group, formed deep purple solutions of liposome under similar synthetic conditions to those applied to **41**; the related ester **20** also turned purple after irradiation but much precipitation of the product occurred leaving a very dilute solution of the PDA. The visible spectrum of PDA-liposome poly-**41** shows typical bands of the blue form, with λ_{max} ca. 630 and 580 nm (cf. Ref. 25); similar visible absorption bands were observed for the products from **24** and **20** (dilute solution), but an additional band with λ_{max} in the region 510–540 nm indicates the presence of some red form, contributing to the overall purple colours (see Table 1). Addition of 2 M aq NaOH to aqueous solutions of liposomes derived from **24**, as for **41**, caused a colour change to red and a shift in the main absorption band to ca. 530 nm (cf. Refs. 26, 30). A band at λ_{max} 271 nm (in poly-**24**) or 263 nm (in poly-**20**), not observed in poly-**41**, may be assigned to absorption by the thymine group. PDA-liposomes were subsequently

Table 1. PDA-liposomes prepared in this work

Liposome	λ_{max} (nm)
poly-**41**	632, 586, 235
poly-**24**	624, 576, 518, 271
poly-**20**	623, 536, 263, (220)[a]
copoly-**41** (95%)+copoly-**24** (5%)}	630, 582, 254
poly-**41** (95%)+poly-**20** (5%)}	631, 584, 230[b]
{poly-**41**+added **27** (5%)}	631, 587, 254
{poly-**41**+added **23** (5%)}	640, 591, 240[b]
copoly-**41** (95%)+copoly-**36** (5%)}	634, 586, 259
poly-**41** (95%)+poly-**38** (5%)}	623, 578, 240[b]
{poly-**41**+added **30** (5%)}	633, 587, 260
{poly-**41**+ added **35** (5%)}	625, 579, 363, 256, (222)[a]

Variations in λ_{max} of up to 10 nm are observed for samples from different preparations.
[a] Dilute solution.
[b] Mainly matrix, poly-**41**.

produced from two component mixtures of **41** with **24** and, separately, **20**, to investigate the ability of forming copolymeric structures: at 5% (by weight) **24**, the resulting deep blue liposome was similar in appearance to the matrix poly-**41** but exhibited a strong UV absorption at λ_{max} 254 nm, supporting the incorporation of the mono-thymine units into this product; however, with **20** (5%), the resulting blue liposome showed a UV band λ_{max} ca. 230 nm, similar to that of the matrix alone, providing no evidence of incorporation of the thymine unit. PDA-liposome formation was also carried out on mixtures of matrix material **41** with varying proportions of the non-polymerizable *N*-stearoyl thymine-monomers **27** and, separately, **23**; mixtures containing the acid **27** (5%) showed evidence, from the UV spectrum, for the inclusion of the thymine-containing component into the resulting blue liposome, but not in the blue product of similar mixtures containing **23** with an ester-head group. These results indicate that thymine-containing, carboxylic acid lipids can be successfully doped into liposomes derived from 10,12-pentacosadiynoic acid as the matrix, either through covalent bonding within the polymer chain (e.g., with **24**) or by secondary interactions with the structure (e.g., with **27**).

The formation of liposomes from two component mixtures of acid-terminated, T_{10}-oligomeric PNAs, **30**, **35**, **36** or **38** (5%) and the diacetylene matrix **41** (95%) was also investigated. Dark blue solutions of liposomes were obtained from samples containing diacetylene-PNA-containing lipids **36**, **38** or the stearoyl-PNA lipid **30**; these solutions were relatively stable, although some precipitation was observed after several days at rt; UV–vis spectra were typical of PDA-lipsomes but only samples containing **30** or **36** as the minor component showed a UV absorption at λ_{max} ca. 260 nm assignable to incorporated pyrimidine (Table 1). The mixture of **35** (5%) and **41** (95%) gave only a dilute, light blue solution of liposome and much precipitation occurred during the preparation.

These results prove that blue PDA-liposomes containing thymine-based PNAs can be prepared and indicate that production of liposomes with other PNA systems are viable. With the propensity of tailor-made PNAs to hybridize strongly with complementary ss-DNA or RNA, such PNA-containing PDA-liposomes have potential to act as biosensors for specific nucleic acids. Further studies in this area are in progress.

3. Experimental

3.1. General

All starting materials were purchased either from Avocado Research, Lancaster Research or Sigma-Aldrich Chemical companies. Anhydrous solvents were dried according to the procedures described by Casey and Leonard.[31] HPLC grade *N*,*N*-dimethylformamide (DMF) was used as supplied. Analytical TLC was carried out on aluminium plates pre-coated with silica gel 60 F_{254} (Merck). Products were visualized either by UV light or by staining the plate with a 7% phosphomolybdic acid solution in methanol (w/v) followed by heating. Column chromatography refers to the method of Still et al.[32] and was carried out using silica gel 60 (200–400 mesh). Analytical HPLC was performed on a Phenomenex Atlantis 300 Å C_{18}-reverse phase column using a Gilson HPLC system. Buffer A was 0.5% TFA in water and buffer B was 0.5% TFA in acetonitrile. Analysis and purification of oligomers was carried out by applying the following elution protocol: 0% buffer B for 10 min at a flow rate of 1.0 mL/min followed by a linear gradient from 0 to 100% buffer B over 30 min. The eluents were monitored at 260 nm. In all cases, unmodified PNA oligomers had retention times between 17 and 18 min whereas stearoyl- or diacetylene-functionalized PNA oligomers had longer retention times between 29 and 33 min. Melting points were determined using an electrothermal instrument and are uncorrected. IR spectra were recorded on Perkin-Elmer FTIR 1600 or 1430 instruments and calibrated against polystyrene. ^{1}H and $^{13}C\{^{1}H\}$ NMR spectra were recorded on Bruker AC 200 or DPX 400 spectrometers; chemical shifts (ppm) are reported with respect to $SiMe_4$ as reference (positive shifts to high frequency/low field). UV–vis spectra were recorded on a Shimadzu UV-160 spectrophotometer. Mass spectra were measured on an upgraded VG MS9 instrument; high resolution mass spectra (HRMS) were also recorded at the EPSRC National Mass Spectrometer Service Centre, University of Wales, Swansea. MALDI-TOF spectra were recorded on an Amersham Biosciences instrument using the matrix 3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid). Experimental errors of the MALDI-TOF peaks are estimated to be $\pm 0.1\%$. Elemental analyses were performed at Heriot-Watt University.

3.1.1. 1-Amino-10,12-pentacosadiyne 10. This compound was prepared by the following sequence:

10,12-Pentacosadiynoyl chloride—Thionyl chloride (1.8 g, 15 mmol) was added dropwise to 10,12-pentacosadiynoic acid (1.0 g, 26.7 mmol) in THF (4 mL). The mixture was heated under reflux for 60 min until no more HCl gas evolved. The solvent was evaporated under reduced pressure to yield 10,12-pentacosadiynoyl chloride as an orange oil, which was used without further purification. δ_H ($CDCl_3$) 0.90 (t, $J=6.2$ Hz, 3H, CH_3), 1.2–1.8 (m, 32H, CH_2), 2.25 (t, $J=6.4$ Hz, 4H, $CH_2C{\equiv}C$), 2.85 (t, 2H, $J=7.2$ Hz, CH_2COCl). δ_C ($CDCl_3$) 14.0 (CH_3), 19.1, 22.6, 24.9, 26.0, 28.1, 28.3, 28.6, 28.7, 28.8, 29.0. 29.2, 29.4, 29.5, 31.8, 47.0 (CH_2), 65.0, 65.3, 77.1 (C≡C), 173.7 (CO). ν_{max} (Nujol, cm^{-1}) 2924, 2854 (CH str), 1803 (CO).

10,12-Pentacosadiynoyl amide—10,12-Pentacosadiynoyl chloride (0.58 g, 15 mmol) was added to cold aqueous ammonia (sp. gr. 0.88, 20 mL) and the mixture was stirred for 3 h. The product was then extracted with DCM (5× 100 mL). The organic extract was dried ($MgSO_4$) and the solvent evaporated under reduced pressure to yield a yellow solid, which was purified by flash column chromatography on silica gel using CH_2Cl_2/MeOH (97:3) as eluent to yield 10,12-pentacosadiynoyl amide as a colourless solid (0.34 g, 62%), mp 100–102 °C. Found: C, 80.30; H, 11.85; N, 3.82%. $C_{25}H_{43}NO$ requires C, 80.37; H, 11.60; N, 3.75%. δ_H ($CDCl_3$) 0.85 (t, 3H, $J=6.3$ Hz, CH_3), 1.2–1.7 (m, 32H, CH_2), 2.20–2.30 (m, 6H, $CH_2C{\equiv}C$ and CH_2CO), 5.40 (br s, 2H, NH_2). δ_C ($CDCl_3$) 14.1 (CH_3), 19.1, 22.6, 25.4, 28.2,

28.3, 28.6, 28.8, 29.0, 29.3, 29.4, 29.5, 31.8, 35.8 (CH_2), 65.1, 77.4 (C≡C), 175.8 (CO). ν_{max} (Nujol, cm^{-1}) 3402 and 3188 (NH str), 2923, 2853 (CH str), 1646 (CO). HRMS (ES) *m/z* $(M+H)^+$ 374.3425 ($C_{25}H_{44}NO$ requires 374.3423).

1-Amino-10,12-pentacosadiyne—To a suspension of 10,12-pentacosadiynoyl amide (0.20 g, 0.54 mmol) in diethyl ether (20 mL) was added $LiAlH_4$ (0.20 g, 5.4 mmol) at 0 °C. The mixture was stirred for 17 h, after which time wet diethyl ether was added slowly until effervescence ceased. The mixture was filtered, the filtrate was washed with water and then dried ($MgSO_4$). The solvent was evaporated under reduced pressure to yield the title compound **10** as a colourless solid (0.13 g, 69%), which turned blue in ambient light, mp 60–62 °C. δ_H ($CDCl_3$) 0.80 (t, 3H, $J=6.4$ Hz, CH_3), 1.2–1.5 (m, 36H, CH_2), 2.18 (t, 4H, $J=6.8$ Hz, $CH_2C{\equiv}C$), 2.6 (br s, 2H, NH_2). δ_C ($CDCl_3$) 14.1 (CH_3), 19.2, 22.7, 28.3 (×2), 28.8 (×2), 29.0, 29.1, 29.3, 29.4 (×2), 29.5, 29.6 (×3), 31.9, 33.6, 42.1 (CH_2), 65.2, 65.3, 77.2, 77.5 (C≡C). ν_{max} (KBr, cm^{-1}) 3352 (NH str) 2954, 2917, 2850 (CH str), 1644, 1595, 1471. HRMS (ES) *m/z* $(M+H)^+$ 360.3626 ($C_{25}H_{46}N$ requires 360.3625).

3.1.2. *N*-[*N′*-(2-*t*-Butoxycarbonylaminoethyl)-*N′*-(thymin-1-ylacetyl)-*N′*-(2-(2-(2-methylamidoethoxy)ethoxy)ethyl)]-10,12-pentacosadiynamide 6. To *N*-(2-*t*-butoxycarbonylaminoethyl)-*N*-(thymin-1-ylacetyl)glycine **5** (0.66 g, 1.72 mmol) in DMF (50 mL) was added EDC (0.36 g, 1.88 mmol) and HOBt (0.25 g, 1.88 mmol) at 0 °C. The mixture was stirred for 1 h at 0 °C, then at rt for 30 min and finally cooled again to 0 °C. To this solution was added a cooled solution of the amide **9**[12] (0.82 g, 1.63 mmol) and DIPEA (0.3 mL, 1.88 mmol) in DMF (50 mL). The mixture was stirred for 17 h at rt, after which the solvent was evaporated under reduced pressure. The residue was dissolved in DCM (50 mL) and the solution was washed successively with saturated sodium bicarbonate solution (3×25 mL), citric acid solution (2×25 mL), water (25 mL) and finally brine (25 mL). The organic phase was dried ($MgSO_4$) and the solvent was evaporated under reduced pressure to leave a crude glassy solid, which was further purified by column chromatography on silica gel with CH_2Cl_2/MeOH (19:1) as eluent to yield the title compound **6** as a glassy solid (0.92 g, 66%). δ_H ($CDCl_3$) (two rotational isomers in a 2:1 ratio were observed) 0.90 (t, 3H, $J=6.4$ Hz, CH_2CH_3), 1.2–1.7 (m, 32H, CH_2), 1.40 (s, 9H, $(CH_3)_3$), 1.90 (s, 3H, thymine-CH_3), 2.20 (t, 2H, $J=8.0$ Hz, CH_2CH_2-CONH), 2.25 (t, 4H, $J=6.7$ Hz, $CH_2C{\equiv}C$), 3.25 (minor) and 3.35 (major) (t, 2H, $J=5.0$ Hz, CH_2), 3.45 (t, 4H, $J=4.9$ Hz, CH_2), 3.55 (m, unresolved, 2H, CH_2), 3.60 (m, unresolved, 4H, CH_2), 3.65 (m, unresolved, 4H, CH_2), 4.00 (major) and 4.10 (minor) (s, 2H, NCH_2CONH), 4.50 (minor) and 4.55 (major) (s, 2H, NCH_2CON), 5.35 (minor) and 5.90 (major) (t, 1H, BocN*H*), 6.45 (minor) and 6.60 (major) (t, 1H, NH), 6.95 (major) and 7.10 (minor) (s, 1H, thymine-H), 7.15 (major) and 7.50 (minor) (t, 1H, NH). δ_C ($CDCl_3$) (two rotational isomers in a 2:1 ratio were observed) 12.2 and 12.3 (thymine-CH_3), 14.1 (CH_2CH_3), 19.1 (×2), 22.6, 25.7, 28.3 (×2) (CH_2), 28.4 (×2) ($(CH_3)_3$), 28.8, 28.8, 28.9, 29.0, 29.2, 29.2, 29.3, 29.4, 29.6, 31.9, 36.5, 36.5, 38.7, 38.9, 39.1, 39.3, 39.4, 45.9, 48.4, 48.6, 48.9, 50.8, 65.2 and 65.3 (C≡C), 69.4, 69.6, 69.8, 69.9, 70.0 (CH_2), 77.3 and 77.4 (C≡C), 77.6 and 79.8 (C–O), 110.8 (thymine C-5), 141.2 (thymine C-6), 151.4 (CO), 156.2 (CONH), 164.4 (CO), 167.6 and 168.1 (CON), 168.2 and 168.6 (CONH), 173.6 (CONH). ν_{max} (thin film, cm^{-1}) 3320, 3066 (NH str), 2926, 2854 (CH str), 1678 (CO). HRMS (ES) *m/z* $(M+H)^+$ 871.5906 ($C_{47}H_{79}N_6O_9$ requires 871.5909).

3.1.3. (Amino-10,12-pentacosadiynoyl)-*N*-(2-*t*-butoxycarbonylaminoethyl)-*N*-(thymin-1-ylacetyl)glycinate 7. To *N*-(2-*t*-butoxycarbonylaminoethyl)-*N*-(thymin-1-ylacetyl) glycine **5** (0.057 g, 0.14 mmol) in DMF (10 mL) was added DIPEA (73 μl, 0.42 mmol) and 1-amino-10,12-pentacosadiyne (0.050 g, 0.14 mmol), followed by HBTU (0.053 g, 0.14 mmol). The mixture was stirred for 17 h at rt, after which the solvent was evaporated under reduced pressure. The residue was dissolved in DCM (25 mL) and the solution was washed successively with 1 M aq $NaHCO_3$ (3×25 mL), 1 M aq $KHSO_4$ (2×25 mL), water (25 mL) and finally brine (25 mL). The organic phase was then dried ($MgSO_4$) and the solvent evaporated under reduced pressure to yield a crude product, which was purified by flash column chromatography on silica gel using CH_2Cl_2/MeOH (97:3) as eluent to yield the title compound **7** as a glassy solid (0.047 g, 45%). δ_H ($CDCl_3$) (two rotational isomers in a 2:1 ratio were observed) 0.85 (t, 3H, $J=6.4$ Hz, CH_2CH_3), 1.2–1.5 (m, 34H, CH_2) 1.40 (s, 9H, $(CH_3)_3$), 1.90 (s, 3H, thymine-CH_3), 2.20 (t, 4H, $J=6.7$ Hz, $CH_2C{\equiv}C$), 3.1–3.5 (m, unresolved, 4H, $NHCH_2CH_2N$), 3.85 (major) and 4.18 (minor) (s, 2H, NCH_2CONH), 4.42 (minor) and 4.50 (major) (s, 2H, NCH_2CON), 5.72 (t, 1H, NH,), 6.35 (t, 1H, NH) 6.90 (major) and 7.0 (minor) (s, 1H, thymine-H), 8.90 (br s, 1H, NH). δ_C ($CDCl_3$) (two rotational isomers in a 2:1 ratio were observed) 12.3 (×2) (thymine-CH_3), 14.1 (CH_2CH_3), 19.2 (×2), 22.7, 26.8, 26.9, 28.3 (×2) (CH_2), 28.4 (×2) ($(CH_3)_3$), 28.8 (×2), 29.0, 29.1, 29.2, 29.3, 29.4, 29.5, 29.6 (×3), 29.7, 31.2, 38.8, 39.8, 40.0, 48.3, 48.7, 49.1, 51.4, 51.9 (CH_2), 65.2, 65.3, 77.2, 77.4 77.5, 77.6 (C≡C), 77.6 and 79.9 (C–O), 110.7 and 110.8 (thymine), 141.1 and 141.2 (C6), 151.1 and 151.2 (CO), 156.2 (CONH), 164.2 and 164.3 (CO), 167.6 and 167.7 (CON), 168.1 and 168.4 (CONH). ν_{max} (thin film, cm^{-1}) 3348, 3306, 3164 (NH str), 2926, 2854 (CH str), 1693, 1658 (CO). HRMS (ES) *m/z* $(M+NH_4)^+$ 743.5436 ($C_{41}H_{71}N_60_6$ requires 743.5430).

3.1.4. *N*-[*N′*-(Aminoethyl)-*N′*-(thymin-1-ylacetyl)-*N′*-(2-(2-(2-methylamidoethoxy)ethoxy)ethyl)]-10,12-pentacosadiynamide hydrochloride 8. Six molar (6 M) aq HCl (5 mL) was added to compound **6** (0.067 g, 0.077 mmol) in DCM (5 mL) and the mixture was stirred at rt for 2 h. The product was evaporated under reduced pressure to afford the title compound **8** as a glassy solid (0.055 g, 89%). δ_H (d_6-DMSO) (two rotational isomers in a 2:1 ratio were observed) 0.82 (t, 3H, $J=6.4$ Hz, CH_2CH_3), 1.2–1.5 (m, 32H, CH_2) 1.72 (s, 3H, thymine-CH_3), 2.05 (t, 2H, $J=7.3$ Hz, CH_2CH_2CONH), 2.25 (t, 4H, $J=6.4$ Hz, $CH_2C{\equiv}C$), 3.2–3.7 (m unresolved, 16H, CH_2), 3.95 and 4.10 (s, 2H, NCH_2-CONH), 4.45 and 4.75 (s, 2H, NCH_2CON), 7.42 (s, 1H, thymine-H), 7.80 (t, 1H, CH_2CH_2CONH), 7.98 and 8.25 (br s, 3H, NH_3), 8.35 and 8.48 (t, 1H, NCH_2CONH), 11.30 and 11.32 (s, 1H, thymine-NH). δ_C (DMSO) (two rotational isomers in a 2:1 ratio were observed) 11.9 (×2) (thymine-CH_3), 13.9 (CH_2CH_3), 18.2, 18.3, 22.1, 25.2, 27.7 (×2), 28.1, 28.2, 28.4, 28.6 (×2), 28.7, 28.8, 28.9, 29.0, 31.3,

35.3, 36.8, 36.9, 38.4, 38.8, 40.8, 45.1, 45.3, 47.7, 48.0, 49.2, 49.9 (CH_2), 65.3 (C≡C), 68.8, 69.1, 69.5, 69.6 (CH_2), 77.9 (×2) (C≡C), 108.0 (×2) (C5), 142.1 (×2) (C6), 151.0 and 151.1 (CO), 164.3 and 164.4 (CO), 167.4 and 168.3 (CON), 168.6, 169.5, 172.2 (CONH). ν_{max} (thin film, cm^{-1}) 3305 (NH str), 2920, 2850 (CH str), 1672, 1554, 1468. HRMS (ES) *m/z* $(M-Cl)^+$, 771.5378 ($C_{42}H_{71}N_6O_7$ requires 771.5379).

3.1.5. 2-(2-(2-*t*-Butoxycarbonylaminoethoxy)ethoxy)-ethylamine 14 and bis-1,2-(2-*t*-butoxycarbonylamino-ethoxy)ethane 13. To a stirred solution of 1,8-diamino-3,6-dioxaoctane (5.0 g, 33.2 mmol) in DCM (40 mL) was added a solution of di-*t*-butyl dicarbonate (3.6 g, 16.9 mmol) in diethyl ether (30 mL) over 30 min. Stirring was continued for an additional 2 h, after which the solvent was evaporated under reduced pressure to yield a crude yellow oil. This oil was subjected to separation by flash column chromatography on silica gel using DCM/MeOH (19:1–17:3) as eluent to yield the two title compounds **14** and **13** as clear oils. (These compounds were not purified to analytical standard).

Compound **14** (1.89 g, 45%, R_f=0.12 [DCM/MeOH 95:5]). δ_H ($CDCl_3$) 1.40 (s, 9H, CH_3), 1.60 (s, 2H, NH_2), 2.83 (t, 2H, J=5.2 Hz, C$H_2$$NH_2$), 3.25 (apparent q, 2H, J=5.2 Hz, BocNHC$H_2$$CH_2$), 3.49 (m, unresolved, 4H, CH_2), 3.58 (s, 4H, OCH_2CH_2O), 5.15 (br s, 1H, BocN*H*). δ_C ($CDCl_3$) 28.3 (CH_3), 40.2 (CH_2), 41.6 (CH_2), 70.1 (CH_2), 73.2 (CH_2), 79.0 (C–O), 155.9 (CO).

Compound **13** (1.07 g, 37%, R_f=0.23 [DCM/MeOH 95:5]). δ_H ($CDCl_3$) 1.42 (s, 18H, CH_3), 3.30 (apparent q, 4H, J=5.0 Hz, BocNHCH_2), 3.51 (t, 4H, J=5.3 Hz, BocNHCH_2-CH_2), 3.54 (s, 4H, OCH_2CH_2O), 5.03 (br s, 2H, BocN*H*). δ_C ($CDCl_3$) 28.3 (CH_3), 40.3 (CH_2), 70.2 (CH_2), (CH_2), 79.2 (C–O), 155.9 (C=O).

3.1.6. *N*-(2-(2-(2-*t*-Butoxycarbonylaminoethoxy)ethoxy) ethyl)-10,12-pentacosadiynamide 15. A solution of 2-(2-(2-*t*-butoxycarbonylaminoethoxy)ethoxy)ethylamine **14** (0.67 g, 2.70 mmol) and *N*-succinimidyl-10,12-pentacosadiynoate[12] (0.10 g, 0.21 mmol) in DCM (50 mL) was stirred at rt for 17 h. The mixture was then washed with water (50 mL) and the solvent was evaporated from the organic fraction to yield a solid, which was subjected to flash column chromatography on silica gel using CH_2Cl_2/MeOH (19:1) as eluent. The title compound **15** (1.10 g, 87%) was isolated as a colourless solid, mp 52–54 °C, which turned blue in ambient light. δ_H ($CDCl_3$) 0.80 (t, 3H, J=6.2 Hz, CH_2CH_3), 1.20–1.60 (m, 32H, CH_2), 1.40 (s, 9H, *t*-Bu), 2.10 (t, 2H, J=7.9 Hz, CH_2CO), 2.15 (t, 4H, J=6.7 Hz, CH_2C≡C), 3.25 (apparent q, 2H, J=5.0 Hz, BocNHCH_2-CH_2), 3.40 (m, 2H, CH_2), 3.50 (t, 4H, J=5.3 Hz, CH_2), 3.60 (s, 4H, OCH_2), 5.0 (br s, 1H, NH), 6.0 (br s, 1H, NH). δ_C ($CDCl_3$) 14.0 (CH_2CH_3), 19.1, 22.6, 25.6 (CH_2), 28.3 ($C(CH_3)_3$), 28.7, 28.8, 28.9, 29.0, 29.1, 29.4, 29.5, 31.8, 36.6, 39.0, 40.2 (CH_2), 65.1 (×2) (C≡C and CH_2), 70.1 (×2) (CH_2), 77.5 (C≡C), 79.3 (C–O), 155.9 (Boc*C*ONH), 173.1 (*C*ONHCH$_2$). ν_{max} (KBr, cm^{-1}) 3352, 3310, 3082 (NH str), 2920, 2848 (CH str), 1685 (CO), 1644 (CO), 1553, 1540, 1466. HRMS (ES) *m/z* $(M+H)^+$605.4896 ($C_{36}H_{65}N_20_5$ requires 605.4893).

3.1.7. *N*-(2-(2-(2-Aminoethoxy)ethoxy)ethyl)-10,12-pentacosadiynamide hydrochloride 16. *N*-(2-(2-(2-*t*-Butoxycarbonylaminoethoxy)ethoxy)ethyl)-10,12-pentacosadiynamide **15** (0.16 g, 0.26 mmol) was dissolved in DCM (2 mL) and 6 M aq HCl (2 mL) was added. The mixture was stirred for 30 min and then the solvents were evaporated under reduced pressure to yield the title compound **16** (0.14 g) in quantitative yield. This compound was used in situ without further purification. δ_H (d_6-DMSO) 0.83 (t, 3H, J=6.0 Hz, CH_3), 1.2–1.7 (m, 32H, CH_2), 2.15 (t, 2H, J=7.2 Hz, CH_2CO), 2.25 (t, 4H, J=6.3 Hz, CH_2C≡C), 2.95 (q, 2H, J=5.4 Hz, CH_2), 3.20 (q, 2H, J=5.6 Hz, CH_2), 3.40 (t, 2H, J=5.4 Hz, CH_2), 3.55 (s, 4H, CH_2O), 3.60 (t, 2H, J=5.2 Hz, CH_2), 7.90 (t, 1H, NH), 8.05 (br s, NH).

3.1.8. (Aminoethyl)-*N*-(thymin-1-ylacetyl)glycine ethyl ester hydrochloride. *N*-(2-*t*-Butoxycarbonylaminoethyl)-*N*-(thymin-1-ylacetyl)-glycine ethyl ester **4**[14,15] (0.55 g, 1.33 mmol) was dissolved in ethyl acetate (25 mL) and 6 M aq HCl (25 mL) was added. The mixture was stirred for 30 min after which time the solvent was evaporated under reduced pressure to yield the title compound as a glassy solid. This was then used in situ without further purification.

3.1.9. *N*-(2-Pentacosa-10,12-diynoylaminoethyl)-*N*-(thymin-1-ylacetyl)glycine ethyl ester 11. *Method A*. To a solution of *N*-succinimdyl-10,12-pentacosadiynoate[12] (0.63 g, 1.34 mmol) in DMF (10 mL) was added (aminoethyl)-*N*-(thymin-1-ylacetyl)glycine ethyl ester hydrochloride (0.45 g, 1.30 mmol) and DIPEA (0.70 mL, 3.9 mmol) in DMF (10 mL) at 0 °C. The mixture was stirred for 17 h at rt, then the solvent was evaporated under reduced pressure. The resulting residue was purified by flash column chromatography on silica gel with DCM/MeOH (19:1) as eluent to yield the title compound **11** as a white foam (0.40 g, 50%).

Method B. To a solution of (aminoethyl)-*N*-(thymin-1-ylacetyl)glycine ethyl ester hydrochloride (0.064 g, 0.16 mmol) in DMF (10 mL) was added DIPEA (85 µl, 0.49 mmol) followed by a solution of 10,12-pentacosadiynoyl fluoride[13] (0.061 g, 0.16 mmol) in DCM (5 mL). The mixture was stirred at rt for 17 h, then worked up as above (Method A) to yield the title compound **11** as a foam (0.096 g, 90%). δ_H ($CDCl_3$) (two rotational isomers in a 2:1 ratio were observed) 0.88 (t, 3H, J=6.8 Hz, $CH_2CH_2CH_3$), 1.2–1.7 (m, 35H, 16×CH_2 and 1×$CO_2CH_2CH_3$), 1.90 (s, 3H, thymine-CH_3), 2.15 and 2.30 (t, 2H, J=7.3 Hz, CH_2CONH), 2.22 (t, 4H, J=7.2 Hz, CH_2C≡C), 3.40 (minor) and 3.44 (major) (apparent q, 2H, J=5.6 Hz, NHC$H_2$$CH_2$) 3.55 (t, 2H, J=5.7 Hz, $NHCH_2CH_2$), 4.05 and 4.15 (s, 2H, CH_2CONH), 4.21 (major) and 4.28 (minor) (q, 2H, J=7.2 Hz, C$H_2$$CH_3$), 4.41 (minor) and 4.55 (major) (s, 2H, CH_2CON), 6.45 (minor) and 6.90 (major) (t, 1H, N*H*), 6.95 (major) and 7.05 (minor) (s, 1H, thymine-H), 9.38 (major) and 9.42 (minor) (s, 1H, thymine-NH). δ_C ($CDCl_3$) (two rotational isomers in a 2:1 ratio were observed) 12.3 (thymine-CH_3), 14.1 (×2) (CH_2CH_3), 18.4, 19.2, 19.2, 22.7, 25.5, 25.5, 25.6, 28.3, 28.4, 28.4, 28.7, 28.8, 28.4, 28.9, 28.9, 29.0, 29.1, 29.2, 29.2, 29.3, 29.3, 29.3, 29.3, 49.5, 29.6, 29.6, 29.7, 29.7, 29.7, 32.0, 36.3, 36.4, 37.6, 37.7, 48.1, 48.3, 48.4, 48.4, 48.9, 50.2, 61.8, 62.4 (CH_2), 65.2, 65.3, 77.2, 77.5, 77.6, 77.7 (C≡C), 110.8 and 110.9

(thymine-C5), 141.0 and 141.2 (thymine-C6), 151.3 (CO), 164.3 and 164.3 (CO), 167.4 and 168.2 (CON), 169.4 and 169.6 (CONH), 174.1 and 174.5 (CO_2Et). ν_{max} (thin film, cm^{-1}) 3319 (NH str), 2924, 2852 (CH str), 1738, 1709, 1689, 1666, 1650, 1548, 1468. HRMS (ES) *m/z* $(M+NH_4)^+$ 686.4862 ($C_{38}H_{64}N_5O_6$ requires 686.4857).

3.1.10. *N*-(2-Pentacosa-10,12-diynoylaminoethyl)glycine methyl ester 18. To *N*-succinimdyl-10,12-pentacosadiynoate (1.28 g, 2.71 mmol) in DMF was added *N*-(2-aminoethyl)-glycine methyl ester dihydrochloride (0.57 g, 2.77 mmol) and DIPEA (1.43 mL, 10.3 mmol) in DMF. The mixture was stirred at rt for 17 h. The solvent was evaporated under reduced pressure and the pink-white residual solid was partitioned with 1 M aq $KHSO_4$ (150 mL) and ethyl acetate (150 mL). Subsequently, the pH of the aqueous phase was adjusted to 8.5 using solid $NaHCO_3$ and a saturated aqueous solution of $NaHCO_3$. The mixture was then extracted with DCM (3×150 mL), the combined organic fractions were dried ($MgSO_4$) and the solvent evaporated under reduced pressure to afford the title compound **18** (0.76 g, 65%) as a colourless solid, mp 59–63 °C, which turned blue in ambient light. δ_H ($CDCl_3$) 0.88 (t, 3H, J=6.5 Hz, CH_2CH_3), 1.2–1.7 (m, 32H, CH_2), 2.15 (t, 2H, J=7.5 Hz, CH_2CH_2CO), 2.23 (t, 4H, J=6.8 Hz, $CH_2C{\equiv}C$), 2.75 (t, 2H, J=5.7 Hz, $CH_2CH_2NHCH_2$), 3.35 (q, 2H, J=5.6 Hz, $CONHCH_2$), 3.42 (s, 2H, $NHCH_2CO$), 3.72 (s, 3H, OCH_3), 6.20 (br s, 1H, NH). [1×NH not observed] δ_C ($CDCl_3$) 14.0 (CH_2CH_3), 19.1, 22.6, 25.0, 25.6, 28.3, 28.8, 28.8, 29.0, 29.1, 29.2, 29.4, 29.5, 31.8, 26.6, 38.7, 48.3, 49.8 (CH_2), 51.9 (OCH_3), 64.1, 77.4 (C≡C), 172.7, 173.4 (CO). ν_{max} (KBr, cm^{-1}) 3296, 3089 (NH str), 2920, 2048 (CH str), 1742, 1640 (CO str), 1558, 1465. HRMS (ES) *m/z* $(M+H)^+$ 489.4049 ($C_{30}H_{53}N_2O_3$ requires 489.4056).

3.1.11. *N*-Succinimidyl octadecanoate. *N*-Hydroxysuccinimide (0.76 g, 6.56 mmol), followed by EDC (1.25 g, 6.56 mmol) were added to a solution of stearic acid (1.87 g, 6.56 mmol) in DCM (50 mL) and the mixture was stirred at rt for 21 h. The solvent was evaporated under reduced pressure. The residue was dissolved in DCM (100 mL) and the solution washed with water (100 mL); the aqueous phase was then back-extracted with DCM (2× 100 mL). The combined organic phase was dried ($MgSO_4$) and the solvent evaporated under reduced pressure to yield the title compound as a colourless solid (2.45 g, 98%). This compound was characterized by NMR spectroscopy but was not purified to analytical standard. It was used without further purification. δ_H ($CDCl_3$) 0.87 (t, 3H, J=6.4 Hz, CH_3), 1.10–1.80 (m, 30H, CH_2), 2.33 (t, 2H, J=7.5 Hz, CH_2COO), 2.82 (s, 4H, $COCH_2CH_2CO$). δ_C ($CDCl_3$) 14.0 (CH_3), 22.6, 24.5, 25.5, 28.7, 28.9, 29.3, 29.3, 30.8, 31.8, 33.7 (CH_2), 168.6, 169.1 (CO).

3.1.12. *N*-(-2-Octadecanoylaminoethyl)glycine methyl ester 19. To a solution of *N*-succinimidyl octadecanoate (2.65 g, 6.95 mmol) in DCM (100 mL) was added a solution of *N*-(2-aminoethyl)glycine methyl ester dihydrochloride (1.42 g, 6.95 mmol) and DIPEA (2.9 mL, 20.85 mmol). The mixture was stirred at rt for 20 h then the solvent was evaporated under reduced pressure. The residue was partitioned between ethyl acetate (100 mL) and 1 M aq $KHSO_4$ (100 mL). The aqueous phase was separated and adjusted to pH 8.5 with solid $NaHCO_3$. The aqueous phase was then extracted with DCM (3×100 mL), the combined organic extract was dried ($MgSO_4$) and the solvent was evaporated under reduced pressure. The crude residue was then purified by flash column chromatography [silica gel, CH_2Cl_2/MeOH 19:1 as eluent] to yield the title compound **19** as a colourless solid (1.66 g, 60%). Found: C, 69.09; H, 11.81; N, 6.99%. $C_{23}H_{46}N_2O_3$ requires C, 69.30; H, 11.63; N, 7.03%. δ_H ($CDCl_3$) 0.86 (t, 3H, J=6.4 Hz, CH_2CH_3), 1.18–1.70 (m, 30H, CH_2), 2.17 (t, 2H, J=7.5 Hz, CH_2-CONH), 2.75 (t, 2H, J=5.8 Hz, CH_2NHCH_2), 3.35 (apparent q, 2H, J=5.5 Hz, $CONHCH_2$), 3.40 (s, 2H, CH_2CO_2Me), 2.72 (s, 3H, OCH_3), 6.10 (br s, 1H, NH). [1×NH not observed] δ_C ($CDCl_3$) 14.0 (CH_3), 22.6, 25.7, 29.3, 29.4, 29.6, 30.8, 31.8, 36.7, 388, 48.3, 50.1 (CH_2), 51.8 (CH_3O), 172.9, 173.3 (CO). HRMS (ES) *m/z* $(M+H)^+$ 399.3585 ($C_{23}H_{47}N_2O_3$ requires 399.3586).

3.1.13. *N*-(2-Pentacosa-10,12-diynoylaminoethyl)-*N*-(thymin-1-ylacetyl)glycine methyl ester 20. To a solution of *N*-(2-pentacosa-10,12-diynoylaminoethyl)glycine methyl ester **18** (1.00 g, 2.05 mmol) and triethylamine (0.84 mL, 6.03 mmol) in DMF (25 mL) was added thymin-1-yl acetic acid (0.38 g, 2.06 mmol) followed by HATU (0.78 g, 2.05 mmol). The mixture was stirred at rt for 17 h then the solvent was evaporated under reduced pressure. The residue was dissolved in DCM (25 mL) and the solution was washed successively with 1 M aq $NaHCO_3$, 1 M aq $KHSO_4$, water, brine and finally dried ($MgSO_4$). The solvent was evaporated under reduced pressure to leave a white foam, which was purified by flash column chromatography [silica gel, CH_2Cl_2/MeOH 17:3 as eluent] to yield the title compound **20** (0.80 g, 63%) as a white foam, mp 59–63 °C, which turned purple in ambient light. Found: C, 67.92; H, 9.07; N, 8.42%. $C_{37}H_{58}N_4O_6$ requires C, 67.86; H, 8.93; N, 8.56%. δ_H ($CDCl_3$) (two rotational isomers in a 2:1 ratio were observed) 0.88 (t, 3H, J=6.6 Hz, CH_2CH_3), 1.20–1.65 (m, 32H, CH_2), 1.88 (major) and 2.00 (minor) (s, 3H, thymine-CH_3), 2.15 (minor) and 2.25 (major) (t, 2H, J=7.5 Hz, CH_2CONH), 2.22 (t, 4H, J=6.0 Hz, $CH_2C{\equiv}C$), 3.40 (minor) and 3.45 (major) (m, unresolved, 2H, $NHCH_2CH_2$), 3.45 (minor) and 3.55 (major) (m, unresolved, 2H, $NHCH_2$-CH_2), 3.75 (major) and 3.80 (minor) (s, 3H, OCH_3), 4.05 (major) and 4.20 (minor) (s, 2H, CH_2CO_2Me), 4.35 (minor) and 4.50 (major) (s, 2H, CH_2CON), 6.15 (minor) and 6.77 (major) (t, 1H, J=5.8 Hz, CONH), 6.95 (major) and 6.99 (minor) (s, 1H, thymine-H). [1×NH not observed] δ_C ($CDCl_3$) (two rotational isomers in a 2:1 ratio were observed) 12.3 (thymine-CH_3), 14.1 (CH_2CH_3), 19.2, 19.2, 22.7, 25.5, 28.3, 28.3, 28.8, 28.8, 28.9, 29.1, 29.2, 29.2, 29.3, 29.3, 29.4, 29.6, 29.6, 29.6, 31.9, 36.3, 36.5, 37.5, 37.6, 47.9, 48.2, 48.3, 48.4, 48.7, 50.0 (CH_2), 52.5 and 52.9 (OCH_3), 65.2, 65.3, 77.2, 77.4, 77.6, 77.6 (C≡C), 110.8 and 110.9 (thymine-C5), 140.8 and 141.0 (thymine-C6), 151.1 (CO), 164.0 and 164.0 (CO), 167.4 and 168.1 (CON), 169.7 and 170.0 (CONH), 173.9 and 174.4 (CO_2Me). ν_{max} (thin film, cm^{-1}) 3321, 3191, 3060 (NH str), 2926, 2854 (CH str), 1746, 1672, 1548, 1530, 1467 (CO). HRMS (ES) *m/z* $(M+H)^+$ 655.4435 ($C_{37}H_{59}N_4O_6$ requires 655.4435).

3.1.14. *N*-(2-Pentacosa-10,12-diynoylaminoethyl)-*N*-(N^4-(benzyloxycarbonyl)cytosine-1-ylacetyl)glycine methyl ester 21. (N^4-(Benzyloxycarbonyl)cytosin-1-yl)acetic

acid[18] (0.061 g, 0.20 mmol) and triethylamine (0.061 g, 0.60 mmol) were added to a solution of *N*-(2-pentacosa-10,12-diynoylaminoethyl)glycine methyl ester **18** (0.10 g, 0.20 mmol) in DMF (10 mL) followed by HATU (0.081 g, 0.22 mmol). The solution was stirred for 17 h at rt, then the solvent was evaporated under reduced pressure. The residue was dissolved in DCM (20 mL) and the solution was washed successively with 1 M aq $NaHCO_3$ (2×20 mL), 1 M aq $KHSO_4$ (2×20 mL), water (10 mL) and finally brine (10 mL). The organic solution was dried ($MgSO_4$) and the solvent was evaporated under reduced pressure to yield the title compound **21** (0.11 g, 71%) as a colourless solid, mp 110 °C. δ_H ($CDCl_3$) (two rotational isomers in a 2:1 ratio were observed) 0.85 (t, 3H, $J=6.3$ Hz, CH_2CH_3), 1.2–1.45 (m, 32H, CH_2), 2.10 (minor) and 2.25 (major) (t, 2H, $J=7.5$ Hz, CH_2CONH), 2.25 (t, 4H, $J=7.3$ Hz, $CH_2C{\equiv}C$), 3.30–3.60 (m unresolved, 4H, $NHCH_2CH_2N$), 3.70 (major) and 3.75 (minor) (s, 3H, OCH_3), 4.05 (major) and 4.35 (minor) (s, 2H, CH_2CO_2Me), 4.50 (minor) and 4.65 (major) (s, 2H, CH_2CON), 5.2O (s, 2H, CH_2OCO), 6.15 (minor) and 6.90 (major) (t, 1H, N*H*), 7.20–7.70 (m unresolved, 7H, 5× Ar-*H*, 2×cytosinyl-*H*). [1×NH not observed] δ_C ($CDCl_3$) (two rotational isomers in a 2:1 ratio were observed) 14.1 (CH_2CH_3), 19.2 (×2), 22.7, 28.3, 28.4, 28.8, 28.9 (×2), 29.1, 29.2, 29.3, 29.5, 29.6 (×3), 31.9, 36.3, 31.2, 28.7, 41.1, 48.2, 48.5, 49.9 (CH_2), 52.5 and 52.9 (OCH_3), 65.2 and 65.3 (C≡C), 67.9, 68.1, 68.2 (CH_2), 77.2, 77.5, 77.6, 77.7 (C≡C), 95.1 (cytosinyl-C5), 128.3, 128.4, 128.7, 128.8, 130.9, 132.5, 134.9, 149.7, 152.1 (NHCOO), 155.7 (cytosinyl-C6), 162.9 (cytosinyl-CO), 167.0 and 167.7 (CON), 169.8 (CONH), 173.8 and 174.3 (CO_2Me). HRMS (ES) $(M+H)^+$774.4808 ($C_{44}H_{63}N_5O_7$ requires 774.4805).

3.1.15. *N*-(2-Pentacosa-10,12-diynoylaminoethyl)-*N*-(N^6-(benzyloxycarbonyl)adenin-9-ylacetyl)glycine methyl ester 22. To a solution of *N*-(2-pentacosa-10,12-diynoylaminoethyl)glycine methyl ester **18** (0.83 g, 1.71 mmol) in DMF (25 mL) and triethylamine (0.71 mL, 5.12 mmol) was added (N^6-(benzyloxycarbonyl)adenin-9-yl)acetic acid[18] (0.56 g, 1.71 mmol) followed by HATU (0.71 g, 1.88 mmol). The mixture was stirred at rt for 17 h, then the solvent was evaporated under reduced pressure. The residue was dissolved in DCM (25 mL) and the solution was washed successively with 1 M aq $NaHCO_3$, 1 M aq $KHSO_4$, water and brine The solution was dried ($MgSO_4$) and the solvent was evaporated under reduced pressure to leave a white foam, which was purified by flash column chromatography [silica gel, CH_2Cl_2/MeOH 97:3 as eluent] to yield the title compound **22** as a white foam (1.10 g, 80%). δ_H ($CDCl_3$) (two rotational isomers in a 2:1 ratio were observed) 0.85 (t, 3H, $J=6.4$ Hz, CH_2CH_3), 1.20–1.70 (m, 32H, CH_2), 2.00 (minor) and 2.26 (major) (t, 2H, $J=7.5$ Hz, CH_2CONH), 2.16 (t, 4H, $J=7.1$ Hz, $CH_2C{\equiv}C$), 3.40 (minor) and 3.50 (major) (apparent q, 2H, $J=6.0$ Hz, $NHCH_2$), 3.55 (minor) and 3.65 (major) (t, 2H, $J=5.6$ Hz, CH_2CH_2N), 3.68 (major) and 3.76 (minor) (s, 3H, OCH_3), 3.99 (major) and 4.23 (minor) (s, 2H, CH_2CO_2Me), 4.88 (minor) and 5.20 (major) (s, 2H, CH_2CON), 5.24 (s, 2H, CH_2–O), 5.90 (minor) and 6.67 (major) (t, 1H, $J=6.1$ Hz, $NHCH_2$), 7.25–7.40 (m, 5H, Ar-*H*), 7.90 (major) and 7.98 (minor) (s, 1H, adenine C-8–H), 8.55 (br s, 1H, CbzN*H*), 8.69 (major) and 8.71 (minor) (s, 1H, adenine-C2–H). δ_C ($CDCl_3$) (two rotational isomers in a 2:1 ratio were observed) 14.1 (CH_2CH_3), 19.1, 19.2, 22.6, 25.4, 25.4, 28.3, 28.3, 28.7, 28.8, 28.9, 29.1, 29.1, 29.2, 29.2, 29.3, 29.4, 29.6, 29.6, 29.6, 29.7, 31.9, 26.5, 36.5, 36.6, 37.4, 37.5, 43.7, 43.9, 48.3, 48.7, 48.9, 50.0 (CH_2), 52.6 and 53.1 (OCH_3) 65.2 and 65.3 (C≡C), 67.7 and 67.8 (CH_2), 77.2, 77.4, 77.6 (C≡C), 121.3 (adenine-C5), 128.5, 128.5, 128.6 (CH), 135.4, 135.4, 143.8 (adenine-C6), 149.3 (NHCOO), 151.5, 152.7, 152.8, 166.4, 167.2 (CON), 169.5 and 170.2 (CONH), 174.2 (CO_2Me). HRMS (ES) $(M+H)^+$798.4911 ($C_{45}H_{64}N_7O_6$ requires 798.4918).

3.1.16. *N*-(2-Octadecanoylaminoethyl)-*N*-(thymin-1-ylacetyl)glycine methyl ester 23. Thymine-1-acetic acid (0.36 g, 1.95 mmol) was dissolved in DMF (10 mL) and HBTU (0.6 g, 1.57 mmol) was added. The mixture was stirred for 15 min and then added to a solution of *N*-(-2-octadecanoylaminoethyl)glycine methyl ester **19** (0.63 g, 1.57 mmol) and DIPEA (2.47 mL, 17.8 mmol) in DMF (10 mL). The mixture was stirred for 19 h, then the solvent was evaporated under reduced pressure. The residue was dissolved in DCM and the solution was washed successively with 1 M aq $NaHCO_3$, 1 M aq $KHSO_4$, water and brine and was finally dried ($MgSO_4$). The solvent was evaporated under reduced pressure to leave an orange oil, which was purified by flash column chromatography [silica gel, CH_2Cl_2/methanol 17:3 as eluent] to yield the title compound **23** (0.76 g, 86%) as a colourless solid, mp 123–127 °C. Found: C, 63.4; H, 9.4; N, 9.8%. $C_{30}H_{52}O_6N_4$ requires C, 63.8; H 9.2; N, 9.9%. δ_H ($CDCl_3$) (two rotational isomers in a 2:1 ratio were observed) 0.76 (t, 3H, $J=6.4$ Hz, CH_2CH_3), 1.10–1.60 (m, 30H, CH_2), 1.88 (s, 3H, thymine-CH_3), 2.10 (minor) and 2.20 (major) (t, 2H, $J=7.5$ Hz, CH_2CONH), 3.40 (minor) and 3.48 (major) (q, 2H, $J=5.8$ Hz, $NHCH_2$), 3.55 (t, 2H, $J=4.9$ Hz, CH_2CH_2N), 3.74 (major) and 3.80 (minor) (s, 3H, OCH_3), 4.05 (major) and 4.21 (minor) (s, 2H, CH_2CO_2Me), 4.38 (minor) and 4.55 (major) (s, 2H, CH_2CON), 6.10 (minor) and 6.70 (major) (t, 1H, CON*H*), 6.95 (major) and 7.03 (minor) (s, 1H, thyminyl C-6–*H*), 8.84 (minor) and 9.05 (major) (s, 1H, thyminyl-N3–*H*). δ_C ($CDCl_3$) (two rotational isomers in a 2:1 ratio were observed) 12.3 (thyminyl-CH_3), 14.1 (CH_2CH_3), 22.7, 25.6, 29.3, 29.4, 29.5, 29.6, 29.7, 30.8, 31.9, 36.5, 36.6, 37.5, 37.6, 47.9, 48.3, 48.5, 48.8, 50.0 (CH_2), 52.6 and 53.0 (OCH_3), 110.8 and 110.9 (thyminyl-C5), 140.8 (thyminyl-C6), 150.9 (CO), 163.9 (CO), 167.3 and 168.1 (CON), 170.0 and 170.1 (CO_2Me), 173.9 and 174.4 (CONH). ν_{max} (KBr, cm^{-1}) 3293, 3190, 3043 (NH str), 2919, 2850 (CH str), 1741, 1682, 1644 (CO), 1548, 1472, 1432, 1416. HRMS (ES) *m/z* (M+H)+565.3958 ($C_{30}H_{53}O_6N_4$ requires 565.3959).

3.1.17. *N*-(2-Pentacosa-10,12-diynoylaminoethyl)-*N*-(thymin-1-ylacetyl)glycine 24. *N*-(2-Pentacosa-10,12-diynoylaminoethyl)-*N*-(thymin-1-ylacetyl)glycine methyl ester **20** (0.15 g, 0.23 mmol) was dissolved in THF (5 mL) and 1 M aq LiOH (3 mL) and the mixture was stirred at rt for 2 h. Water (5 mL) was then added and the aqueous layer was separated and washed with DCM (2×10 mL) before being acidified to pH 2.0 with 1 M aq HCl. This acidic aqueous solution was extracted with DCM (5×25 mL), the combined organic phase was dried ($MgSO_4$) and the solvent was evaporated to yield the title compound **24** (0.14 g, 97%).as a white foam. δ_H (d_6-DMSO) (two rotational isomers in a 2:1 ratio were observed) 0.80 (t, 3H, $J=6.4$ Hz,

CH_2CH_3), 1.20–1.45 (m, 32H, CH_2), 1.75 (s, 3H, thymine-CH_3), 2.00 (minor) and 2.10 (major) (t, 2H, $J=7.3$ Hz, CH_2CONH), 2.25 (t, 4H, $J=6.4$ Hz, $CH_2C{\equiv}C$), 3.15 (minor) and 3.25 (major) (m, unresolved, 2H, $NHCH_2CH_2$), 3.25 (minor) and 3.38 (major) (m, unresolved, 2H, $NHCH_2$-CH_2), 3.95 (major) and 4.15 (minor) (s, 2H, CH_2CO_2H), 4.45 (minor) and 4.65 (major) (s, 2H, CH_2CON), 7.25 (minor) and 7.30 (major) (s, 1H, thymine-H), 7.70 (minor) and 7.85 (major) (t, 1H, $J=5.8$ Hz, $NHCH_2$), 11.20 (minor) and 11.22 (major) (s, 1H, OH). δ_C (d_6-DMSO) (two rotational isomers in a 2:1 ratio were observed), 11.8 and 11.9 (thymine-CH_3), 13.9 (CH_3), 18.3, 22.1, 25.0, 25.1, 27.7, 27.7, 28.1, 28.2, 28.3, 28.4, 28.7, 28.7, 28.8, 28.9, 28.9, 31.3, 35.3, 35.4, 36.2, 36.8, 40.7, 46.5, 46.6, 47.4, 47.7, 54.9 (CH_2), 65.3, 65.3, 77.9, 77.9 (C≡C), 108.0 and 108.1 (thymine-C5), 142.0 and 142.1 (thymine-C6), 151.1 (CO), 164.4 (CO), 167.1 and 167.6 (CON), 170.4 and 170.8 (CONH), 172.2 and 172.7 (CO_2H). ν_{max} (KBr, cm^{-1}) 3351, 3169 (NH str), 2923, 2850 (CH str), 1736, 1692, 1660, 1601, 1422. HRMS (ES) m/z $(M+H)^+$641.4278 ($C_{36}H_{57}N_4O_6$ requires 641.4278).

3.1.18. *N*-(2-Pentacosa-10,12-diynoylaminoethyl)-*N*-(N^4(benzyloxycarbonyl)cytosin-1-ylacetyl)glycine 25. *N*-(2-Pentacosa-10,12-diynoylaminoethyl)-*N*-(N^4-(benzyl-oxycarbonyl)cytosin-1-ylacetyl)glycine methyl ester **21** (0.10 g, 0.13 mmol) was dissolved in THF (10 mL) and 1 M aq LiOH (5 mL) and the mixture was stirred for 3 h. Water (20 mL) was added and the solution was washed with DCM (2×20 mL) and then acidified to pH 1.0 using a 0.7 M solution of citric acid. The solution was extracted with DCM (6×10 mL), the extract was washed with brine (10 mL) and then dried ($MgSO_4$). The solvent was evaporated under reduced pressure to yield the title compound **25** as a colourless solid (0.094 g, 95%). δ_H (d_6-DMSO) (two rotational isomers in a 2:1 ratio were observed) 0.85 (t, 3H, $J=7.1$ Hz, CH_3), 1.2–1.6 (m, 32H, CH_2), 2.10 (m unresolved, 2H, CH_2CONH), 2.30 (t, 4H, $J=6.2$ Hz, $CH_2C{\equiv}C$), 3.4 (m, 4H, NCH_2CH_2N), 3.95 (major) and 4.2 (minor) (s, 2H, CH_2CO_2H), 5.15 (s, 2H, CH_2Ph), 7.0 (2×d) and 7.9 (2×d) (2H, $J=7.2$ Hz, cytosinyl-H), 7.4 (m, 5H, Ph). HRMS (ES) $(M+H)^+$760.4647 ($C_{43}H_{62}N_5O_7$ requires 760.4649).

3.1.19. *N*-(2-Pentacosa-10,12-diynoylaminoethyl)-*N*-(N^6-(benzyloxycarbonyl)adenin-9-ylacetyl)glycine 26. *N*-(2-Pentacosa-10,12-diynoylaminoethyl)-*N*-(N^6-(benzyl-oxycarbonyl)adenin-9-ylacetyl)glycine methyl ester **22** (0.24 g, 0.30 mmol) was dissolved in THF (5 mL) and 1 M aq LiOH (3 mL) and the mixture was stirred at rt for 2 h. Water (5 mL) was then added and the solution was washed with DCM (2×10 mL) before being acidified to pH 2.0 with 1 M aq HCl. The aqueous phase was extracted with DCM (5×10 mL), the extract was dried ($MgSO_4$) and the solvent was evaporated under reduced pressure to yield the title compound **26** as a white foam (0.16 g, 69%), which turned purple in ambient light. δ_H ($CDCl_3$) (two rotational isomers in a 2:1 ratio were observed) 0.80 (t, 3H, $J=6.4$ Hz, CH_3), 1.20–1.50 (m, 32H, CH_2), 1.95 (minor) and 2.05 (major) (t, 2H, $J=7.3$ Hz, CH_2CONH), 2.25 (t, 4H, $J=6.4$ Hz, $CH_2C{\equiv}C$), 3.15 (minor) and 3.35 (major) (m, unresolved, 2H, $NHCH_2$), 3.35 (minor) and 3.50 (major) (m, unresolved, 2H, CH_2CH_2N), 4.00 (major) and 4.32 (minor) (s, 2H, CH_2CO_2H), 5.13 (minor) and 5.34 (major) (s, 2H, CH_2CON), 5.20 (s, 2H, CH_2O), 7.30–7.50 (m, 5H, Ar-*H*), 7.75 (minor) and 8.00 (major) (t, 1H, N*H*), 8.32 (s, 1H, adeninyl-C8–H), 8.60 (s, 1H, adeninyl-C2–H), 10.65 (br s, 1H, OH). δ_C ($CDCl_3$) (two rotational isomers in a 2:1 ratio were observed) 13.9 (CH_3), 18.3, 22.1, 25.0, 25.1, 27.7, 27.7, 28.1, 28.2, 28.3, 28.4, 28.7, 28.7, 28.8, 28.9, 29.0, 31.3, 35.4, 36.1, 36.9, 40.5, 43.9, 44.1, 46.7, 47.5, 49 (CH_2), 65.3 and 65.3 (C≡C), 66.2 (CH_2), 72.2 and 77.9 (C≡C), 122.9 (adenine-C5), 127.8, 127.9, 128.4 (CH), 136.4, 145.2 (adenine-C6), 149.3 (NHCOO), 151.4, 152.1, 152.3, 166.5 and 167.0 (CON), 170.3 and 170.8 (CONH), 172.3, 172.8 (CO_2H). MS (ES) m/z 784.6 $(M+H)^+$(70%).

3.1.20. *N*-(2-Octadecanoylaminoethyl)-*N*-(thymin-1-ylacetyl)-glycine 27. *N*-(2-Octadecanoylaminoethyl)-*N*-(thymin-1-ylacetyl)glycine methyl ester **23** (0.56 g, 0.63 mmol) was dissolved in THF (60 mL) and 1 M aq LiOH (30 mL). The mixture was stirred for 3 h, then the solution was washed with DCM. The aqueous phase was separated, acidified with 1 M aq HCl and then extracted with ethyl acetate (5×50 mL). The ethyl acetate extract was dried ($MgSO_4$) and the solvent evaporated under reduced pressure to yield the title compound **27** as a colourless solid (0.34 g, 97%, mp 145–147 °C). δ_H (d_6-DMSO) (two rotational isomers in a 2:1 ratio were observed) 0.84 (t, 3H, $J=6.4$ Hz, CH_2CH_3), 1.20–1.50 (m, 30H, CH_2), 1.74 (s, 3H, thymine-CH_3), 2.08 (m, unresolved, 2H, CH_2CONH), 3.37 (m, 4H, NCH_2CH_2N), 3.98 (major) and 4.20 (minor) (s, 2H, CH_2CO_2H), 4.48 (minor) and 4.64 (major) (s, 2H, CH_2CON), 7.35 (minor) and 7.42 (major) (s, 1H, thymine-C6–*H*), 7.65 (minor) and 7.88 (major) (s, 1H, $NHCH_2$), 11.30 (s, 1H, OH). [1×NH not observed] δ_C (d_6-DMSO) (two rotational isomers in a 2:1 ratio were observed) 12.3 (thymine-CH_3), 14.1, 22.7, 25.6, 29.3, 29.4, 29.5, 29.6, 29.7, 30.8, 31.9, 36.5, 36.6, 37.5, 37.6, 47.9, 48.3, 48.5, 48.8 (CH_2), 50.0 (CH_3), 110.8 and 110.9 (thymine-C5), 150.9 (CO), 163.9 (CO), 167.3 and 168.1 (CON), 170.0 and 170.1 (CONH), 173.9 and 174.4 (CO_2H). ν_{max} (KBr, cm^{-1}) 3319, 3066 (NH str), 2918, 2850 (CH str), 1667, 1644 (CO), 1549, 1469, 1415. HRMS (ES) m/z $(M+H)^+$551.3803 ($C_{29}H_{51}N_4O_6$ requires 551.3806).

3.1.21. Ethyl (N^6-bis(*t*-butoxycarbonyl)adenin-9-yl)acetate 28b. To a solution of ethyl adenin-9-ylacetate[18] **28a** (1.00 g, 4.52 mmol) in THF (25 mL) was added DMAP (1.66 g, 13.59 mmol) followed by di-*t*-butyl dicarbonate (2.96 g, 13.56 mmol) and the mixture was stirred at rt for 17 h. The solvent was evaporated and the crude residue was purified by flash column chromatography [silica gel, CH_2Cl_2/MeOH (0–1%) as eluent] to yield the title compound **28b** as a colourless oil (1.19 g, 62%). δ_H ($CDCl_3$) 1.28 (t, 3H, $J=7.2$ Hz, CH_2CH_3), 1.44 (s, 9H, $(CH_3)_3$), 4.26 (q, 2H, $J=7.1$ Hz, CH_2CH_3), 5.05 (s, 2H, CH_2CO_2Et), 8.14 (s, 1H, H-8), 8.36 (s, 1H, H-2). δ_C ($CDCl_3$) 14.1 (CH_2CH_3), 27.8 ($(CH_3)_3$), 44.4, 62.5 (CH_2), 83.7 (C–O), 128.3 (C5), 144.9 (C6), 150.3, 150.5, 152.3, 153.4 (CON), 166.7 (CO_2Et). ν_{max} (thin film, cm^{-1}) 2981, 2937 (CH str), 1787, 1753 (CO), 1603, 1581, 1508, 1452. HRMS (ES) $(M+H)^+$422.2032 ($C_{19}H_{28}O_6N_5$ requires 422.2034).

3.1.22. (N^6-Bis(*t*-butoxycarbonyl)adenin-9-yl)acetic acid

28c. A solution of ethyl (N^6-bis(*t*-butoxycarbonyl)adenin-9-yl)acetate **28b** (0.53 g, 1.26 mmol) in THF (15 mL) and 1 M aq LiOH (10 mL) was stirred at rt for 1 h. The solution was then partitioned between 1 M aq $KHSO_4$ (15 mL) and ethyl acetate (15 mL). The aqueous phase was separated and re-extracted with ethyl acetate (4×15 mL) and the combined extract was dried ($MgSO_4$). The solvent was evaporated to yield the title compound **28c** (0.36 g, 72%) as a colourless solid, mp 104–105 °C. δ_H (d_6-DMSO) 1.38 (s, 9H, CH_3), 5.15 (s, 2H, CH_2), 8.60 (s, 1H, H-8), 8.83 (s, 1H, H-2), 10.10 (br s, 1H, OH). δ_C (d_6-DMSO) 27.4 ($(CH_3)_3$), 44.5 (CH_2), 83.4 (C–O), 127.4 (C-5), 147.4, 149.0, 150.0, 151.6, 153.3 (CON), 168.9 (CO_2H). ν_{max} (KBr, cm^{-1}) 3420 (OH str), 2981, 2936 (CH str), 1790, 1740 (CO), 1652, 1609, 1585, 1511, 1472. HRMS (ES) $(M+H)^+$394.1721 ($C_{17}H_{24}O_6N_5$ requires 394.1721).

3.1.23. *N*-(2-Pentacosa-10,12-diynoylaminoethyl)-*N*-(N^6-(bis-*t*-butoxycarbonyl)adenin-9-ylacetyl)glycine methyl ester 28d. To a solution of *N*-(2-pentacosa-10,12-diynoyl-aminoethyl)glycine **18** (0.15 g, 0.31 mmol) in DMF (10 mL) was added DIPEA (0.15 mL, 0.84 mmol), (N^6-bis(*t*-butoxycarbonyl)adenin-9-yl)acetic acid **28c** (0.11 g, 0.28 mmol) and HBTU (0.11 g, 0.28 mmol). The mixture was stirred at rt for 17 h, then the solvent was evaporated under reduced pressure. The residue was dissolved in DCM (50 mL) and this solution was washed successively with 1 M aq $NaHCO_3$ (3×50 mL), 1 M aq $KHSO_4$ (50 mL), water (50 mL) and finally brine (50 mL). The solution was dried ($MgSO_4$) and the solvent was evaporated under reduced pressure to give the crude product, which was purified by flash column chromatography [silica gel, CH_2Cl_2/methanol (97:3) as eluent] to yield the title compound **28d** as a glassy solid (0.11 g, 44%). δ_H ($CDCl_3$) (two rotational isomers in a 2:1 ratio were observed) 0.85 (t, 3H, J=6.5 Hz, CH_2CH_3), 1.22 (s, 9H, $(CH_3)_3$), 1.4–1.7 (m, 32H, CH_2), 2.05 (minor) and 2.35 (major) (t, 2H, J=7.6 Hz, CH_2CONH), 2.22 (t, 4H, J=6.8 Hz, $CH_2C{\equiv}C$), 3.35 (minor) and 3.50 (major) (q, 2H, J=5.6 Hz, $NHCH_2$), 3.55 (minor) and 3.65 (major) (t, 2H, J=5.5 Hz, CH_2CH_2N), 3.73 (major) and 3.84 (minor) (s, 3H, OCH_3), 4.07 (major) and 4.30 (minor) (s, 2H, CH_2CO_2Me), 5.00 (minor) and 5.18 (major) (s, 2H, CH_2CON), 5.95 (minor) and 6.70 (major) (t, 1H, NH), 8.10 (major) and 8.18 (minor) (s, 1H, H-8), 8.78 (major) and 8.79 (minor) (s, 1H, H-2). δ_C ($CDCl_3$) (two rotational isomers in a 2:1 ratio were observed) 14.1 (CH_2CH_3), 19.2 (×2), 22.7 (CH_2), 27.8 and 28.3 ($(CH_3)_3$), 28.3, 28.7, 28.8, 28.9, 29.1, 29.3, 29.4, 29.6 (×3), 31.9, 36.6, 37.6, 44.0, 48.6 (CH_2), 48.9, 52.6 (OCH_3), 65.2 and 65.3 (C≡C), 77.4 and 77.6 (C≡C), 83.7 and 83.8 (C–O), 128.3 (purine-C5), 145.7 (purine-C6), 150.3 (×2), 151.9, 153.5 (NCO_2), 166.4 (CON), 170.2 (CONH), 174.2 (CO_2Me). ν_{max} (thin film, cm^{-1}) 3375 (NH str), 2927, 2854 (CH str), 1786, 1751 (CO), 1670, 1604, 1581, 1540, 1457, 1415. HRMS (ES) $(M+H)^+$886.5422 ($C_{47}H_{74}N_7O_8$ requires 886.5413).

3.1.24. *N*-(2-Pentacosa-10,12-diynoylaminoethyl)-*N*-(adenin-9-ylacetyl)-glycine hydrochloride 28e. To a solution of *N*-(2-pentacosa-10,12-diynoylaminoethyl)-*N*-(N^6-bis(*t*-butoxycarbonyl)adenin-9-ylacetyl)glycine methyl ester **28d** (0.106 g, 0.12 mmol) in DCM (10 mL) was added 6 M aq HCl (10 mL) and the mixture was stirred at 40 °C for 17 h. The mixture was evaporated under reduced pressure to leave the title compound **28e** as a glassy solid (0.083 g, 99%). δ_H (d_6-DMSO) (two rotational isomers in a 2:1 ratio were observed) 0.85 (t, 3H, J=6.5 Hz, CH_2CH_3), 1.2–1.5 (m, 32H, CH_2), 2.00 (minor) and 2.15 (major) (t, 2H, J=7.0 Hz, CH_2CONH), 2.25 (t, 4H, J=6.0 Hz, $CH_2C{\equiv}C$), 3.2–3.6 (m, unresolved, 4H, $NHCH_2CH_2N$), 4.00 (major) and 4.32 (minor) (s, 2H, CH_2CO_2H), 5.15 (minor) and 5.35 (major) (s, 2H, CH_2CON), 7.80 (minor) and 8.05 (major) (t, 1H, NH), 8.25 (s, 1H, H-8), 8.35 (s, 1H, H-2). δ_C (d_6-DMSO) (two rotational isomers in a 2:1 ratio were observed) 13.9 (CH_2CH_3), 18.2, 22.0, 25.0, 25.1, 27.7 (×2), 28.1, 28.2, 28.3 (×2), 28.6, 28.7, 28.8, 28.9, 29.0, 31.2, 25.3, 36.1, 36.9, 44.1, 46.7, 47.5 (CH_2), 65.3 (×2), 77.9 (C≡C), 143.7, 147.8, 152.4, 166.3 and 166.7 (CON), 170.2 and 170.7 (CONH), 172.3 and 172.8 (CO_2H). ν_{max} (thin film, cm^{-1}) 3074 (NH str), 2926, 2854 (CH str), 1660, 1605, 1563, 1552, 1524, 1467, 1422. HRMS (ES) $(M+H)^+$650.4387 ($C_{36}H_{56}N_7O_4$ requires 650.4388).

3.2. General solid phase method for attachment of *N*-terminal stearoyl group to PNA oligomers. Synthesis of $CH_3(CH_2)_{16}CO$-$(T')_{10}$-$LysNH_2$ 29 and $CH_3(CH_2)_{16}CO$-$(T')_{10}$-$AspNH_2$ 30

The *N*-Boc protected PNA T'_{10}-mer was assembled manually on either a Boc-Lys(2-Cl–Z)- or Boc-Asp(Bz)-derivatized MBHA resin, respectively, following the strategy described by Christensen et. al.[21] (0.200 g of resin (dry weight), loading factor=ca. 0.15 mmol/g). Subsequently, the following procedure was performed: (1) *N*-Boc deprotection using a solution of TFA:*m*-cresol (95:5, v/v), 2×4 min. (2) Washing with DMF:DCM (1:1, v/v), 3×20 s; washing with pyridine, 2×20 s (3) Coupling using a solution of octadecanoyl fluoride[13] (0.029 g, 0.10 mmol) and DIPEA (0.052 mL, 0.30 mmol) in DMF:DCM (1:1, v/v) (1 mL). Coupling was allowed to proceed for 20 min at rt. (4) Washing with DMF:DCM (1:1, v/v), 2×20 s. Steps 3 and 4 were repeated a further two times. After the final washing, the resins were dried under vacuum and the PNA oligomers were concomitantly deprotected and cleaved from the solid support using the TFMSA method reported by Christensen et al.[21] The crude products afforded were purified by RP-HPLC to give the pure PNA oligomers **29** and **30** as white pellets. The identities of oligomers **29** and **30** were verified by MALDI-TOF mass spectroscopy (Table 1).

3.3. General method for solution phase coupling of a stearoyl or diacetylene group to a PNA oligomer. Synthesis of Ac-$(T')_{10}$-$Lys\{CO(CH_2)_{16}CH_3\}NH_2$ 32, Ac-$(T')_{10}$-$Lys\{CO(CH_2)_8C_4(CH_2)_{11}CH_3\}NH_2$ 33, AcAsp-$(T')_{10}$-$Lys\{CO(CH_2)_{16}CH_3\}NH_2$ 35, AcAsp-$(T')_{10}$-$Lys\{CO(CH_2)_8C_4(CH_2)_{11}CH_3\}NH_2$ 36, $CH_3(CH_2)_{11}C_4(CH_2)_8CO$-$(T')_{10}$-$AspNH_2$ 38, and $CH_3(CH_2)_{11}C_4(CH_2)_8CO$-PEG-$(T')_{10}$-$AspNH_2$ 40**

The appropriate parent PNA oligomers {that is **31** for preparation of **32** and **33**, **34** for **35** and **36**, **37** for **38** and **39** for **40** (Scheme 7)} were assembled manually according to the solid phase strategy developed by Christensen et al.[21] Following deprotection and cleavage from the resin, the identities of the crude PNA oligomers afforded were verified

Table 2. MALDI-TOF mass spectral data for oligomers **29–40** (Scheme 7)

		Calculated average mass	Found (*m/z*)
29	$CH_3(CH_2)_{16}CO$-$(T')_{10}$-$LysNH_2$	3097.2 $[M+Na]^+$	3096.4 $[M+Na]^+$
30	$CH_3(CH_2)_{16}CO$-$(T')_{10}$-$AspNH_2$	3084.1 $[M+Na]^+$	3082.9 $[M+Na]^+$
31	Ac-$(T')_{10}$-$LysNH_2$	2850.8 $[M+H]^+$	2850.3 $[M+H]^+$
32	Ac-$(T')_{10}$-Lys$\{CO(CH_2)_{16}CH_3\}NH_2$	3139.2 $[M+Na]^+$	3136.5 $[M+Na]^+$
33	Ac-$(T')_{10}$-Lys$\{CO(CH_2)_8C_4(CH_2)_{11}CH_3\}NH_2$	3229.4 $[M+Na]^+$	3227.6 $[M+Na]^+$
34	AcAsp-$(T')_{10}$-$LysNH_2$	2965.9 $[M+H]^+$	2963.1 $[M+H]^+$
35	AcAsp-$(T')_{10}$-Lys$\{CO(CH_2)_{16}CH_3\}NH_2$	3254.3 $[M+Na]^+$	3252.5 $[M+Na]^+$
36	AcAsp-$(T')_{10}$-Lys$\{CO(CH_2)_8C_4(CH_2)_{11}CH_3\}NH_2$	3344.4 $[M+Na]^+$	3342.5 $[M+Na]^+$
37	H-$(T')_{10}$-$AspNH_2$	2817.6 $[M+Na]^+$	2816.0 $[M+Na]^+$
38	$CH_3(CH_2)_{11}C_4(CH_2)_8CO$-$(T')_{10}$-$AspNH_2$	3174.2 $[M+Na]^+$	3174.2 $[M+Na]^+$
39	H-PEG-$(T')_{10}$-$AspNH_2$	2940.8 $[M+H]^+$	2939.2 $[M+H]^+$
40	$CH_3(CH_2)_{11}C_4(CH_2)_8CO$-PEG-$(T')_{10}$-$AspNH_2$	3319.4 $[M+Na]^+$	3319.2 $[M+Na]^+$

The matrix used was 3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid).

by MALDI-TOF mass spectrometry (Table 1). Subsequently, the required PNA oligomer was dissolved in DMF:pyridine (1:1, v/v) (1 mL/0.01 g) and either octadecanoyl fluoride[13] or 10,12-pentacosadiynoic acid fluoride[13] (10 equiv) was added followed by DIPEA (30 equiv). The resulting mixture was left to stir at rt for 24 h before the solvent was removed in vacuo. The remaining residue was re-dissolved in TFA (1 mL) and the crude product was precipitated from this solution by addition of diethyl ether (10 mL). The suspension was centrifuged and the supernatant was decanted off from the pellet yielded. The pellet was then re-suspended in fresh diethyl ether (10 mL) and the centrifugation and decanting processes were repeated. This step was performed a further 5 times before the pellet was dried using a gentle stream of dry nitrogen gas. Finally, the crude product afforded was purified by RP-HPLC to give the pure PNA oligomers **32**, **33**, **35**, **36**, **38** and **40** as white pellets. The identities of oligomers **32**, **33**, **35**, **36**, **38** and **40** were confirmed by MALDI-TOF mass spectroscopy (Table 2).

3.4. General method for formation of polymerized liposomes (cf. Refs. .9, 28, 29)

The requisite amphiphilic compounds, including at least one diacetylene as a component, dissolved in chloroform (ca. 1 mL) were mixed in the desired ratios in a test tube. The chloroform was then evaporated under a gentle stream of nitrogen to leave a thin coating on the surface of the tube. To this solid was added the required amount of deionised water to give a total solute concentration of 1 mM and the mixture was sonicated at 80 °C for 30 min using a Sonozap 25 kHz battery-operated microprobe ultrasonic processor. The colourless solution containing liposomes was immediately filtered while hot (Whatman 0.8 μm nylon filter) and stored at 4 °C for 17 h. After this time, the solution was purged with nitrogen before polymerization by irradiating with a 'mineralight lamp' low intensity short wave UV lamp (254 nm) for up to 1 h to afford solutions of coloured PDA-liposomes.

Acknowledgements

We thank Heriot-Watt University for support (to E.M.), Dr. A.S.F. Boyd for recording NMR spectra, Mrs. Christina Graham and Mr. Gareth Evans for elemental analysis and the EPSRC Mass Spectrometry Service Centre, Swansea, UK, for HRMS data.

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Tetrahedron 61 (2005) 8888–8894
Tetrahedron

A concise sequential photochemical-metathesis approach for the synthesis of (+)-castanospermine and possible uniflorine-A stereoisomers

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Received 31 May 2005; revised 5 July 2005; accepted 6 July 2005

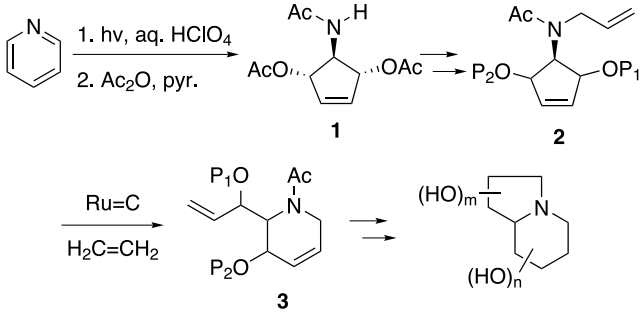
Available online 28 July 2005

Abstract—A recently developed strategy for polyhydroxylated indolizidine ring construction has been applied to the synthesis of (+)-castanospermine and possible isomers of uniflorine-A. The routes to these targets rely on the use of the earlier discovered photocyclization reaction of pyridinium perchlorate in a concise route for preparation of a key *N*-allylacetamidocyclopentendiol intermediate. Ring rearrangement metathesis of this substance gives an allyl-tetrahydropyridine, which is then transformed to the targets by execution of regio- and stereo-controlled hydroxylation processes followed by indolizidine ring construction.

1. Introduction

Members of the polyhydroxylated indolizidine, natural product family have received continuous intense scrutiny from synthetic chemists owing to their densely functionalized, stereochemically rich structures and their biomedically relevant physiological properties.[1] Recently,[2] we described a unique and stereochemically flexible strategy for synthesis of functionalized indolizidines that relies on the combined use of photochemical and ring rearrangement metathesis (RRM) reactions in key ring building steps. The overall approach, outlined in Scheme 1, begins with the preparation of 4-amino-3,5-cyclopentendiol derivative **1** through photocyclization reaction of pyridinium perchlorate.[3,4] Transformation of this substances to *N*-acetamido-*N*-allylcyclopentenes **2** is then followed by ruthenium alkylidene[5] catalyzed RRM reactions[6] to generate the corresponding 6-allyltetrahydropyridines **3**. The RRM derived products contain an array of exocyclic and endocyclic alkene and potentially differentiated alcohol functionalities, which can be manipulated to both introduce the groups required for indolizidine ring formation and install target functionality.

In an earlier publication,[2] we described the advent of this strategy and its application to the synthesis of the potent



Scheme 1.

α-D-mannosidase and mannosidase II inhibitor, (−)-swainsonine (**8**). In the approach to this target (Scheme 2), regiocontrolled RRM reaction of allylacetamidocyclopentene **4** was employed to produce the tetrahydropyridine **5**. As a result of $A^{1,3}$-strain between the *N*-acetyl and 6-allyl groups, the corresponding acetate derivative exists predominantly in the diaxial conformation **6**, in which both faces of the endocyclic alkene group are blocked. Consequently, dihydroxylation of the exocyclic olefin in **6** takes place selectively to produce the requisite precursor **7** in the route to (−)-swainsonine.

Broad application of this strategy to a variety of naturally occurring, biomedically important indolizidines requires that methods be available to selectively manipulate the endocyclic alkene moiety in the RRM derived 6-allyl-tetrahydropyridine intermediates. (+)-Castanospermine, an

Keywords: Pyridinium salt photochemistry; Ring rearrangement metathesis; Polyhydroxylated indolizidines.
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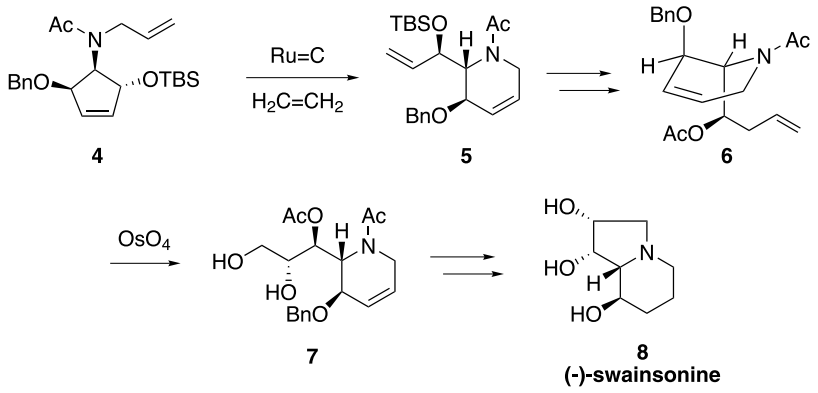
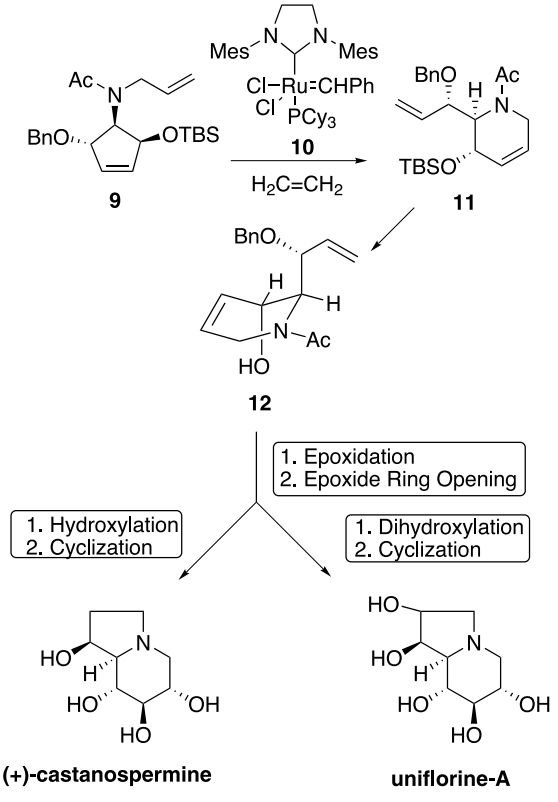
doi:10.1016/j.tet.2005.07.014



Scheme 2.

excellent glycosidase inhibitor,[7] is one of a group of targets, which can be approached by using this design variation. As depicted in Scheme 3, a possible route to (+)-castanospermine would employ RRM reaction of the *N*-acetamido-*N*-allylcyclopentene **9** to generate the tetrahydropyridine **11**. Based on $A^{1,3}$-strain considerations, the alcohol derivative **12** should exist in the diaxial conformation, in which the C-5 hydroxyl is perfectly aligned to guide selective α-face epoxidation of the internal alkene moiety. Trans-diaxial epoxide ring opening would then set the stage for cyclization to produce the tetrahydroxylated indolizidine skeleton of castanospermine. Importantly, this general strategy can be employed to design concise, stereoselective routes for the preparation of pentahydroxylated indolizidines that are potentially related to the incorrectly identified glycosidase inhibitor, uniflorine-A.[8] For this purpose, hydroxyl guided epoxidation of **12** and epoxide ring opening would be followed by dihydroxylation of the exocyclic olefin and indolizidine ring formation (Scheme 3).



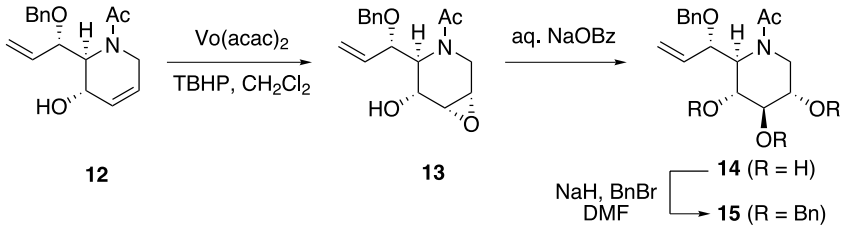
Scheme 3.

2. Results and discussion

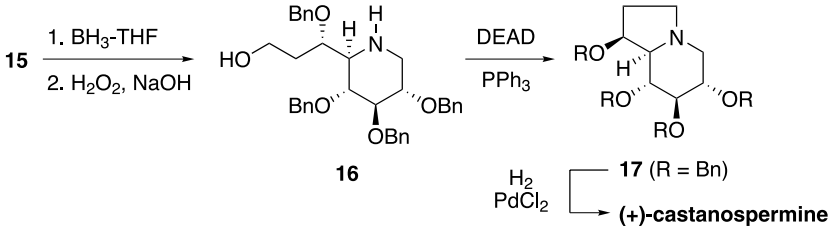
2.1. Synthesis of (+)-castanospermine

Earlier, we reported that RRM reaction of the enantiomerically enriched *cis*,*-trans*-*N*-allylacetamidocyclopentendiol derivative **9**, produced from pyridine by using a photochemical-enzymatic desymmetrization-alcohol inversion sequence, proceeds smoothly (93%) to furnish the corresponding 6-allyltetrahydropyridine **11**.[2] This highly regioselective process is performed by using the second generation, Grubbs ruthenium alkylidene catalyst **10**[5] in the presence of ethylene (Scheme 3). X-ray crystallographic analysis of endocyclic allylic alcohol **12**, generated by treatment of **11** with TBAF, clearly demonstrates that it exists in the diaxial conformation. This preference results from relief of $A^{1,3}$-strain caused by interactions between the *N*-acetyl and 6-allyl side chain in the alternative diequatorial conformation. A consequence of this preference is that the endocyclic hydroxyl group in **12** can be used advantageously to guide regio- and stereo-selective dihydroxylation reaction of the endocyclic alkene moiety. In the context of the current targets, the hydroxyl group is employed to direct selective epoxidation of **12** to produce the corresponding epoxy-alcohol **13**. Trans-diaxial ring opening of **13** under mild basic conditions then furnishes the *trans*,*trans*,*trans*-trihydroxy-piperidine **14**. Benzylation of **14** provides the tetrabenzyl ether **15** (Scheme 4), which serves as the key intermediate in routes to castanospermine and the possible uniflorine-A diastereomers.

Several approaches were explored to transform **15** to the corresponding terminal alcohol. The best method (albeit not fully satisfactory) for performing this operation was found to be one using the hydroboration–oxidation protocol. Accordingly, treatment of **15** with the BH_3–THF complex followed by NaOH–H_2O_2 oxidation produces the alcohol **16** (Scheme 5). Surprisingly the *N*-acetyl group is fortuitously removed under these conditions.[9] As expected this process also forms a minor amount of the *N*-ethyl analog of **16** by way of reduction of the amide group. An attempt to minimize this unwanted side reaction by using 9-BBN was not successful; **15** is recovered when treated with this



Scheme 4.

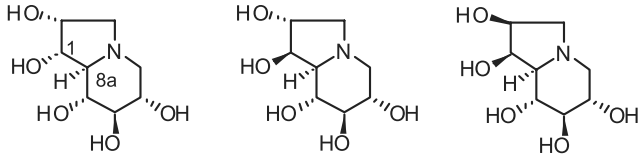


Scheme 5.

hydroboration agent. Indolizidine ring formation takes place when **16** is subjected to typical Mitsunobo cyclization conditions (DEAD–PPh_3) to yield indolizidine **17**. Hydrogenolytic removal of the benzyl protecting groups then generates the target, (+)-castanospermine. The physical and spectroscopic properties of the synthetic material match those reported for the natural product.[10]

2.2. Pentahydroxylated indolizidines potentially related to uniflorine-A

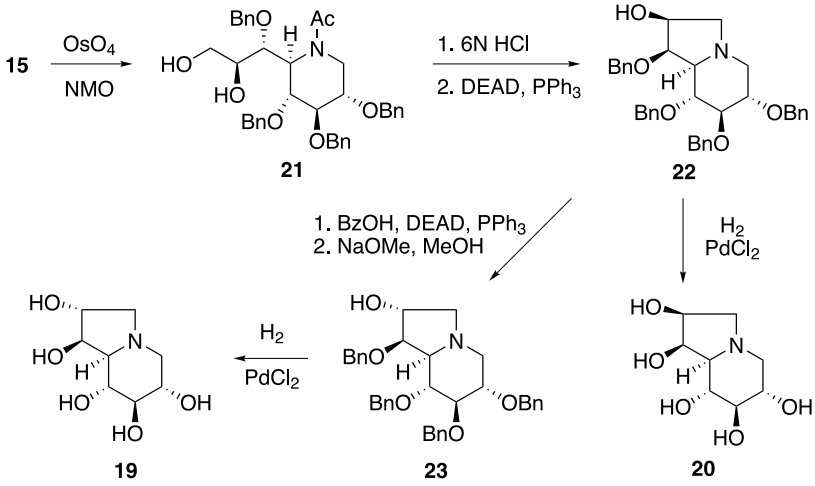
A novel pentahydroxylated indolizidine, uniflorine-A, was recently isolated from the tree *Eugenia uniflora L.* whose leaves are used in folk medicine as antidiarrhetics, antirheumatics and antidiabetics.[8a] The structure/stereochemistry of this substance was assigned by Arisawa and his co-workers[8a] as **18** based on NMR spectroscopic data. Recently, Pyne and his co-workers[8b] synthesized the putative uniflorine-A **18** by using an elegent pathway starting with L-xylose. However, the ^1H and ^{13}C NMR data of the synthetic material did not match those reported for the natural product by the Arisawa group. One significant difference between the natural and synthetic materials was found in their ^1H NMR spectra. Specifically, the H_1–H_{8a} coupling constant reported for uniflorine-A is 4.5 Hz while that of the synthetic material **18** is 7.7 Hz, as expected for the *anti*-relationship of these protons. This difference led Pyne to make the reasonable suggestion that 'uniflorine-A, if it is an indolizidine alkaloid, has the same H-1 stereochemistry as castanospermine'; that is, that H-1 and H-8a have a *syn* relationship.[8b]



Based on Pyne's conclusion, uniflorine-A could be one of the two pentahydroxylated indolizidines **19** or **20**, both of which have the trans,trans,trans-piperidine ring stereochemistry and *syn* relationships between H-1 and H-8a. The preparation of one of these, indolizidine **20**, was reported in 1996 by Fleet and his co-workers.[11] A comparison of their spectroscopic data with those reported by Arisawa (Table 1) indicates that uniflorine-A is not **20**. To our knowledge, indolizidine **19** has not been prepared previously. Owing to the potentially interesting biomedical properties of uniflorine-A and the fact that its structure/stereochemistry remains undefined, we have applied the RRM based strategy described above to the synthesis of the respective

Table 1. ^1H NMR spectroscopic data (D_2O) for uniflorine-A and the pentahydroxy-indolizidines **18**–**20**

	Chemical shifts (ppm)					Coupling constants (Hz)			
	Uniflorine-A[8a]	**18**[8b]	**19**	**20**[11]		Uniflorine-A[8a]	**18**[8b]	**19**	**20**[11]
H_1	4.18	3.82	4.08	4.06	H_1–H_2	4.5	7.3	0	5.3
H_2	4.35	4.11	4.25	4.24	H_1–H_{8a}	4.5	7.5	6.3	3.5
H_3	2.98	3.26	2.63	2.54	H_2–H_3	5.1	6.8	6.3	8.1
H_3	3.04	2.20	2.88	2.71	H_2–H_3	5.1	6.8	4.4	2.1
H_5	3.61	3.01	2.96	2.95	H_{8a}–H_8	7.7	9.2	10.2	9.7
H_5	3.76	2.09	1.91	1.91	H_8–H_7	7.7	9.0	9.1	9.5
H_6	2.76	3.46	3.42	3.42	H_7–H_6	9.0	9.0	9.1	9.1
H_7	3.81	3.20	3.13	3.12	H_6–H_5	6.4	10.9	9.1	10.6
H_8	3.94	3.25	3.48	3.47	H_6–H_5	3.8	5.5	5.2	5.1
H_{8a}	3.14	2.08	2.01	2.01	H_3–H_3	12.1	10.5	10.3	10.7
					H_5–H_5	11.8	10.7	10.9	10.6



Scheme 6.

unknown and known pentahydroxylated indolizidines **19** and **20**.

The divergent route to both targets (Scheme 6) begins with the protected piperidine **15**. Stereocontrolled catalytic osmylation of this substance cleanly produces the corresponding diol **21**. Amide hydrolysis and Mitsunobo cyclization is then employed to transform **21** to the pentahydroxy-indolizidine derivative **22**, which serves as a direct precursor (benzyl deprotection) of the known indolizidine **20**.[11] Indolizidine **19**, is also generated from **22** by using Mitsunobo hydroxyl inversion to form **23** followed by hydrogenolytic benzyl deprotection.

The physical and spectroscopic properties of **19** do not match those reported for uniflorine-A. Significant differences are found between the melting points and optical rotations of the synthetic material (mp 114–116 °C, $[\alpha]_0^{25}$ +22.7 (*c*, 0.07, H_2O))[8a] and naturally occurring substance (mp 175–178 °C, $[\alpha]_0$ −4.4 (*c*, 1.2, H_2O)). Equally different are the ^1H and ^{13}C NMR spectra of **19** and uniflorine-A (Table 1). Moreover, the properties reported for the natural product are not equivalent to those reported earlier for **20** by Fleet and his co-workers (Table 1).[11] To insure that the differences between the properties of the natural product and those determined for **19** and **20** are not a consequence of different protonation state, the hydrochloride salts of the of **19** and **20** were prepared. As with their neutral counterparts, the spectroscopic data for these salts do not match those obtained for uniflorine-A. Based on these results, we conclude that the glycosidase inhibitor uniflorine-A isolated by Arisawa and his co-workers does not have the structure and/or stereochemistry found in either of the three pentahydroxy indolizidines **18**–**20**.

3. Conclusions

The studies described above demonstrate the unique preparative potential of the strategy for functionalized indolizidine synthesis, that is, based on pyridinium salt photochemistry and ring rearrangement metathesis chemistry. In addition, the ability to develop concise stereocontrolled routes to members of the polyhydroxylated indolizidine family has been exploited in the synthesis of the interesting glycosidase inhibitor (+)-castanospermine. Finally, by using this chemistry we have demonstrated that the natural product uniflorine-A does not possess the pentahydroxylated indolizidine stereostructures represented by **19** and **20**.

4. Experimental

4.1. General

Each reaction was run under a dry nitrogen atmosphere. All reagents were obtained from commercial sources and used without further purification, and all solvents were dried by using the standard procedures. ^1H and ^{13}C NMR spectra were recorded by using $CDCl_3$ solutions, unless specified otherwise, and chemical shifts are reported in ppm relative to $CHCl_3$ (7.24 ppm for ^1H and 77.0 ppm for ^{13}C), which was used as a chemical shift internal standard for samples in $CDCl_3$. For spectra recorded on d_6-acetone solutions, chemical shifts are reported in ppm relative to d_5-acetone (2.05 ppm for ^1H and 29.92 ppm for ^{13}C). For spectra recorded using d_4-methanol, chemical shifts are reported in ppm relative to d_3-methanol (3.31 ppm for ^1H and 49.15 ppm for ^{13}C). ^{13}C NMR resonance assignments were aided by the use of the DEPT-135 technique to determine numbers of attached hydrogens. All compounds were isolated as oils unless otherwise specified and their purities were determined to be >90% by ^1H and ^{13}C NMR analysis. Column chromatography was performed by using 230–400 mesh silica gel as absorbent.

4.1.1. 1-Acetyl-2-(1-benzyloxyallyl)-3-hydroxy-4,5-epoxypiperidine 13. To a stirred solution of the known[2] allylic alcohol **12** (29 mg, 0.10 mmol) in 3 mL of CH_2Cl_2 at 0 °C was added vanadyl acetylacetonate (2 mg, 0.01 mmol) and 0.07 mL (5–6 M in decane) of *t*BuOOH. The resulting mixture was stirred at 25 °C for 5 h, diluted with 1 mL of satd $NaHSO_3$ and $CHCl_3$. The $CHCl_3$ layer was separated, washed with satd $NaHSO_3$, satd $NaHCO_3$ and satd NaCl,

dried and concentrated in vacuo giving a residue, which was subjected to column chromatography (silica gel, 1:1 acetone/hexane) to yield 15 mg (50%) of the epoxide **13**. $[\alpha]_D^{25}$ +26.6 (*c*, 0.13, $CHCl_3$); ^1H NMR (mixture of rotamers) 7.34–7.20 (m, 5H), 5.72–5.68 (m, 1H), 5.46 (d, *J*=10.0 Hz, 1H), 5.36 (d, *J*=16.6 Hz, 1H), 4.80 (d, *J*=15.4 Hz, 1H), 4.58 (d, *J*=11.9 Hz, 1H), 4.23 (dd, *J*=3.9, 11.9 Hz, 1H), 3.98–3.94 (m, 1H), 3.76 (m, 1H), 3.42 (m, 1H), 3.41 (m, 1H), 2.91 (d, *J*=15.3 Hz, 1H), 2.71 (d, *J*=9.0 Hz, 1H), 2.07 (s, 3H); ^{13}C NMR rotamer A 172.3, 137.3, 134.7, 128.4, 127.9, 127.8, 127.6, 121.5, 78.4, 70.1, 62.6, 61.8, 52.6, 51.1, 36.5, 21.7; rotamer B 172.4, 137.3, 134.4, 128.4, 127.9, 127.8, 127.6, 119.5, 80.4, 71.1, 64.2, 58.5, 52.6, 49.7, 42.1, 21.9; HRMS (FAB) *m/z* 326.1358 (M+Na), calcd for $C_{17}H_{23}NO_5Na$ 326.1363.

4.1.2. 1-Acetyl-2-(1-benzyloxyallyl)-3,4,5-hydroxypiperidine 14. A solution of epoxide **13** (46 mg, 0.15 mmol) in 1 mL of water containing sodium benzoate (6 mg, 0.04 mmol) was stirred at 130 °C for 12 h, cooled and concentrated in vacuo giving a residue, which was subjected to column chromatography (silica gel, 3:2 acetone/hexane) to yield 27 mg (55%) of the triol **14**. $[\alpha]_D^{25}$ +7.2 (*c*, 0.1, CH_3OH); ^1H NMR (mixture of rotamers) 7.51–7.23 (m, 5H), 5.86–5.82 (m, 1H), 5.48–5.41 (m, 2H), 4.59 (d, *J*=11.9 Hz, 1H), 4.47–4.40 (m, 1H), 4.29–4.25 (m, 1H), 3.96 (d, *J*=10 Hz, 1H), 3.85 (s, 1H), 3.80 (s, 1H), 3.65 (d, *J*=15.2 Hz, 1H), 3.31 (s, 1H), 2.83 (d, *J*=14.1 Hz, 1H), 2.19 (s, 3H); ^{13}C NMR rotamer A 175.7, 139.8, 137.1, 129.5, 129.1, 128.7, 121.4, 77.3, 71.2, 70.5, 69.9, 69.8, 66.1, 39.7, 22.2; rotamer B 174.3, 140.0, 137.4, 129.4, 129.1, 128.7, 120.5, 78.3, 71.9, 71.2, 71.1, 70.0, 60.0, 46.3, 22.2; HRMS (FAB) *m/z* 344.1482 (M+Na), calcd for $C_{17}H_{23}NO_5Na$ 344.1468.

4.1.3. 1-Acetyl-2-(1-benzyloxyallyl)-3,4,5-benzyloxypiperidine 15. To a solution of triol **14** (180 mg, 0.60 mmol) in 10 mL DMF at 0 °C was added sodium hydride (95%, 86 mg, 3.4 mmol), After stirring at 0 °C for 30 min, 0.5 mL of benzyl bromide was added and the solution was stirred for 2 h, diluted with ethyl acetate, washed with satd NaCl, dried and concentrated in vacuo giving a residue, which was subjected to column chromatography (silica gel, 1:2 EA/hexane) to give 330 mg (94%) of **15**. $[\alpha]_D^{25}$ +35.8 (*c*, 0.17, $CHCl_3$); ^1H NMR (mixtures of rotamers) 7.32–7.12 (m, 20H), 5.78–5.70 (m, 0.4H), 5.62–5.55(m, 0.6H), 5.31 (d, *J*=10.2 Hz, 0.6H), 5.25 (d, *J*=10.1 Hz, 0.4H), 5.16 (t, *J*=15.3 Hz, 1H), 4.72 (d, *J*=11.7 Hz, 1.3H), 4.68 (d, *J*=12.4 Hz, 1.2H), 4.60 (d, *J*=12.1 Hz, 1H), 4.54 (m, 2.2H), 4.41 (m, 2.9H), 4.21(d, *J*=12.0 Hz, 0.6H), 4.07 (m, 1.5H), 3.92 (d, *J*=9.2 Hz, 0.6H), 3.77 (m, 0.4H), 3.61 (m, 2.5H), 3.51 (m, 1H), 2.75 (d, *J*=14.7 Hz, 0.6H), 2.15 (s, 2.3H), 2.11 (s, 1.3H); ^{13}C NMR rotamer A 171.6, 138.1, 137.8, 128.0, 127.7, 127.1, 120.7, 75.5, 73.1, 73.0, 71.2, 70.7, 70.2, 69.5, 61.6, 35.6, 21.6; rotamer B 170.6, 137.7, 137.4, 127.9, 127.5, 127.4, 118.2, 81.1, 78.6, 78.3, 74.3, 73.4, 72.8, 70.8, 56.2, 45.1, 21.3; HRMS (FAB) *m/z* 614.2879 (M+Na), calcd for $C_{38}H_{41}NO_5Na$ 614.2877.

4.1.4. 2-(1-Benzyloxy-3-hydroxypropyl)-3,4,5-benzyloxypiperidine 16. To a solution of **15** (110 mg, 0.186 mmol) in 3 mL of THF was added $BH_3\cdot THF$ (0.5 mmol) at 0 °C. The resulting solution was stirred at 0 °C for 3 h and 0.5 mL of 3 M NaOH and 1 mL of 30% hydrogen peroxide were added. The solution was stirred at 25 °C for 3 h and extracted with $CHCl_3$. The $CHCl_3$ extracts were dried and concentrated in vacuo giving a residue, which was subjected to column chromatography (silica gel, 1:2 acetone/hexane) to give 33 mg (31%) of **16**. ^1H NMR 7.36–7.21 (m, 20H), 4.99 (d, *J*=10.7 Hz, 1H), 4.91 (d, *J*=11.1 Hz, 1H), 4.82 (d, *J*=10.7 Hz, 1H), 4.69 (d, *J*=10.9 Hz, 1H), 4.64 (d, *J*=11.7 Hz, 1H), 4.50 (d, *J*=11.1 Hz, 1H), 4.35 (d, *J*=11.1 Hz, 1H), 4.09 (d, *J*=11.1 Hz, 1H), 4.05 (d, *J*=6 Hz, 1H), 3.75 (t, *J*=11.4 Hz, 2H), 3.58 (t, *J*=9 Hz, 1H), 3.51 (m, 1H), 3.46 (t, *J*=9.5 Hz, 1H), 3.35 (m, 1H), 3.19 (dd, *J*=8.1 Hz, 13.8 Hz, 1H), 2.49–2.43 (m, 2H), 2.16 (m,1H); ^{13}C NMR 138.5, 138.3, 138.2, 137.9, 128.4, 128.0, 127.9, 127.9, 127.7, 127.5, 87.1, 80.8, 79.8, 75.8, 75.2, 72.8, 72.8, 70.6, 62.3, 55.3, 46.6, 34.2; HRMS (FAB) *m/z* 590.2894 (M+Na), calcd for $C_{36}H_{41}NO_5Na$ 590.2877.

4.1.5. 1,6,7,8-Tetrabenzyloxyindolizidine 17. A solution of **16** (20 mg, 0.035 mmol) in 2 mL THF containing PPh_3 (14 mg, 0.053 mmol), DEAD (9 mg, 0.05 mmol) was stirred at 25 °C for 12 h, diluted with satd $NaHCO_3$, and extracted with ethyl acetate. The ethyl acetate extracts were washed with satd NaCl, dried and concentrated in vacuo giving a residue, which was subjected to column chromatography (silica gel, 1:2 EA/hexane) to yield 18 mg (93%) of **17**. $[\alpha]_D^{25}$ +35.2° (*c*, 0.01, $CHCl_3$) (lit.[10] $[\alpha]_D^{25}$ +32 (*c*, 1.0, $CHCl_3$)); the spectroscopic data for this substance matched those previously reported.[10] ^1H NMR (C_6D_6) 7.43–7.16 (m, 20H), 5.18 (d, *J*=6.5 Hz, 1H), 5.15 (d, *J*=6.9 Hz, 1H), 5.01 (d, *J*=11.3 Hz, 1H), 4.92 (d, *J*=11.7 Hz, 1H), 4.57 (abq, *J*=12 Hz, 2H), 4.40 (d, *J*=11.7 Hz, 1H), 4.27 (t, *J*=9.2 Hz, 1H), 4.18 (d, *J*=11.7 Hz, 1H), 4.02–3.97 (m, 1H), 3.89 (ddd, *J*=5.0, 4.2, 9.5 Hz,1H), 3.76 (t, *J*=8.9 Hz, 1H), 3.24 (dd, *J*=5.0, 10.4 Hz, 1H), 2.97 (m, 1H), 2.08 (dd, *J*=4.9, 9.4 Hz, 1H), 1.99 (t, *J*=10.3 Hz, 1H), 1.89 (dd, *J*=8.6, 17.3 Hz, 1H), 1.86–1.79 (m,1H), 1.75–1.69 (m,1H); ^{13}C NMR 139.1, 138.9, 138.4, 138.1, 128.3, 128.3, 128.2, 128.1, 127.8, 127.4, 87.5, 79.1, 77.3, 75.6 (2), 74.2, 72.8, 71.6, 70.5, 54.5, 52.4, 29.7.

4.1.6. (+)-Castanospermine. A solution of **17** (15 mg, 0.027 mmol) in 3 mL ethyl acetate and 3 mL MeOH containing palladium chloride (22 mg, 0.013 mmol) under an atmosphere of hydrogen (1 atm) was stirred for 4 h at 25 °C, filtered through Celite. The filtrate was concentrated in vacuo giving a residue, which was subjected to ion-exchange chromatography (Dowex 1-X8, OH^- form, 100–200 mesh, eluted with water) to yield 5 mg (91%) of (+)castanospermine. $[\alpha]_D^{25}$ +20.6 (*c*, 0.05, H_2O) (lit.[10] $[\alpha]_D^{25}$ +70 (*c*, 0.33, H_2O)). The ^1H and ^{13}C NMR spectra of the synthetic material matched those reported previously.[10] ^1H NMR (D_2O) 4.24 (m, 1H), 3.64 (m, 2H), 3.35 (m, 1H), 3.19 (m, 1H), 3.14 (m, 1H), 2.35(m, 1H), 2.23 (m, 1H), 2.10–2.02 (m, 2H), 1.74–1.72 (m, 1H); ^{13}C NMR 79.1, 71.4, 70.2, 69.7, 69.0, 55.4, 51.6, 32.8.

4.1.7. 2-(1-Benzyloxy-2,3-dihydroxypropyl)-3,4,5-benzyloxypiperidine 21. To a solution of **15** (290 mg, 0.049 mmol) and NMO (172 mg, 1.47 mmol) in 30 mL acetone was added OsO_4 (0.7 mL, 7 mg in 10 mg/ml 1:1 acetone/water). The resulting solution was stirred for 4 h at 25 °C diluted with 3 mL satd $Na_2S_2O_5$, stirred for 30 min,

and filtered. The filtrate was concentrated in vacuo giving a residue, which was subjected to column chromatography (silica gel, 1:1 acetone/hexane) to yield 240 mg (78%) **21** along with 20 mg (6.5%) of the uncharacterized diastereomeric diol. $[\alpha]_D^{25}$ +50.8 (*c*, 0.14, $CHCl_3$); ^1H NMR 7.35–7.17 (m, 20H), 5.43 (s, 1H), 4.99 (d, $J=11.7$ Hz, 1H), 4.80 (abq, $J=6.5$ Hz, 2H), 4.63 (dd, $J=4.2$, 11.6 Hz, 2H), 4.55 (d, $J=9.2$ Hz, 1H), 4.50 (d, $J=8.3$ Hz, 1H), 4.39 (d, $J=11.4$ Hz, 1H), 4.07 (d, $J=11.4$ Hz, 1H), 3.75 (d, $J=6.4$ Hz, 1H), 3.73–3.66 (m, 7H), 3.38 (br s, 1H), 2.16 (s, 3H); ^{13}C NMR 173.2, 138.1, 137.9, 137.4, 128.5, 128.4, 128.0, 127.8, 127.6, 127.3, 85.3, 81.7, 79.2, 75.1, 74.7, 74.6, 74.3, 71.4, 70.1, 63.5, 57.0, 47.2, 21.3; HRMS (FAB) *m/z* 648.2912 (M+Na), calcd for $C_{38}H_{43}NO_7Na$ 648.2932.

4.1.8. 1,6,7,8-Tetrabenzyloxy-2-hydroxyindolizidine 22. A solution of **21** (120 mg, 0.19 mmol) in 4 mL THF and 4 mL 6 N HCl was stirred at 70 °C for 4 h, cooled and concentrated in vacuo to give a residue. A solution of the residue in 3 mL anhydrous pyridine containing 0.2 g molecular sieves, PPh_3 (63 mg, 0.24 mmol) and DEAD (42 mg, 0.24 mmol) was stirred at 0 °C for 4 h, diluted with satd $NaHCO_3$ and extracted with $CHCl_3$.The $CHCl_3$ extracts were washed with satd NaCl, dried and concentrated in vacuo to yield a residue, which was subjected to column chromatography (silica gel, 1:2 EA/hexane) to give 80 mg (74%) of **22**. $[\alpha]_D^{25}$ +40 (*c*, 0.032, $CHCl_3$); ^1H NMR 7.49–7.18 (m, 20H), 5.07 (d, $J=11.3$ Hz, 1H), 5.03 (d, $J=11.0$ Hz, 1H), 4.83 (d, $J=10.9$ Hz, 1H), 4.69–4.64 (m, 4H), 4.58 (d, $J=11.0$ Hz, 1H), 4.32 (br s, 1H), 4.18 (t, $J=5.6$ Hz, 1H), 3.90 (m, 1H), 3.78 (m, 1H), 3.62 (m, 1H), 3.29 (dd, $J=4.8$, 10.5 Hz, 1H), 3.22 (br s, 1H), 3.04 (d, $J=8.9$ Hz, 1H), 2.35 (dd, $J=6.3$, 10.3 Hz, 1H), 2.15 (m, 1H), 1.94 (t, $J=11.9$ Hz, 1H); ^{13}C NMR 138.9, 138.7, 138.3, 137.2, 128.5, 128.4, 128.4, 127.9, 127.1, 87.6, 79.1, 78.1, 76.8, 75.5, 74.4, 74.2, 72.8, 70.4, 69.9, 62.7, 54.2; HRMS (FAB) *m/z* 588.2702 (M+Na), calcd for $C_{36}H_{39}NO_5Na$ 588.2720.

4.1.9. 1,2,6,7,8-Pentahydroxyindolizidine 20. A solution of **22** (32 mg, 0.057 mmol) in 3 mL EA and 3 mL MeOH containing palladium chloride (22 mg, 0.013 mmol) under an atmosphere of hydrogen (1 atm) was stirred at 25 °C for 4 h and filtered through Celite. The filtrate was concentrated in vacuo giving a residue, which was subjected to ion-exchange chromatography (Dowex 1-X8, OH^- form, 100–200 mesh, eluted with water) to yield 11 mg (94%) of **20**. $[\alpha]_D^{25}$ +37.9 (*c*, 0.013, H_2O) (lit.[11] $[\alpha]_D^{25}$ +66.5 (*c*, 1.33, H_2O)). The ^1H and ^{13}C NMR spectra of this substance matched those reported earlier.[11] ^1H NMR (D_2O) 4.26 (m, 1H), 4.08 (m, 1H), 3.50–3.41 (m, 2H), 3.13 (t, $J=9.1$ Hz, 1H), 2.97 (dd, $J=5.1$, 10.8 Hz, 1H), 2.73 (dd, $J=2.1$, 11.0 Hz, 1H), 2.48 (dd, $J=8.1$, 10.7 Hz, 1H), 2.00 (dd, $J=3.5$, 9.7 Hz, 1H), 1.90 (t, $J=10.6$ Hz, 1H); ^{13}C NMR (D_2O) 81.1, 72.6, 72.2, 72.0, 71.6, 71.1, 61.8, 57.6.

4.1.10. 1,6,7,8-Tetrabenzyloxy-2-hydroxyindolizidine 23. A solution of **22** (60 mg, 0.11 mmol), benzoic acid (26 mg, 0.22 mmol), PPh_3 (58 mg, 0.022 mmol) and DEAD (37 mg, 0.22 mmol) in 3 mL THF was stirred at 25 °C for 12 h, diluted with satd $NaHCO_3$ and extracted with $CHCl_3$. The $CHCl_3$ extracts were washed with satd NaCl, dried and concentrated in vacuo to give a residue, which was subjected to column chromatography (silica gel, 1:2 EA/hexane) to yield 62 mg of the inverted benzoate ester. ^1H NMR 8.06 (d, $J=8.1$ Hz, 2H), 7.56–7.50 (m, 1H), 7.50–7.34 (m, 2H), 7.33–7.13 (m, 20H), 5.49 (m, 1H), 5.01 (d, $J=11.0$ Hz, 1H), 4.93 (d, $J=11.5$ Hz, 1H), 4.86 (t, $J=11.5$ Hz, 2H), 4.71 (d, $J=11.0$ Hz, 1H), 4.59 (abq, $J=11.0$ Hz, 2H), 4.37 (m, 1H), 4.18 (d, $J=4.5$ Hz, 1H), 3.92 (t, $J=9.1$ Hz, 1H), 3.85–3.70 (m, 1H), 3.65–3.55 (m, 2H), 3.31 (dd, $J=4.2$ Hz, 9.4 Hz, 1H), 2.5 (dd, $J=4.1$, 9.5 Hz, 1H), 2.35 (dd, $J=5.2$, 10.2 Hz, 1H), 2.12 (t, $J=10.3$ Hz, 1H); ^{13}C NMR 165.6, 138.7 (2), 138.2, 137.3, 133.2, 129.5, 128.3, 128.2, 128.1, 127.8, 127.7, 127.6, 127.5, 127.3, 87.2, 82.1, 78.8, 76.4, 76.2, 75.5, 74.5, 72.8, 71.3, 69.3, 58.7, 54.2.

A solution of the inverted ester (60 mg, 0.11 mmol) in 10 mL MeOH containing sodium methoxide (54 mg, 1 mmol) was stirred for 12 h at 25 °C, diluted with 1 mL of water, and concentrated in vacuo giving a residue, which was subjected to column chromatography (silica gel, 1:1 acetone/hexane) to yield 44 mg (73%) of **23**. $[\alpha]_D^{25}$ +16.2 (*c*, 0.04, $CHCl_3$); ^1H NMR 7.33–7.20 (m, 20H), 4.99 (d, $J=10.9$ Hz, 1H), 4.84 (abq, $J=11.1$ Hz, 2H), 4.68–4.60 (m, 4H), 4.50 (d, $J=11.6$ Hz, 1H), 4.32 (t, $J=6.2$ Hz, 1H), 3.89 (dt, $J=4.6$, 9.2 Hz, 2H), 3.73–3.68 (m, 1H), 3.60–3.52 (m, 2H), 3.26 (dd, $J=4.9$, 10.5 Hz, 1H), 2.45 (dd, $J=4.6$, 9.3 Hz, 1H), 2.08–2.03 (m, 2H); ^{13}C NMR 139.0, 138.8, 138.3, 137.6, 128.3, 128.3, 127.9, 127.8, 127.3, 87.4, 85.2, 78.9, 77.0, 75.6, 75.3, 72.8, 71.3, 69.3, 61.7, 54.3; HRMS (FAB) *m/z* 588.2740 (M+Na), calcd for $C_{36}H_{39}NO_5Na$ 588.2720.

4.1.11. 1,2,6,7,8-Pentahydroxyindolizidine 19. A solution of **23** (42 mg, 0.074 mmol) in 3 mL EA and 3 mL MeOH containing palladium chloride (22 mg, 0.013 mmol). The mixture under an atmosphere of hydrogen (1 atm) was stirred at 25 °C for 4 h and filtered through Celite. The filtrate was concentrated in vacuo giving a residue, which was subjected to ion-exchange chromatography (Dowex 1-X8, OH^- form, 100–200 mesh, eluted with water) to yield 15 mg (98%) of **19**, mp 114–116 °C, $[\alpha]_D^{25}$ +22.7 (*c*, 0.07, H_2O); ^1H NMR (D_2O) 4.21 (t, $J=6.3$ Hz, 1H), 4.12 (d, $J=4.3$ Hz, 1H), 3.64–3.58 (m, 2H), 3.51 (dd, $J=7.1$, 10.3 Hz, 1H), 3.38 (t, $J=9.1$ Hz, 1H), 3.19 (dd, $J=5.2$, 10.9 Hz, 1H), 2.37 (dd, $J=4.4$, 10.2 Hz, 1H), 2.19–2.14 (m, 2H); ^{13}C NMR 81.2, 79.6, 78.9, 72.3, 71.7, 70.9, 61.9, 57.7; HRMS (FAB) *m/z* 228.0841 (M+Na), calcd for $C_8H_{15}NO_5Na$ 228.0842.

Acknowledgements

Financial support for this work by the National Science Foundation and a generous gift from Dijindo Molecular Technologies is greatly appreciated. We thank Professor Stephen G. Pyne for informative discussion about this effort and Professor George W. J. Fleet for kindly providing spectra for indolizidine **20**.

Supplementary data

Supplementary data associated with this article can be

found, in the online version, at doi:10.1016/j.tet.2005.07.014. Contained in the supplementary data are ^{1}H and ^{13}C NMR spectra for compounds **13–17**, **19–23**, and synthetic (+)-castanospermine.

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Tetrahedron 61 (2005) 8895–8903

Mukaiyama aldolisation reactions of α,β-epoxyaldehydes in aqueous media

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Received 11 May 2005; revised 4 July 2005; accepted 6 July 2005

Available online 28 July 2005

Abstract—The Mukaiyama aldolisation reaction in aqueous media of cis and trans α,β-epoxyaldehydes with *tert*-butyldimethylsilyl ketene acetal in the presence of Lewis's acids was studied. $Sc(OTf)_3$ gave the best results in terms of selectivity. The same reaction of cis and trans α,β-epoxyaldehydes with the enoxysilane of ethyl pyruvate resulted in epoxy substituted ulosonic derivatives issued from a double sequential condensation of the pyruvate on the epoxy derivatives.

1. Introduction

Use of water as a reaction solvent has received considerable attention in synthetic organic chemistry especially in relation to today's environmental concerns and the aim of reducing the use of harmful organic reagents. Numerous reactions have been performed in the last 10 years in this economical solvent, generally realised under mild conditions with respect to organic solvents. Recent developments include pericyclic reactions, transition metal catalyzed reactions, radical ones, oxidations and reductions, carbonyl additions.[1] Nucleophilic additions to carbonyl compounds, one of the major fields in organic synthesis make use of strong nucleophiles or weak ones in the presence of Lewis acid activators. While Lewis acid promoted reactions in organic solvents need strictly anhydrous conditions, during the last years rare earth metal triflates and some other metal salts have been found to catalyze aldol reactions in water systems.[2]

In the course of our ongoing research programme directed towards construction of 1,2,3 polyhydroxylated frames, useful synthons in the elaboration of ulosonic compounds and peptidonucleosides,[3] we developed a method based on the stereocontrolled addition on the α,β-epoxyaldehydes. In that respect, we have extensively studied the issue and the diastereoselectivity of the aldol reaction of various α,β-epoxyaldehydes[4a] with lithium enolates issued from acetates. The same general trends in terms of selectivity were observed under Mukaiyama conditions in organic solvents.[4b] On the other hand, we have reported a direct synthesis of bicyclic precursors of ulosonic esters[5a] and furanosidic fluorinated ulosonic analogues[5b] by reaction of cis and trans α,β-epoxyaldehydes, respectively, with ethyl 2-(trimethylsilyloxy)-2-propenoate in the presence of boron trifluoride diethyl etherate.

Terminal epoxides have recently been used in water reactions in order to derivatize sucrose by grafting a α,β(or α)-di (or mono)hydroxylated fatty chain.[6]

We wish to describe in this report, our first results on the aqueous Mukaiyama aldol reactions of α,β-epoxyaldehydes with enol silanes issued from *tert*-butyl acetate and ethyl pyruvate, an issue that has never been addressed before.

α,β-Epoxyaldehydes **4** and **5** were synthesized as reported previously[4a] while benzylated racemic cis epoxyaldehyde **6** was obtained by reaction with *m*-cpba.[7]

tert-Butyldimethylsilyl ketene acetal **7** was effectively obtained in high yield (93%) by reacting lithium *tert*-butyl acetate with *tert*-butyldimethyl silyl chloride in THF at 0 °C in the presence of HMPA.[4b]

Ethyl 2-(trimethylsilyloxy)-2-propenoate **13** was prepared in 70% yield from the corresponding pyruvate by treatment with trimethylsilylchloride and triethylamine in the presence of a catalytic amount of DMAP.[8]

Lewis acid catalysis of the aldolisation reaction in water

Keywords: Mukaiyama aldolisation reaction; Ulosonic esters; α,β-Epoxyaldehydes.
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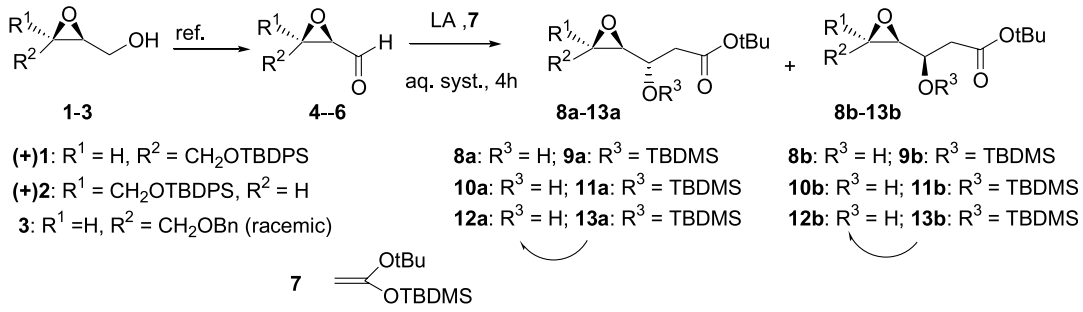
doi:10.1016/j.tet.2005.07.027



Scheme 1.

(Scheme 1), was first examined in the reaction of cis α,β-epoxyaldehyde **4** with ketene silyl acetal **7**. Different experimental conditions were evaluated (Table 1). Where lanthanide triflates were used in water, the reaction operated in the presence of an anionic surfactant, sodium dodecyl sulfate (SDS). When the commercialized, scandium tris (dodecylsulfate) (STDS) or when a mixture THF/H_2O were used, SDS was not introduced.

A typical experimental procedure consisted in adding a surfactant solution containing the Lewis acid to the α,β-epoxyaldehyde, followed by the addition of ketene silyl acetal **7** and allowing the reaction to proceed for 4 h before quenching with saturated aq NaCl. The crude material was purified on silica gel and/or analyzed by analytical chromatography. In the latter case, yields were calculated from analytical data and by ^{1}H NMR spectroscopy.

While the reaction proceeded sluggishly in the presence of a catalytic amount of a lanthanide triflate, a certain amount of organic solvent (THF) and in the absence of any micellar system (Table 1, entry 1), it proceeded smoothly when SDS was used (Table 1, entries 2–5). In fact, the SDS/H_2O system led to the obtention of the aldol adducts in moderate yields. Europium and lanthanum triflates afforded same results in terms of yields (25 and 26%, respectively), and diastereoselectivity of the reaction (*anti*/*syn* 90/10 and 91/9, respectively). The aldolisation reaction in the presence of $Yb(OTf)_3$ led to a slightly better yield and diastereoselectivity, while the best results were obtained when operating in the presence of a catalytic amount of $Sc(OTf)_3$ in the H_2O/SDS micellar system (41% yield and 94:6 *anti*/*syn* diastereoface preference). In each case studied, about 15% of unreacted aldehyde was observed by ^{1}H NMR spectroscopy. Additional amount of ketene silylacetal **7** (2 equiv) did not lead to better results. On the other hand, prolonged reaction times (till 15 h) afforded degradation products that do not bear the epoxide ring and the yield of the aldol adducts highly decreased in each case. Finally, it is noteworthy, that when the aldolisation reaction in the presence of $Sc(OTf)_3$/SDS/H_2O system was stopped after 2 h (40% of unreacted aldehyde detected by analytical liquid chromatography), the same *anti* diastereoface preference was observed (*anti*/*syn* 94/6).

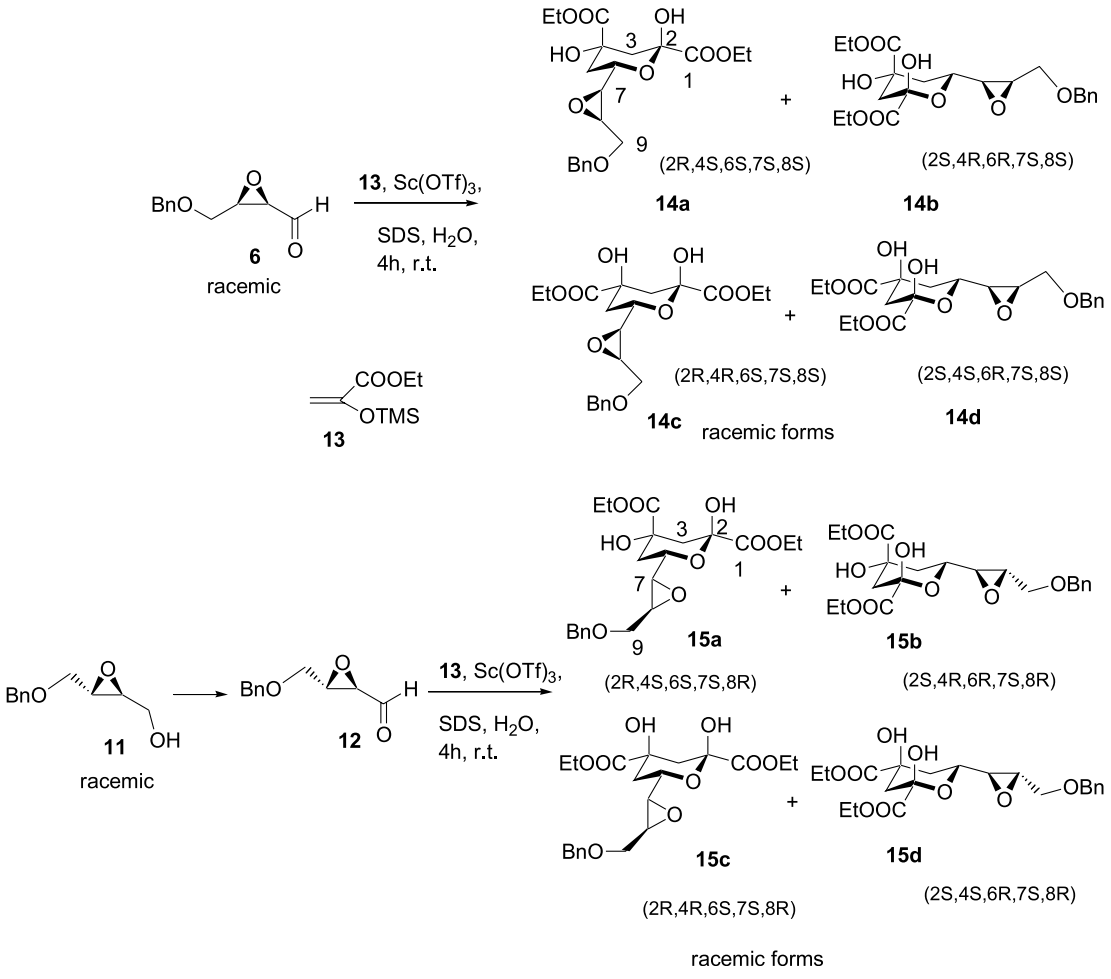
Some other systems have also been examined. Use of the Lewis acid-surfactant combined catalyst (LASC) scandium tris (dodecyl sulfate) STDS,[9] led to similar yields of aldol adducts as for $Sc(OTf)_3$ but to a lower diastereoselectivity (Table 1, entry 6). We also explore the possibility to enhance the reactivity of the reaction by adding a Brönsted acid. This has been recently reported in the case of aldol reactions in water between a benzaldehyde and silyl enolates in the presence of LASC catalysts.[10] Disappointingly, in the case of α,β-epoxyaldehyde **4**, reactions in the presence of a Brönsted acid (HCl, TsOH) and a lanthanide triflate or STDS were very sluggish (Table 1, entries 7–9), leading to degradation products and to invariably low yields of aldol adducts. Moreover, when the reaction mixture was quenched and examined in shorter periods (1 h), results showed essentially starting material and degradation products (absence of the epoxide ring by ^{1}H NMR spectroscopy).

Reaction was also tested with α,β-epoxyaldehydes **5**, **6**, by operating in the most favorable systems in terms of reactivity and selectivity. For trans epoxyaldehyde **5** reaction in the presence of $Sc(OTf)_3$ or $La(OTf)_3$ gave in

Table 1. Aqueous Mukaiyama aldol reaction of aldehydes **4–6** with **7** under different systems

Entry	Aldehyde	Lewis acid	System used	Yield%	*Anti*/*syn*
1	**4**	$Yb(OTf)_3$	H_2O/THF	<5	nd
2	**4**	$Eu(OTf)_3$	H_2O/SDS	25	90/10
3	**4**	$La(OTf)_3$	H_2O/SDS	46[a]	91/9
4	**4**	$Sc(OTf)_3$	H_2O/SDS	41[a]	94/6
5	**4**	$Yb(OTf)_3$	H_2O/SDS	33	94/6
6	**4**	STDS	H_2O	38	90/10
7	**4**	$La(OTf)_3$	H_2O/SDS/HCl	<5	nd
8	**4**	$La(OTf)_3$	H_2O/SDS/TsOH	<5	nd
9	**4**	STDS	H_2O/HCl	<5	nd
10	**5**	$Sc(OTf)_3$	H_2O/SDS	35[a]	73/27
11	**5**	$La(OTf)_3$	H_2O/SDS	33	67/33
12	**6**	$Sc(OTf)_3$	H_2O/SDS	40[a]	93/7
13	**6**	$La(OTf)_3$	H_2O/SDS	35	90/10

[a] After silica gel purification.



Scheme 2.

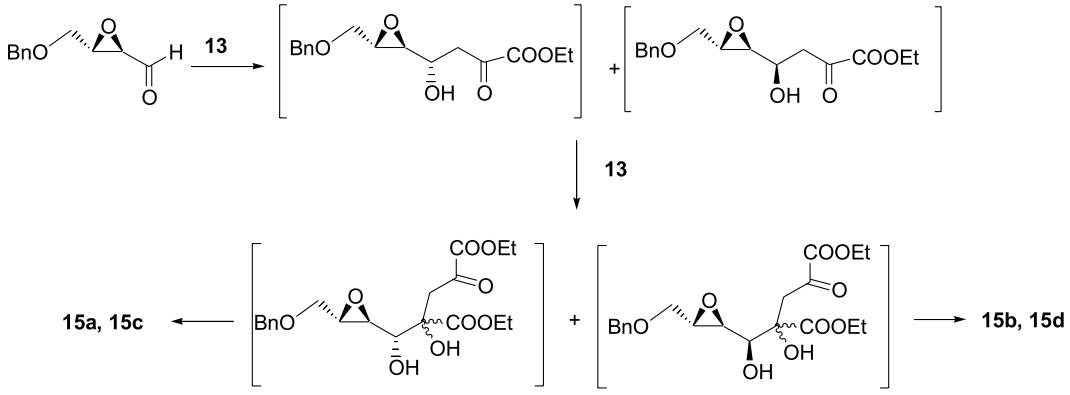
moderate yields the aldol compounds in a 3:1 diastereoisomeric ratio in favor of the *anti* derivative (Table 1, entries 10 and 11). Concerning the aldol reaction with cis benzylated epoxyaldehyde **6**, in order to better analyze the results, after determining by ^{1}H NMR spectroscopy the amount of non reacted aldehyde, the mixture was treated with SiO_2 supported *n*-Bu_4NF; the two separable hydroxy containing diastereoisomers were then analyzed. The $Sc(OTf)_3$/SDS/H_2O system afforded in 40% total yield the aldol adducts in a 93/7 *anti*/*syn* ratio, results similar to that obtained with aldehyde **4**.

The Mukaiyama aldolisation reaction in aqueous media was also examined by reacting enol silane issued from ethyl pyruvate with cis α,β-epoxyaldehyde **6** (Scheme 2). The reaction is triggered by adding ethyl 2-(trimethylsilyloxy)-2-propenoate **13** in a mixture of SDS, $Sc(OTf)_3$, and aldehyde in water and allowed to proceed for 4 h before quenching. After two silica gel purifications of the complex reaction mixture, two fractions were isolated. Analytical chromatography of each fraction (C18 column $d=3$ mm, eluant H_2O/CH_3CN 65:35, flow rate 0.6 ml/mn, UV 220 nm) revealed the presence of two compounds. They have been identified by mass spectrometry and NMR as the modified ulosonic esters **14a**, **14b** and **14c**, **14d** obtained in 35% yield and in a ratio of 6:3:1:0.5.

The same reaction has also been performed with trans α, β-epoxyaldehyde **12** obtained in its racemic form in five steps and 56% total yield from the *cis*-buten-1,4-diol. Again, purification of the complex reaction mixture afforded two fractions revealing the presence of two compounds in each one. The products have been identified as before, as the modified ulosonic esters[11] **15a**, **15b**, and **15c**, **15d** obtained in 29% yield and in a ratio of 10:4:2:0.4.

Compounds **15a**–**15b** have been analyzed through extensive NMR analysis (^{1}H, 2D COSY, ^{13}C, ^{13}C DEPT, ^{1}H–^{13}C HMBC, and HSQC). Each compound shows large coupling constants between H_6 and H_{5ax} protons indicating a trans axial disposition of these two protons. A ^{4}J coupling constant appears between H_{3eq} and H_{5eq} protons. Chemical shifts for proton and carbon atoms for the couple (**a**,**b**) are almost identical indicating that axial/equatorial position of the substituents are the same. We may thus conclude that compounds **a**,**b** are in a chair conformation where the lateral epoxide ring is in equatorial position.

The ^{13}C chemical shift of the C2 carbon atom ($\delta=94.8$ ppm for **a** and **b**) is relevant of a pyranosidic ulosonic functionality while the C4 quaternary carbon atom resonates at $\delta=71.3$ ppm. Assuming that in ulosonic esters, the most favorable position of the C1 ester group is equatorial, the



Scheme 3.

relative configuration of the C4 quaternary carbon atoms is determined by the C3 proton chemical shifts. In fact, the chemical shifts of H3a, H3eq protons are inverted when changing the axial/equatorial position of the C2/C4 hydroxy groups (δ_{H3eq} greater than δ_{H3ax}), to an axial/axial disposition. This is in accordance with the findings in ulosonic acids[12] and with our previous work on modified methyl quinate compounds.[13] Nevertheless, we cannot still determine if compounds **a/b** are issued from the *anti*/*syn* aldolisation adducts.

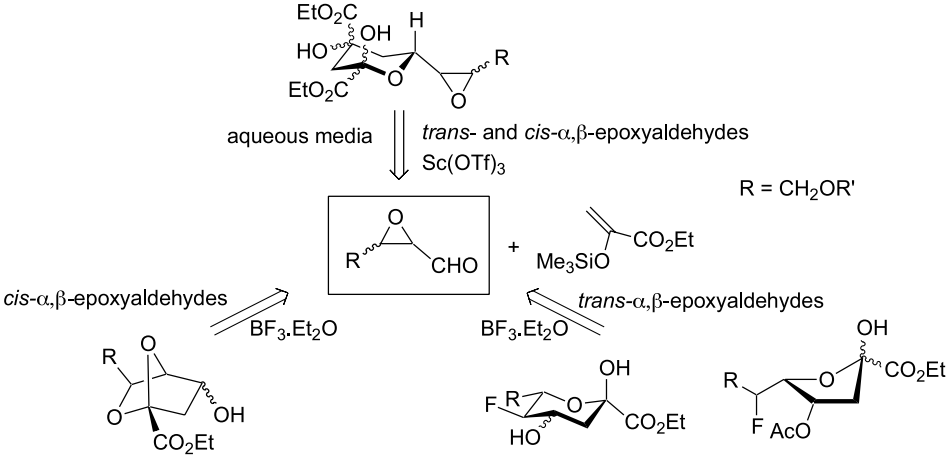
These modified ulosonic esters are issued from the sequential double condensation of the ethyl 2-(trimethylsilyloxy)-2-propenoate **13** on the aldehyde. Mukaiyama aldolisation reaction leads first to the two *anti*/*syn* adducts. A second reaction occurs between the carbonyl group and **13** leading to four diastereoisomeric intermediates that can then favorably cyclise affording the final adducts (Scheme 3).

Other reaction conditions have been used in order to isolate a monocondensation adduct (1 equiv of **13**, its portionwise addition, reaction time). The five-fold (0.2 equiv) portionwise addition of **13** (time interval 1 h) led to a complex reaction mixture possessing a weak peak in mass spectrometry ($m/z=328$ M+18), corresponding to the monocondensation adduct (that could not be isolated). The known propensity of pyruvate to autocondense is operating in the system studied here.[14]

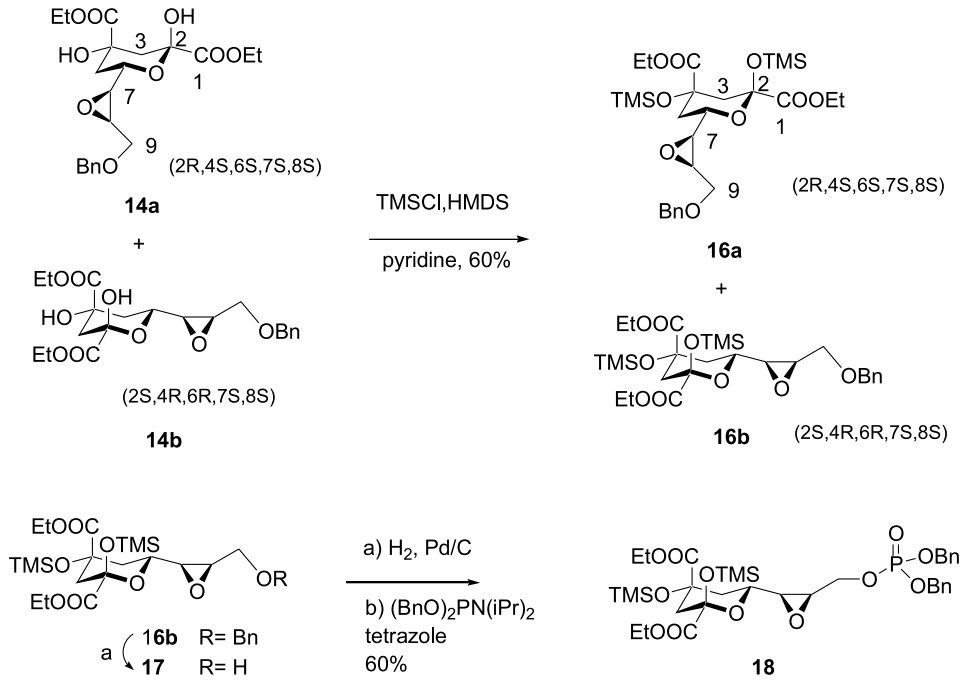
The aqueous Mukaiyama aldol reaction leads in this case to results completely different from the reaction in organic media (Scheme 4). In fact, we have shown earlier that in CH_2Cl_2 and when using $Et_2O\cdot BF_3$ (the only Lewis acid effectively operating), reaction with trans α,β-epoxyaldehydes yields essentially fluorinated ulosonic compounds,[5b] while cis α,β-epoxyaldehydes afford in a most efficient manner bicyclic compounds.[5a] Both adducts are issued from a monocondensation reaction of enolpyruvate with the corresponding aldehyde. This represents one of the rare examples where a Mukaiyama aldolisation reaction between same reactants, offers three different reaction pathways and final products depending on the geometry of the starting allylic alcohol (affording cis or trans α,β-epoxyaldehyde) and the reaction medium (organic, aqueous).

Some further functionnalisations have been performed on the **14a**,**b** compounds (Scheme 5). Silylation of the tertiary hydroxy functions was accomplished by reacting the mixture with TMSCl/HMDS in pyridine. The fully silylated compounds **16a** and **16b** were obtained individually after purification by silica gel chromatography in 60% total yield. Compound **16b** was then debenzylated and the primary alcohol **17** was phosphorylated in the presence of *N*,*N*-diisopropylamino-dibenzyl phosphoramidite followed by *m*-cpba oxidation, affording in 55% yield (two steps) the phosphorylated modified ulosonic ester **18**.

In summary, we have further extended the study on the



Scheme 4.



Scheme 5.

aldolisation reaction involving α,β-epoxyaldehydes. Rare earths functioning as Lewis acids in aqueous media can induce the aldolisation reaction of ketene silyl acetal. The issue and the diastereoselectivities observed concerning the aldol adducts remain the same in comparing with reactions operating in organic media (nucleophilic or Mukaiyama aldolisation reaction), while yields are lower. Rare earths can also perform the aqueous aldolisation reaction with the labile enoxysilane of ethylpyruvate, leading to new derivatives of ulosonic esters.

Further studies are in progress in order to optimize and extend the utility of the aqueous Mukaiyama aldol reaction involving α,β-epoxyalehydes.

2. Experimental

2.1. General remarks

The following solvents and reagents were dried prior to use: methylene chloride, dimethylformamide (from calcium hydride, stored over 4 Å molecular sieves), triethylamine (from calcium hydride, stored over potassium hydroxide pellets), THF (freshly distilled from sodium/benzophenone). Thin-layer chromatography (TLC) reaction monitoring was carried out with Macherey-Nagel ALUGRAM® SIL G/UV$_{254}$ (0.2 mm) plates visualized with 10% phosphomolybdic acid in ethanol or Dragendorff reagent as dipping solutions. Standard column chromatography was performed with SDS 70–200 μm silica gel. Medium-pressure liquid chromatography was performed with a Jobin-Yvon apparatus using Merck 15–40 μm or Amicon 6–35 μm silica gel. Analytical liquid chromatography was performed with a Spectroflow 400Kratos pump equipped with a UV 759A Applied Biosystems detector and a silica Novapack (15 cm) column (pressure 19 bars). NMR spectroscopic data were obtained with Bruker AC200, AC250, and AC400 instruments operating with ^{1}H spectra at 200, 250, and 400 MHz, respectively, ^{13}C spectra at 50, 63, and 100 MHz, respectively. Chemical shifts are quoted in parts per million (ppm) downfield from tetramethylsilane and coupling constants are in Hertz. Infrared (IR) spectra were recorded on a Perkin-Elmer FT-IR 1725X spectrometer. Mass spectrometry (MS) data were obtained on a NERMAG R10-10 spectrometer.

Synthesis of (2*S*,3*S*) and (2*S*,3*R*)-4-(*tert*-butyldiphenylsilyloxy)-2,3-epoxybutan-1-als **4** and **5** is accomplished in three (and five) steps, respectively, from *cis*-buten-1,4-diol, by using (+)DET as the chiral agent in the Sharpless asymmetric epoxidation reaction.[4a]

The general procedure for synthesis of racemic α,β-epoxyalcohols **3** and **11** has been previously reported.[7] The Döering method[15] has been used to obtain the corresponding aldehydes **6** and **12** as for compounds **4** and **5** starting from the corresponding epoxyalcohols.

2.1.1. (2*R*,3*R*) and (2*S*,3*S*)-4-(Benzyloxy)-2,3-epoxybutan-1-al 6. Aldehyde (1.58 g) was obtained through Döering oxidation of 2 g (10.3 mmol) of alcohol **3** (yield 80%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 9.44 (d, 1H, $J_{1/2}$=4.8 Hz, H1), 7.36–7.28 (m, 5H, phenyl), 4.56 (s, 2H, PhCH_2O), 3.87 (dd, 1H, $J_{4a/3}$=3.5 Hz, $J_{4a/4b}$=11.5 Hz, H4a), 3.73 (dd, 1H, $J_{4b/3}$=4.5 Hz, $J_{4b/4a}$=11.5 Hz, H4b), 3.49 (m, 1H, H3), 3.40 (m, 1H, H2).

^{13}C NMR (50 MHz, $CDCl_3$) δ 197.8 (C1), 137.8 (Cq phenyl), 128.6, 127.8 (CH phenyl), 73.6 (CH_2OPh), 66.2 (C4), 58.0 and 57.4 (C2 and C3).

MS (DCI, NH_3): 210 (MNH_4^+, 100%). IR ($CHCl_3$) ν_{max} 3065–3033 (CH arom.), 2864 (CHO), 1723 (C=O), 1095 (C–O) cm^{-1}. Anal. Calcd for $C_{11}H_{12}O_3$: C, 68.73; H, 6.29. Found: C, 68.41; H, 6.29.

2.1.2. (2*R*,3*R*) and (2*S*,3*S*)-4-(Benzyloxy)-2,3-epoxybutan-1-ol 11. *m*-CPBA epoxidation of the starting allylic alcohol (3 g, 16.84 mmol) afforded 2.95 g (90% yield) of compound **11** after silica gel purification (petroleum ether/ether 3:7).

^1H NMR (250 MHz, $CDCl_3$) δ 7.35–7.28 (m, 5H, phenyl), 4.54 and 4.60 (AB syst, 2H, $J=11.9$ Hz PhCH_2O), 3.92 (dd, 1H, $J_{4a/3}=2.5$ Hz, $J_{4a/4b}=12.5$ Hz, H4a), 3.77 (dd, 1H, $J_{1a/2}=3.05$ Hz, $J_{1a/1b}=11.51$ Hz, H1a), 3.64 (dd, 1H, $J_{4b/4a}=12.73$ Hz, $J_{4b/3}=4.18$ Hz, H4b), 3.53 (dd, 1H, $J_{1b/2}=5.45$ Hz, $J_{1b/1a}=11.52$ Hz, H1b), 3.22–3.24 and 3.09–3.11 (2m, 2H, H2, and H3).

^{13}C NMR (50 MHz, $CDCl_3$) δ 137.8, 128.5, 127.9 (C arom., OBn), 73.4 (CH_2OBn), 69.7 (C4), 61.3 (C1), 55.7 and 54.2 (C2 and C3).

Anal. Calcd for $C_{11}H_{14}O_3$: C, 68.02; H, 7.28. Found: C, 67.99; H, 7.28.

2.1.3. (2*S*,3*R*) and (2*R*,3*S*)-4-(Benzyloxy)-2,3-epoxybutan-1-al 12. Aldehyde (1.50 g) was obtained through Döering oxidation of 2 g (10.3 mmol) of alcohol **11** (yield 75%).

^1H NMR (250 MHz, $CDCl_3$) δ 9.05 (d, 1H, $J_{1/2}=6.26$ Hz, H1), 7.36–7.28 (m, 5H, phenyl), 4.58 (s, 2H, PhCH_2O), 3.85 (dd, 1H, $J_{4a/3}=2.62$ Hz, $J_{4a/4b}=11.67$ Hz, H4a), 3.58 (dd, 1H, $J_{4b/3}=4.96$ Hz, $J_{4b/4a}=11.66$ Hz, H4b), 3.48 (m, 1H, H3), 3.34 (dd, 1H, $J_{2/3}=1.97$ Hz, $J_{2/1}=6.23$ Hz, H2).

^{13}C NMR (50 MHz, $CDCl_3$) δ 197.7 (C1), 137.4 (Cq phenyl), 128.6, 128.1, 127.9 (CH phenyl), 73.6 (CH_2OPh), 66.2 (C4), 58.0 and 57.4 (C2 and C3).

IR ($CHCl_3$) ν_{max} 3068–3030 (CH arom.), 2869 (CHO), 1724 (C=O), 1090 (C–O) cm^{-1}.

Anal. Calcd for $C_{11}H_{12}O_3$: C, 68.73; H, 6.29. Found: C, 68.91; H, 6.25.

2.1.4. Ethyl 2-(trimethylsilyloxy)-2-propenoate 13. To 200 ml of anhydrous toluene was added successively 24 ml of TMSCl (189.1 mmol, 1.3 equiv), 0.888 g of DMAP (7.27 mmol, 0.05 equiv) and 15.94 ml of ethyl pyruvate (145.46 mmol, 1 equiv). The solution was heated under reflux and 26.3 ml of Et_3N (189.1 mmol, 1.3 equiv) were added dropwise. After 2 h under reflux, the mixture was cooled, filtered rapidly then toluene evaporated off. The remaining crude product was distilled under reduced pressure (90 °C, 50 mbar) affording the desired product (19.2 g, 70% yield).

^1H NMR (250 MHz, $CDCl_3$) δ 5.52 (d, 1H, $J=1.10$ Hz, H3a), 4.88 (d, 1H, $J=1.04$ Hz, H3b), 4.22 (q, 2H, $J=7.18$ Hz, OCH_2CH$_3$), 1.31 (t, 3H, $J=7.18$ Hz, OCH$_2$CH_3), 0.23 (s, 9H, Si(CH_3)$_3$).

^{13}C NMR (50 MHz, $CDCl_3$) δ 164.4 (C1), 147.1 (C2), 103.9 (C3), 61.1 (CH_2 ethyl), 14.1 (CH_3 ethyl), −0.5 (TMS).

IR (neat) ν_{max} 2963 (C=H), 1731 (C=O), 1627 (C=C) cm^{-1}. MS (DCI, NH_3): 206 (MNH_4^+, 100%).

2.2. Procedure for the aldolisation reaction with *tert*-butyl dimethylsilyl ketene acetal 7

A water solution (6 ml) containing the α,β-epoxyaldehyde (1.2 mmol), the Lewis acid (0.1 equiv), SDS (69.2 mg, 0.238 mmol, 0.2 equiv) and (or not) a Brönsted acid (0.1 equiv), was stirred for 10 min. Ketene silyl acetal **7** (0.553 g, 2.4 mmol, 2 equiv) was then added to the mixture, which was stirred vigorously (1250 rpm) for 4 h. The reaction was then quenched by adding 6 ml aq saturated NaCl. Extraction was operated with ethyl acetate (3× 10 ml). The organic phase was dried over $MgSO_4$ and solvent evaporated. Purification and identification of compounds **8–11** was done as previously reported.[4]

Reaction in the absence of a surfactant was performed as before in a THF–H_2O solution (6 ml, 2/1).

Reaction in the presence of STDS was done as before in the absence of SDS and in 12 ml of water.

Concerning the reaction with aldehyde **6**, the final reaction mixture was treated by TBAF on silica gel (1.7 g at about 1.25 mol of fluoride/g; 1.1 mmol) in anhydrous THF (15 ml). The reaction mixture was vigorously stirred for 7 h and filtered. Silica gel was washed several times with ethyl acetate and the combined organic phases concentrated. The crude product was purified by MPLC chromatography affording the known products **12a**, **12b**.[4a]

2.3. Procedure for the aldolisation reaction with ethyl 2-(trimethylsilyloxy)-2-propenoate 13

A water solution (40 ml) containing 1.5 g of α,β-epoxyaldehyde (7.81 mmol, 1 equiv), 0.445 g of SDS (1.54 mmol, 0.19 equiv), 0.385 g of scandium triflate (0.78 mmol, 0.1 equiv), was stirred for 10 min. Compound **13** (2.4 g, 12.7 mmol, 1.6 equiv) was then added and the mixture was vigorously stirred for 5 h, before quenching with potassium chloride (10 equiv) and diluting with ethyl acetate (45 ml). The organic phase obtained was extracted with H_2O (3×30 ml), dried over $MgSO_4$, filtered and the solvent evaporated.

2.3.1. Ethyl 2,4-dihydroxy-4-carbethoxy-6-(9-(benzyloxy)-7,8-*cis*-epoxy)-tetrahydropyran-2-carboxylate 14a–14d. The crude product was first purified by medium pressure silica gel chromatography (eluant petroleum ether/ether 55:45) affording 0.3 g of starting aldehyde. The remaining mixture was then purified by inverse phase chromatography (silica, Hyperprep C18, eluant H_2O/CH_3CN 70:30). Two fractions could be isolated containing compounds **14a**,**14b** (792 mg) and **14c**,**14d** (132 mg).

R_f: 0,4 (PE/AcOEt 5:5).

Analytical HPLC: C18, 3 mm column; eluant: H_2O/CH_3CN

65:35, flow rate = 0.6 ml/min, UV 220 nm. Retention time **14a/14b** = 4.26/2.62 min. **14c/14d** = 3.66/6.37 min.

^{1}H NMR (400 MHz, $CDCl_3$) δ (**14a**,**14b**) 7.33–7.31 (m, 5H, phenyl), 4.61–4.51 (m, 2H, PhCH_2O), 4.29–4.20 (m, 4H, CH_2 ethyl), 4.09 (ddd, 1H, $J_{6/7}$ = 7.90 Hz, $J_{6/5ax}$ = 12.35 Hz, $J_{6/5eq}$ = 2.39 Hz, H_6), 3.76 (dd, 1H, $J_{9a/8}$ = 4.42 Hz, $J_{9a/9b}$ = 11.18 Hz, H9a), 3.63 (dd, 1H, $J_{9b/8}$ = 6.48 Hz, $J_{9b/9a}$ = 11.19 Hz, H9b), 3.29–3.24 (m, 1H, H_8), 3.08 (dd, 1H, $J_{7/6}$ = 7.77 Hz, $J_{7/8}$ = 4.52 Hz, H_7), 2.39 (dd, 1H, $J_{3eq/3ax}$ = 13.04 Hz, $^4J_{3eq/5eq}$ = 2.09 Hz, H3eq), 2.19 (td, 1H, $J_{5eq/3eq} = J_{5eq/6}$ = 2.10 Hz, $J_{5eq/5ax}$ = 12.90 Hz, H5eq), 2.16 (d, $J_{3ax/3eq}$ = 13.01 Hz, H3ax), 1.60 (t, 1H, $J_{5ax/5eq} = J_{5ax/6}$ = 12.68 Hz, H5ax), 1.31–1.24 (m, 6H, CH_3 ethyl).

δ (**14c** or **14d**) 7.34–7.31 (m, 5H, phenyl), 4.61 and 4.52 (2d, 2H, J = 11.71 Hz, PhCH_2O), 4.29–4.25 (m, 4H, CH_2 ethyl), 4.03 (ddd, 1H, $J_{6/7}$ = 7.79 Hz, $J_{6/5ax}$ = 11.37 Hz, $J_{6/5eq}$ = 2.72 Hz, H_6), 3.90 (dd, 1H, $J_{9a/8}$ = 3.39 Hz, $J_{9a/9b}$ = 11.74 Hz, H9a), 3.55 (dd, 1H, $J_{9b/8}$ = 6.82 Hz, $J_{9b/9a}$ = 11.74 Hz, H9b), 3.28–3.21 (m, 1H, H_8), 3.04 (dd, 1H, $J_{7/6}$ = 7.76 Hz, $J_{7/8}$ = 4.20 Hz, H_7), 2.47 (d, 1H, $J_{3ax/3eq}$ = 14.08 Hz, H3ax), 2.05–1.90 (m, 3H, H3eq, H5eq, H5ax), 1.31–1.24 (m, 6H, CH_3 ethyl).

δ (**14d**,**14c**) 7.34–7.31 (m, 5H, phenyl), 4.46 and 4.58 (2d, 2H, J = 11.71 Hz, PhCH_2O), 4.29–4.20 (m, 4H, CH_2 ethyl), 4.14 (ddd, 1H, $J_{6/7}$ = 7.38 Hz, $J_{6/5ax}$ = 12.25 Hz, $J_{6/5eq}$ = 2.37 Hz, H_6), 3.67 (dd, 1H, $J_{9a/8}$ = 5.91 Hz, $J_{9a/9b}$ = 11.07 Hz, H9a), 3.63 (dd, 1H, $J_{9b/8}$ = 6.82 Hz, $J_{9b/9a}$ = 11.09 Hz, H9b), 3.28–3.21 (m, 1H, H_8), 3.09 (dd, 1H, $J_{7/6}$ = 7.36 Hz, $J_{7/8}$ = 4.48 Hz, H_7), 2.46 (d, 1H, $J_{3ax/3eq}$ = 14.09 Hz, H3ax), 2.05–1.90 (m, 2H, H3eq and H5ax), 1.70 (td, 1H, $J_{5eq/5ax}$ = 13.4 Hz, $J_{5eq/6} = {}^4J_{5eq/3eq}$ = 2.32 Hz, H5eq), 1.31–1.24 (m, 3H, CH_3 ethyl).

^{13}C NMR (63 MHz, $CDCl_3$) δ (**14a**,**14b**) 173.9, 173.7, 168.0, 169.2 (CO_2Et), 137.8 (Cq phenyl), 128.5, 128.2, 127.9, 127.8, 127.7 (CH phenyl), 94.7 (C2), 73.3 and 73.1 (O*C*H_2Ph), 70.8 and 72.1 (C4), 69.6 and 68.4 (C6), 68.2 and 68.0 (C9), 62.8, 62.7, and 62.1 (CH_2 ethyl), 57.3 and 56.8 (C8), 55.7 and 54.5 (C7), 40.7, 40.5, and 36.7, 35.0 (C5 and C3), 14.0 and 13.9 (CH_3 ethyl).

δ (**14c** and **14b**) 173.7, 173.6, 168.8, and 169.7 (CO_2Et), 137.9 and 137.6 (Cq phenyl), 128.5, 128.4, 128.0, 127.9, 127.8, 127.7 (CH phenyl), 94.8 (C2), 73.4 and 73.2 (O*C*H_2Ph), 72.2 and 72.0 (C4), 68.1 and 68.0 (C9), 66.2 and 64.7 (C6), 62.6, 62.5 and 62.4 (CH_2 ethyl), 57.3, 56.7 (C8), 55.9 and 54.5 (C7), 37.2, 36.9, 35.2 (C5 and C3), 14.1, 14.0 (CH_3 ethyl).

MS (DCI, NH_3): 442 (M+18). IR (neat) ν_{max} 3431 (OH), 3060 (CH arom.), 2983 (CH), 1738 (C=O) cm^{-1}.

Anal. Calcd for $C_{21}H_{28}O_9$: C, 59.43; H, 6.60. Found: C, 59.64; H, 6.48.

2.3.2. Ethyl 2,4-dihydroxy-4-carbethoxy-6-(9-(benzyloxy)-7,8-*trans*-epoxy)-tetrahydropyran-2-carboxylate 15a–15d. The crude product was first purified by medium pressure silica gel chromatography (eluant petroleum ether/ethyl acetate 6:4) affording 0.25 g of starting aldehyde. The remaining mixture was then purified by inverse phase chromatography (silica, hyperprep C18, eluant H_2O/CH_3CN 55:45). Two fractions could be isolated containing compounds **15a**, **15b** (0.73 g), and **15c**, **15d** (0.125 g).

R_f: 0,28 (PE/AcOEt 5:5).

Analytical HPLC: C18, 3 mm column, eluant: H_2O/CH_3CN 65:35, flow rate = 0.6 ml/min, UV 220 nm. Retention time **15a/15b** = 6.72/3.77 min. **15c/15d** = 4.14/2.69 min.

^{1}H NMR (400 MHz, $CDCl_3$) δ (**15a** or **15b**) 7.31–7.30 (m, 5H, phenyl), 4.56 and 4.51 (2d, 2H, J = 12.00 Hz, PhCH_2O), 4.24–4.18 (m, 4H, CH_2 ethyl), 4.11 (ddd, 1H, $J_{6/7}$ = 5.48 Hz, $J_{6/5ax}$ = 12.15 Hz, $J_{6/5eq}$ = 2.37 Hz, H6), 3.73 (dd, 1H, $J_{9a/8}$ = 2.97 Hz, $J_{9a/9b}$ = 11.71 Hz, H9a), 3.48 (dd, 1H, $J_{9b/8}$ = 5.33 Hz, $J_{9b/9a}$ = 11.56 Hz, H9b), 3.20 (ddd, 1H, $J_{8/9a} = J_{8/7}$ = 2.71 Hz, $J_{8/9b}$ = 5.19 Hz, H_8), 3.03 (dd, 1H, $J_{7/6}$ = 5.48 Hz, $J_{7/8}$ = 2.23 Hz, H7), 2.39 (dd, 1H, $J_{3eq/3ax}$ = 13.2 Hz, $^4J_{3eq/5eq}$ = 2.22 Hz, H3eq), 2.27 (td, 1H, $J_{5eq/6} = {}^4J_{5eq/3eq}$ = 2.22 Hz, $J_{5eq/5ax}$ = 12.88 Hz, H5eq), 2.15 (d, 1H, $J_{3ax/3eq}$ = 13.19 Hz, H3ax), 1.65 (t, 1H, $J_{5ax/5eq} = J_{5ax/6}$ = 12.59 Hz, H5ax), 1.30–1.24 (m, 6H, CH_3 ethyl).

δ (**15b** or **15a**) 7.31–7.30 (m, 5H, phenyl), 4.56 and 4.51 (d, 2H, J = 12.00 Hz, PhCH_2O), 4.24–4.18 (m, 4H, CH_2 ethyl), 4.14 (ddd, 1H, $J_{6/7}$ = 4.64 Hz, $J_{6/5ax}$ = 12.15 Hz, $J_{6/5eq}$ = 2.51 Hz, H_6), 3.76 (dd, 1H, $J_{9a/8}$ = 2.81 Hz, $J_{9a/9b}$ = 11.55 Hz, H9a), 3.43 (dd, 1H, $J_{9b/8}$ = 5.63 Hz, $J_{9b/9a}$ = 11.55 Hz, H9b), 3.16 (ddd, 1H, $J_{8/9b}$ = 5.26 Hz, $J_{8/9a} = J_{8/7}$ = 2.67 Hz, H_8), 2.99 (dd, 1H, $J_{7/6}$ = 4.37 Hz, $J_{7/8}$ = 2.15 Hz, H_7), 2.38 (dd, 1H, $J_{3eq/3ax}$ = 13.19 Hz, $^4J_{3eq/5eq}$ = 2.30 Hz, H3eq), 2.27 (td, 1H, $J_{5eq/6} = {}^4J_{5eq/3eq}$ = 2.22 Hz, $J_{5eq/5ax}$ = 12.88 Hz, H5eq), 2.16 (d, 1H, $J_{3ax/3eq}$ = 13.19 Hz, H3ax), 1.61 (dd, 1H, $J_{5ax/6}$ = 12.15 Hz, $J_{5ax/5eq}$ = 12.88 Hz, H5ax), 1.30–1.24 (m, 3H, CH_3 ethyl).

δ (**15c** or **15d**) 7.31–7.23 (m, 5H, phenyl), 4.56 and 4.51 (2d, 2H, J = 11.60 Hz, PhCH_2O), 4.27–4.18 (m, 5H, CH_2 ethyl and H6), 3.74 (dd, 1H, $J_{9a/8}$ = 2.79 Hz, $J_{9a/9b}$ = 11.67 Hz, H9a), 3.43 (dd, 1H, $J_{9b/8}$ = 5.60 Hz, $J_{9b/9a}$ = 11.68 Hz, H9b), 3.13 (ddd, 1H, $J_{8/9a} = J_{8/7}$ = 2.65 Hz, $J_{8/9b}$ = 5.10 Hz, H_8), 3.02 (dd, 1H, $J_{7/6}$ = 4.72 Hz, $J_{7/8}$ = 2.18 Hz, H_7), 2.45 (d, 1H, $J_{3eq/3ax}$ = 14.06 Hz, H3ax), 1.98–1.90 (m, 2H, H3eq and H5ax), 1.73 (dt, 1H, $J_{5ax/5eq}$ = 13.4 Hz, $J_{5eq/6} = {}^4J_{5eq/3eq}$ = 2.37 Hz, H5eq), 1.31–1.27 (m, 6H, CH_3 ethyl).

^{13}C NMR (100 MHz, $CDCl_3$) δ (**15a** or **15b**) 173.5 and 169.3 (CO_2Et), 137.9 (Cq phenyl), 128.6 and 127.9 (CH phenyl), 94.8 (C2), 73.4 (OCH_2Bn), 71.3 (C4), 69.6 (C9), 69.4 (C6), 62.9 (CH_2 ethyl), 62.1 (CH_2 ethyl), 56.9 (C8), 54.3 (C7), 40.6 (C5), 35.3 (C3), 14.1 (CH_3 ethyl).

δ (**15b** or **15a**) 173.5 and 169.3 (CO_2Et), 137.9 (Cq phenyl), 128.6 and 127.9 (CH phenyl), 94.7 (C2), 73.4 (OCH_2Bn), 71.3 (C4), 69.7 (C9), 68.5 (C6), 62.9 (CH_2 ethyl), 62.1 (CH_2 ethyl), 56.6 (C8), 55.1 (C7), 40.6 (C5), 35.3 (C3), 14.1 (CH_3 ethyl).

δ (**15c** or **15d**) 173.9 and 168.6 (CO_2Et), 137.8 (Cq phenyl), 128.4, 128.0, 127.9, 127.8 (CH phenyl), 94.9 (C2), 73.2 (OCH_2Bn), 72.2 (C4), 69.5 (C9), 66.1 (C6), 62.6 (CH_2

ethyl), 62.4 (CH_2 ethyl), 56.4 (C8), 55.3 (C7), 37.3 and 34.8 (C_5+C_3), 14.1 and 19.2 (CH_3 ethyl).

δ (**15d** or **15c**) 173.9 and 168.6 (CO_2Et), 137.8 (Cq phenyl), 128.4, 128.0, 127.9, 127.8 (CH phenyl), 94.9 (C2), 73.5 (OCH_2Bn), 72.1 (C4), 69.5 (C9), 66.3 (C6), 62.7 (CH_2 ethyl), 62.4 (CH_2 ethyl), 57.3 (C8), 53.5 (C7), 36.9 and 34.7 (C_5+C_3), 14.1 and 19.2 (CH_3 ethyl).

MS (DCI, NH_3): 442 (M+18). IR (neat) ν_{max} 3431 (OH), 3065 (CH arom.), 2984 (CH), 1732 (C=O) cm^{-1}.

Anal. Calcd for $C_{21}H_{28}O_9$: C, 59.43; H, 6.60. Found: C, 59.24; H, 6.69.

2.3.3. Ethyl 2,4-di-trimethylsilyloxy-4-carbethoxy-6-(9-(benzyloxy)-7,8-*cis*-epoxy)-tetrahydropyran-2-carboxylate 16a,16b. To a solution of compounds **14a**, **14b** (0.171 g, 0.4 mmol) in pyridine (1 ml), was added under stirring and nitrogen TMSCl (0.186 ml, 1.45 mmol, 3.6 equiv) and HMDS (0.223 ml, 1.05 mmol, 1 equiv), the reaction mixture was stirred at rt for 8 h before adding methylene chloride (2 ml) and an aq solution of NH_4Cl 20% (2 ml). The aqueous phase was extracted with ether (3× 5 ml) and the combined organic phases were dried over $MgSO_4$, filtered, and concentrated. The crude product was purified on silica gel (eluant petroleum ether/ether 6:4) affording **16a** (65 mg) and **16b** (53 mg) along with 25 mg of starting material (yield 60%).

R_f: **16a**:0,44 (PE/Et_2O 6:4); **16b**:0,32 (PE/Et_2O 6:4).

^{1}H NMR (250 MHz, $CDCl_3$) δ (**16a**) 7.37–7.26 (m, 5H, CH arom.), 4.66 and 4.56 (2d, 2H, $J=11.9$ Hz, $PhCH_2O$), 4.22–4.06 (m, 5H, CH_2 ethyl and H6), 3.96 (dd, 1H, $J_{9a/9b}=11.6$ Hz, $J_{9a/8}=2.7$ Hz, H9a), 3.56 (dd, 1H, $J_{9b/9a}=11.6$ Hz, $J_{9b/8}=7.3$ Hz, H9b), 3.29 (m, 1H, H8), 3.07 (dd, 1H, $J_{7/8}=4.2$ Hz, $J_{7/6}=7.1$ Hz, H7), 2.13 (dd, 1H, $J=14.2$, 1.8 Hz, H3eq), 2.01–1.92 (m, 3H, H3ax, H5eq, and H5ax), 1.32–1.26 (m, 6H, CH_3 ethyl), 0.11 (s, 18H, OTMS).

δ (**16b**) 7.36–7.27 (m, 5H, CH arom.), 4.60 and 4.54 (2d, 2H, $J=11.7$ Hz, $PhCH_2O$), 4.25–4.05 (m, 5H, CH_2 ethyl, and H6), 3.67 and 3.65 (2 s, 2H, H9a, and H9b), 3.27 (m, 1H, H8), 3.17 (dd, 1H, $J_{7/8}=4.5$ Hz, $J_{7/6}=7.9$ Hz, H7), 2.15 (dd, 1H, $J_{3eq/3ax}=14.3$ Hz, $^4J_{3eq/5eq}=2.0$ Hz, H3eq), 2.07 (d, 1H, $J_{3ax/3eq}=14.3$ Hz, H3ax), 1.89 (dd, 1H, $J_{5ax/5eq}=13.6$ Hz, $J_{5ax/6}=11.8$ Hz, H5ax), 1.76 (td, 1H, $J_{5eq/6}=^4J_{5eq/3eq}=2.1$ Hz, $J_{5eq/5ax}=13.6$ Hz, H5eq), 1.34–1.23 (m, 6H, CH_3 ethyl), 0.17 (s, 9H, OTMS), 0.12 (s, 9H, OTMS).

^{13}C NMR (63 MHz, $CDCl_3$) δ (**16a**) 173.6 and 170.1 (CO_2Et), 137.9 (Cq phenyl), 128.4, 127.8, 127.7 (CH phenyl), 95.8 (C2), 74.0 (C4), 73.2 (OCH_2Ph), 68.7 (C9), 64.0 (C6), 61.8 (CH_2 ethyl), 61.5 (CH_2 ethyl), 56.9 (C8), 56.4 (C7), 40.9, and 37.6 (C_5 and C_3), 14.1 (CH_3 ethyl), 1.8 and 1.4 (OTMS).

δ (**16b**) 173.4 and 170.6 (CO_2Et), 137.7 (Cq phenyl), 128.5, 127.9, 127.8 (CH phenyl), 96.3 (C2), 73.9 (C4), 73.5 (OCH_2Ph), 68.4 (C9), 66.4 (C6), 61.8 (CH_2 ethyl), 61.6 (CH_2 ethyl), 57.3 (C8), 54.2 (C7), 40.1 and 37.0 (C_5 and C_3), 14.1 (CH_3 ethyl), 1.9 and 1.3 (OTMS).

MS (DCI, NH_3): 586 (M+18).

Anal. Calcd for $C_{27}H_{44}O_9Si_2$: C, 57.04; H, 7.73. Found: C, 57.28; H, 7.92.

2.3.4. Ethyl 2,4-di-trimethylsilyloxy-4-carbethoxy-6-(9-(dibenzylphosphate)-7,8-*cis*-epoxy)-tetrahydropyran-2-carboxylate 18. Compound **16b** (45 mg, 0.079 mmol) was hydrogenolyzed in the presence of 8 mg of Pd/C 10% in freshly distilled ethyl acetate (4 ml). After 24 h under stirring, the mixture was filtered off and concentrated affording 37 mg (97% yield) of the epoxyalcohol **17**, used without purification.

R_f: 0,2 (PE/Et_2O 5:5).

^{1}H NMR (250 MHz, $CDCl_3$) δ 4.33 (ddd, 1H, $J_{6/7}=7.0$ Hz, $J_{6/5ax}=11.7$ Hz, $J_{6/5eq}=2.2$ Hz, H6), 4.25–4.12 (m, 4H, CH_2 ethyl), 3.81 (m, 2H, H9a, and H9b), 3.24 (m, 2H, H8, and H7), 2.17 (dd, 1H, $J_{3eq/3ax}=14.3$ Hz, $^4J_{3eq/5eq}=2.1$ Hz, H3eq), 1.95 (d, 1H, $J_{3ax/3eq}=14.3$ Hz, H3ax), 1.89 (dd, 1H, $J_{5ax/5eq}=13.6$ Hz, $J_{5ax/6}=11.8$ Hz, H5ax), 1.74 (td, 1H, $J_{5eq/6}=^4J_{5eq/3eq}=2.2$ Hz, $J_{5eq/5ax}=13.5$ Hz, H5eq), 1.34–1.26 (m, 6H, CH_3 ethyl), 0.17 (s, 9H, OTMS), 0.13 (s, 9H, OTMS).

^{13}C NMR (63 MHz, $CDCl_3$) δ 173.4 and 170.5 (CO_2Et), 96.1 (C2), 73.9 (C4), 65.9 (C6), 61.9 (CH_2 ethyl), 61.6 (CH_2 ethyl), 60.9 (C9), 57.9, 56.2 (C7 and C8), 40.2, 36.9 (C_5 and C_3), 14.1, 14.0 (CH_3 ethyl), 1.9 and 1.2 (OTMS).

MS (DCI, NH3): 496 (MNH_4^+). IR (neat) ν_{max} 3465 (OH); 2960 (CH); 1741 (C=O) cm^{-1}.

To a solution of epoxyalcohol **17** (37 mg, 0.077 mmol) in methylene chloride (1.3 ml) containing tetrazole (6 mg, 0.0857 mmol, 1.11 equiv), was added dropwise *N*,*N*-diisopropyl dibenzyl phosphoramidite (28 mg, 0.081 mmol, 1.05 equiv). The mixture was stirred for 3 h at rt then cooled to −40 °C before adding *m*-cpba (77.3 mg, 0.317 mmol, 4 equiv). The temperature was then allowed to reach 0 °C and stirring was continued for 45 min. The reaction was then quenched with a 10% $Na_2S_2O_3$ aq solution (3 ml). After 15 min, it was diluted with methylene chloride (15 ml). The organic phase obtained was washed successively with 10% $Na_2S_2O_3$ (5 ml), saturated aq $NaHCO_3$ (5 ml), saturated aq NaCl (5 ml), dried over $MgSO_4$, filtered and concentrated. The crude product was purified on silica gel (eluant petroleum ether/CH_2Cl_2/AcOEt 5:8:2) affording 35 mg (60% yield) of the desired compound **18**.

R_f: 0,16 (PE/Et_2O 5:5).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (m, 10H, Ph), 4.25 (ddd, 1H, $J_{6/7}=7.0$ Hz, $J_{6/5ax}=11.7$ Hz, $J_{6/5eq}=2.2$ Hz, H6), 4.20–4.05 (m, 6H, CH_2 ethyl, CH_2O), 3.23 (m, 1H, H8), 3.14 (dd, 1H, $J_{7/8}=4.5$, $J_{7/6}=7.1$ Hz, H7), 2.17 (dd, 1H, $J_{3eq/3ax}=14.3$ Hz, $^4J_{3eq/5eq}=2.1$ Hz, H3eq), 1.92 (d, 1H, $J_{3ax/3eq}=14.3$ Hz, H3ax), 1.84 (dd, 1H, $J_{5ax/5eq}=13.6$ Hz, $J_{5ax/6}=11.8$ Hz, H5ax), 1.64 (td, 1H, $J_{5eq/6}=^4J_{5eq/3eq}=2.2$ Hz, $J_{5eq/5ax}=13.5$ Hz, H5eq), 1.26–1.20 (m, 6H, CH_3 ethyl), 0.12 (s, 9H, OTMS), 0.11 (s, 9H, OTMS).

^{13}C NMR (63 MHz, $CDCl_3$) δ 173.2 and 170.3 (CO_2Et), 135.7, 135.6 (Cq phenyl), 128.6, 128.4, 128.0, 127.4 (CH phenyl), 96.2 (C2), 73.8 (C4), 69.5 (d, $^2J_{C/P}=5.5$ Hz, CH_2OPh), 66.3 (d, $^2J_{C9/P}=5.5$ Hz, C9), 65.6 (C6), 61.9 (CH_2 ethyl), 61.6 (CH_2 ethyl), 57.8 (C7), 54.4 (d, $^3J_{C8/P}=8.1$ Hz, C8), 40.0 and 37.0 (C_5 and C_3), 14.0 (CH_3 ethyl), 1.9 and 1.2 (OTMS).

^{31}P NMR (81 MHz, $CDCl_3$) δ −0.77.

MS (DCI, NH3): 756 (MNH_4^+). IR (neat) ν_{max} 2961 (CH alcane), 1743 (C=O), 1251 (P=O), 1020 (P–O) cm^{-1}.

Anal. Calcd for $C_{34}H_{51}O_{12}PSi_2$: C, 55.26; H, 6.91. Found: C, 55.14; H, 6.72.

Acknowledgements

We are grateful to the CNRS and the 'Université Paul Sabatier' for financial support and the 'Ministère de la Recherche et des Technologies' for grant (YR).

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Tetrahedron 61 (2005) 8904–8909
Tetrahedron

First successful synthesis, isolation and characterization of open-chain 1,2-diazidoethenes☆

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Received 29 April 2005; revised 30 June 2005; accepted 6 July 2005

Available online 28 July 2005

Abstract—Open-chain 1,2-diazidoethenes have been obtained from the recently reported acceptor-substituted propargyl azides by one-pot reactions with tetramethylguanidinium azide (TMGA) and in some cases via a two-step procedure starting with in situ production of the corresponding azidoallene followed by addition of hydrazoic acid with the help of TMGA. In a different synthetic pathway, the substitution reactions between 2-azido-3-haloacroleins and hexadecyltributylphosphonium azide (QN_3) have been used. In order to have more evidence for their structures, some diazido compounds were converted to their bis-triazole derivatives through 1,3-dipolar cycloaddition.

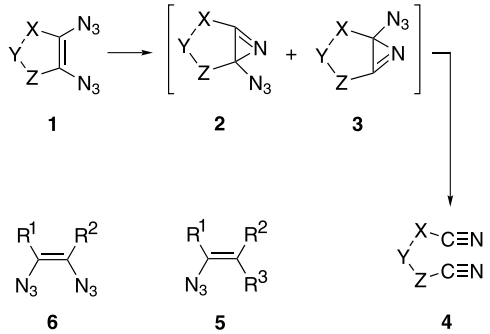
1. Introduction

Cyclic 1,2-diazidoethenes **1** have been known for many decades, and they have been used for various synthetic purposes (Scheme 1).[2–13] However, all attempts to isolate or to observe the open-chain analogous compounds **6** failed.[14,15] In fact, cyclic 1,2-diazidoethenes **1** can be synthesized on the one hand via Diels–Alder reactions of 2,3-diazido-1,3-butadiene derivatives with dienophiles,[4,5] and on the other hand by nucleophilic substitution of cyclic 1,2-dihaloalkenes activated by two flanking, electron-withdrawing substituents.[6–8,13] With regard to their reactivity, it is well-known that cyclic 1,2-diazidoethenes of type **1** undergo thermal[4–10,16] and photochemical[3–5] degradation to form the corresponding dicyano derivatives **4**. In order to rationalize this degradation process, several intermediates had been postulated.[7–10] By photolysis of 1,2-diazidobenzene in an inert matrix at low temperature, *ortho*-phenylene-bisnitrene was generated.[3] However, 2-azido-2*H*-azirines **2** and **3** are the most plausible intermediates for the transformation of **1** to yield **4**.[17] Nevertheless, direct proof of such intermediates had not yet been obtained. Most probably, **2** and **3** could not be observed spectroscopically due to their anti-Bredt structures. It emerges that open-chain 1,2-diazidoethenes **6** may led to spectroscopically observable 2-azido-2*H*-azirines. Such observation should constitute evidence to the mechanism of the fragmentation of 1,2-diazidoethenes to yield cyano derivatives. Unfortunately, open-chain 1,2-diazidoethenes **6** were up to now only known as spontaneously decomposing compounds.[14,15] In this paper, we report the first access to the open-chain 1,2-diazidoethenes. Moreover, we show how the stability of these compounds can be improved.

2. Results and discussion

2.1. Open-chain 1,2-diazidoethenes from acceptor-substituted propargyl azides

Treatment of the recently accessible propargyl azide **7**[18] with tetramethylguanidinium azide (TMGA) yielded regioselectively a mixture of the open-chain 1,2-diazidoethenes



Scheme 1.

☆ See Ref. 1.

Keywords: Propargyl azides; 1,2-Diazidoethenes; Triazoles; Huisgen's 1,3-dipolar cycloaddition; Nucleophilic addition; Nucleophilic substitution.
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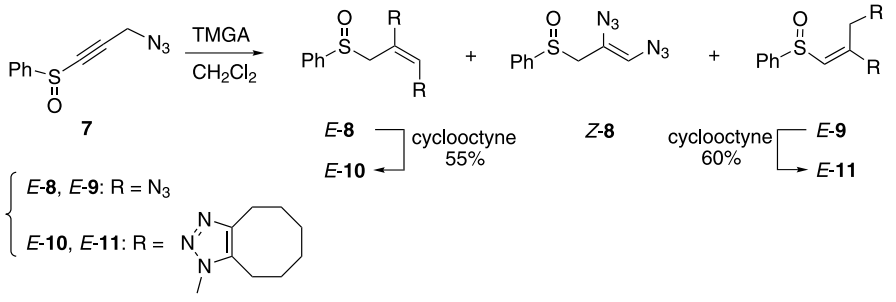
doi:10.1016/j.tet.2005.07.025

E/*Z*-**8** (*E*/*Z*=5:1) and traces of the expected isomer *E*-**9** (Scheme 2). Fortunately, this mixture could be separated on silica gel without any remarkable decomposition. The overall yield was 72%. Surprisingly, *E*-**8** isomerized slowly to *Z*-**8**. A plausible rationalization of this isomerization may be the formation of *E*-**9** as an intermediate. However, during the ^{1}H NMR monitoring of this isomerization, *E*-**9** could not be observed. To prove the presence of two azido groups in the structures of *E*-**8** and *E*-**9**, they both were treated with cyclooctyne to yield the corresponding bis-triazole derivatives *E*-**10** (55%) and *E*-**11** (60%), respectively, as products of Huisgen's 1,3-dipolar cycloaddition reaction.

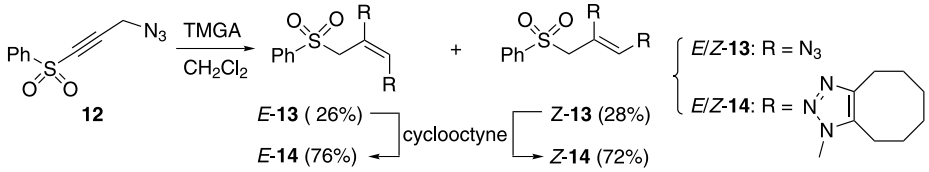
The reaction of the propargyl azide **12**[18] with TMGA preceded highly regioselectively and afforded only the desired open-chain derivatives *E*/*Z*-**13** (*E*/*Z*=1:1, Scheme 3). Separation of the mixture of isomers on silica gel was performed without any special precautions to obtain 26% of *E*-**13** and 28% of *Z*-**13**. The presence of two azido groups in the structures of *E*-**13** and *Z*-**13** was also verified by their treatment with cyclooctyne yielding the bis-triazole compounds *E*-**14** (76%) and *Z*-**14** (72%), respectively.

In contrast to the two precedent reactions, treatment of the propargyl azide **15**[18] with TMGA afforded a mixture containing the four possible isomeric products (*E*/*Z*-**16** and *E*/*Z*-**17**) of the addition of HN_3 (Scheme 4). The chromatography of this mixture on silica gel yielded firstly *E*-**16** as minor compound in a mixture (1:4) with *E*-**17** and secondly *Z*-**16** as minor compound in a mixture (1:6) with *Z*-**17**. The overall yield was 51%. Attempts to isolate the desired products *E*-**16** and *Z*-**16** by crystallization were unsuccessful. Moreover, we were not able to isomerize *E*/*Z*-**17** to **16** with the help of bases. This can be ascribed to the significant lowering of the acidity of the allyl protons by the additional methyl group. By storing a solution of *E*-**16**/*E*-**17** or *Z*-**16**/*Z*-**17** in chloroform at room temperature for several days, *E*-**16** and *Z*-**16** could be singularly decomposed to phenylsulfonylacetonitrile and acetonitrile (compare to **4** in Scheme 1). This serves as proof of the existence of an 1,2-diazidoethene unit in the structure of the latter compounds. After starting with the mixture of *E*-**16** and *E*-**17** and decomposition of *E*-**16**, the presence of two azido groups in the structure of remaining *E*-**17** was proved by its treatment with cyclooctyne giving the bis-triazole compound *E*-**18** with about 90% yield.

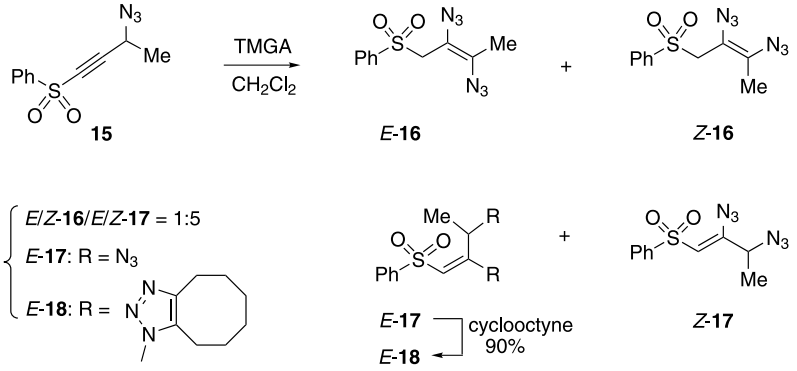
Due to the difficult separation of the desired open-chain 1,2-diazidoethenes *E*-**16** and *Z*-**16** from the mixture obtained after using the one-pot procedure described



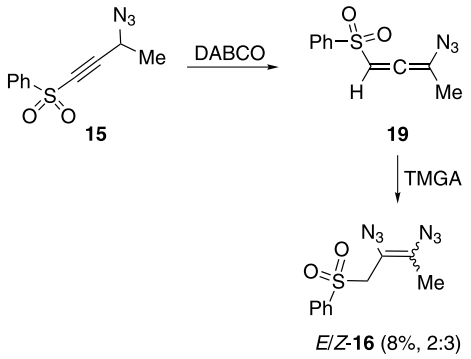
Scheme 2.



Scheme 3.



Scheme 4.



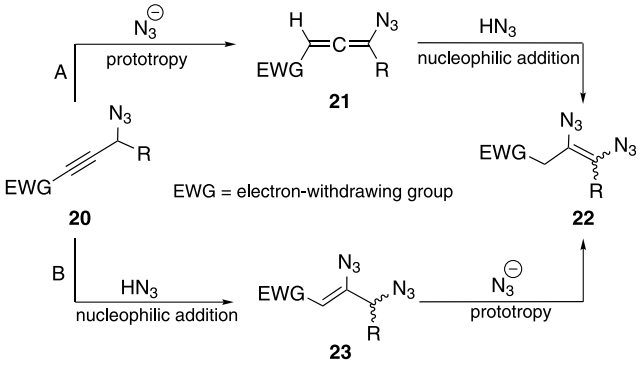
Scheme 5.

above, a two-step procedure via **19** was also executed giving the products *E*/*Z*-**16** (8%, *E*/*Z*=2:3, Scheme 5). Neither *E*- nor *Z*-**17** could be observed in the reaction mixture.

The formation of open-chain 1,2-diazidoethenes of type **22** by the reactions of the acceptor-substituted propargyl azides of type **20** with TMGA can be rationalized by the pathways A and B (Scheme 6). An evidence for the pathway A was obtained by the formation of *E*/*Z*-**16** from **15** via **19** (Scheme 5). Indeed the pathway B is also very plausible, its evidence could not be proved directly since treatment of *E*/*Z*-**17** (Scheme 4) with bases failed to yield the corresponding open-chain 1,2-diazidoethenes. However, we cannot omit this pathway because similar transformations are known.[18]

2.2. Possible factors influencing the stability of 1,2-diazidoethenes

Noteworthy, the solutions of the 1,2-diazidoethenes in dichloromethane described above were relatively stable at room temperature and could be stored at low temperature for several months without decomposition. But why did former attempts to prepare open-chain 1,2-diazidoethenes lead to their spontaneous decomposition? It is well-known that normal vinyl azides **5** (Scheme 1) with strong electron-donating groups R^1, R^2 and R^3 (e.g., R^1, R^2, R^3=Ph, OR, NR_2) decomposed spontaneously at temperatures near 20 °C.[19] In the structures of 1,2-diazidoethenes **6**, the substituent R^3 (see **5**) is replaced by an additional azido group. However, the azido group is also known as strong electron-donating group comparable to alkoxy groups.[20] In

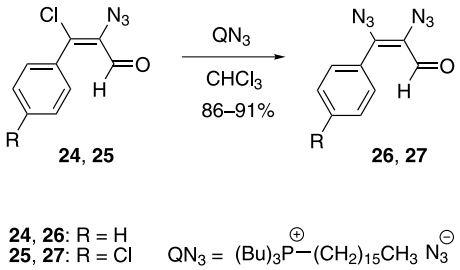


Scheme 6.

this context, considering one azido group as strong electron-donating, we easily conclude that the stability of the 1,2-diazidoethenes just like the one of the traditional vinyl azides is decreased by electron-donating substituents. Moreover, exploring the structures of the known cyclic 1,2-diazidoethenes,[2–13] it is remarkable that all of them enclose at least one electron-withdrawing group related to the 1,2-diazidoethene unit, even if in some cases[4,5] these substituents have only a weak acceptor character. Probably, the understanding of the influence of the electron-withdrawing groups on the stabilization of these compounds suffered from the fact that acceptor substituents were commonly introduced into the starting materials just with the aim of facilitating the nucleophilic substitution of the leaving groups by the azide anion.

2.3. Open-chain 1,2-diazidoethenes from 2-azido-3-halopropenals

In order to confirm that the stability of the 1,2-diazidoethenes can be improved by acceptor substituents, we undertook the synthesis of derivatives bearing at least one strong electron-withdrawing group. In this connection, 2-azido-3-halopropenals *Z*-**24** and *Z*-**25**[21] were treated with nucleophiles containing the azide anion (Scheme 7). Both the aldehydes *Z*-**24** and *Z*-**25** are bearing a *β*-chloro substituent, which can be substituted by the azide anion via the well-known addition–elimination mechanism. Such reactions are known to proceed with retention of the configuration at the C=C unit. However, using sodium azide as source of the azide anion, only poor yields were obtained. This can be ascribed to the lowering of the nucleophilicity of the azide anion through the strong association with the sodium cation. Fortunately, using hexadecyltributylphosphonium azide (QN_3),[22] the desired products *Z*-**26** (from *Z*-**24**) and *Z*-**27** (from *Z*-**25**) were formed with a yield of 86 and 91%, respectively. In fact, unlike sodium azide, the bulky Q^+ cation of QN_3 is weakly associated to the azide anion, which is consequently very reactive. The compounds *Z*-**26** and *Z*-**27** proved to be relatively stable at room temperature confirming that the thermal stability of 1,2-diazidoethenes can be improved by electron-withdrawing groups.



Scheme 7.

3. Conclusion

The synthesis of open-chain 1,2-diazidoethenes has been accomplished for the first time starting with acceptor-substituted propargyl azides and TMGA or 2-azido-3-halopropenals and QN_3. Although the open-chain 1,2-azidoethenes were only known as spontaneously decomposing compounds

hitherto, those described in this paper could be synthesized and isolated at room temperature without any special precautions. Moreover, evidence was obtained that the thermal stability of these open-chain 1,2-diazidoethenes can be improved by electron-withdrawing groups. The photochemical and thermal transformations of these 1,2-diazidoethenes are currently being examined in our laboratory for purpose of mechanistic investigations.

4. Experimental

4.1. General remarks

^{1}H and ^{13}C NMR spectra were recorded at 300 and 75 MHz, respectively, in $CDCl_3$, unless otherwise noted. Chemical shifts (δ) are reported in parts per million (ppm) downfield from TMS. Coupling constants (J) are reported in hertz (Hz), and spin multiplicities are indicated by the following symbols: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet). Assignments of stereochemistry were supported by homonuclear NOE experiments in the case of *E*/*Z*-**8**, *E*-**9**, *E*/*Z*-**13**, *E*/*Z*-**16**, *E*/*Z*-**17** and *Z*-**24**, whereas the stereochemistry of compounds *E*-**10**, *E*-**11**, *E*/*Z*-**14**, *E*-**18**, *Z*-**26** and *Z*-**27** was deduced from that of the respective precursors. Infrared spectra were recorded as solutions in $CDCl_3$. TLC was performed on Macherey-Nagel precoated silica gel Polygram Sil G/UV$_{254}$ plates and viewed by UV. Chromatography refers to flash chromatography,[23] carried out on Fluka silica gel 60. For the elemental analyses, Vario El (Elementar Analysensystem GmbH) was employed. Mariner 5229 from Applied Biosystems was used for MS-spectra. The method applied was the electrospray ionization. For UV/vis spectra, Lambda 40 (Perkin-Elmer) was used.

Warning: Elemental analyses of azides could not be performed because of explosive decomposition. Caution should be exercised during isolation of azide, which may be explosive. In the case of solutions of tetrabutylammonium azide in CH_2Cl_2, an explosion probably caused by diazidomethane was reported.[24] Therefore, special caution is also necessary in the case of tetramethylguanidinium azide (TMGA) in CH_2Cl_2, although we never observed any incident.

The known compounds **7**,[18] **12**,[18] **15**,[18] **19**,[18] and **25**[21] were synthesized according to the literature.

4.1.1. *E*/*Z*-(2,3-Diazidoprop-2-enylsulfinyl)-benzene (*E*/*Z*-8) and *E*-(2,3-diazidoprop-1-enylsulfinyl)-benzene (*E*-9). Compound **7**[18] (150 mg, 0.732 mmol) in CH_2Cl_2 (10 mL) was cooled down to 0 °C and treated in small portions with TMGA (150 mg, 0.949 mmol). Stirring was continued at room temperature for additional 40 min. The solvent was partially removed (neat substance can be very explosive) and the residue was chromatographed on silica gel with Et_2O/*n*-hexane 4:1 to give a mixture (yellow oil, 115 mg, 0.464 mmol, 63%) of *E*-**8** and *Z*-**8** (*E*/*Z*=3:1), pure *Z*-**8** (5.00 mg, 0.020 mmol, 2.8%, yellow oil) and pure *E*-**9** (10.0 mg, 0.040 mmol, 6%, yellow oil). Addition of TMGA to **7** in CH_2Cl_2 at room temperature instead of 0 °C, led to a mixture of *E*-**8** and *Z*-**8** (*E*/*Z*=5:1) and only traces of *E*-**9**.

E-(2,3-Diazidoprop-2-enylsulfinyl)-benzene (*E*-**8**): IR ($CDCl_3$, *E*/*Z*-**8**=5:1): 2094 (N_3), 1049 (SO) cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 3.54 (d, ^2J=13.2 Hz, 1H, H-1′), 3.70 (d, ^2J=13.2 Hz, 1H, H-1′), 6.29 (s, 1H, H-3′), 7.55 (m, 3H, Ph), 7.69 (m, 2H, Ph). ^{13}C NMR ($CDCl_3$): δ 55.28 (t, C-1′), 119.38 (d, C-3′), 120.02 (s, C-2′), 124.22 (d, 2C, Ph), 129.13 (d, 2C, Ph), 131.73 (d, *p*-Ph), 142.83 (s, *i*-Ph).

Z-(2,3-Diazidoprop-2-enylsulfinyl)-benzene (*Z*-**8**): IR ($CDCl_3$): 2115 (N_3), 1022 (SO) cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 3.32 (d, ^2J=13.5 Hz, 1H, H-1′), 3.39 (d, ^2J=13.5 Hz, 1H, H-1′), 5.88 (s, 1H, H-3′), 7.56 (m, 3H, Ph), 7.63 (m, 2H, Ph). ^{13}C NMR ($CDCl_3$): δ 59.97 (t, C-1′), 116.07 (s, C-2′), 119.37 (d, C-3′), 123.96 (d, 2C, Ph), 129.39 (d, 2C, Ph), 131.68 (d, *p*-Ph), 142.39 (s, *i*-Ph).

E-(2,3-Diazidoprop-1-enylsulfinyl)-benzene (*E*-**9**): IR ($CDCl_3$): 2111 (N_3), 1262 (N_3), 1039 (SO) cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 4.11 (d, ^2J=13.8 Hz, 1H, H-3′), 4.43 (d, ^2J=13.8 Hz, 1H, H-3′), 6.09 (s, 1H, H-1′), 7.55 (m, 3H, Ph), 7.65 (m, 2H, Ph). ^{13}C NMR ($CDCl_3$): δ 48.75 (t, C-3′), 123.55 (d, C-1′), 124.12 (d, 2C), 129.60 (d, 2C), 131.27 (d, *p*-Ph), 143.37 (s, *i*-Ph), 146.51 (s, C-2′).

4.1.2. (*E*-3-Phenylsulfinylprop-1-en-1,2-diyl)-bis-4,5,6,7,8,9-hexahydro-1*H*-cyclooctatriazole (*E*-10). To a stirred solution of *E*/*Z*-**8** (10.0 mg, 0.038 mmol, *E*/*Z*=5:1) in CH_2Cl_2 (1 mL), cyclooctyne (13 mg, 0.122 mmol) was added. Stirring was continued at room temperature for 2 h. The solvent and the rest of cyclooctyne were removed at 10^{-3} Torr, and the residue was chromatographed on silica gel with Et_2O/MeOH (97:3) yielding *E*-**10** (10.0 mg, 0.021 mmol, 55%) as yellow oil. The presence of *Z*-**10** in the remaining fractions could not be proved. ^{1}H NMR ($CDCl_3$): δ 1.48 (m, 8H), 1.79 (m, 8H), 2.70 (m, 2H), 2.89 (m, 6H), 4.56 (d, ^2J=13.0 Hz, 1H, H-3′), 4.93 (d, ^2J=13.0 Hz, 1H, H-3′), 7.04 (s, 1H, H-1′), 7.40 (m, 3H), 7.60 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 21.74 (t), 22.41 (t), 24.14 (t), 24.18 (t), 24.24 (t), 24.99 (t), 25.32 (t), 25.47 (t), 26.03 (t), 27.32 (t), 27.69 (t), 28.00 (t), 60.02 (t, C-3′), 123.43 (d), 123.94 (d, 2C), 125.16 (s), 128.96 (d, 2C), 131.06 (d), 134.35 (s), 134.80 (s), 142.98 (s), 144.53 (s), 145.17 (s). MS (ESI); *m*/*z*: 465.20 [M+H$^+$].

4.1.3. (*E*-3-Phenylsulfinylprop-2-en-1,2-diyl)-bis-4,5,6,7,8,9-hexahydro-1*H*-cyclooctatriazole (*E*-11). Compound *E*-**11** (60%, yellow oil) was obtained from *E*-**9** by analogy to the procedure described for *E*-**10**. ^{1}H NMR ($CDCl_3$): δ 1.18–1.80 (m, 18H), 2.35 (m, 2H), 2.73 (m, 4H), 5.85 (d, ^2J=15.0 Hz, 1H, H-3′), 6.02 (d, ^2J=15.0 Hz, 1H, H-3′), 6.46 (s, 1H, H-1′), 7.60 (m, 3H), 8.02 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 21.26 (t), 21.30 (t), 24.07 (t), 24.18 (t), 24.30 (t), 24.82 (t), 25.10 (t), 25.60 (t), 25.91 (t), 26.76 (t), 28.10 (t, 2C), 46.29 (t, C-3′), 124.37 (d, 2C), 129.63 (d, 2C), 131.61 (d), 134.47 (s), 135.01 (s), 135.51 (d), 137.29 (s), 142.78 (s), 144.36 (s), 145.82 (s). MS (ESI); *m*/*z*: 465.20 [M+H$^+$].

4.1.4. *E*- and *Z*-(2,3-Diazidoprop-2-enylsulfonyl)-benzene (*E*-13 and *Z*-13). Compound **12**[18] (600 mg, 2.71 mmol) in CH_2Cl_2 (25 mL) was cooled to 15 °C and treated portionwise with TMGA (600 mg, 3.80 mmol). Stirring was continued at the same temperature for additional 90 min. The solvent was partially removed

(neat substance can be very explosive) and the residue was chromatographed on silica gel with Et_2O/*n*-hexane 1:9 to afford *E*-**13** (181 mg, 0.69 mmol, 26%, yellow oil), *Z*-**13** (200 mg, 0.76 mmol, 28%, yellow oil) and a mixture of *E*-**13** and *Z*-**13** (11 mg, 0.04 mmol, 1.5%) after very careful removal of the solvent under vacuum.

E-(2,3-Diazidoprop-2-enylsulfonyl)-benzene (*E*-**13**): IR ($CDCl_3$): 2096 (N_3), 1322 (SO_2), 1153 (SO_2) cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.97 (s, 2H, H-1′), 6.23 (s, 1H, H-3′), 7.60 (m, 2H, *m*-Ph), 7.70 (m, 1H, *p*-Ph), 7.96 (m, 2H, *o*-Ph). ^{13}C NMR ($CDCl_3$): δ 54.22 (t, C-1′), 118.30 (s, C-2′), 120.29 (d, C-3′), 128.78 (d, 2C), 128.98 (d, 2C), 134.28 (d, *p*-Ph), 138.15 (s, *i*-Ph).

Z-(2,3-Diazidoprop-2-enylsulfonyl)-benzene (*Z*-**13**): IR ($CDCl_3$): 2117 (N_3), 1324 (SO_2), 1157 (SO_2) cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.71 (s, 2H, H-1′), 5.89 (s, 1H, H-3′), 7.61 (m, 2H, *m*-Ph), 7.70 (m, 1H, *p*-Ph), 7.92 (m, 2H, *o*-Ph). ^{13}C NMR ($CDCl_3$): δ 58.52 (t, C-1′), 115.27 (s, C-2′), 120.73 (d, C-3′), 128.51 (d, 2C), 129.39 (d, 2C), 134.39 (d, *p*-Ph), 137.82 (s, *i*-Ph).

4.1.5. (*E*-3-Phenylsulfonylprop-1-en-1,2-diyl)-bis-4,5,6,7,8,9-hexahydro-1*H*-cyclooctatriazole (*E*-14). Compound *E*-**14** (76%) was obtained from *E*-**13** by analogy to the procedure described for *E*-**10**. White solid: mp (CH_2Cl_2/Et_2O) 158–159 °C. ^1H NMR ($CDCl_3$): δ 1.52 (m, 8H), 1.82 (m, 8H), 2.65 (m, 2H), 2.92 (m, 6H), 5.36 (s, 2H, H-3′), 7.02 (s, 1H, H-1′), 7.45 (m, 2H, *m*-Ph), 7.54 (m, 1H, *p*-Ph), 7.81 (m, 2H, *o*-Ph). ^{13}C NMR ($CDCl_3$): δ 21.73 (t), 22.55 (t), 24.19 (t), 24.20 (t), 24.30 (t), 25.01 (t), 25.41 (t), 25.54 (t), 26.03 (t), 27.48 (t), 27.70 (t), 28.10 (t), 57.51 (t, C-3′), 121.86 (s), 124.36 (d), 128.09 (d, 2C), 128.95 (d, 2C), 133.81 (d), 134.40 (s), 134.79 (s), 138.78 (s, *i*-Ph), 144.57 (s), 145.30 (s). Anal. Calcd for $C_{25}H_{32}N_6O_2S$ (480.63): C, 62.47; H, 6.71; N, 17.49. Found: C, 62.67; H, 6.61; N, 17.47.

4.1.6. (*Z*-3-Phenylsulfonylprop-1-en-1,2-diyl)-bis-4,5,6,7,8,9-hexahydro-1*H*-cyclooctatriazole (*Z*-14). Compound *Z*-**14** (72%) was obtained from *Z*-**13** by analogy to the procedure described for *E*-**10**. White solid: mp (CH_2Cl_2/Et_2O) 184–185 °C. ^1H NMR ($CDCl_3$): δ 1.43 (m, 10H), 1.70 (m, 4H), 1.78 (m, 2H), 2.27 (br s, 2H), 2.63 (m, 2H), 2.84 (m, 4H), 4.56 (s, 2H, H-3′), 7.16 (s, 1H, H-1′), 7.54 (m, 2H, *m*-Ph), 7.65 (m, 1H, *p*-Ph), 7.82 (m, 2H, *o*-Ph). ^{13}C NMR ($CDCl_3$): δ 21.44 (t), 21.93 (t), 23.96 (t), 24.10 (t), 24.19 (t), 24.36 (t), 24.69 (t), 25.70 (t), 25.91 (t), 26.04 (t), 27.04 (t), 27.99 (t), 60.94 (t, C-3′), 119.52 (s), 122.91 (d), 127.85 (d, 2C), 129.42 (d, 2C), 134.32 (d), 134.35 (s), 135.53 (s), 138.35 (s, *i*-Ph), 144.13 (s), 144.43 (s). Anal. Calcd for $C_{25}H_{32}N_6O_2S$ (480.63): C, 62.47; H, 6.71; N, 17.49. Found: C, 61.81; H, 6.72; N, 17.30.

4.1.7. *E*/*Z*-(2,3-Diazidobut-2-enylsulfonyl)-benzene (*E*/*Z*-16). Compound **15**[18] (1.12 g, 4.77 mmol) in $CHCl_3$ (10 mL) was treated with DABCO (1.07 g, 9.5 mmol) at 18 °C. Stirring was continued at the same temperature for additional 90 min. The solvent was partially removed, and the residue was filtrated through silica gel with Et_2O. The etheric phase, which contained the allenyl azide **19**,[18] was concentrated and dissolved rapidly in CH_2Cl_2 (10 mL). Then TMGA (0.75 g, 4.77 mmol) was added. After stirring for 20 min at room temperature, the solvent was partially removed. The residue was filtrated through silica gel with Et_2O, and the solvent was partially evaporated. By standing at −18 °C, the product crystallized slowly. The solid was filtrated and washed with Et_2O/*n*-hexane (3:2). The filtrate was concentrated, dissolved in a small amount of Et_2O, and crystallization was repeated for several times to give the desired products *E*/*Z*-**16** (100 mg, 0.36 mmol, 7.6%, *E*/*Z*=2:3) as yellow-orange solid. IR ($CDCl_3$, mixture): $\tilde{\nu}$=2110 (N_3), 1325 (SO_2), 1139 (SO_2) cm^{-1}. ^1H NMR ($CDCl_3$, mixture): δ 1.77 (s, 3H, H_3C, *E*-isomer), 1.83 (s, 3H, H_3C, *Z*-isomer), 3.92 (s, 2H, H-1′, *Z*-isomer), 4.12 (s, 2H, H-1′, *E*-isomer), 7.61 (m, 2×2H, *m*-Ph), 7.71 (m, 2×1H, *p*-Ph), 7.91 (m, 2×2H, *o*-Ph). ^{13}C NMR ($CDCl_3$, mixture): δ 12.68 (q, H_3C), 14.47 (q, H_3C), 53.30 (t, C-1′), 56.30 (t, C-1′), 109.61 (s), 111.36 (s), 126.79 (s), 127.33 (s), 128.27 (d, 2C), 128.62 (d, 2C), 128.66 (d, 2C), 129.48 (d, 2C), 134.41 (d), 134.59 (d), 136.92 (s, 2C).

4.1.8. *E*/*Z*-(2,3-Diazidobut-2-enylsulfonyl)-benzene (*E*/*Z*-16) and *E*/*Z*-(2,3-Diazidobut-1-enylsulfonyl)-benzene (*E*/*Z*-17). Compound **15**[18] (400 mg, 1.70 mmol) in CH_2Cl_2 (25 mL) was treated with TMGA (0.30 g, 1.90 mmol) at −10 °C over a period of 2 min. Stirring was continued at −5 °C for 2 h. The solvent was partially removed, and the residue was chromatographed on silica gel with Et_2O/*n*-hexane (3:2). *E*-**17** was obtained as major product in a mixture with *E*-**16** (150 mg, 0.54 mmol, 32%, *E*-**17**/*E*-**16**=18:1) and then *Z*-**17** as major product in a mixture with *Z*-**16** (90.0 mg, 0.324 mmol, 19%, *Z*-**17**/*Z*-**16**=6:1). When the reaction was executed at 0 °C instead of room temperature, *E*-**17**/*E*-**16** (4:1) and *Z*-**17**/*Z*-**16** (6:1) were obtained after chromatography. For the data of *E*/*Z*-**16**, see Section 4.1.7.

E-(2,3-Diazidobut-1-enylsulfonyl)-benzene (*E*-**17**): IR ($CDCl_3$, mixture of *E*-**17**/*E*-**16**): 2113 (N_3), 1246 (N_3), 1307 (SO_2), 1153 (SO_2) cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.40 (d, ^3J=6.6 Hz, 3H, H_3C), 5.49 (q, ^3J=6.6 Hz, 1H, H-3′), 6.02 (s, 1H, H-1′), 7.58 (m, 2H, *m*-Ph), 7.66 (m, 1H, *p*-Ph), 7.91 (m, 2H, *o*-Ph). ^{13}C NMR ($CDCl_3$): δ 17.36 (q, H_3C), 52.16 (d, C-3′), 115.70 (d, C-1′), 127.14 (d, 2C), 129.57 (d, 2C), 133.84 (d, *p*-Ph), 141.32 (s, *i*-Ph), 154.35 (s, C-2′).

Z-(2,3-Diazidobut-1-enylsulfonyl)-benzene (*Z*-**17**): IR ($CDCl_3$, mixture of *Z*-**17**/*Z*-**16**=6:1): 2121 (N_3), 1309 (SO_2), 1151 (SO_2) cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.51 (d, ^3J=6.6 Hz, 3H, H_3C), 4.19 (q, ^3J=6.6 Hz, 1H, H-3′), 5.98 (s, H-1′), 7.55 (m, 2H, *m*-Ph), 7.63 (m, 1H, *p*-Ph), 7.98 (m, 2H, *o*-Ph). ^{13}C NMR ($CDCl_3$): δ 17.82 (q, H_3C), 58.50 (d, C-3′), 115.82 (d, C-1′), 127.72 (d, 2C), 129.03 (d, 2C), 133.58 (d, *p*-Ph), 141.29 (s, *i*-Ph), 148.77 (s, C-2′).

4.1.9. (*E*-1-Phenylsulfonylbut-1-en-2,3-diyl)-bis-4,5,6,7,8,9-hexahydro-1*H*-cyclooctatriazole (*E*-18). A mixture (85.0 mg, 0.306 mmol) of *E*-**16** and *E*-**17** (1:18) in CH_2Cl_2 (10 mL) was stored at room temperature over a period of 48 h. The ^1H NMR spectra proved the total decomposition of *E*-**16**. The mixture was then treated with cyclooctyne according to the procedure described for *E*-**10** and chromatographed to give *E*-**18** (136 mg, 0.275 mmol, 90%) as yellow oil. ^1H NMR ($CDCl_3$): δ 1.27–1.90 (m, 18H), 2.03 (d, ^3J=7.2 Hz, 3H, H_3C), 2.76 (m, 6H), 6.33 (s,

1H, H-1′), 6.87 (q, $^3J=7.2$ Hz, 1H, H-3′), 7.62 (m, 2H, *m*-Ph), 7.70 (m, 1H, *p*-Ph), 7.94 (m, 2H, *o*-Ph). ^{13}C NMR ($CDCl_3$): δ 18.69 (q, H_3C), 21.20 (t), 21.48 (t), 23.87 (t), 24.21 (t), 24.36 (t), 24.46 (t), 25.40 (t), 25.82 (t), 25.98 (t), 26.05 (t), 27.29 (t), 28.22 (t), 51.81 (d, C-3′), 127.64 (d, 2C), 129.79 (d, 2C), 132.42 (d), 134.06 (s), 134.85 (d), 135.00 (s), 138.97 (s), 143.86 (s), 144.47 (s), 145.29 (s). HRMS (ESI): *m*/*z* calcd for $C_{26}H_{34}N_6O_2S$ [M+H$^+$]: 495.2463; found: 495.2451.

4.1.10. *Z*-2-Azido-3-chloro-3-phenyl-propenal (*Z*-24). Compound *Z*-**24** (70%, yellow, light-sensitive solid after recrystallization from Et_2O) was obtained from 2-azido-1-phenyl-ethanone[25] by analogy to the procedure described by Perumal and co-workers;[21] decomposition above room temperature. IR ($CDCl_3$): 2113 (N_3), 1674 (CHO) cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 7.42–7.45 (m, 4H, Ph), 7.47–7.51 (m, 1H, *p*-Ph), 9.41 (s, 1H, C*H*O). ^{13}C NMR ($CDCl_3$): δ 128.54 (d), 130.27 (d), 130.86 (d), 133.43 (s), 140.21 (s), 184.14 (d, *C*HO). One ^{13}C NMR signal could not be detected.

4.1.11. *Z*-2,3-Diazido-3-phenyl-propenal (*Z*-26). To a stirred solution of *Z*-**24** (0.94 g, 4.53 mmol) in $CHCl_3$ (16 mL), QN_3 (2.28 g, 4.86 mmol) was added. The mixture was stirred over a period of 90 min. The solvent was partially removed, and the residue was purified by flash chromatography on silica gel with Et_2O yielding *Z*-**26** (0.83 g, 3.87 mmol, 86%) as yellow, light-sensitive solid after recrystallization from Et_2O; decomposition above room temperature. IR (CCl_4): 2102 (N_3), 1669 (CHO) cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 7.34–7.42 (m, 2H, Ph), 7.49–7.58 (m, 3H, Ph), 9.16 (s, 1H, C*H*O). ^{13}C NMR ($CDCl_3$): δ 123.31 (s), 127.81 (s), 129.12 (d), 129.61 (d), 131.38 (d), 145.43 (s), 185.70 (d, *C*HO). UV/vis (cyclohexane): λ_{max}/nm (log ε) 326 (3.96), 251 (3.89).

4.1.12. *Z*-2,3-Diazido-3-(4-chlorophenyl)-propenal (*Z*-27). For the synthesis of *Z*-**27** (91%, yellow, light-sensitive solid after recrystallization from Et_2O) from **25**[21] see the procedure for *Z*-**26**; decomposition above room temperature. IR ($CDCl_3$): 2107 (N_3), 1666 (CHO) cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 7.36 (AA′BB′, 2H, Ph), 7.51 (AA′BB′, 2H, Ph), 9.20 (s, 1H, C*H*O). ^{13}C NMR ($CDCl_3$): δ 125.26 (s), 127.48 (s), 129.52 (d), 131.23 (d), 137.74 (s), 143.59 (s), 184.66 (d, *C*HO). UV/vis (cyclohexane): λ_{max}/nm (log ε) 328 (3.89), 260 (3.95), 243 (3.99).

Acknowledgements

Joseph Rodolph Fotsing gratefully thanks the German Academic Exchange Service (DAAD) for a generous fellowship. The research was supported by the Fonds der Chemischen Industrie.

References and notes

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Tetrahedron 61 (2005) 8910–8915
Tetrahedron

Isolation of naturally occurring dactylomelane metabolites as *Laurencia* constituents

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Received 29 April 2005; revised 28 June 2005; accepted 6 July 2005

Abstract—Dactylomelol **1**, initially isolated from molluscs of the genus *Aplysia*, was the first example of a monocarbocyclic diterpene skeleton with a C-6–C-11 cyclisation. This paper reports on the identification and structural elucidation of six new diterpenes, compounds **4–9**, isolated from specimens of *Laurencia* sp., together with dactylomelol, all with this particular carbon backbone that we named dactylomelane. This result is the first to confirm a red alga as the biogenetic origin of this class of compounds.

1. Introduction

Marine diterpenes have been isolated from both animals and plants. The coelenterates, principally class Actinozoa, molluscs of the genus *Aplysia*, selected species of sponges, tropical and subtropical algae such as green and brown algae are the main marine sources. Although, red algae of the genus *Laurencia* produce principally bromine-containing natural metabolites with sesquiterpene skeletons, there are several examples of bromine diterpenes as well. Formally, the biosynthesis of these cyclic diterpenes is initiated by the formation of a bromonium ion that evolves to their characteristic cyclic carbon skeletons. Within this group, diterpenes that present in their carbon skeleton only one carbon cyclisation between C-6 and C-11 are unusual and, as far as we are aware, only three examples have been reported with this carbon backbone (Fig. 1).[1–4] The first example of this type of carbon skeleton was that of dactylomelol **1**, isolated from the digestive glands of the shell-less mollusc *Aplysia dactylomela* whose structure was secured by X-ray diffraction methods. It was proposed as a potentially useful defensive substance.[5] The second example, sphaerolabdadiene-3,14-diol **2**, was isolated from the red alga *Sphaerococcus coronopifolius*, in the course of a programme aimed at the isolation and characterisation of bioactive compounds along the coast of Morocco. Its structure and stereochemistry were determined by spectroscopical methods.[6] Recently, from a sea hare *Aplysia punctata* collected in Sardinia (Italy) was isolated puctatene acetate **3**, a compound closely related to dactylomelol **1**, whose structure was determined by spectral methods.[7]

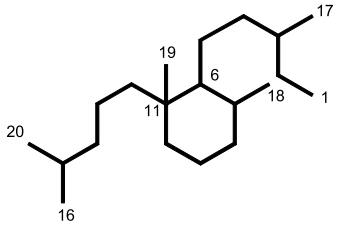

Figure 1. The dactylomelanes arise by cyclisation between carbons C-6 and C-11 of the phytane skeleton.

2. Results and discussion

We wish to report here on the isolation and structural determination from specimens of *Laurencia* sp. of several new diterpene metabolites possessing a carbon skeleton that we named dactylomelane. The alga was collected in April 2002 at Paraiso Floral (South Tenerife, Canary Islands), and extracted with dichloromethane/methanol (1:1) at room temperature. The crude extract was successively chromatographed on Sephadex LH-20 and medium pressure silica gel columns whereas the final purification was achieved by HPLC on μ-Porasil. This chromatographic study afforded, in addition to dactylomelol **1**, new relatively unstable hydroperoxide compounds such as *E* and *Z* dactylohydroperoxides A **4–5**, dactylohydroperoxide B **6** and its

Keywords: Dactylomelane; *Laurencia* sp.; *Aplysia*.
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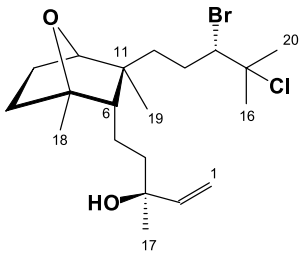
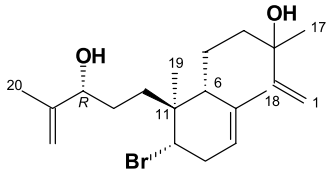
doi:10.1016/j.tet.2005.07.024

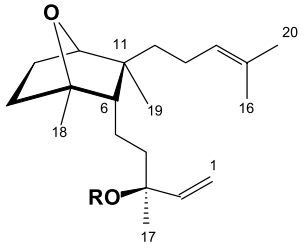
corresponding alcohol **7**, dactylohydroperoxide C **8** and puctatene **9**.



1 Dactylomelol



2 Sphaerolabdadiene-3,14-diol



3 Puctatene acetate; R=Ac
9 Puctatene, R=H

Compound **4**, *E*-dactylohydroperoxide A, was obtained as an amorphous white solid. Mass spectral analysis of this metabolite showed a molecular ion at *m/z* 338.2464 consistent with the molecular formula $C_{20}H_{34}O_4$ (Calcd 338.2457). Assignment of the structure for this metabolite was aided by its spectroscopical analogy to dactylomelol **1** (Tables 1 and 2). The NMR spectrum of compound **4** was nearly identical with that of **1** with the exception of the data belonging to fragment C-11→C-15, including the methyl groups at carbon C-16 and C-20, which were centred at δ_H 1.35 and 1.34, respectively. Thus, the proton chemical shift for H_2-12 (δ_H 2.12, dd, J=7.9, 13.0 Hz; 2.04, dd, J=7.8, 13.0 Hz), H-13 (δ_H 5.80, ddd, J=7.8, 7.9, 15.7 Hz) and H-14 (δ_H 5.50, d, J=15.7 Hz) were correlated in the HSQC experiment with the carbon signals centred at δ_C 46.7, 128.7 and 135.8, thus, establishing the presence of an *E* double bond between carbons C-13 and C-14. Furthermore, the appearance in compound **4** of a new singlet proton at δ_H 8.45 as well as that of a quaternary oxygen-bearing carbon at δ_C 81.7 suggested the presence at this carbon of a hydroperoxide group. This was located at C-15 by its carbon signal correlations in the HMBC experiment with the protons H-13, H-14, H_3-16 and H_3-20, while the methylene protons H_2-12 were correlated with the quaternary carbon at C-11 (δ_C 45.1). All these data permit to us to unequivocally establish the structure for the C-11→C-15 fragment. The relative configurations of the stereocentres of the carbons C-3, C-6, C-7, C-10 and C-11 of **4** were determined to be identical with those found in compound **1**, based on the connectivities observed in the ROESY experiment. Thus, in addition to the ROE connectivity between H-6 (δ_H 1.39) and H-13 (δ_H 5.80), a significant ROE enhancement between the proton H-14 and protons H_3-19 (δ_H 0.89) was also observed. This fact can only be explained by the presence of an intramolecular hydrogen bond between the hydroxyl group at C-3 and the hydroperoxide group at C-15 that fixed a cyclic-like ring structure as shown in Figure 2 and established that the conformation form of *E*-dactylohydroperoxide A **4** in solution may not be extended as in dactylomelol **1**.[8]

Compound **5** proved to be an isomer of **4** and differed from it in the C-13=C-14 double bond. Thus, the allylic methylene protons H_2-12 appear centred at δ_H 2.08 (H-12α, dd, J=4.0, 12.0 Hz; H-12β, dd, J=12.0, 12.0 Hz) and connected in the COSY experiment with H-13 at δ_H 5.59 (ddd, J=4.0, 10.0, 12.0 Hz) that involves the *Z* olefin at H-14 (δ_H 5.63, d, J=10.0 Hz). Moreover, the carbon signal for C-15, shifted from δ_C 81.7 to 71.2 in accordance with the *Z* geometry of the olefin.[9–11] These data establish that compound **5** is *Z*-dactylohydroperoxide A. The correlations observed for this compound in the ROESY experiment showed that the relative stereochemistry was identical with that observed for compound **4**. Moreover, the presence of a H-bond between

Table 1. ^{1}H NMR data for compounds **1** and **4–9** in $CDCl_3$

Carbon	1	4	5	6	7	8	9
1	5.15/5.01	5.21/5.08	5.20/5.07	5.20/5.08	5.21/5.07	5.22/5.08	5.19/5.05
2	5.84	5.90	5.89	5.90	5.90	5.90	5.88
4	1.47/1.40	1.54/1.45	1.52/1.44	1.54/1.42	1.55/1.47	1.52/1.46	1.53/1.45
5	1.27/1.17	1.34/1.18	1.34/1.17	1.31/1.19	1.31/1.16	1.31/1.22	1.29/1.15
6	1.13	1.39	1.25	1.17	1.17	1.19	1.15
8	1.59/1.14	1.64/1.19	1.64/1.15	1.63/1.21	1.64/1.20	1.67/1.21	1.63/1.17
9	1.75/1.53	1.81/1.61	1.79/1.60	1.75/1.61	1.77/1.63	1.78/1.64	1.76/1.60
10	3.87	3.95	3.90	3.90	3.93	3.91	3.94
12	1.82/1.30	2.12/2.04	2.08 (2H)	1.41 (2H)	1.50/1.25	1.75/1.71	1.42/1.31
13	2.23/1.61	5.80	5.59	1.56/1.41	1.51 (2H)	2.41 (2H)	1.88 (2H)
14	3.91	5.50	5.63	4.25	4.01		5.06
16	1.72	1.35	1.32	5.03/5.01	4.93/4.84	1.25	1.65
17	1.23	1.29	1.28	1.29	1.29	1.30	1.27
18	1.33	1.37	1.39	1.38	1.38	1.39	1.36
19	0.81	0.89	0.84	0.83	0.84	0.85	0.84
20	1.60	1.34	1.31	1.73	1.72	1.25	1.58
–OOH		8.45	10.13	7.91		9.77	

Bruker 500 AMX, chemical shifts refer to TMS δ_H 0.

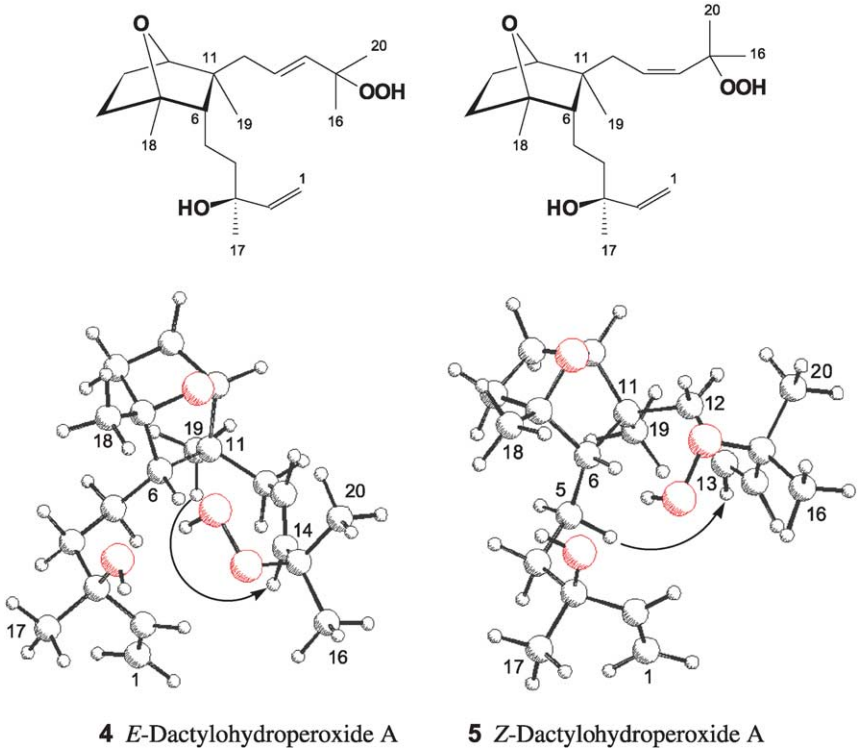
Table 2. ^{13}C NMR data for compounds **1** and **4–9** in $CDCl_3$

Carbon	1	4	5	6	7	8	9
1	112.0	112.0	112.7	111.8	112.3	112.1	112.1
2	144.9	144.7	146.5	145.6	145.3	144.7	145.3
3	72.4	73.2	73.7	73.1	73.7	73.5	73.5
4	42.1	41.9	42.5	42.1	42.3	41.9	42.4
5	21.2	20.9	22.7	21.3	21.6	21.7	21.5
6	56.0	53.8	55.7	55.9	56.6	55.8	56.6
7	87.9	88.3	88.3	87.9	88.1	88.0	88.1
8	29.1	29.0	29.3	29.1	29.4	29.1	29.4
9	26.6	26.6	26.3	26.8	26.9	26.6	26.8
10	84.1	84.3	84.7	83.4	84.2	83.5	84.0
11	45.1	45.4	45.8	44.8	45.3	44.9	45.7
12	42.8	46.7	46.7	39.7	40.2	35.6	44.5
13	30.0	128.7	123.7	25.6	30.2	40.0	23.2
14	66.6	135.8	141.7	90.1	77.0	202.8	125.1
15	73.2	81.7	71.2	143.3	145.6	91.1	131.5
16	33.3	25.1	29.2	115.0	111.9	29.6	25.7
17	27.8	27.8	29.5	27.9	28.2	28.1	28.1
18	21.7	21.2	22.0	21.7	22.2	21.9	21.9
19	17.1	18.6	17.3	17.0	17.5	17.0	17.2
20	27.0	24.7	29.2	17.3	17.8	29.6	18.1

Bruker 400 Avance, chemical shifts refer to $CDCl_3$ δ_C 77.0.

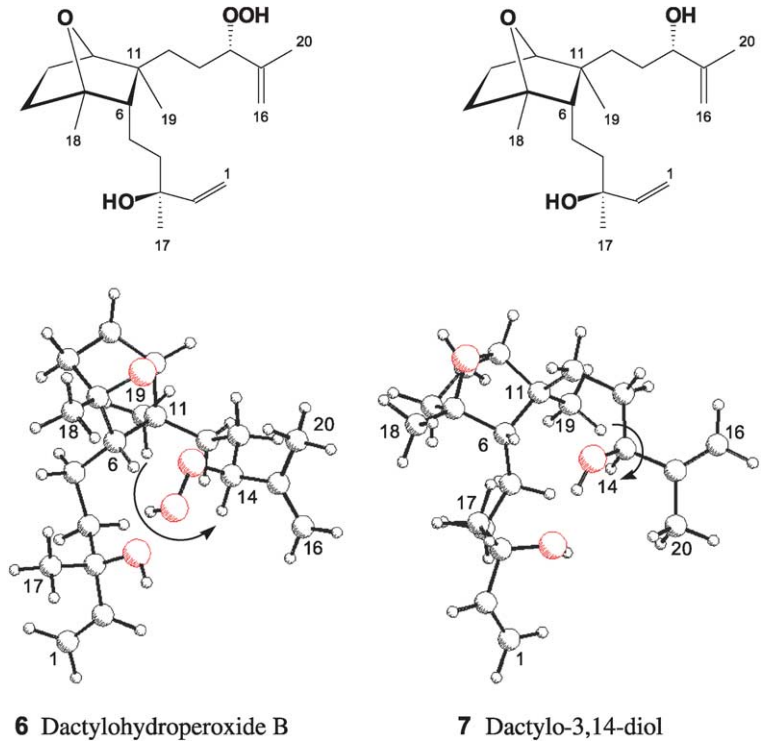
the hydroperoxide and the hydroxyl group was supported by the ROE correlations between the proton H-13 and the protons H_3-19 (δ_H 0.84) and H-5 (δ_H 1.34) generating a cyclic-like ring comparable to compound **4** (Fig. 2).

The next compound, dactylohydroperoxide B **6**, proved to be an isomer of compounds **4** and **5**, as established by HRMS, and the detailed comparison of their spectral data showed that the differences between them were also located at fragment C-11→C-15. Thus, the methyl groups attached to a hydroperoxide-bearing carbon in **4** and **5** were substituted in **6** by an olefinic methylene centred at δ_H 5.03/5.01 and by a vinylic methyl group at δ_H 1.73. Likewise, instead of the olefinic methine signals, which disappeared in compound **6**, a new proton signal was observed at δ_H 4.25 that correlated in the HSQC with a carbon signal centred at δ_C 90.1. Furthermore, this proton signal together with those of H_2-16 (δ_H 5.03 and 5.01) and H_3-20 (δ_H 1.73) showed correlation, in the HMBC experiment, with the quaternary carbon C-15 (δ_C 143.3), establishing the structural proposal. Similarly to compounds **4** and **5**, the ROE correlations observed for dactylohydroperoxide B **6** pointed to a relative stereochemistry identical to that of dactylomelol **1**. As in the previous examples, the intense dipolar correlation between proton H-14 and protons H_3-19 can only be explained by the



4 *E*-Dactylohydroperoxide A **5** *Z*-Dactylohydroperoxide A

Figure 2. Stereo view of proposed 3D structures and significant ROE correlations for dactylohydroperoxides A.



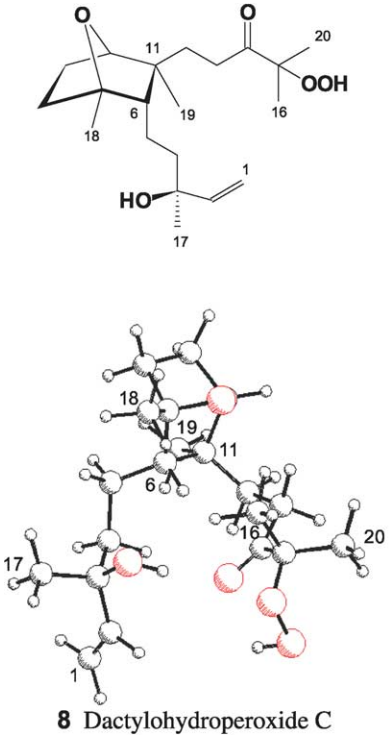
6 Dactylohydroperoxide B **7** Dactylo-3,14-diol

Figure 3. Lowest energy conformations and most significant ROE correlations for compounds **6** and **7**.

presence of an intramolecular hydrogen bond between the hydroxyl group at C-3 and the hydroperoxy moiety with an *S** relative configuration at C-14, as illustrated in Figure 3.

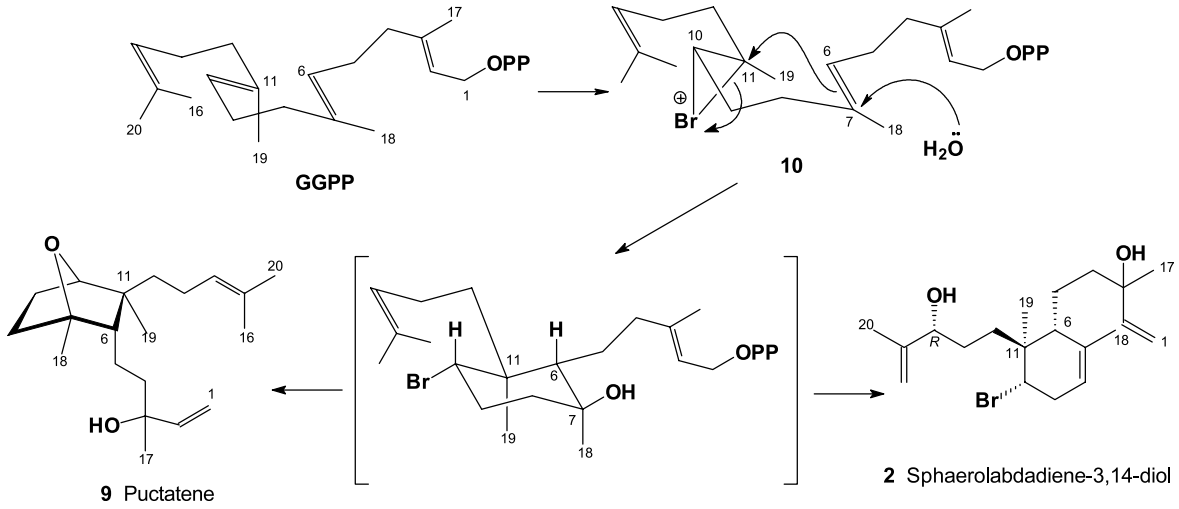
Compound **7**, dactylo-3,14-diol, was easily identified through its HRMS and by comparison of the NMR spectral data with those of **6**, which showed that the differences between them were located around carbon C-14. Thus, in the ^{1}H NMR spectrum of **6**, the H-14 proton signal was centred at δ_H 4.25 while in compound **7**, that signal was centred at δ_H 4.01 (dd, $J=5.6$, 5.9 Hz) and the hydroperoxide proton signal at δ_H 7.91 disappeared. More clearly, the chemical shift of the carbon bearing an oxygen atom C-14 in the ^{13}C NMR was centred at δ_C 90.1 in compound **6**, while in compound **7** it was centred at δ_C 77.0. These differences together with the HRMS suggested the presence at C-14 of a hydroxy group in compound **7** instead the hydroperoxy group of compound **6**. On the other hand, the ROESY correlations observed for compound **7** showed an identical stereochemistry with that of compound **6**, including the H-bond between the hydroxyl groups at C-3 and at C-14 (Fig. 3).

Compound **8** proved to be a new example of a dactylohydroperoxide derivative, the differences with the dactylohydroperoxides A and B being located in the fragment C-11→C-15. In accordance with the presence of a strong band at 1732 cm^{-1} in the IR and the carbon signal centred at δ_C 202.8 in the ^{13}C NMR spectrum, the presence of a carbonyl group was suggested. This was confirmed by the proton signals centred at δ_H 2.41 (2H, dd, $J=8.0$, 8.3 Hz) with a typical chemical shift for a methylene α to a carbonyl group, was correlated in the COSY experiment with the signals from an isolated methylene group centred at δ_H 1.75 and 1.71, thus, establishing the fragment ■–CH_2–CH_2–CO–■. This fragment was located between C-12 and C-14 due to the HMBC correlation of the proton signals centred at δ_H 1.75 and 1.71 (H_2-12) and the carbon signal for C-11 at δ_C 44.9. On the other hand, the hydroperoxide proton signal shifted downfield (δ_H 9.77) in comparison with compound **6** and was located at C-15 (δ_C 91.1) in accordance with the correlation of this carbon with



8 Dactylohydroperoxide C

Figure 4. Lowest energy conformation for dactylohydroperoxide C.



Scheme 1. Possible biogenesis for the dactylomelane skeleton.

the protons H_3-16 and H_3-20 at δ_H 1.25. Finally, the ROESY correlations established that the structure of the side chain and the relative stereochemisty of the chiral centres are as shown in Figure 4.[12]

Puctatene **9** was isolated as a colourless oil. Its spectroscopic data were closely related to those of puctatene acetate **3** isolated from the sea hare *Aplysia punctata*. Interpretation of COSY, HMQC, HMBC and ROESY data allowed assignments of ^1H and ^{13}C signals (Tables 1 and 2), which readily demonstrated that the sole difference between them was the absence of signals corresponding to the acetate moiety present in compound **3**. In addition, treatment of dactylomelol **1** with Zn/AcOH at 0 °C yielded puctatene **9**. This result led to the conclusion that the absolute configurations at C-3, C-6, C-7, C-10 and C-11 in compound **9** are *S*, *S*, *S*, *R* and *S*, respectively.

From a biogenetic point of view, the basic skeleton of dactylomelane can be viewed as resulting from the C-6–C-11 cyclisation of the geranylgeraniol bromonium ion derivative **10** yielding the cation at C-7, which is quenched with water. This intermediate could evolve by S_N2 nucleophilic substitution of the bromine atom at C-10 to give puctatene **9** or an *E*2 elimination that yielded the sphaerolabdadiene-3,14-diol **2** (Scheme 1). Puctatene could progress to the formation of dactylomelol **1** or, on the other hand, by the action of peroxidase enzymes to produce the compounds **4**–**7**, as shown in Scheme 2.

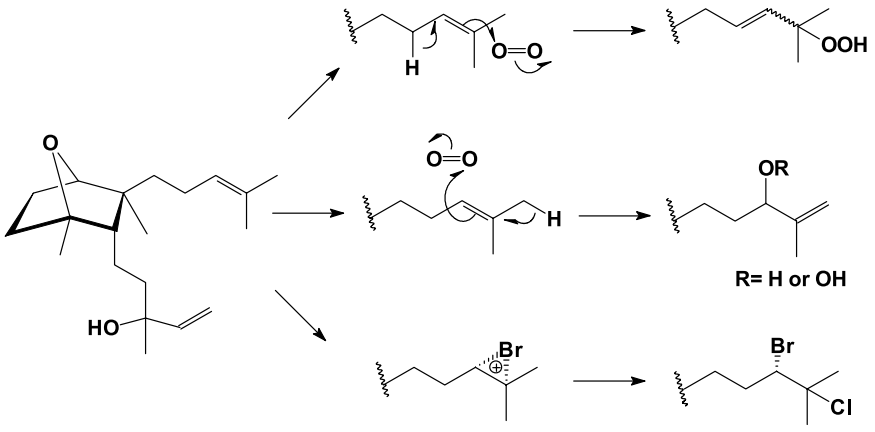
3. Experimental

3.1. General methods

Optical rotations were determined on a Perkin-Elmer 241 polarimeter. IR spectra were measured on a Bruker IFS55 spectrometer. The NMR spectra were obtained with Bruker 400 Avance and 500 AMX instruments. Chemical shifts are reported relative to TMS and coupling constants are given in Hz. HREIMS were performed on a VG AutoSpec FISON spectrometer. HPLC was carried out with a LKB 2248 system equipped with a differential diffractometer detector. Silica gel CC and TLC were performed on Silica gel Merck 60 G. TLC plates were visualised by spraying with H_2SO_4/H_2O/AcOH (1:4:20) and heating.

3.2. Plant material

Specimens of *Laurencia* sp. were collected in April 2002 in the intertidal zone at Paraíso Floral (Tenerife, Canary Islands). Dried material of sterile plants, sporophytes and



Scheme 2. Plausible biogenetic pathway for compounds **1** and **4**–**7**.

gametophytes was filed at TFC Phyc. 9860 (Herbario de la Universidad de La Laguna, Departamento de Biología Vegetal, Botánica, Tenerife).

3.3. Extraction

The dried alga (0.3 kg) was extracted with CH_2Cl_2/MeOH (1:1) at room temperature. The combined extracts were evaporated in vacuo to leave a dark-green viscous oil (19.5 g, 1.3% dry weight).

3.4. Chromatographic separation

The crude extract was chromatographed on a Sephadex LH-20 column using as eluent *n*-hexane/$CHCl_3$/MeOH (2:1:1). Selected fractions exhibiting similar TLC profiles were combined and rechromatographed on medium pressure silica gel Si 60 columns eluted with EtOAc/*n*-hexane at 1:4 concentrations. Final purification was carried out by HPLC on a μ-Porasil column using similar eluent mixes in different proportions affording the pure new compounds dactylomelol **1** (279.1 mg), *E*-dactylohydroperoxide A **4** (3.1 mg), *Z*-dactylohydroperoxide A **5** (0.4 mg), dactylohydroperoxide B **6** (1.5 mg) and its corresponding alcohol **7** (1.1 mg), dactylohydroperoxide C **8** (3.0 mg) and puctatene **9** (43.4 mg).

3.4.1. Compound 4. Amorphous white solid; $[\alpha]_D^{25}$ +1.3 (*c* 0.30, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3629, 3363, 2969, 1749, 1458, 1381, 993, 919 cm^{-1}; for ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Tables 1 and 2; EIMS *m/z* 338, 320, 305, 304, 287, 269, 223, 109; HREIMS *m/z* 338.2464 (Calcd for $C_{20}H_{34}O_4$, 338.2457).

3.4.2. Compound 5. Amorphous white solid; $[\alpha]_D^{25}$ −70.0 (*c* 0.01, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3627, 3544, 2967, 1683, 1652, 1456, 1057 cm^{-1}; for ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Tables 1 and 2; EIMS *m/z* 338, 307, 176, 154, 138, 124; HREIMS *m/z* 338.2466 (Calcd for $C_{20}H_{34}O_4$, 338.2457).

3.4.3. Compound 6. Amorphous white solid; $[\alpha]_D^{25}$ −73.3 (*c* 0.03, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3629, 3599, 2918, 2850, 1089 cm^{-1}; for ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Tables 1 and 2; EIMS *m/z* 338, 307, 302, 267, 253, 223; HREIMS *m/z* 338.2466 (Calcd for $C_{20}H_{34}O_4$, 338.2457).

3.4.4. Compound 7. Amorphous white solid; $[\alpha]_D^{25}$ −33.3 (*c* 0.03, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3627, 3598, 2924, 1085 cm^{-1}; for ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Tables 1 and 2; EIMS *m/z* 322, 307, 289, 224, 124; HREIMS *m/z* 322.2521 (Calcd for $C_{20}H_{34}O_3$, 322.2508).

3.4.5. Compound 8. Amorphous white solid; $[\alpha]_D^{25}$ +6.1 (*c* 0.15, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3419, 2925, 1732, 1456, 1382, 1261, 1022 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) see Tables 1 and 2; EIMS *m/z* 355, 341, 313, 305, 283, 237, 223; HREIMS *m/z* 355.2402 (Calcd for $C_{20}H_{34}O_5$, 355.2406).

3.4.6. Compound 9. Colourless oil; $[\alpha]_D^{25}$ +3.4 (*c* 4.60, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3418, 2968, 2880, 1749, 1458, 1381, 995 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) see Tables 1 and 2; EIMS *m/z* 306, 288, 223, 206; HREIMS *m/z* 306.2518 (Calcd for $C_{20}H_{34}O_2$, 306.2559).

3.5. Preparation of puctatene 9 from dactylomelol 1. To a solution of 2 mg of dactylomelol **1** in 0.5 mL of EtOH at 0 °C was added 2 mg of Zn powder and 0.1 mL of AcOH. The mixture was stirred for 3 h, filtered off and the solvent evaporated to yield 1.3 mg of compound **9**.

Acknowledgements

The authors acknowledge financial support from the MEC (PPQ2002-04361-C04-04; AGL2004-08241-C04-01) and MEC-FSE (Programa Ramón y Cajal, MLS). L.V.G is grateful to the MEC for a research fellowship.

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Search for strategies by computer: the CONAN approach. Application to steroid and taxane frameworks

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Received 28 April 2005; revised 5 July 2005; accepted 6 July 2005

Available online 28 July 2005

Abstract—We describe the CONAN (CONnectivity ANalysis) program the aim of which is to search for strategies by simplification of the target skeleton by different types of disconnection. The options available for the disconnections of the target are: add one bond, delete one or two bonds, search for linear precursors, for convergent strategies, for precursors containing a given number of rings and linear chains, and then disconnection of rings by formal [$x+y$] reaction types like [2+2], [4+2], etc. Taxane and steroid skeletons have been studied. CONAN runs on PC's microcomputers.

1. Introduction

In 1988 we developed the computer-aided organic synthesis (CAOS) MARSEIL program for Macintosh.[1] In this article an approach to CAOS by means of several programs was proposed; MARSEIL being the first one. An approach to search for the key step of a synthesis by coding the target and reactions at a skeleton level was, then, developed.[2] More recently, we described a computer program, which proposed new strategies by disconnecting the skeleton of the target by means of 'pseudo reactions', which delete and/or add bonds.[3] This program was called CONAN from Wender's chapter dealing with CONnectivity ANalysis[4] who shows the importance to work on the target skeleton as in the Hendrickson's approach for multiple constructions.[5] The results of this first version of CONAN were interesting, nevertheless there was room for improvements. For example, when the user wanted to delete two bonds, all the combinations were displayed, without selection. Also, all the transforms were coded by means of substructures and the associated connectivity tables were matched with the connectivity table of the target, even the ones for adding or deleting bonds, the overall process being relatively slow. We present now an improved version of CONAN: for deleting bonds the program does not use transforms, this is done by direct computing. This is faster than the previous version. Also, this new version of CONAN includes new options such as searching for convergent disconnections of the target, searching for linear precursors and searching to disconnect rings by means of formal standard reactions ([4+2], [2+2], [2+2+2], etc). The disconnections generated by CONAN are 'naked' solutions and the chemist has to 'dress' them by searching for the reactions which will allow to built the target and adding the necessary functional groups. CONAN runs on PC's microcomputers.

2. Program and results

The screen is divided in four windows. The first one is devoted to the drawing of the target, which is user friendly by means of mouse and menus.

Two menus for disconnecting the target are available in CONAN. The first one deals with the different strategies: disconnection of one or two bonds, addition of one bond between two atoms, convergent disconnections, search for linear precursors and search for precursors with a number of rings and linear chains requested by the user. The second menu concerns the disconnection of rings by means of formal [$x+y$] reaction type ([2+2], [4+2], etc.). Some examples will illustrate these options.

One-bond disconnection. For this option and the following one, the program ranks the bonds from their 'complexity'. We call 'complexity' of a bond the sum of the connectivity (i.e., the number of neighbours, excepted hydrogen atoms) of the two atoms of the bond. For example, in the case of

Keywords: Computer-aided organic synthesis; Retrosynthesis; Strategy; Steroid; Taxane.
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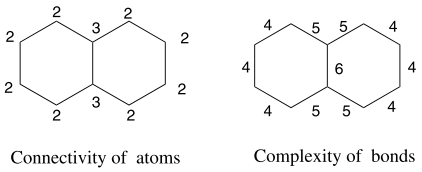
doi:10.1016/j.tet.2005.07.029



Scheme 1.

bicyclo[4.4.0] decane these values are displayed in Scheme 1.

Bonds with the greatest complexity may be considered as strategic: if one deletes them, they lead to the greatest decrease of complexity.[4] An example is given in Figure 1 in the case of bicyclo[4.4.0] decane. This identification of strategic bonds is different from Corey's approach, which is based upon chemical rules.[6]

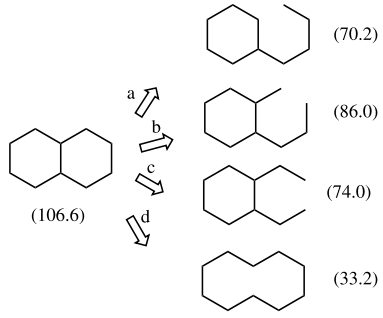
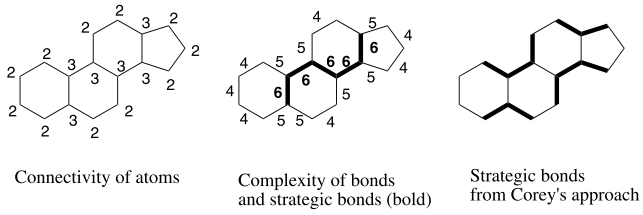


Figure 1. Numbers in parenthesis are the values of complexities calculated from Bertz's approach[7a–c] by Hendrickson's CPXCAL program.[7d] Solution a corresponds to the strategic bond according to Corey's approach and solution d to the best graphical solution with the greatest decrease in complexity.[4]

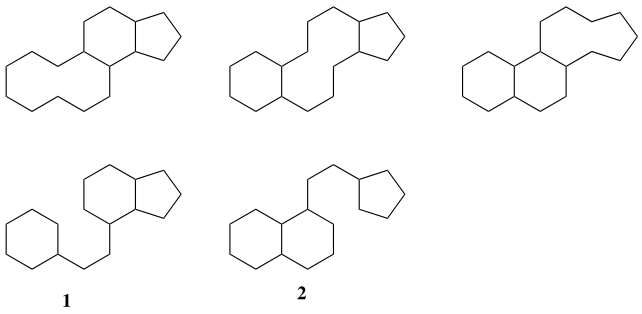
From this approach the strategic bonds of steroids are displayed in Scheme 2 (bold bonds, those with the greatest complexity), where the strategic bonds provided by Corey's analysis are also given for comparison.



Scheme 2.

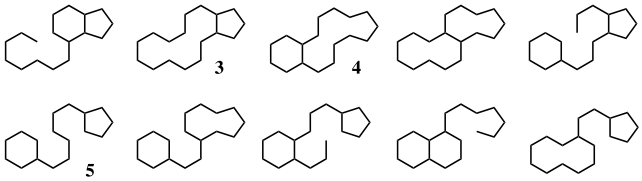
When this option is selected, the user has to choose the rank in complexity of the bonds to delete. So the user may see the solutions according to their degree of complexity change instead of seeing all the solutions indiscriminately as in the first version of CONAN.[3] With the steroid skeleton, there are 3 levels of complexity. When the chemist selects the bonds of highest priority the solutions of Scheme 3 are displayed. Ring closures as in structures **1** and **2** are found in several syntheses (see below, Scheme 4 and Fig. 4).

Two-bonds disconnection. Again, when this option is selected the user has to choose the ranks of the bonds to delete. The user may combine rank 1 with rank 1, or rank 1



Scheme 3.

with rank 2, or rank 2 with rank 3, etc. All the possible combinations can be seen. For example with a steroid skeleton, which contains 20 bonds there are 190 ways to cut two bonds. There are 5 bonds of complexity 6 (rank 1), 8 of complexity 5 (rank 2) and 7 of complexity 4 (rank 3) (Scheme 2). Figure 2 shows the number of solutions for each combination.



Scheme 4.

Since it is not possible to display all the 190 solutions, the 10 solutions generated by combining rank 1 with rank 1 are shown in Scheme 4. Structures **3** and **4** are found in strategies involving transannular Diels–Alder reaction.[8] Disconnection of structure **5** was reported in several syntheses.[9]

Addition of one bond. This strategy corresponds, in the forward direction, to fragmentation reaction. Adding a bond creates a new ring in the target and it yields a more complex skeleton. Nevertheless this strategy may be interesting if the new precursor opens the way to an easier method of building the substructure.[10] This option was available in the previous version, but the solutions were found by matching the substructure of the reaction to the target, the process was relatively slow. Now the combinations are directly computed, the program starts with atom 1 and it adds a bond to atom 2, then to atom 3 and so on, then from atom 2, and so on. To improve the flexibility of the program, an option allows to select the starting atom, that is the atom from which the bonds are added. When a solution is displayed on the screen, the user may analyse it as a target, avoiding the tedious operation of saving the precursor to disk to analyse it later. Nevertheless this possibility is also

	1(5)	2(8)	3(7)
1(5)	10	40	35
2(8)		28	56
3(7)			21

Figure 2. Number of solutions generated for each possible combination. Rank 1 (5 bonds) combined with rank 1 generates 10 solutions, with rank 2 (8 bonds) it generates 40 solutions and 35 solutions with rank 3.

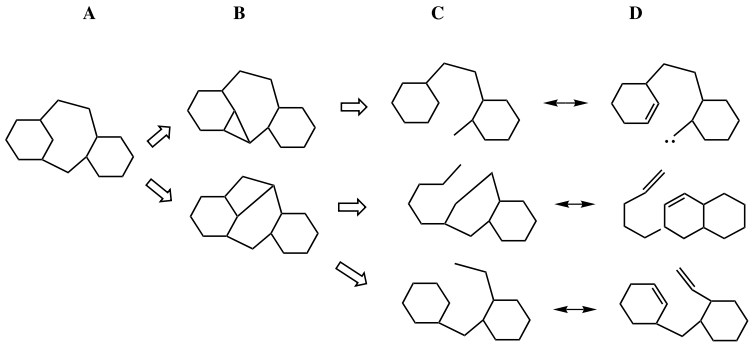
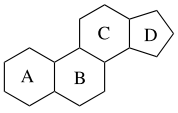


Figure 3. Column A, target; Column B, some precursors generated by the addition of one bond; Column C, some precursors generated by [2+1], [2+2] disconnections; Column D, solutions suggested from column C.

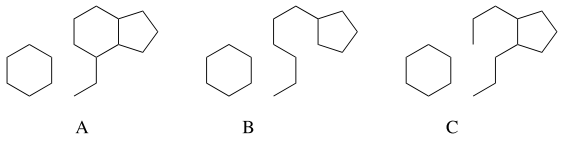
available. Figure 3 shows some examples with taxane skeleton. More examples of this strategy applied to taxane skeleton are available in Ref. 3.

Convergent disconnection. This option allows to cut the target in two pieces of approximately equal size. This strategy was developed in Hendrickson's SYNGEN program to build the shortest route of a synthesis.[11] To perform this disconnection an analysis of the target is done to search for the rings present. They are analysed to determine those which are the core of the target. The program then cuts successively two and three bonds of these rings. To save time, the program does not delete bonds of the peripheral rings, that is rings with are connected to only one ring. For example, for the steroid skeleton peripheral rings are rings A and D and core rings are rings B and C (Scheme 5).



Scheme 5.

When the program deletes three bonds it discards solutions which involve solutions found for the two bonds disconnection. For example, if in the first part solution A is found, in the second part solutions B and C are not saved as convergent since they derive from solution A (Scheme 6).



Scheme 6.

Figure 4 shows the results given by the program for the steroid skeleton. There are 46 possible ways to cut the core rings of the skeleton by deleting two and three bonds. With the method described above, among them, only 16 are considered as convergent. By deleting two bonds 6 solutions are convergent and 10 more by deleting three bonds.

Disconnections **6**,[12] **7**,[13] **8**[14] have been widely described. They correspond, in fact, to the opening of a cyclobutane

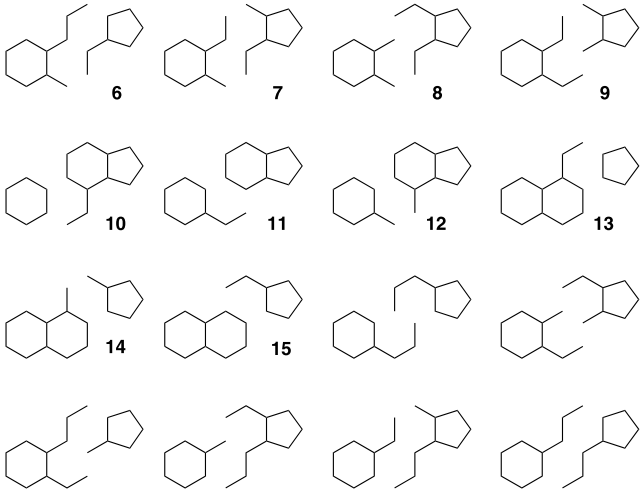
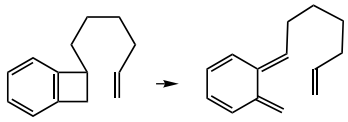


Figure 4. Convergent strategies for the steroid skeleton.

system (Scheme 7). Solutions involving disconnections **9**,[15] **10**,[16] **11**,[17] **12**,[18] **13**,[19] **14**[20] and **15**[21] are known.

In order to see solutions such as B and C (Scheme 6) another option is available in which the user indicates the number of bonds to be deleted (presently they are in the range of 2–4). In this case, if the user selects to delete 3 bonds, the program no longer discards solutions found for 2 bonds deletion, in contrast with what it was doing in the above option, and the 40 possibilities are displayed.

Linear disconnection. Another interesting strategy is to try to start from linear precursors, such as in Johnson's synthesis of steroid compounds,[22a–c] which was one of the earliest spectacular example of holosynthetic strategies.[23] To search for linear precursors, if there are *n* rings in the structure, the program has to delete *n* bonds. For each generated precursor the program counts the number of neighbours of each atom: when there are two atoms with only one neighbour (i.e., terminal atoms of a chain) and the connectivity table contains only one molecule, a linear



Scheme 7.

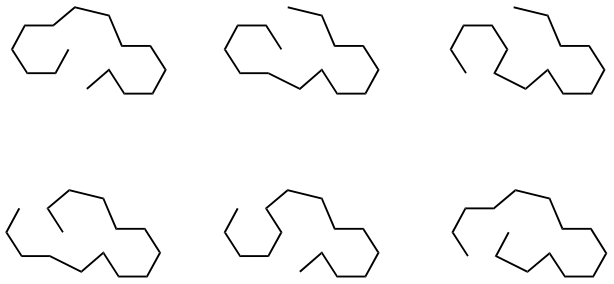


Figure 5. Linear precursors for taxane skeleton.

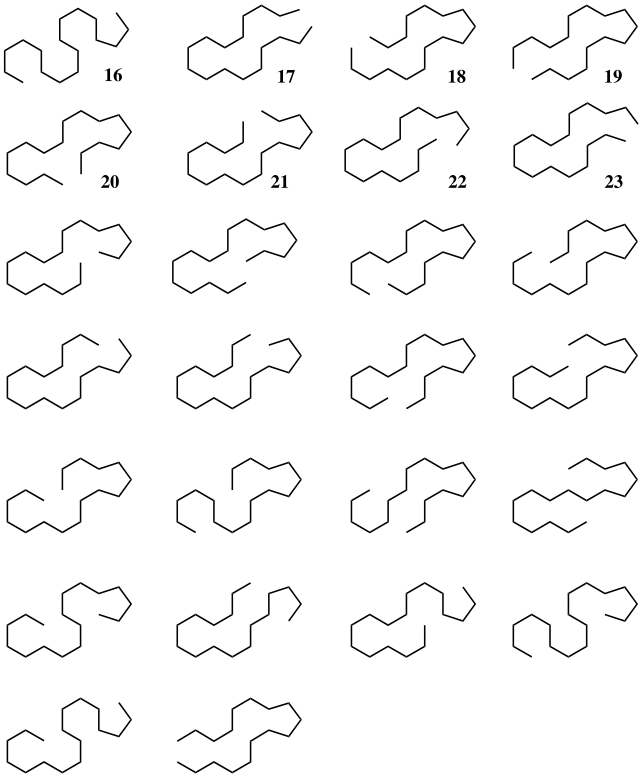
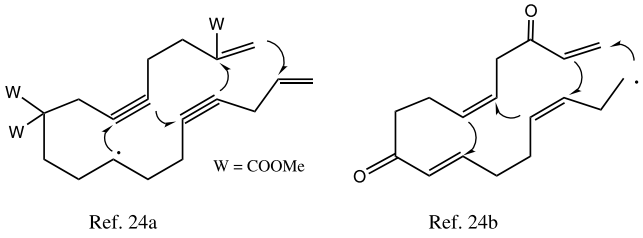
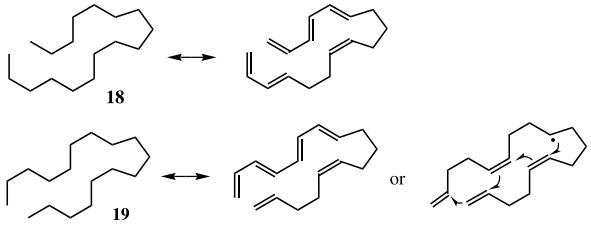


Figure 6. Linear precursors for the steroid skeleton.

precursor is found. This option allows the user to see all the possible linear skeletons as potential precursors. Figure 5 shows the results provided for the taxane skeleton and Figure 6 those for the steroid skeleton. Solution **16** has been widely studied by Johnson and others[22] Solution **17** has been realised by two radical centered approaches[24] (Scheme 8).



Scheme 8.



Scheme 9.

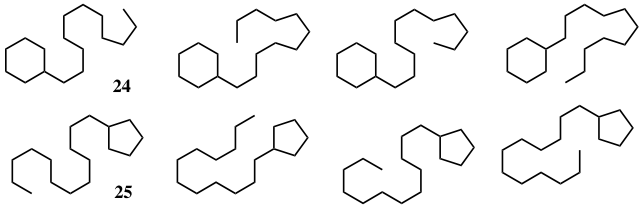


Figure 7. Precursors containing one ring and one chain for steroid skeleton. Solutions such as **24** and **25** have been reported.[26,27]

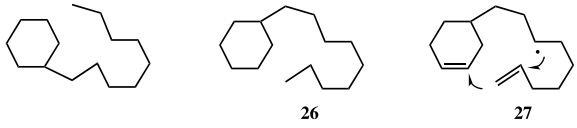
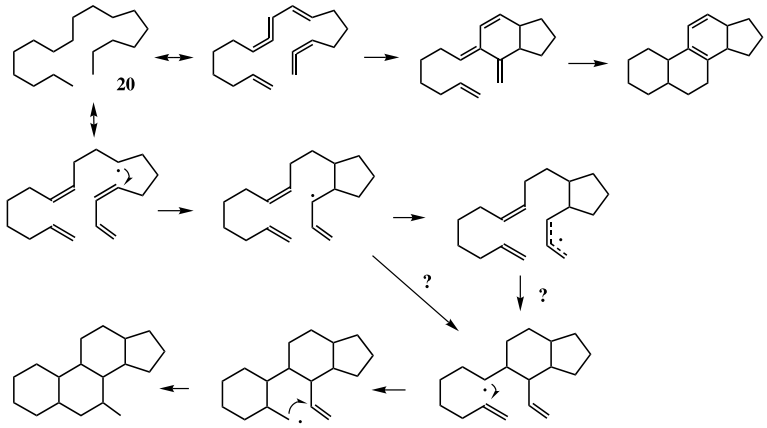


Figure 8. Precursors containing one ring and one chain for taxane skeleton. Solution **26** suggests a radical reaction (solution **27**).

Solutions **18** and **19** (Fig. 6) suggest an hypothetical tandem Diels–Alder reaction. Solution **19** suggests also a radical cascade similar to **17** (Scheme 9). Solution **20** suggests a



Scheme 10.

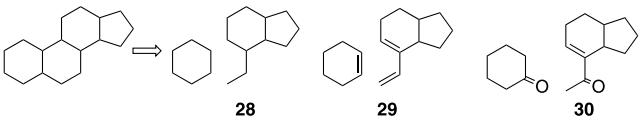
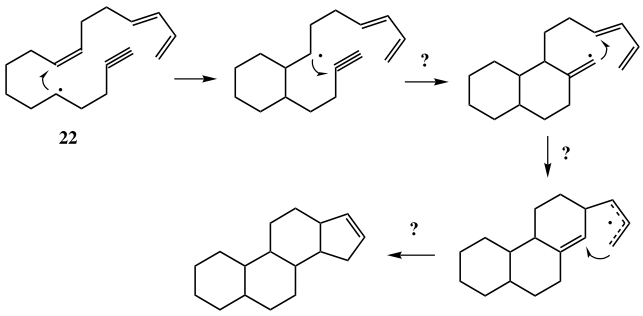


Figure 9. Example of a solution for a [4+2] reaction scheme. CONAN deletes the corresponding bonds. Solution **28** is a solution among the 18 possible. This solution may be interpreted as a Diels–Alder reaction (**29**) or a Robinson reaction (**30**).

more hypothetical tandem Diels–Alder reaction via two allenic carbons or a radical cascade (Scheme 10). Similar reactions could be considered with solution **21**. With solution **22** another hypothetical radical cascade could be put forth (Scheme 11). A domino [4+2] [3+2] could be considered with solution **23**. For the other intermediates no simple reactions seem possible since they involve less common disconnections such as [3+3] or [5+1] intramolecular schemes, which would need complicated functionalized precursors.

This option has also been developed in the SLIP[25] program.

X ring(s) plus Y chain(s) disconnection. Another way to simplify the skeleton is to search for precursors with a given number of rings and a given number of chains. The standard



Scheme 11.

option being one ring and one chain, but the user may modify these numbers at will. The solutions found for the steroid skeleton (one ring, one chain) are given in Figure 7, solutions **24** and **25** have been described.[26,27] The solutions for taxane skeleton are shown in Figure 8.

If the user chooses zero ring and one chain this option is similar to the previous one: linear precursors are found.

Disconnection by formal [$x+y$] *reaction scheme*. A list of several disconnections is available: rings of size 3 by a

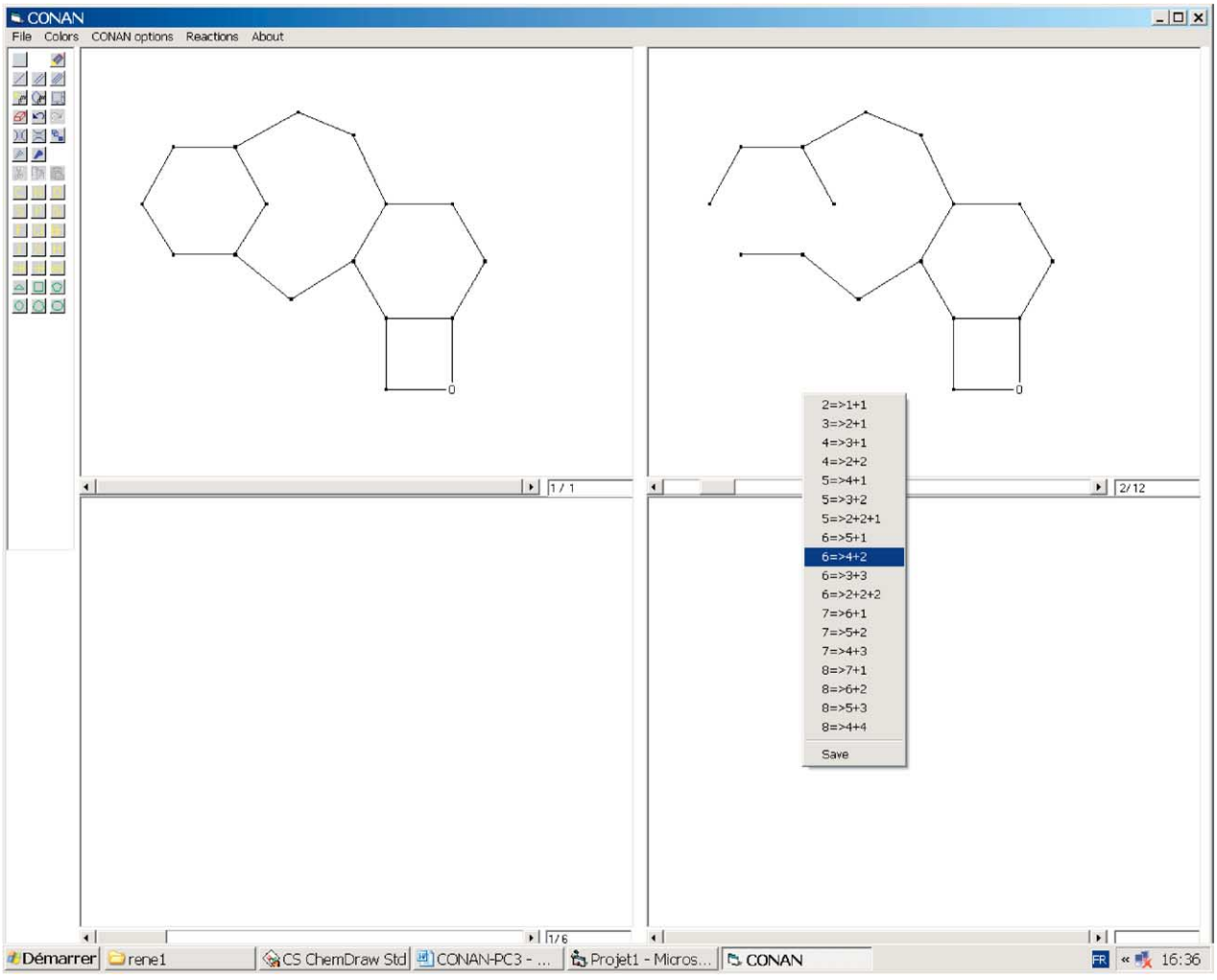


Figure 10. Example of tandem [4+2] reaction applied to taxane skeleton. When the precursor is displayed, a click on the right button allows the user to select a reaction in the pop up menu. The result is shown in Figure 11.

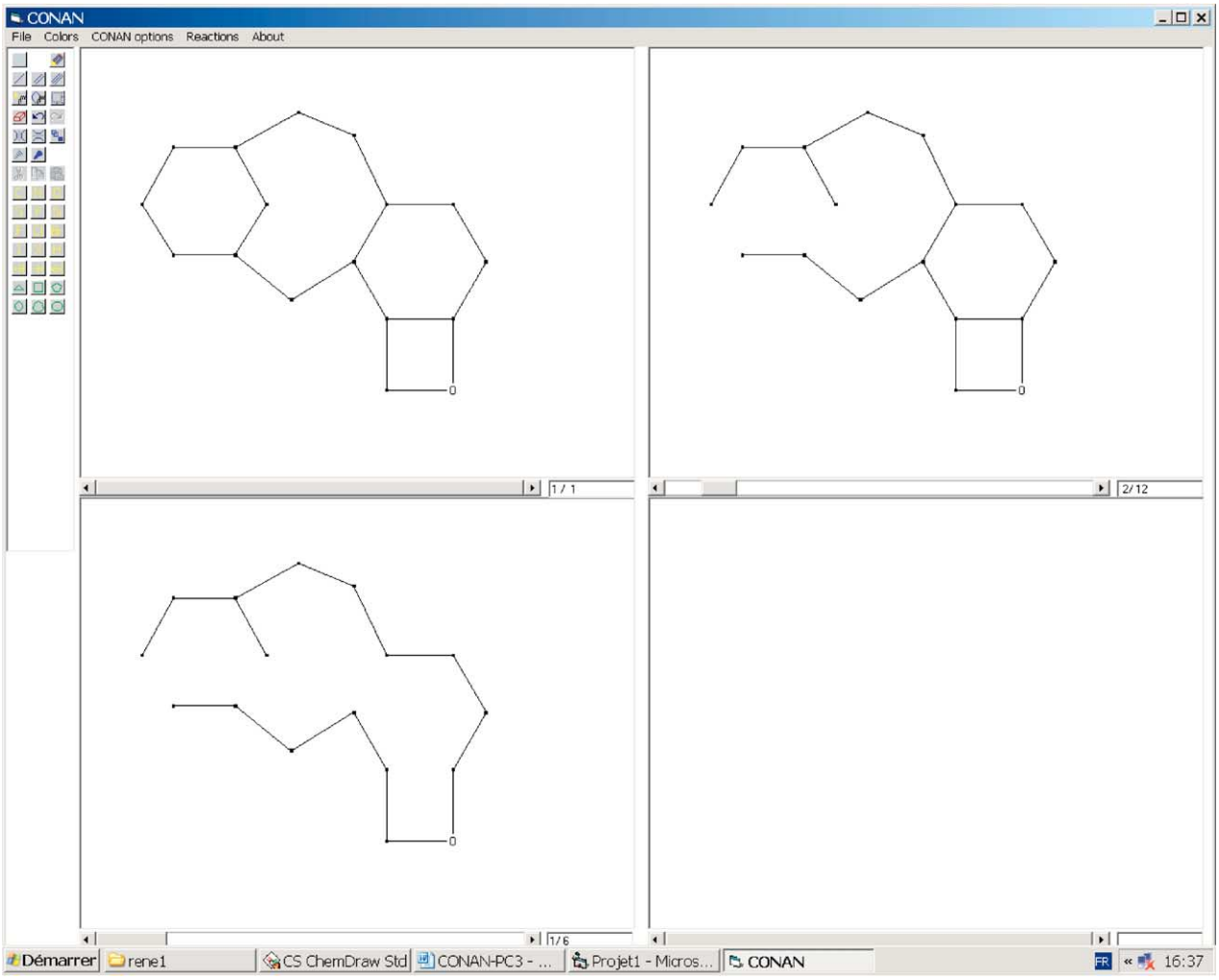


Figure 11. Example of tandem [4+2] reaction applied to taxane skeleton.

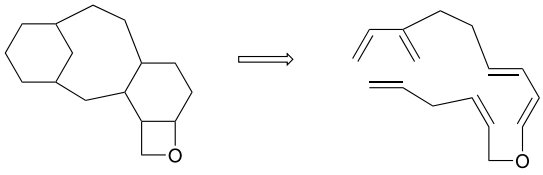
[2+1] scheme, rings of size 4 by a [2+2] or [3+1] scheme, etc. In this option, CONAN simply deletes the bonds. For a [4+2] disconnection it does not draw a Diels–Alder scheme, since a [4+2] scheme is also found in the Robinson reaction for example, Figure 9 shows an example of a [4+2] reaction applied to steroid skeleton.

The user selects a reaction scheme thanks to a pop-up menu and the corresponding disconnection is applied to the target. The user may visualize the solutions with the horizontal scroll bar. When a precursor is displayed, the user may save it for further analysis, or he may analyse it directly by the same or another transform in a new window (Fig. 10). This retroanalysis may be conducted up to three levels. This three steps retroanalysis may give to the chemist general lines of strategies by tandem, domino or cascade reactions.[28]

Figure 11 shows the dissection of taxane framework by two [4+2] reactions.

Solution of Figure 11 suggests a tandem Diels–Alder reaction to build simultaneously the four rings of taxane. (Scheme 12). Rings A, B, C have already been built by Winkler[29] by two Diels–Alder reactions.

Application of [2+2+2] scheme to taxane suggests a new possibility to built rings B, C, D in one step (Fig. 12). A similar strategy has been proposed by Malacria.[30] Figure 13 shows a solution from a tandem [4+2] applied to the steroid skeleton. It could be interpreted by tandem Diels–Alder (solution **31**), which seems not very probable, nevertheless it could suggest interesting new strategies for derivatives of steroids (solutions **32** and **33**).



Scheme 12.

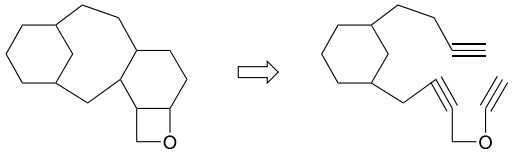


Figure 12. New strategies suggested by CONAN for the taxane skeleton by [2+2+2] reaction.

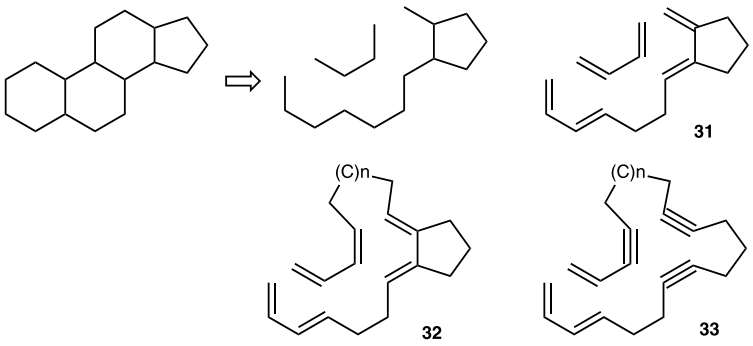


Figure 13. New strategies suggested by CONAN for the steroid skeleton.

3. Conclusion

The computer-aided connectivity analysis based on the graphs of compounds allows the user to see all the possible disconnections of a target. It allows a global perception of dissections, which would be tedious to do without computer. These disconnections are potential solutions that the chemist has to analyse with his own experience and his aesthetic prejudices. They may give new ideas of synthesis since the solutions are not directly chemistry based, each solutions being in fact a question: is it possible to perform concisely this synthesis? Does a reaction exist to create these bonds in one step? How would these rings be built simultaneously in one step?

Acknowledgements

The authors thank Professor Paul Wender for helpful discussions.

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Metalation of sulfoxides in the benzodiazine series. Diazines. Part 44

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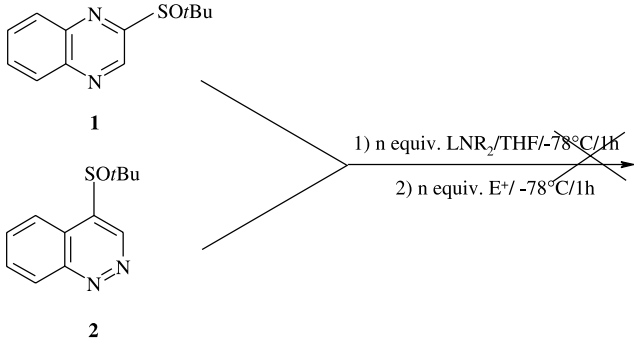
Received 29 March 2005; revised 20 June 2005; accepted 6 July 2005

Abstract—The metalation of 12 benzodiazines sulfoxides was tested. This reaction was effective with 3-sulfinylcinnolines, 5-phenylsulfinyl-2-*tert*-butylquinazoline and 8-*tert*-butylsulfinyl-2-*tert*-butyl-4(3*H*)-quinazolinone.

1. Introduction

The metalation of benzene sulfoxides was previously studied[1–3] and it was demonstrated that the sulfinyle group was a powerful *ortho*-directing group. In the naphthalene series few results were described and the yields were low.[4,5] In the pyridine series Furukawa and Snieckus[1,6–8] tested successfully the phenyl and *tert*-butylsulfinyl groups as *ortho*-directing groups, they obtained moderate to good yields (20–90%) of the functionalized compounds. More recently in our laboratory, Pollet[9] metalated diazine sulfoxides with good yields.

We have recently described the syntheses of new sulfoxides in the benzodiazine series;[10] this paper describes the metalation tests performed on these compounds. The metalation can take place either on the diazine ring or on the benzene ring following the studied compound. The first part will focus on the metalation of the diazine ring.



Scheme 1.

1.1. Metalation of the diazine ring

The first tests were performed with 2-*tert*-butylsulfinylquinoxaline **1** (Scheme 1, Table 1).

Table 1. Metalation of 2-*tert*-butylsulfinylquinoxaline **1**

Entry	*n* (equiv)	LNR_2	E^+	Result
1	1.1	LTMP	PhCHO	58% of **1**
2	2.1	LTMP	PhCHO	—
3	2.1	LDA	PhSSPh	63% of **1**
4	3.1	LTMP	PhCHO	—

We could not obtain any disubstituted product and in the case of entries 1 and 3 some starting material was recovered, in the other cases, (entries 2 and 4) untreatable tars were found.

The same tests were performed with 4-*tert*-butylsulfinylcinnoline **2** without success (Scheme 1). However, when diphenyl disulfide was used as electrophile an unexpected result was obtained (Scheme 2, Table 2).

A mixture of two sulfanylated products **3** and **4** was isolated. The formation of these compounds can be explained by the reaction pathways shown on Scheme 3.

These reactions suppose a great lability of the sulfoxide group of **2**. In order to validate this hypothesis, lithium

Keywords: Metalation; Sulfoxides; Benzodiazines; Cinnoline; Quinoxaline; Quinazoline; Phtalazine.
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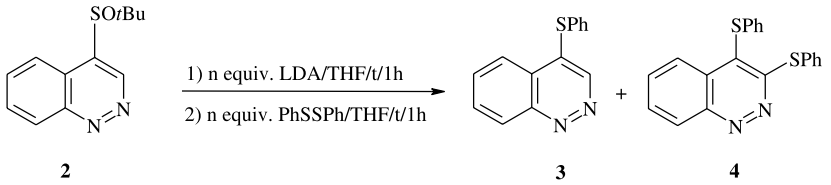
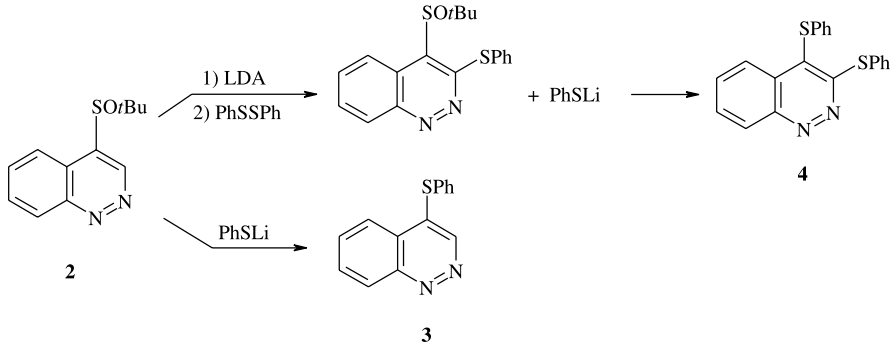
0040–4020/$ - see front matter © 2005 Published by Elsevier Ltd.
doi:10.1016/j.tet.2005.07.028



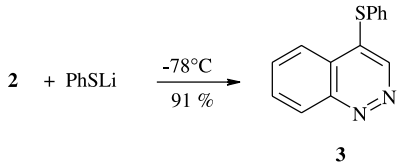
Scheme 2.

Table 2. Metalation of **2** and reaction with diphenyl disulfide

Entry	*n* (equiv)	*t* (°C)	**2**	**3** (%)	**4**	Global yield (%)
1	1.2	−78	63%	3	—	66
2	2.1	−78	—	52	28%	80
3	2.1	−100	—	23	55%	78
4	3.2	−78	—	48	31%	79



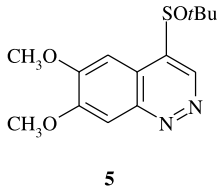
Scheme 3.



Scheme 4.

thiolate was reacted with **2** at −78 °C in THF and after 1 h the substitution was effective (Scheme 4).

The sensitivity of the sulfoxide group to nucleophilic substitution in the diazine series may explain the failure of the metalation of compounds **1** and **2**. Taking into account this property, we have tested the metalation of a cinnoline with two electrondonating groups: 6,7-dimethoxy-4-*tert*-butylsulfinylcinnoline **5** (Scheme 5).



Scheme 5.

In spite of the better stability of this sulfoxide, its metalation was also unsuccessful.

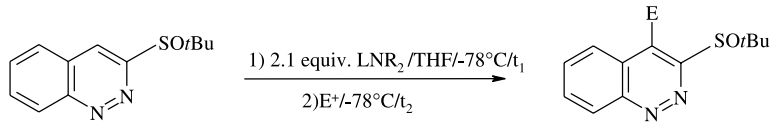
The 4-substituted cinnolines are very prone to undergo nucleophilic substitution but the cinnolines with leaving group in 3 position are much less reactive to nucleophilic substitution.[11] Thus, the 3-*tert*-butylsulfinylcinnoline **6** was tested and metalated with success (Scheme 6, Table 3).

The use of 2.1 equiv of LTMP allowed us to obtain the *ortho*-functionalized molecules with good yields. When the electrophile was prochiral (R–CHO), an asymmetric induction was observed, the induction was correlated with the steric hindrance of the aldehyde: low for acetaldehyde (de = 14%) and very high with pivaldehyde (de > 98%) (Scheme 6, Table 3). Having highlighted the importance of the steric hindrance of the aldehyde it was interesting to use a less hindered sulfinyl derivative. The 3-*p*-tolylsulfinylcinnoline **14** was metalated and reacted with DCl and *p*-anisaldehyde as electrophiles (Scheme 7, Table 4).

The diastereoisomeric excess was 26% whereas with *tert*-butylsulfinyl group it was 62% with the same electrophile. These results indicate that the bulkiness of the sulfinyl group plays also a role in the chiral induction.

The presence of the substituent at 5 position could bring a *peri* steric hindrance for the metalation at 4 position and therefore increase the asymmetric induction, so we attempted to metalate 5-chloro-3-*tert*-butylsulfinylcinnoline **17** (Scheme 8) but the metalation failed and the starting material was recovered or untreatable tars obtained when the experimental conditions were more severe.

In conclusion, for the metalation of the diazine moiety, the



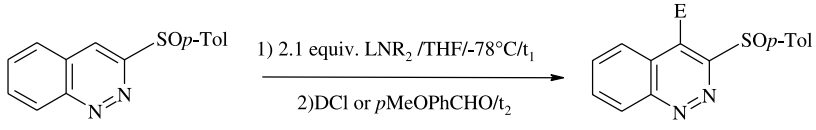
6 **7 - 13**

7: E = D
8: E = CH_3CHOH
9: E = PhCHOH
10: E = *p*-MeOPhCHOH
11: E = *t*BuCHOH
12: E = I
13: E = $SnBu_3$

Scheme 6.

Table 3. Metalation of 3-*tert*-butylsulfinylcinnoline **6**

Entry	LNR_2/t_1	E^+/t_2	**6**	Product	Yield (%)	de
1	LDA/0.5 h	DCl/5 min	12%	**7**	77	—
2	LTMP/1 h	CH_3CHO/1 h	—	**8**	90	14%
3	LTMP/1 h	PhCHO/1 h	—	**9**	94	54%
4	LTMP/1 h	*p*-MeOPhCHO/1 h	—	**10**	90	62%
5	LTMP/1 h	$(CH_3)_3CCHO$/1 h	16%	**11**	70	>98%
6	LTMP/1 h	I_2/1 h	—	**12**	85	—
7	LTMP/1 h	nBu_3SnCl	19%	**13**	67	—



14

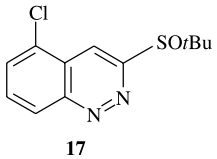
15 E = D
16 E = *p*-MeOPhCHOH

Scheme 7.

Table 4. Metalation of 3-*p*-tolylsulfinylcinnoline **14**

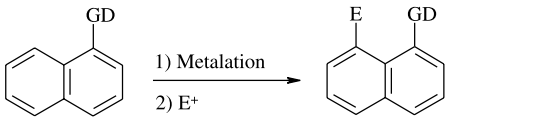
Entry	LNR_2/t_1	E^+/t_2	Product	Yield (%)	de
1	LTMP/1 h	DCl/5 min	**15**	56	—
2	LDA/0.5 h	DCl/5 min	**15**	81	—
3	LDA/0.5 h	*p*-MeOPhCHO/1 h	**16**	82	26%

easy nucleophilic substitution in the case of quinoxaline and 4-sulfinylcinnoline made the metalation reaction unsuccessful. 3-Sulfinylcinnoline, which are less prone to nucleophilic substitution were successfully metalated and a diastereoisomeric excess was observed.



17

Scheme 8.



$GD = OMe, NMe_2$ $E^+ = CO_2, Me_3SnCl, Me_3SiCl$

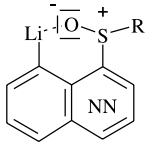
Scheme 9.

1.2. Metalation of the benzene ring

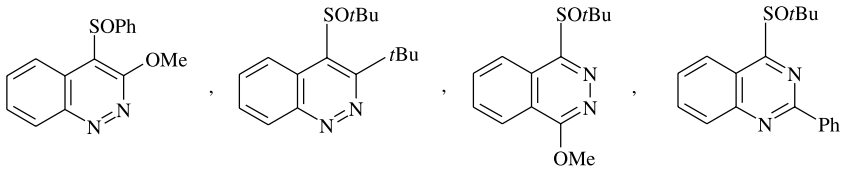
In the naphthalene series, *peri* metalation was described with methoxy or dimethylamino directing groups[4,5] (Scheme 9).

The *peri* metalation was tested without success in the benzodiazine series[12] using the same ODG's but not with sulfinyl groups. This group would allow a strong stabilisation of the lithio derivative by formation of a six membered ring with the *peri* lithiated compound (Scheme 10).

In order to favour *peri* metalation it is mandatory to use compounds which cannot be *ortho*-metalated on the diazine

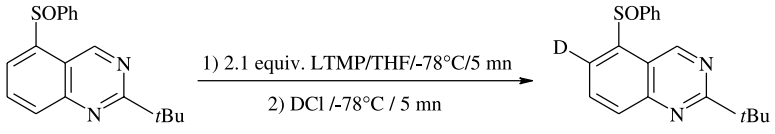


Scheme 10.



18 **19** **20** **21**

Scheme 11.



22 **23**

Scheme 12.

ring, we tested four compounds completely substituted on the diazine ring (**18**–**21**) (Scheme 11).

We could not isolate any *peri* metalated product; in some cases the starting material was recovered. The failure of these attempts may be due to the great sensitivity of the sulfoxide group on the diazine ring towards nucleophilic substitution as was mentioned before. To prevent this, a solution would be the positioning of the sulfoxide group on the benzene ring. 5-Phenylsulfinyl-2-*tert*-butylquinazoline **22** was tested; this product could be metalated at the 4 *peri* position or at the 6 *ortho* position. We never could observe a *peri* metalation at 4 position, however, an *ortho* metalation at 6 position occurred (Scheme 12).

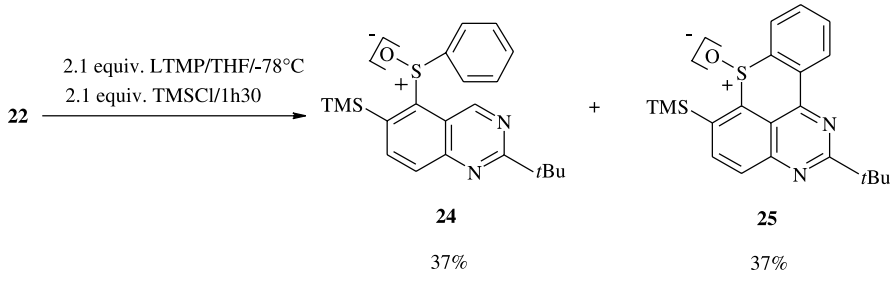
The first attempts using the usual metalation time: t_1 (0.5–1 h) gave no results. The shortening of t_1 to 5 min allowed us to obtain a good yield of *ortho* deuterated compound **23** (86%); but the same experimental conditions used with aldehyde as electrophiles (acetaldehyde, benzaldehyde, hexanal) gave mainly tarry products.

In view to understanding what happened in the reaction medium, we used chlorotrimethylsilane as electrophile, which was able to trap the lithiated species as soon as they were formed, an unexpected result was observed (Scheme 13).

Product **24** was the result of the expected *ortho*-directed metalation, whereas product **25** was the result of a second *ortho*-directed metalation on the upper phenyl ring followed by a nucleophilic addition of the lithio derivative at 4 position of the quinazoline, after an air oxidation the aromatised product **25** was obtained (Scheme 14).

This competitive metalation could explain the unsatisfactory results obtained when using aldehydes as electrophiles.

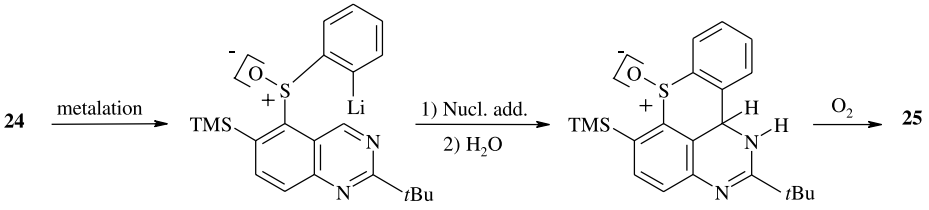
It must be noticed that we could not isolate any product



22 **24** **25**

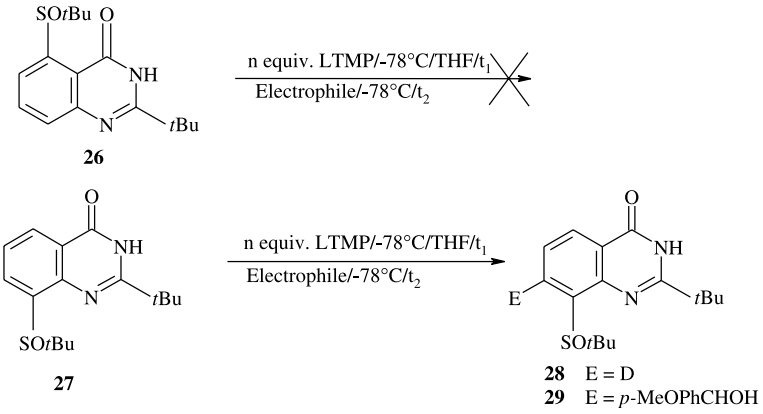
37% 37%

Scheme 13.



24 **25**

Scheme 14.



Scheme 15.

Table 5. Metalation of 8-*tert*-butylsulfinyl-2-*tert*-butyl-4(3*H*)-quinazolinone **27**

Entry	*n* (equiv)/t_1	Electrophile/t_2	**27** (%)	Product	Yield (%)
1	2.1/1 h	DCl/5 min	100	—	
2	3.1/1 h	DCl/5 min	26	**28**	34
3	4.1/1 h	DCl/5 min	32	**28**	68
4	3.1/2 h	DCl/5 min	9	**28**	91
5	2.1/2 h	*p*-MeOPhCHO/1 h	—	**29a**+**29b**	88

arising from a metalation of the free 4 position on the diazine ring; this is a very unexpected result if we take into account the great acidity of the diazine hydrogens.

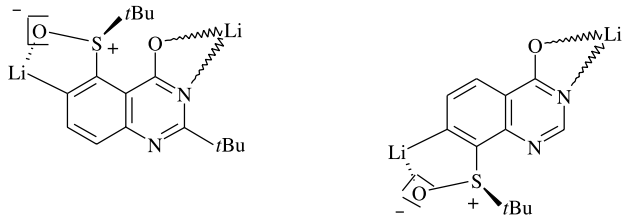
Then, two quinazolinones with sulfoxide groups at 5 position (**26**) or at 8 position (**27**) were tested (Scheme 15).

The metalation tests of **26** were unsuccessful and starting material was recovered besides large amount of tars. The metalation of **27** was performed with LTMP as metalating agent (Table 5).

Using *p*-anisaldehyde as electrophile (entry 5), two diastereoisomers **29a** and **29b** were formed and separated by column chromatography. The diastereoisomeric excess was 28%.

The comparison of the metalation reaction of **26** and **27** can be correlated to the heat of formation of their lithio derivatives calculated by Li/PM_3,[13] the lithio derivative **27Li** is much more stable than **26Li** (Scheme 16).

This difference could be explained by the steric hindrance of the *peri* oxygen atom with the *tert*-butyl group of the sulfoxide in **26Li**, which could prevent the formation of the planar lithio derivative. On the contrary there is no such steric hindrance for **27Li**.



26Li

ΔHf = -49.5 kcal/mole

27Li

ΔHf = -56.98 kcal/mole

Scheme 16.

2. Conclusion

We could never highlight a *peri* metalation even in the favourable case of compound **22**. The metalation of compounds bearing a labile sulfoxide group at 4 position of cinnoline and at 2 position of quinoxaline gave very poor results. The metalation of sulfoxides on the diazine ring but not at labile position like 3-cinnoline was effective, such as was the metalation of sulfoxides on the benzene ring if the planar conformation of the lithio derivative was not hampered.

3. Experimental

3.1. General data

Melting points were measured on a Kofler hot-stage apparatus. NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 with a Bruker Avance 300 spectrometer (^{1}H at 300 MHz and ^{13}C at 75 MHz). IR spectra were obtained as potassium bromide pellets with a Perkin Elmer Paragon 500 spectrometer. Elemental analyses were performed on a Carlo Erba 1106 apparatus. Mass spectra were recorded with a Jeol JMS-AX500 spectrometer.

Tetrahydrofuran (THF) was distilled from benzophenone sodium and used immediately (water content <60 ppm). Column chromatography were performed with silica gel Merck (70–230 mesh ASTM).

3.2. General procedure for direct lithiation by lithium alkylamide (LTMP or LDA)

A solution of *n*-butyllithium (1.6 M or 2.5 M in hexane) was added to cold (−50 °C), stirred, anhydrous tetrahydrofuran (10 ml) under an atmosphere of nitrogen. Then 2,2,6,6-tetramethylpiperidine (TMPH) or diisopropylamine (DIPAH) was added. The mixture was warmed to 0 °C. After 20 min, the temperature was lowered to *T* and the substrate to metalate dissolved in 5 ml of THF was added. After a time t_1 at *T*, the corresponding electrophile was introduced and stirring was continued for a time t_2 at *T*. Hydrolysis was then carried out at *T* using a mixture of water, ethanol and tetrahydrofuran (1:4:5). The reaction solution was warmed to room temperature and the solvent was evaporated. The aqueous layer was extracted with dichloromethane and the combined organic layers were dried over magnesium sulphate and evaporated. The crude product was purified by column chromatography on silica gel.

3.2.1. 4-Phenylsulfanylcinnoline (3) and 3,4-bisphenylsulfanylcinnoline (4). Metalation of **2** (0.15 g, 0.64 mmol) according to the general procedure for direct lithiation with *n*-BuLi 1.6 M (0.8 ml, 1.34 mmol), DIPAH (0.18 ml, 1.34 mmol), $t_1=1$ h, $T=-100$ °C, followed by reaction with diphenyldisulfide (0.29 g, 1.34 mmol), $t_2=1$ h, gave after purification by column chromatography on silica gel (petroleum ether/ethyl acetate (12:3)) 0.055 g (23%) of **3**[10] as a yellow solid and 0.12 g (55%) of **4** as a yellow solid. Mp 112 °C. ^{1}H NMR ($CDCl_3$): δ 8.33 (m, 1H, H_8); 8.17 (m, 1H, H_5); 7.62 (m, 2H); 7.52 (m, 2H); 7.31 (m, 3H, SPh); 7.13 (m, 5H, SPh). ^{13}C NMR ($CDCl_3$): δ 161.4 (C); 149.5 (C); 135.6 (CH); 134.3 (C); 132.9 (CH); 131.0 (CH); 130.9 (C); 130.1 (CH); 129.9 (CH); 129.7 (CH); 129.4 (CH); 129.2 (CH); 128.9 (C); 127.4 (CH); 127.1 (C); 124.7 (CH). IR (KBr) (cm^{-1}): 3057, 1609, 1505, 1477, 1267, 1161, 1056, 760, 742, 686. Anal. Calcd for $C_{20}H_{14}N_2S_2$: C, 69.33; H, 4.07; N, 8.08; S, 18.51. Found: C, 69.03; H, 4.16; N, 8.21; S, 18.38%.

3.2.2. 4-Deutero-3-*tert*-butylsulfinylcinnoline (7). Metalation of **6** (0.15 g, 0.64 mmol) according to the general procedure for direct lithiation with *n*-BuLi 1.6 M (0.84 ml, 1.34 mmol), DIPAH (0.19 ml, 1.34 mmol), $t_1=30$ min, $T=-78$ °C, followed by reaction with DCl in D_2O (1 ml), $t_2=5$ min, gave after purification by column chromatography on silica gel (ethyl acetate) 0.133 g (89%) of an orange solid containing (^{1}H NMR) 77% of **7** and 12% of **6**.

3.2.3. 1-(3-*tert*-Butylsulfinylcinnolin-4-yl)ethanol (8). Metalation of **6** (0.15 g, 0.64 mmol) according to the general procedure for direct lithiation with *n*-BuLi 1.6 M (0.84 ml, 1.34 mmol), TMPH (0.23 ml, 1.34 mmol), $t_1=1$ h, $T=-78$ °C, followed by reaction with acetaldehyde (1 ml), $t_2=1$ h, gave after purification by column chromatography on silica gel (ethyl acetate/dichloromethane (6:4)) 0.161 g (90%) of **8** as a mixture of two diastereoisomers (ratio 57:43 determined by ^{1}H NMR) as a pale yellow solid.

Major diastereoisomer. ^{1}H NMR ($CDCl_3$): δ 8.48 (d, $J=8.4$ Hz, 1H, H_8); 8.25 (d, $J=8.3$ Hz, 1H, H_5); 7.80 (m, 2H, H_6+H_7); 6.09 (d, $J=9.4$ Hz, 1H, OH); 5.90 (m, 1H, CH); 1.66 (d, $J=6.8$ Hz, 3H, CH_3); 1.36 (s, 9H, *t*-Bu). ^{13}C NMR ($CDCl_3$): δ 155.4 (C); 150.0 (C); 143.4 (C); 132.5 (CH); 132.1 (CH); 131.1 (CH); 124.9 (C); 124.2 (CH); 64.8 (CH); 61.7 (C); 25.5 (CH_3); 24.4 (CH_3).

Minor diastereoisomer. ^{1}H NMR ($CDCl_3$): δ 8.77 (d, $J=7.7$ Hz, 1H, H_8); 8.13 (d, $J=7.5$ Hz, 1H, H_5); 7.80 (m, 2H, H_6+H_7); 5.90 (m, 1H, CH); 5.49 (s, 1H, OH); 1.60 (d, $J=6.8$ Hz, 3H, CH_3); 1.19 (s, 9H, *t*-Bu). ^{13}C NMR ($CDCl_3$): δ 152.7 (C); 150.8 (C); 141.7 (C); 131.8 (CH); 131.7 (CH); 131.0 (CH); 126.9 (CH); 124.4 (C); 64.4 (CH); 59.3 (C); 24.4 (CH_3); 24.2 (CH_3). Anal. Calcd for $C_{14}H_{18}N_2O_2S$: C, 60.19; H, 6.49; N, 10.03; S, 11.48. Found: C, 59.77; H, 6.34; N, 9.87; S, 11.19%.

3.2.4. (3-*tert*-Butylsulfinylcinnolin-4-yl)phenylmethanol (9). Metalation of **6** (0.15 g, 0.64 mmol) according to the general procedure for direct lithiation with *n*-BuLi 1.6 M (0.84 ml, 1.34 mmol), TMPH (0.23 ml, 1.34 mmol), $t_1=1$ h, $T=-78$ °C, followed by reaction with benzaldehyde (0.13 ml, 1.34 mmol), $t_2=1$ h, gave after purification by column chromatography on silica gel (ethyl acetate) 0.195 g (94%) of **9** as two diastereoisomers (ratio 77:23 determined by ^{1}H NMR).

A first fraction gave the minor diastereoisomer (69 mg, 32%) as a yellow oil. ^{1}H NMR ($CDCl_3$): δ 8.27 (d, $J=8.7$ Hz, 1H); 8.22 (d, $J=8.7$ Hz, 1H); 7.76 (dd, $J=7.3$, 7.7 Hz, 1H); 7.58 (dd, $J=7.7$, 7.9 Hz, 1H); 7.15 (m, 6H); 5.61 (d, $J=5.7$ Hz, 1H); 1.17 (s, 9H). ^{13}C NMR ($CDCl_3$): δ 154.0 (C); 151.3 (C); 142.2 (C); 140.0 (C); 131.9 (CH); 131.8 (CH); 130.8 (CH); 128.6 (CH); 127.8 (CH); 127.7 (CH); 126.8 (CH); 125.2 (C); 68.1 (CH); 59.5 (C); 24.4 (CH_3). IR (KBr) (cm^{-1}): 3325, 2855–2963, 1725, 1451, 1366, 1039, 732. HRMS (CI^+) calcd for (MH^+) $C_{19}H_{21}N_2O_2S$: *m/z* 341.1324, found 341.1337.

A second fraction gave the major diastereoisomer (136 mg, 62%) as a pale yellow solid. Mp > 260 °C. ^{1}H NMR ($CDCl_3$): δ 8.51 (d, $J=8.3$ Hz, 1H); 8.02 (d, $J=8.7$ Hz, 1H); 7.82 (dd, $J=7.5$, 8.5 Hz, 1H); 7.64 (dd, $J=7.0$, 8.5 Hz, 1H); 7.18 (m, 5H); 6.94 (d, $J=9.1$ Hz, 1H); 6.25 (d, $J=9.1$ Hz, 1H); 1.37 (s, 9H). ^{13}C NMR ($CDCl_3$): δ 156.4 (C); 150.4 (C); 142.4 (C); 140.2 (C); 132.6 (CH); 132.1 (CH); 131.0 (CH); 128.9 (CH); 128.1 (CH); 126.7 (CH); 125.6 (C); 125.2 (CH); 69.6 (CH); 61.7 (C); 25.4 (CH_3). IR (KBr) (cm^{-1}): 3307, 2854–3058, 1729, 1273, 1037. Anal. Calcd for $C_{19}H_{20}N_2O_2S$: C, 67.03; H, 5.92; N, 8.23; S, 9.42. Found: C, 66.54; H, 5.95; N, 8.52; S, 9.47%.

3.2.5. (4-Methoxyphenyl)(3-*tert*-butylsulfinylcinnolin-4-yl)methanol (10). Metalation of **6** (0.15 g, 0.64 mmol) according to the general procedure for direct lithiation with *n*-BuLi 1.6 M (0.84 ml, 1.34 mmol), TMPH (0.23 ml, 1.34 mmol), $t_1=1$ h, $T=-78$ °C, followed by reaction with *p*-anisaldehyde (0.19 ml, 1.5 mmol), $t_2=1$ h, gave after purification by column chromatography on silica gel (ethyl acetate/dichloromethane (1:1)) 0.214 g (90%) of **10** as a mixture of two diastereoisomers (ratio 81:19 determined by ^{1}H NMR) as a yellow solid from which the NMR data of the major isomer can be obtained.

Major diastereoisomer. ^{1}H NMR ($CDCl_3$): δ 8.43 (d,

J=8.7 Hz, 1H); 8.10 (d, J=8.7 Hz, 1H); 7.74 (dd, J=7.5, 7.5 Hz, 1H); 7.56 (dd, J=7.2, 7.9 Hz, 1H); 7.08 (d, J=8.3 Hz, 2H); 6.86 (d, J=6.8 Hz, 1H); 6.64 (d, J=8.3 Hz, 2H); 6.30 (d, J=7.2 Hz, 1H); 3.59 (s, 3H); 1.29 (s, 9H). ^{13}C NMR ($CDCl_3$): δ 159.2 (C); 155.1 (C); 150.8 (C); 140.0 (C); 134.7 (C); 132.3 (CH); 132.1 (CH); 130.7 (CH); 127.9 (CH); 126.1(CH); 125.2 (C); 114.1 (CH); 69.2 (CH); 60.9 (C); 55.5 (CH_3); 24.9 (CH_3). Anal. Calcd for $C_{20}H_{22}N_2O_3S$: C, 64.94; H, 5.99; N, 7.56. Found: C, 64.61; H, 6.15; N, 7.72%.

3.2.6. 2,2-Dimethyl-1-(3-*tert*-butylsulfinylcinnolin-4-yl) propan-1-ol (11). Metalation of **6** (0.15 g, 0.64 mmol) was performed according to the general procedure for direct lithiation modified as described below with *n*-BuLi 1.6 M (0.84 ml, 1.34 mmol), TMPH (0.23 ml, 1.34 mmol), t_1= 1 h, T=−78 °C, followed by reaction with pivaldehyde (0.15 ml, 1.34 mmol), t_2=1 h. After hydrolysis and evaporation of the solvent, the crude product was determined by ^{1}H NMR as a single diastereoisomer. The crude product was washed with water and dichloromethane giving 0.144 g (70%) of **11** as a pale yellow solid. Mp 238 °C. ^{1}H NMR (DMSO-d_6): δ 9.08 (d, J=9.0 Hz, 1H, H_8); 8.59 (d, J=8.3 Hz, 1H, H_5); 8.08 (dd, J=7.5, 6.6 Hz, 1H, H_6); 7.95 (dd, J=8.3, 7.1 Hz, 1H, H_7); 6.44 (d, J=4.9 Hz, 1H); 5.56 (d, J=4.9 Hz, 1H); 1.23 (s, 9H); 1.00 (s, 9H). ^{13}C NMR (DMSO-d_6): δ 154.7 (C, C_3); 151.3 (C, C_9); 137.1 (C, C_4); 132.3 (CH, C_7); 130.9 (CH, C_6); 129.9 (CH, C_5); 129.7 (CH, C_8); 124.9 (C, C_{10}); 76.7 (CH, CHOH); 58.0 (C); 38.8 (C); 27.5 (CH_3); 23.4 (CH_3). IR (KBr) (cm^{-1}): 3316, 2964, 1479, 1099, 1044, 1018, 787. Anal. Calcd for $C_{17}H_{24}N_2O_2S$: C, 63.72; H, 7.55; N, 8.74; S, 10.01. Found: C, 63.43; H, 7.69; N, 8.35; S, 9.78%.

3.2.7. 3-*tert*-Butylsulfinyl-4-iodocinnoline (12). Metalation of **6** (0.15 g, 0.64 mmol) according to the general procedure for direct lithiation with *n*-BuLi 1.6 M (0.84 ml, 1.34 mmol), TMPH (0.23 ml, 1.34 mmol), t_1=1 h, T=−78 °C, followed by reaction with iodine (0.34 g, 1.34 mmol), t_2=1 h, gave after purification by column chromatography on silica gel (ethyl acetate/dichloromethane (1:1)) 0.196 g (85%) of **12** as a yellow solid. Mp 134 °C. ^{1}H NMR ($CDCl_3$): δ 8.49 (d, J=8.4 Hz, 1H); 7.94 (m, 3H); 1.40 (s, 9H). ^{13}C NMR ($CDCl_3$): δ 158.8 (C); 150.2 (C); 133.8 (CH); 133.1 (CH); 131.6 (CH); 130.5 (CH); 129.1 (C); 110.5 (C); 60.7 (C); 23.9 (CH_3). IR (KBr) (cm^{-1}): 2920–3060, 1460, 1377, 1041, 770. Anal. Calcd for $C_{12}H_{13}IN_2OS$: C, 40.01; H, 3.64; N, 7.78; S, 8.90. Found: C, 40.45; H, 3.99; N, 7.75; S, 8.66%.

3.2.8. 3-*tert*-Butylsulfinyl-4-tri-*n*-butylstannylcinnoline (13). Metalation of **6** (0.15 g, 0.64 mmol) according to the general procedure for direct lithiation with *n*-BuLi 1.6 M (0.84 ml, 1.34 mmol), TMPH (0.23 ml, 1.34 mmol), t_1= 1 h, T=−78 °C, followed by reaction with tri-*n*-butyltin chloride (0.37 ml, 1.34 mmol), t_2=1 h, gave after purification by column chromatography on silica gel (dichloromethane/ethyl acetate (7:3)) 0.225 g (67%) of **13** as a yellow oil. ^{1}H NMR ($CDCl_3$): δ 8.49 (d, J=8.3 Hz, 1H); 8.10 (d, J=8.3 Hz, 1H); 7.83 (dd, J=7.5, 7.5 Hz, 1H); 7.74 (dd, J=7.2, 7.9 Hz, 1H); 1.44 (m, 6H); 1.23 (m, 12H); 1.20 (s, 9H); 0.78 (t, J=7.2 Hz, 9H). ^{13}C NMR ($CDCl_3$): δ 164.6 (C); 149.7 (C); 143.2 (C); 134.7 (C); 131.5 (CH); 131.3 (CH); 131.0 (CH); 130.2(CH); 60.0 (C); 29.5 (CH_2); 27.7 (CH_2); 24.6 (CH_3); 17.0 (CH_2); 14.0 (CH_3). IR (KBr) (cm^{-1}): 2870–3425, 1627, 1466, 1041, 1012, 761. Anal. Calcd for $C_{24}H_{40}N_2OSSn$: C, 55.08; H, 7.70; N, 5.35; S, 6.13. Found: C, 55.66; H, 7.74; N, 5.53; S, 6.02%.

3.2.9. 4-Deutero-3-*p*-tolylsulfinylcinnoline (15). Metalation of **14** (0.15 g, 0.56 mmol) according to the general procedure for direct lithiation with *n*-BuLi 1.6 M (0.73 ml, 1.17 mmol), DIPAH (0.16 ml, 1.17 mmol), t_1=30 min, T=−78 °C, followed by reaction with DCl in D_2O (1 ml), t_2=5 min, gave after purification by column chromatography on silica gel (dichloromethane/petroleum ether (2:1)) 0.12 g (81%) of **15**.

3.2.10. (4-Methoxyphenyl)(3-*p*-tolylsulfinylcinnolin-4-yl) methanol (16). Metalation of **14** (0.15 g, 0.56 mmol) according to the general procedure for direct lithiation with *n*-BuLi 1.6 M (0.73 ml, 1.17 mmol), DIPAH (0.16 ml, 1.17 mmol), t_1=30 min, T=−78 °C, followed by reaction with *p*-anisaldehyde (0.19 ml, 1.5 mmol), t_2=1 h, gave after purification by column chromatography on silica gel (ethyl acetate/dichloromethane (1:1)) 0.180 g (82%) of **16** as a mixture of two diastereoisomers (ratio 63:37 determined by ^{1}H NMR) as a yellow solid.

Major diastereoisomer. ^{1}H NMR ($CDCl_3$): δ 8.42 (d, J=8.3 Hz, 1H); 7.95 (d, J=8.7 Hz, 1H); 7.77 (dd, J=7.2, 7.9 Hz, 1H); 7.70 (m, 1H); 7.64 (d, J=7.9 Hz, 2H); 7.13 (d, J=7.9 Hz, 2H); 6.97 (m, 2H); 6.87 (d, J=7.9 Hz, 1H); 6.69 (d, J=9.1 Hz, 2H); 6.13 (d, J=8.7 Hz, 1H); 3.68 (s, 3H); 2.24 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 159.8 (C); 159.4 (C); 150.6 (C); 142.2 (C); 139.8 (C); 138.8 (C); 134.2 (C); 132.6 (CH); 132.1 (CH); 130.9 (CH); 130.4 (CH); 128.3 (CH); 126.0 (CH); 125.6 (C); 125.1 (CH); 114.2 (CH); 69.6 (CH); 55.6 (CH_3); 21.8 (CH_3).

Minor diastereoisomer. ^{1}H NMR ($CDCl_3$): δ 8.23 (d, J=8.2 Hz, 1H); 8.16 (d, J=8.7 Hz, 1H); 7.63 (m, 2H); 7.44 (d, J=7.9 Hz, 2H); 6.97 (m, 5H); 6.67 (m, 2H); 5.77 (d, J=5.7 Hz, 1H); 3.68 (s, 3H); 2.21 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 159.3 (C); 158.0 (C); 151.0 (C); 142.3 (C); 139.7 (C); 137.8 (C); 134.1 (C); 132.2 (CH); 131.9 (CH); 130.8 (CH); 130.2 (CH); 127.9 (CH); 126.5 (CH); 126.0 (CH); 125.1 (C); 114.2 (CH); 69.0 (CH); 55.6 (CH_3); 21.8 (CH_3). Anal. Calcd for $C_{23}H_{20}N_2O_3S$: C, 68.30; H, 4.98; N, 6.93; S, 7.93. Found: C, 68.58; H, 4.66; N, 6.73; S, 7.64%.

3.2.11. 5-Phenylsulfinyl-2-*tert*-butyl-6-trimethylsilylquinazoline (24) and 2-*tert*-butyl-6-trimethylsilyl-7-thia-1,3-diaza-benzo[de]anthracene 7-oxide (25). Compound **22** (0.10 g, 0.32 mmol) dissolved in 5 ml of anhydrous tetrahydrofuran and trimethylsilyl chloride (0.085 ml, 0.67 mmol) were simultaneously introduced at −78 °C into the solution containing the metalating agent (LTMP) prepared according to the general procedure for direct lithiation with *n*-BuLi 1.6 M (0.42 ml, 0.67 mmol) and TMPH (0.12 ml, 0.67 mmol). The mixture was then stirred for 1 h 30 min at −78 °C. The following steps are similar to general procedure for direct lithiation. A purification by column chromatography on silica gel (dichloromethane/ethyl acetate (9:1)) gave **25** (41 mg, 37%) in a first fraction as a yellow solid. Mp 260 °C. ^{1}H NMR ($CDCl_3$): δ 8.96

(m, 1H); 7.81 (d, $J=8.3$ Hz, 1H); 7.63 (d, $J=8.3$ Hz, 1H); 7.46 (m, 1H); 7.37 (m, 2H); 1.53 (s, 9H); 0.49 (s, 9H). ^{13}C NMR ($CDCl_3$): δ 174.3 (C); 157.5 (C); 152.9 (C); 139.4 (C); 137.9 (CH); 135.8 (C); 131.3 (CH); 130.3 (C); 128.3 (C); 128.2 (CH); 126.5 (CH); 125.7 (CH); 122.7 (CH); 117.5 (C); 39.7 (C); 29.6 (CH_3); -1.0 (CH_3). IR (KBr) (cm^{-1}): 2860–3064, 1591, 1528, 1475, 1288, 859, 837. Anal. Calcd for $C_{21}H_{24}N_2OSSi$: C, 66.27; H, 6.36; N, 7.36; S, 8.42. Found: C, 65.93; H, 6.42; N, 7.12; S, 8.01%.

A second fraction gave **24** (42 mg, 37%) as a yellow solid. ^{1}H NMR ($CDCl_3$): δ 9.91 (s, 1H); 8.01 (d, $J=8.7$ Hz, 1H); 7.95 (d, $J=8.7$ Hz, 1H); 7.37 (m, 5H); 1.33 (s, 9H); 0.48 (s, 9H). ^{13}C NMR ($CDCl_3$): δ 172.8 (C); 155.9 (CH); 150.8 (C); 144.9 (C); 143.0 (C); 142.7 (C); 135.6 (CH); 131.2 (CH); 128.9 (CH); 128.1 (CH); 123.3 (CH); 118.7 (C); 38.3 (C); 28.1(CH_3); 0.0 (CH_3).

3.2.12. 8-*tert*-Butylsulfinyl-2-*tert*-butyl-7-[hydroxy-(4-methoxyphenyl)methyl]-4(3*H*)-quinazolinone (29). Metalation of **27** (0.10 g, 0.33 mmol) according to the general procedure for direct lithiation with *n*-BuLi 1.6 M (0.63 ml, 1 mmol), TMPH (0.17 ml, 1 mmol), $t_1=2$ h, $T=-78$ °C, followed by reaction with *p*-anisaldehyde (0.15 ml, 1.2 mmol), $t_2=1$ h, gave after purification by column chromatography on silica gel (ethyl acetate/dichloromethane (1:1)) 0.129 g (88%) of **29a** and **29b** as a mixture of two diastereoisomers (ratio 64:36 determined by ^{1}H NMR) as a white solid.

Major diastereoisomer. ^{1}H NMR ($CDCl_3$): δ 11.59 (s, 1H, NH); 8.15 (d, $J=8.7$ Hz, 1H); 7.68 (d, $J=8.3$ Hz, 1H); 7.57 (s, 1H); 7.45 (d, $J=8.7$ Hz, 2H); 6.69 (d, $J=8.3$ Hz, 2H); 3.64 (s, 3H); 1.36 (s, 9H); 1.31 (s, 9H).

Minor diastereoisomer. ^{1}H NMR ($CDCl_3$): δ 11.59 (s, 1H, NH); 8.12 (m, 1H); 7.26 (d, $J=8.7$ Hz, 2H); 7.06 (d, $J=8.7$ Hz, 1H); 7.00 (s, 1H); 6.84 (d, $J=8.7$ Hz, 2H); 3.75 (s, 3H); 1.40 (s, 9H); 1.38 (s, 9H). Anal. Calcd for $C_{24}H_{30}N_2O_4S$: C, 65.13; H, 6.83; N, 6.33; S, 7.24. Found: C, 64.85; H, 6.85; N, 6.47; S, 7.68%.

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Tetrahedron

Synthesis and properties of coumarin-derived organogelators

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Received 8 June 2005; revised 26 June 2005; accepted 5 July 2005

Abstract—A new family of coumarin derivatives containing amide group with different alkyl chain lengths was synthesized and their properties as organogelators were evaluated. It was found that the organogelation abilities were not obviously affected by the alkyl spacer length of amide group. Helical morphologies formed either in nonpolar or high polar solvents by most of the gelators. Occurrence of reversible and stereoselective photodimerization of the gel formed by 4-(7′-coumarinoxy)-*N*-octadecylbutanamide (**3a**) in cyclohexane was confirmed by ^{1}H NMR, UV absorption, and fluorescence spectra. The photoreaction of the gel proceeded without any dissolution, but the drastic microscopic changes of gel morphologies accompanied with the irradiation were identified using SEM and AFM investigations.

1. Introduction

Organogels are thermally reversible viscoelastic rigid solutions of organic solvents immobilized by the addition of low concentrations of low molecular mass organic gelator molecules.[1] These gelator molecules are able to self-associate to form long two- and three-dimensional networks, entrapping solvent molecules within their void space. Organogels have the good processability to be formed in different shapes with the diversity of gel structures on both a microscopic and mesoscopic scale. Recent discoveries of low molecular weight gelators have stimulated considerable interest not only in academic investigation but also potentially wide applications to industrial such as cosmetics, foods, medical science, and tissue engineering.[2–5]

The photochemical and photophysical properties of coumarin and its derivatives have been intensively investigated. It is well known that direct irradiation ($\lambda > 300$ nm) of coumarin leads exclusively to photochemical dimerization resulting in cyclobutane-type dimers, which revert to the starting compound upon irradiation with light of shorter wavelengths.[6] Another important feature associated with the photodimerization of coumarin is the change in its emission intensity.[7] Coumarin fluoresces upon excitation at 320 nm while its dimers do not. Therefore, these simple photodimerization and photocleavage reactions represent an on–off fluorescence switch that might be reversible photomodulated. Recently, applications of this reversible and thermally stable photochromic system were explored where coumarin and its derivatives have been used as photoreactive materials for applications such as photorecording and photoresists[8] and as photolabile protecting groups for biological applications.[9]

Photodimerization of coumarin and its derivatives has been intensively studied in solution,[10] solid,[11] and self-assembled monolayer,[12] as well as polymeric film.[13] For extending potentially practical applications, it is desirable to determine whether such photochemical behavior is also observable in gel. However, up to the recent literature sources, no contribution was made concerning organogelators with coumarin group, only one study involves the photodimerization of organogelators based on anthracene.[14]

As part of our program aimed at the design and synthesis of new gelators to study their properties,[15] a series of potent gelators composed of coumarin were designed in this study. Here we report the preparation, characterization, and gelation abilities of the gelators (**3a**–**3i**). These coumarin derivatives were easily synthesized with high yield (Scheme 1). The behaviors of photodimerization under >300 nm and subsequent photocleavage under <280 nm were investigated in detail. We demonstrate that the reversible photodimerization of coumarin can be extended to gel, as established by UV, fluorescence, and ^{1}H NMR spectra. This work was also undertaken to study the microstructures of gels by scanning electron microscopy (SEM) and atomic force microscopy (AFM).

Keywords: Organogels; Coumarins; Photodimerization; Fluorescence; Gel microstructure.
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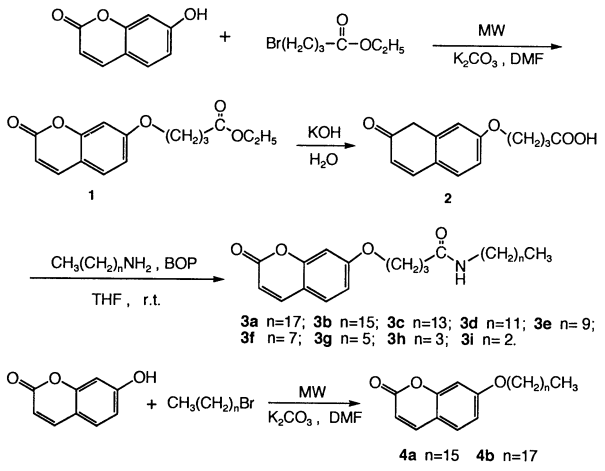
doi:10.1016/j.tet.2005.07.003



Scheme 1.

2. Results and discussion

2.1. Gelation properties

The gelation properties of compounds **3a**–**3i** were tested in a range of solvents by means of the inverted test-tube method. Table 1 summarizes the gelation capability of **3a**–**3i** towards different organic liquids. To explore the effect of dispersive interactions among the flexible alkyl chains to gel stability, the length of alkyl chain moiety of amide was varied ($n=2$–17) with fixing the structure of other part in compounds **3a**–**3i**. The gelation abilities were not very affected by the length of alkyl chain. However, among the nine compounds, **3a**–**3e** act as better gelators than the other four derivatives. The gelators **3a**–**3d** were only sparingly soluble at room temperature in the polar solvents examined, but were moderately soluble in the solvents when heated. On subsequent cooling, gels or precipitates were formed at room temperature. On the other hand, **3e**–**3i** were more soluble in most of the polar solvents and hence cannot rigidify such solutions.

Interestingly, the gels were well formed in both the nonpolar solvents (cyclohexane and *n*-hexane) and the high polar solvents (glycerol, ethylene glycol, and diethylene glycol). It suggests that the stability of its gels may be related to the enhanced π–π and hydrogen bonding interactions in nonpolar and high polar solvents, respectively. The gels given in Table 1 were found to be completely thermo-reversible and stable at least for one year at room temperature.

Structural modifications of the gelators have also been made to compare the relative contribution of hydrogen bonding among the amide groups to gelation stability. Replacement of the amide with alkyl unit in **3a** and **3b**, 7-octadecoxycoumarin (**4a**) and 7-hexadecoxycoumarin (**4b**) were synthesized (Scheme 1). Relative to **3a** and **3b**, which bearing amide group, **4a** and **4b** were found to be more soluble in most of the diverse organic solvents, and did not act as gelators at all. The difference in the structure considerably affected the organogelation ability. The results indicate that the amide group may play an important role in the gel-forming ability in this study.

2.2. Morphology studies

To obtain a visual insight into the aggregation mode of the gels, the morphologies of gels were observed by SEM. Inspection of the fibers formed by **3a**–**3i** revealed that some of them were interestingly helical either in nonpolar or polar solvents (Table 1). One can clearly see that the morphology of the cyclohexane gel of **3d** observed in the SEM possesses the helical ribbon structure (Fig. 1a). The SEM picture revealed that the left-hand and right-hand helical structures coexisted randomly, no domains were formed by only one type of helix (Fig. 5a and b). The gelation of chiral molecules to form helical morphologies has received considerable attention,[16] however, this kind of helical morphologies formed by achiral molecules has been relatively scarcely reported.[17]

Table 1. Gelation properties of the compounds in organic solvents[a]

Solvent	State								
	3a	**3b**	**3c**	**3d**	**3e**	**3f**	**3g**	**3h**	**3i**
	$n=17$	15	13	11	9	7	5	3	2
n-Hexane	G[b]	G[b]	P	P	P	P	P	P	P
Cyclohexane	G[b]	G	G[b]	G[b]	G	G[b]	G[b]	G[b]	Gp
Benzene	P	P	P	S	P	P	P	P	P
Toluene	P	P	P	P	P	P	P	P	P
Carbon tetrachloride	P	P	P	P	P	Gp	Gp	Gp	Gp
Ethyl ether	P	P	P	P	Gp	P	P	P	P
Triethylamine	P	P	Gp	Gp	Gp	Gp	Gp	Gp	Gp
Ethyl acetate	Gp	P	P	S	S	S	S	S	S
Acetone	Gp	P	P	S	S	S	S	S	S
Acetonitrile	Gp	Gp	Gp	S	S	S	S	S	S
Methanol	Gp	Gp	Gp	P	S	S	S	S	S
Ethanol	P	Gp	Gp	P	S	S	S	S	S
1-Propanol	P	P	P	S	S	S	S	S	S
2-Propanol	P	P	P	P	S	S	S	S	S
n-Hexyl alcohol	P	P	P	P	S	S	S	S	S
Diethylene glycol	G	Gp	Gp	Gp	S	S	S	S	S
Ethylene glycol	G	G[b]	Gp	Gp	G	S	S	S	S
Glycerol	P	P	G	G	G	G[b]	G[b]	G[b]	G[b]

[a] G, gel (minimum gel concentration: 0.1 g l^{-1}); S, soluble; P, precipitate; Gp, partial gel.
[b] Helical morphologies were observed.

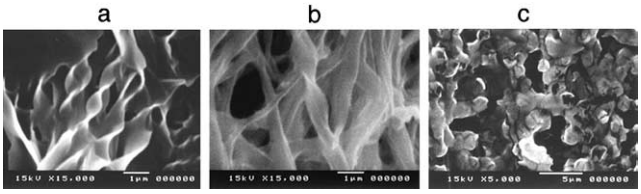


Figure 1. SEM images of (a) the cyclohexane gel of **3d**; (b) glycerol gel of **3f**; and (c) glycerol gel of **3e**.

In the organic solvents tested except glycerol, no significant differences were observed among the gels formed by **3a**–**3i**. All consisted of networks of bundles of fibers. In glycerol, however, the SEM images of **3f**–**3i** gels showed networks of fibers (Fig. 1b), while the images of **3a**–**3e** gels revealed the presence of hollow spheres (capsules) with diameters around 1 μm (Fig. 1c). This feature is quite interesting because a little change in the length of alkyl chains produced completely difference in the morphologies of the aggregation. However, the reason for the formation of different gel microstructures is not clear at the present time.

2.3. Photoreaction

In this work, gelator **3a** was chosen for irradiation with >300 and <280 nm light to investigate their photoreaction properties in gel formed in cyclohexane. We found that **3a** underwent reversible photodimerization of coumarin chromophores in gel phase and irradiation cannot destroy the stable gel. Figure 2 shows the fluorescence spectral changes observed during one photodimerization–reversion cycle.

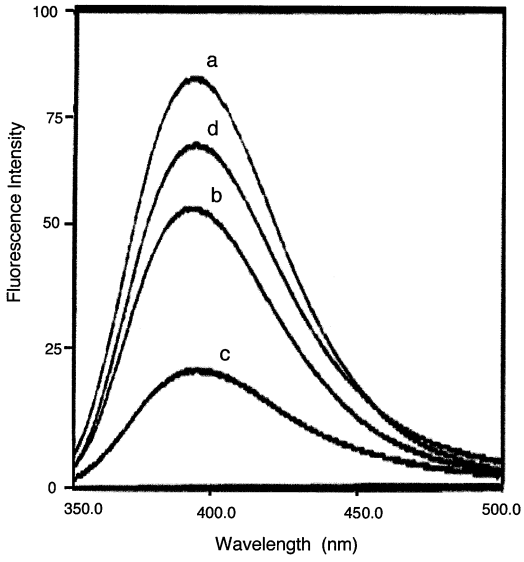


Figure 2. Fluorescence spectral variations of the cyclohexane gel of **3a**; $\lambda_{ex}=320$ nm; (a) before irradiation; (b) after irradiation at >300 nm for 24 h; (c) irradiation at >300 nm for 48 h; (d) irradiation at >300 nm for 24 h and then at <280 nm for 24 h.

Before irradiation, the cyclohexane gel of **3a** showed strong fluorescence emission at 390 nm (Fig. 2a). The emission intensity dropped upon photodimerization by the irradiation with the light at >300 nm for 24 h (Fig. 2b). Prolonged irradiation for 48 h significantly decreased the fluorescence intensity (Fig. 2c). After irradiation at >300 nm for 24 h, upon irradiation with the light at <280 nm for 24 h, the fluorescence intensity increased again by symmetric cleavage of the photodimer (Fig. 2d). However, the peak height was not completely recovered to the initial peak height (Fig. 2a). This indicates that the photocleavage (<280 nm) might be much slower than the photodimerization (>300 nm) for **3a** in gel state.

The absorption spectra of the cyclohexane gel of **3a** before and after irradiation are given in Figure 3. The cyclohexane gel of **3a** after irradiation with an UV light at >300 nm resulted in a decrease of absorbance at 320 nm (Fig. 3b). This suggests the formation of coumarin photodimers due to the disappearance of the 3,4-double bonds on the coumarinyl groups.

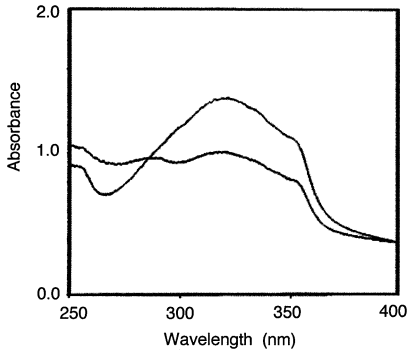


Figure 3. UV absorption spectral change of the cyclohexane gel of **3a** (a) before irradiation and (b) after irradiation with >300 nm light.

Using a high-pressure mercury lamp, the cyclic dimers of **3a** were formed as determined by ^{1}H NMR. The yield of dimer from irradiation of the cyclohexane gel of **3a** could be inferred from integration ratio of phenyl protons in ^{1}H NMR spectra of the sample before and after irradiation; the cyclohexane was removed in advance under vacuum and the obtained sample was dissolved in $CDCl_3$. The configurational assignment of the dimers is primarily based on their ^{1}H NMR spectral data (Fig. 4).

The chemical shifts and splitting patterns of the cyclobutyl protons of the four coumarinyl photodimers (the *syn* head-to-head (H–H), *syn* head-to-tail (H–T), *anti* head-to-head, and *anti* head-to-tail dimers) have been reported.[10,11] In general, the distinct patterns are quite valuable in assigning the dimers configurations. The analogous dimers from the irradiated cyclohexane gel of substituted coumarin **3a** could be clearly associated with the ^{1}H NMR resonances of one of them. The cyclobutyl protons of *syn* dimers resonate around δ 4.0–4.2 (H_{d3} and H_{d4} in Fig. 4b), whereas those of *anti* isomers resonate below δ 3.90 (not found in Fig. 4b). This shielding is caused by the diamagnetic anisotropic effects of a carbonyl or phenyl nucleus in the *anti* configuration. Coumarin *syn* H–T dimers have a characteristic upfield (>0.6 ppm) H_8 proton, deriving from the diamagnetic anisotropy caused by a phenyl ring situated in front of the H_8 proton. In this isomer, in comparison with the same protons of the monomer, the aromatic protons H_{d5}, H_{d6}, and H_{d8} are shifted slightly upfield (Fig. 4b). Cyclobutyl and H_{d8}

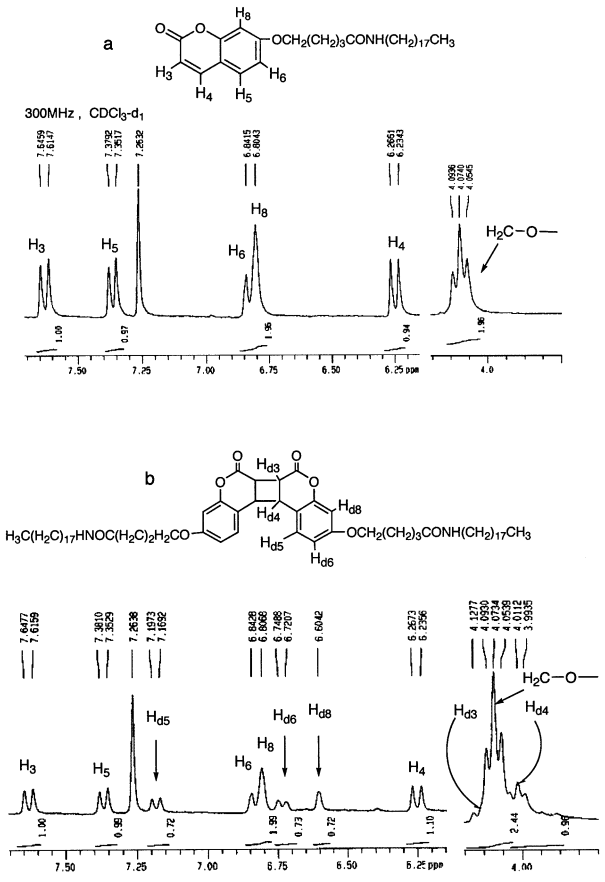


Figure 4. [1]H NMR spectra of (a) **3a**, and (b) the cyclohexane gel of **3a** after irradiation with >300 nm light for 24 h.

protons of the dimer of **3a** exhibited patterns identical with the *syn* H–H dimer of coumarin. These dimers are assumed to have *syn* H–H configuration. The occurrence of stereoselective photodimerization suggests that the coumarin chromophores in the initial gel phase are stacked in head-to-head manner. The irradiation behavior of **3a** at >300 nm in gel state is similar to 7-acetoxycoumarin in solid state.[11] On the other hand, in dichloromethane solution, **3a** dimerized to *syn* head-to-head and *syn* head-to-tail dimer without stereoselectivity under photo-irradiation at >300 nm, the result is very similar to 7-methoxycoumarin in dichloromethane solution.[10]

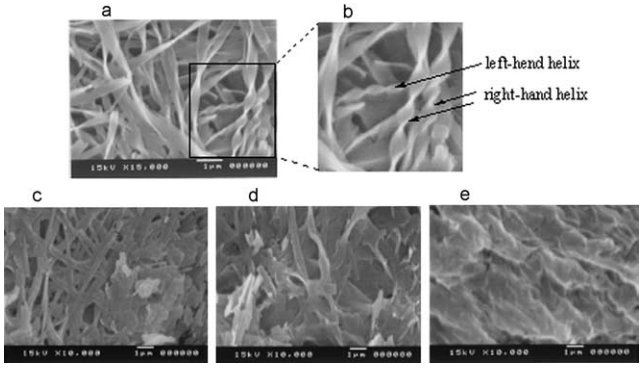


Figure 5. Change of SEM images of the cyclohexane gel of **3a**; (a) before irradiation; (b) enlarged part of (a); (c) after irradiation at >300 nm for 24 h; (d) irradiation at >300 nm for 48 h; (e) irradiation at >300 nm for 24 h and then at <280 nm for 24 h.

A diversity of the gel morphologies of **3a** during irradiation was identified using SEM investigation (Fig. 5). The appearance of the gel surface generally changed under irradiation at >300 nm, the fibers were merged into lumps with extending the irradiation time (Fig. 5c and d). When the gel was irradiated subsequently at <280 nm, the morphologies changed from the lumps to the sponge-like aggregation (Fig. 5e). After irradiation, the appearance of macroscopic gel phase was maintained, but the microscopic morphologies took on drastic changes.

More microscopic morphology change of the cyclohexane gel of **3a** by the photoirradiation was monitored by AFM measurement. Before irradiation, the gel surface is rather smooth (Fig. 6a). The sharp step lines are the boundaries of the gel fibers. When the gel was irradiated with an UV light at >300 nm, the morphology changed drastically as seen in Figure 6b and c, there are numbers of wrinkles on the surface and all the direction of the wrinkles were nearly parallel. These wrinkles almost disappeared on illumination at <280 nm as shown in Figure 6d. At the present time, it is difficult to correlate the morphology change with the photodimerization behavior, because the aggregation mode at molecular level in the gel has never elucidated. Further studies on the molecular arrangements are indispensable for the complete understanding of the gel phase.

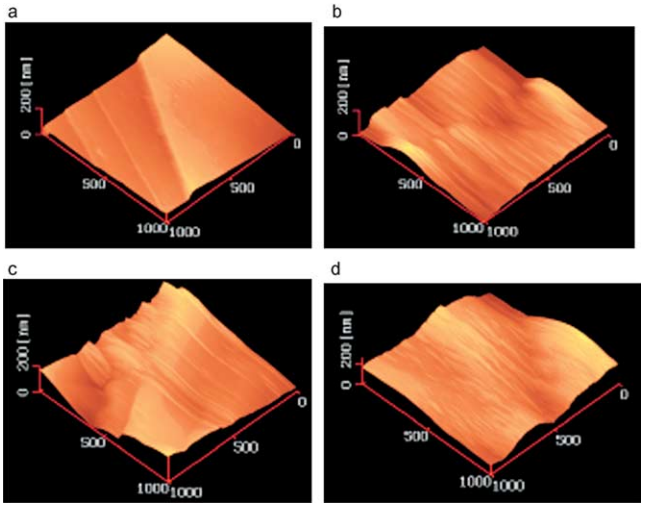


Figure 6. Change of AFM images of the cyclohexane gel of **3a**; (a) before irradiation; (b) after irradiation at >300 nm for 24 h; (c) irradiation at >300 nm for 48 h; (d) irradiation at >300 nm for 24 h and then at <280 nm for 24 h.

3. Conclusion

This work presented the utilization of coumarin building block bonded to amide group with alkyl chain to yield gelating materials with significant photochemical and photophysical properties. The reversible and stereoselective photodimerization of coumarin chromophores in the gel phase proceeded with keeping the visual gel state, but accompanied with the drastic change of microscopic gel morphologies.

4. Experimental

4.1. General

^{1}H NMR spectra were measured on a JEOL JNM-GSX270 spectrometer with tetramethylsilane as an internal standard. IR spectra were recorded on a JASCO FT/IR-300E spectrophotometer. Mass spectra were carried out with a Perkin Elmer ELAN 600. Melting points were not corrected. The solvents for gelation experiments were of analytical grade.

4.1.1. Synthesis of 4-(7′-coumarinoxy)-butyric acid ethyl ester (1). A solution of 7-hydroxycoumarin (6.17 mmol), 4-bromobutyric acid ethyl ester (12.3 mmol) and anhydrous K_2CO_3 (12.3 mmol) in dry DMF (10 ml) was irradiated in a household microwave (MW) oven at 200 W for 4 min. After irradiation, the solid was filtered, 20 ml of water was added to the solution, and the residue was extracted by ethyl acetate (3×10 ml) and CH_2Cl_2 (3×10 ml), the extract was dried over with anhydrous $MgSO_4$, filtered, and evaporated in vacuum. The residue was then washed with hexane and dried to afford **1** as a white solid 1.28 g (75%); mp 64–66 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.64 (d, J=9.3 Hz, 1H), 7.37 (d, J=8.4 Hz, 1H), 6.85 (d, J=2.4 Hz, 1H), 6.82–6.80 (m, 1H), 6.25 (d, J=9.5 Hz, 1H), 4.20–4.13 (m, 2H), 4.08 (t, J=6.0 Hz, 2H), 2.53 (d, J=7.3 Hz, 2H), 2.20–2.11 (m, 2H), 1.27 (t, J=7.1 Hz, 3H); IR (KBr): ν 3080, 2926, 1734, 1611, 1124 cm^{-1}; HRMS calcd for $C_{15}H_{16}O_5$ $(M)^+$276.0998, found 276.0995.

4.1.2. Synthesis of 4-(7′-coumarinoxy)-butyric acid (2). A mixture of **1** (3.2 mmol), KOH (63.9 mmol) and 2 ml water in mortar was ground under heating (below 70 °C) over the period of 30 min. After cooling, the solution of HCl (12 M) was added to the mixture until pH 1. The precipitation was filtered, washed with water and methanol, and dried to afford 0.8 g (99%) of a white solid **2**; mp 198–200 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.67 (d, J=9.4 Hz, 1H), 7.39 (d, J=8.2 Hz, 1H), 6.87 (d, J=2.4 Hz, 1H), 6.83 (s, 1H), 6.26 (d, J=9.3 Hz, 1H), 4.09 (t, J=6.2 Hz, 2H), 2.54 (d, J=7.1 Hz, 2H), 2.19–2.10 (m, 2H); IR (KBr): ν 3445, 3083, 2968, 1724, 1611, 1135 cm^{-1}; HRMS calcd for $C_{13}H_{12}O_5$ $(M)^+$248.0685, found 248.0687.

4.1.3. Synthesis of compounds 3a–h. BOP (0.602 mmol) was added to a stirred solution of octadecylamine (0.524 mmol) and **2** (0.403 mmol) in 10 ml THF. The mixture was stirred at room temperature for 3 h. The residue after concentration under reduced pressure was subjected to column chromatography (silica gel, 10:1 $CHCl_3$/MeOH) to give the product **3a** as a white powder 0.363 g (90%); mp 94–97 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.63 (d, J=9.5 Hz, 1H), 7.37 (d, J=8.4 Hz, 1H), 6.84 (s, 1H), 6.81 (s, 1H), 6.25 (d, J=9.5 Hz, 1H), 5.46 (s, 1H), 4.07 (t, J=5.7 Hz, 2H), 3.28–3.22 (m, 2H), 2.38 (t, J=7.1 Hz, 2H), 2.19–2.15 (m, 2H), 1.47–1.25 (m, 32H), 0.88 (t, J=5.7 Hz, 3H); IR (KBr): ν 3300, 3051, 2920, 1736, 1634, 1163 cm^{-1}; HRMS calcd for $C_{31}H_{49}NO_4$ $(M)^+$499.3662, found 499.3667.

The compounds **3b–3h** were similarly prepared as above:

Compound **3b**. White powder (76%); mp 91–94 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.63 (d, J=9.5 Hz, 1H), 7.36 (d, J=8.3 Hz, 1H), 6.84 (s, 1H), 6.80 (s, 1H), 6.25 (d, J=9.5 Hz, 1H), 5.48 (s, 1H), 4.08 (t, J=5.9 Hz, 2H), 3.28–3.22 (m, 2H), 2.38 (t, J=7.1 Hz, 2H), 2.21–2.17 (m, 2H), 1.47–1.25 (m, 28H), 0.88 (t, J=5.9 Hz, 3H); IR (KBr): ν 3299, 3053, 2921, 1736, 1633, 1163 cm^{-1}; HRMS calcd for $C_{29}H_{45}NO_4$ $(M)^+$471.3349, found 471.3351.

Compound **3c**. White powder (81%); mp 112–113 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.64 (d, J=9.7 Hz, 1H), 7.38 (d, J=8.3 Hz, 1H), 6.84 (d, J=2.2 Hz, 1H), 6.80 (s, 1H), 6.26 (d, J=9.5 Hz, 1H), 5.44 (s, 1H), 4.07 (t, J=5.8 Hz, 2H), 3.28–3.21 (m, 2H), 2.38 (t, J=7.2 Hz, 2H), 2.21–2.14 (m, 2H), 1.47–1.24 (m, 24H), 0.87 (t, J=6.5 Hz, 3H); IR (KBr): ν 3294, 3079, 1734, 1633, 1101 cm^{-1}; HRMS calcd for $C_{27}H_{41}NO_4$ $(M)^+$443.3036, found 443.3034.

Compound **3d**. White powder (66%); mp 106–108 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.64 (d, J=9.3 Hz, 1H), 7.37 (d, J=8.4 Hz, 1H), 6.84 (d, J=2.4 Hz, 1H), 6.81 (t, J=3.4 Hz, 1H), 6.26 (d, J=9.5 Hz, 1H), 5.47 (s, 1H), 4.09 (t, J=5.9 Hz, 2H), 3.28–3.21 (m, 2H), 2.40 (t, J=7.2 Hz, 2H), 2.21–2.12 (m, 2H), 1.47–1.24 (m, 20H), 0.89 (t, J=6.6 Hz, 3H); IR (KBr): ν 3303, 3070, 1743, 1639, 1121 cm^{-1}; HRMS calcd for $C_{25}H_{37}NO_4$ $(M)^+$415.2723, found 415.2724.

Compound **3e**. White powder (74%); mp 104–106 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.64 (d, J=9.5 Hz, 1H), 7.38 (d, J=8.4 Hz, 1H), 6.85 (d, J=2.4 Hz, 1H), 6.81 (d, J=2.0 Hz, 1H), 6.26 (d, J=9.5 Hz, 1H), 5.45 (s, 1H), 4.09 (t, J=5.9 Hz, 2H), 3.28–3.21 (m, 2H), 2.40 (t, J=7.1 Hz, 2H), 2.21–2.12 (m, 2H), 1.47–1.24 (m, 16H), 0.89 (s, 3H); IR (KBr): ν 3298, 3060, 1738, 1639, 1123 cm^{-1}; HRMS calcd for $C_{23}H_{33}NO_4$ $(M)^+$387.2410, found 387.2407.

Compound **3f**. White powder (57%); mp 103–106 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.64 (d, J=9.5 Hz, 1H), 7.38 (d, J=8.4 Hz, 1H), 6.85 (d, J=2.1 Hz, 1H), 6.81 (t, J=2.1 Hz, 1H), 6.26 (d, J=9.5 Hz, 1H), 5.44 (s, 1H), 4.09 (t, J=5.9 Hz, 2H), 3.28–3.21 (m, 2H), 2.40 (t, J=7.1 Hz, 2H), 2.21–2.14 (m, 2H), 1.47–1.25 (m, 12H), 0.89 (t, J=6.6 Hz, 3H); IR (KBr): ν 3294, 3078, 1735, 1634, 1123 cm^{-1}; HRMS calcd for $C_{21}H_{29}NO_4$ $(M)^+$359.2097, found 359.2098.

Compound **3g**. White powder (69%); mp 102–105 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.64 (d, J=9.3 Hz, 1H), 7.38 (d, J=8.4 Hz, 1H), 6.85 (d, J=2.3 Hz, 1H), 6.81 (d, J=2.1 Hz, 1H), 6.26 (d, J=9.5 Hz, 1H), 5.47 (s, 1H), 4.09 (t, J=6.0 Hz, 2H), 3.28–3.21 (m, 2H), 2.40 (t, J=7.1 Hz, 2H), 2.17 (t, J=6.4 Hz, 2H), 1.47–1.26 (m, 8H), 0.86 (s, 3H); IR (KBr): ν 3296, 3087, 1737, 1639, 1158 cm^{-1}; HRMS calcd for $C_{19}H_{25}NO_4$ $(M)^+$331.1784, found 331.1788.

Compound **3h**. White powder (68%); mp 98–99 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.65 (d, J=9.3 Hz, 1H), 7.38 (d, J=8.4 Hz, 1H), 6.84 (s, 1H), 6.81 (t, J=4.7 Hz, 1H), 6.26 (d, J=9.3 Hz, 1H), 5.49 (s, 1H), 4.07 (t, J=5.9 Hz, 2H), 3.29–3.23 (m, 2H), 2.38 (t, J=7.2 Hz, 2H), 2.17 (t, J=6.7 Hz, 2H), 1.49–1.31 (m, 4H), 0.90 (t, J=7.2 Hz, 3H); IR

(KBr): ν 3299, 3084, 1735, 1639, 1125 cm^{-1}; HRMS calcd for $C_{17}H_{21}NO_4$ $(M)^+$303.1471, found 303.1470.

Compound **3i**. White powder (79%); mp 127–129 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 7.65 (d, $J=9.3$ Hz, 1H), 7.38 (d, $J=8.4$ Hz, 1H), 6.84 (s, 1H), 6.80 (t, $J=4.7$ Hz, 1H), 6.25 (d, $J=9.5$ Hz, 1H), 5.83 (s, 1H), 4.07 (t, $J=5.9$ Hz, 2H), 3.29–3.23 (m, 2H), 2.41 (t, $J=7.2$ Hz, 2H), 2.18 (m, 2H), 1.52–1.41 (m, 2H), 0.90 (t, $J=7.2$ Hz, 3H); IR (KBr): ν 3299, 3084, 1735, 1639, 1125 cm^{-1}; HRMS calcd for $C_{16}H_{19}NO_4$ $(M)^+$289.1314, found 289.1319.

4.1.4. Synthesis of compounds 4a–b. A solution of 7-hydroxycoumarin (6.17 mmol), 1-bromooctadecane (12.3 mmol) and anhydrous K_2CO_3 (12.3 mmol) in dry DMF (10 ml) was irradiated in a household MW oven at 200 W for 4 min. After irradiation, the solid was filtered, 20 ml of water was added to the solution, the precipitation was filtered, washed with methanol and dried to afford a white powder **4a** (95%); mp 61–63 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 7.63 (d, $J=9.5$ Hz, 1H), 7.36 (d, $J=8.4$ Hz, 1H), 6.85 (d, $J=2.4$ Hz, 1H), 6.82–6.80 (m, 1H), 6.24 (d, $J=9.3$ Hz, 1H), 4.01 (t, $J=6.4$ Hz, 2H), 1.86–1.76 (m, 2H), 1.46–1.26 (m, 2H), 0.88 (t, $J=6.4$ Hz, 3H); IR (KBr): ν 3052, 2918, 1739, 1619, 1163 cm^{-1}; HRMS calcd for $C_{27}H_{42}O_3$ $(M)^+$414.3134, found 414.3137.

Compound **4b** was obtained in 87% yield from 7-hydroxycoumarin and 1-bromohexadecane as a white powder according to the same procedure as above; mp 57–58 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 7.63 (d, $J=9.5$ Hz, 1H), 7.36 (d, $J=8.4$ Hz, 1H), 6.85 (d, $J=2.4$ Hz, 1H), 6.82–6.79 (m, 1H), 6.24 (d, $J=9.3$ Hz, 1H), 4.01 (t, $J=6.4$ Hz, 2H), 1.86–1.76 (m, 2H), 1.46–1.26 (m, 2H), 0.88 (t, $J=6.4$ Hz, 3H); IR (KBr): ν 3050, 2918, 1738, 1619, 1164 cm^{-1}; HRMS calcd for $C_{25}H_{38}O_3$ $(M)^+$386.2821, found 386.2820.

4.2. Gel test

A gelator (1.0 mg) and appropriate solvent (0.1 ml) were mixed in a closed-capped test tube and the mixture was heated until the solid was dissolved. The solution was subsequently cooled in air to room temperature. Gelation was determined by the absence of flow of the solvent when the vial was inverted.

4.3. Photoreaction

Three test tubes containing the cyclohexane gel of **3a** were irradiated with a 400 W high-pressure mercury lamp (>300 nm irradiation) at 20 °C for 24 h. One of them was irradiated with a 100 W low-pressure mercury lamp (<280 nm irradiation) at 20 °C for 24 h. One of them was continuously irradiated with a 400 W high-pressure mercury lamp at 20 °C for 24 h. The irradiated gels were used to measure UV, fluorescence, and ^1H NMR.

4.4. UV and fluorescence measurements

Absorption spectra were acquired using a Hitachi U-4000 spectrophotometer and emission spectra were obtained on a Hitachi F-4500 fluorospectrometer. Absorption and fluorescence spectra of gels were measured with a sample gel sandwiched by two quartz plates, one of which had 1 mm concavity.

4.5. SEM and AFM observations

A piece of a gel was placed on a SEM stub with a carbon tape, dried, coated with Au, and observed on the stage of JEOL JSM-5300 scanning electron microscope using a 15–20 kV accelerating voltage. AFM observation was carried out with SII SPA 300. The specimens for the observation were prepared by drying a small amount of gel on a glass plate.

Acknowledgements

This research was supported by the Grant-in-Aid for Scientific Research (KAKENHI) in Priority Area 'Molecular Nano Dynamics' from Ministry of Education, Sports, Culture, Science and Technology, and the Shorai Foundation for Science and Technology.

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Tetrahedron 61 (2005) 8939–8946

Enantioselective addition of methyllithium to aromatic imines catalyzed by C_2 symmetric tertiary diamines

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Received 30 May 2005; revised 5 July 2005; accepted 5 July 2005

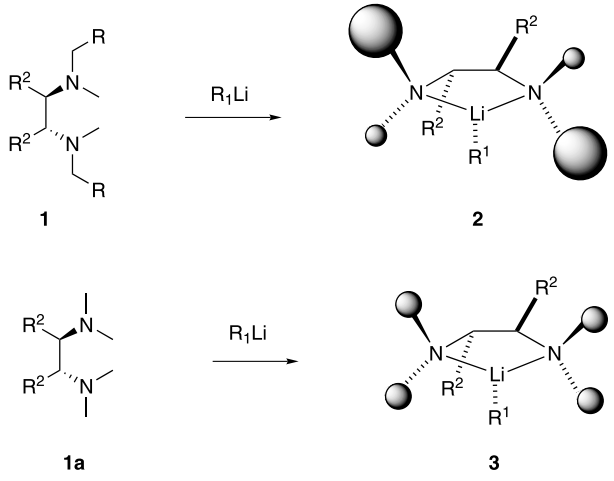
Available online 26 July 2005

Abstract—Enantioselective addition of methyllithium to aromatic imines catalyzed by C_2 symmetric tertiary diamines is described. Eleven diamines have been tested, for which dramatic effect of the nitrogen substitution has been observed. Diamines bearing hindered group close to the nitrogen led to racemic product while homologous hindered diamines led to the best results. Enantiomeric excess up to 74% could be achieved. An explanation of the absolute configuration of the product obtained is given considering the mechanism of the reaction.

1. Introduction

The discovery of new ligands for enantioselective reactions is one of the major goals in asymmetric synthesis. Diamines have often been used as chiral inducers and have turned out to be a powerful tool in inducing high levels of enantioselectivity.[1] Their ability to be associated with several metals makes them even more attractive as a wide range of reactions can be performed in the presence of diamines.[2] We are interested in the continuing development of these ligands by modifying their structures and substituents to improve their selectivities and extend their application.[3] In that context, we recently reported conceptually new chiral tertiary diamines **1** capable to generate, when associated with organolithium reagents, reactive intermediates **2**, which contain a chiral nitrogen atom.[4] In the five membered ring formed with a metallic species, the more bulky nitrogen substituent adopts a trans relationship with the R^2 group of the carbon backbone (Scheme 1). Similar conceptually chiral diether ligands have been studied, for which stereogenic oxygen atoms are involved in the reactive intermediate.[5] Many ligands including (−)-sparteine have been used for the enantioselective addition of organometallic reagents to imines.[6]

In our preliminary study, concerning the catalytic enantioselective addition of methyllithium to aromatic imines, dramatic variations in selectivities have been observed depending on R substituent. On the contrary, in the case of diamine **1a**, both nitrogen atoms bearing four identical substituents cannot become stereogenic centers.[4] We would like to disclose in this article the complete study concerning the enantioselective addition of methyllithium to aromatic imines catalyzed by our diamines. Furthermore, mechanistic considerations will be discussed to explain the stereochemical outcome of the reaction.



Scheme 1.

Keywords: Asymmetry; Diamines; Organolithium; Imines; Catalysis.
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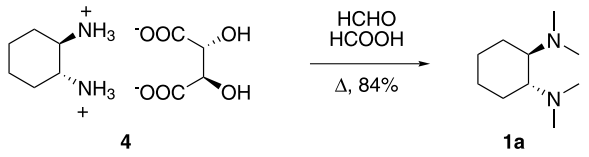
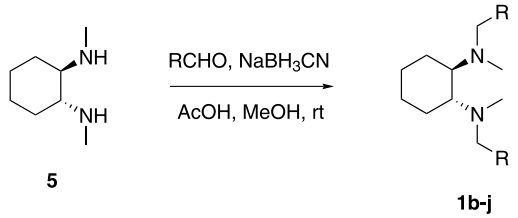
doi:10.1016/j.tet.2005.07.008



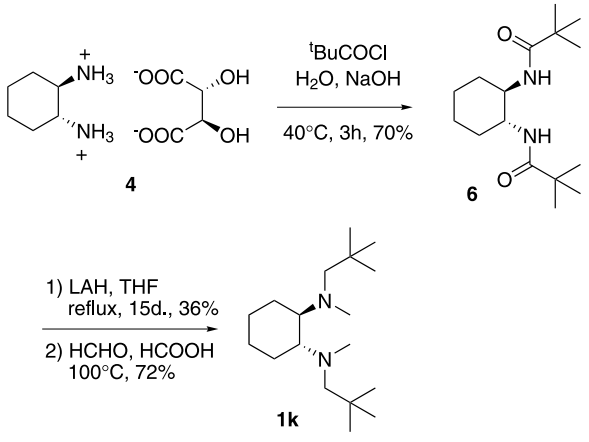
Scheme 2.

2. Synthesis of diamine ligands

Diamines **1a–j** have all been prepared in good yields from the same starting material **4** readily available on a large scale.[7] Ligand **1a** was obtained by an Eschweiler–Clark reaction directly performed on **4** (Scheme 2) while ligands **1b–j** have been obtained by reductive amination of *N*,*N*′-dimethyl-1,2-cyclohexanediamine **5** with the appropriate aldehyde (Scheme 3, Table 1). Diamine **5** can be prepared very easily on a large scale following the two steps procedure previously described.[8] Concerning the synthesis of bulkier ligand **1k**, reductive amination failed: only traces of the product were detected even after several days of reaction. Therefore, a three-step procedure was followed: bis-amide **6** was easily prepared from **4** and reduced with LAH to give the corresponding secondary diamine in moderate yield after 2 weeks. Finally, an Eschweiler–Clark methylation led to the desired ligand **1k** (Scheme 4, Table 1).



Scheme 3.



Scheme 4.

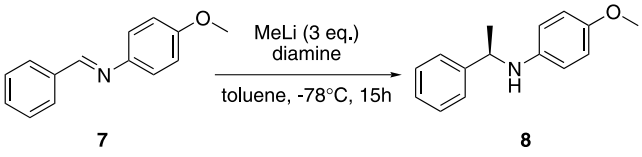
Table 1. Reductive amination of *N*,*N*′-dimethyl-1,2-cyclohexanediamine producing ligands **1b–j**

Entry	Product	R	Yield%
1	**1b**	Et	68
2	**1c**	*n*-Pr	89
3	**1d**	^{i}Pr	72
4	**1e**	^{i}Bu	82
5	**1f**	CH_2^tBu	83
6	**1g**	Ph	49
7	**1h**	CH_2Ph	82
8	**1i**	CH_2CH_2Ph	75
9	**1j**	2,4,6-Me_3–C_6H_2	87

3. Results and discussion

In a preliminary study, the efficiency of diamines were evaluated in the addition of methyllithium to imine **7**. We compared first diamines **1a** and **1g** under stoichiometric conditions and found that the adduct was formed in 20 and 48% ee, respectively, which validates our starting hypothesis.

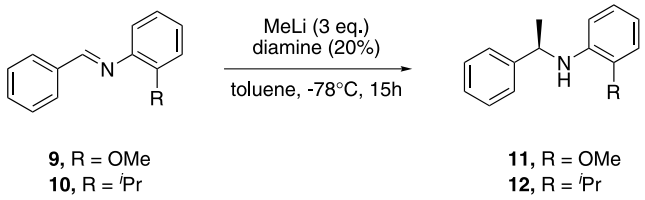
As methyllithium was reacting very slowly with imines at −78 °C without activation (6% yield after 120 min at −78 °C),[9] catalysis was thought to be possible and indeed we observed that 0.2 equiv of diamine was sufficient to maintain good yields without any loss of enantioselectivity. Therefore, all of the other diamines were tested in catalytic amount. A strong influence of the lateral chain was observed. By changing R from H to ethyl and *n*-propyl led to an increase of the ee. When bulkier substituents with ramified alkyl chains were placed close to the nitrogen atom, the products were obtained as a racemic mixture in good yields (Table 2, entries 4, 11, and 12). On the contrary, when the size of the bulky substituent increased in the β position to the nitrogen atom, an increase in ee was observed again. Indeed, the best selectivity was obtained with diamines **1f** and **1h** (Table 2, entries 6 and 9). Moving the bulky substituent another methylene unit away did not improve the selectivity but the corresponding ligand **1i** was still efficient as the product was obtained in 58% ee (Scheme 5).



Scheme 5.

Table 2. Enantioselective addition of MeLi to imine **7** with diamines **1a–k**

Entry	Ligand	Equivalent	Yield%	ee%
1	**1a**	2	78	20 (R)
2	**1b**	0.2	93	24 (R)
3	**1c**	0.2	93	34 (R)
4	**1d**	0.2	94	0
5	**1e**	0.2	94	53 (R)
6	**1f**	0.2	98	67 (R)
7	**1g**	0.2	78	48 (R)
8	**1g**	2	94	40 (R)
9	**1h**	0.2	98	68 (R)
10	**1i**	0.2	93	58 (R)
11	**1j**	0.2	95	0
12	**1k**	0.2	76	0



9, R = OMe
10, R = ^{i}Pr

11, R = OMe
12, R = ^{i}Pr

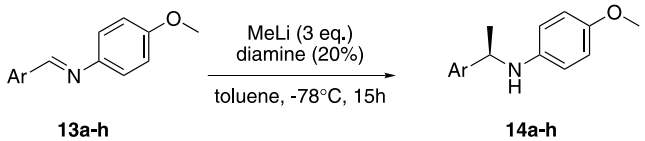
Scheme 6.

We have evaluated the efficiency of our best diamines on other imines. Initially, the *para*-methoxy phenyl moiety was selected because it is an easily removable protecting group of the nitrogen functionality. However, it appears to be more difficult in some cases.[9–11] Nevertheless, we have checked the influence of an *ortho* substituent such as in substrates **9** and **10** (Table 3). The *ortho*-methoxy substituent was found to accelerate considerably the rate of the addition, thus, allowing a fast addition of methyllithium through the non catalyzed process. Therefore, the selectivity obtained with diamine **1f** on imine **9** was very low. Imine **10** has improved the selectivity of the addition compared to the non *ortho*-substituted imines only when diamine **1a** was used (Table 3, entry 3). On the contrary, while **1f** led to 67% ee with **7**, the selectivity dropped to 20% ee with the *ortho* isopropyl substituted imine **10** (Table 3). This result is in sharp contrast with the result obtained with ligand **1a** and with the result reported by Tomioka.[9,10] We can explain these differences by the great sensitivity of our diamines to steric hindrance. However, this behavior had already been observed with (−)-sparteine[12] and when the fine tuning of our diamines **1a–1k** were made (Table 2). In the case of substrates as well, when steric hindrance was increased close to the reaction site, unfavored steric interactions appear with the hindered diamine **1f** (Scheme 6).

Table 3. Enantioselective addition of MeLi to imine **9–10** with diamines **1a** and **1f**

Entry	Substrate	Ligand	Yield%	ee%
1	**9**	**1a**	82	6
2	**9**	**1f**	77	2
3	**10**	**1a**	99	24
4	**10**	**1f**	93	20

The aldehyde part of the imines were changed with other aromatic substitution. Imines **13a–h** were tested under the same experimental conditions as **7** with diamines **1h** or **1f**. Although, both diamines **1f** and **1h** gave the same selectivity for imine **7**, diamine **1h** was usually found to be superior to **1f** with imines **13a–h** (Table 4). Similar levels of enantioselectivity were obtained with *para* substituted



13a-h

14a-h

a, Ar = *p*-Me-C_6H_4
b, Ar = *p*-Cl-C_6H_4
c, Ar = p-CF3-C6H4
d, Ar = 2-furyl
e, Ar = 2-thienyl
f, Ar = 2-pyridyl
g, Ar = 2-naphthyl
h, Ar = piperonyl

Scheme 7.

Table 4. Enantioselective addition of MeLi to imines **7** and **13a–h** with diamines **1f** and **1h**

Entry	Substrate	Ligand	Yield%	ee%[a]
1	**7**	**1h**	98	68 (R)
2	**13a**	**1h**	50	74
3[b]	**13b**	**1f**	70	57 (+)
4	**13b**	**1h**	57	68 (+)
5	**13c**	**1f**	70	38 (−)
6	**13c**	**1h**	35	58 (−)
7	**13d**	**1f**	94	20
8	**13e**	**1f**	93	58
9	**13f**	**1f**	87	4
10[c]	**13g**	**1f**	78	42
11[c]	**13g**	**1h**	42	68
12	**13h**	**1h**	88	48 (+)

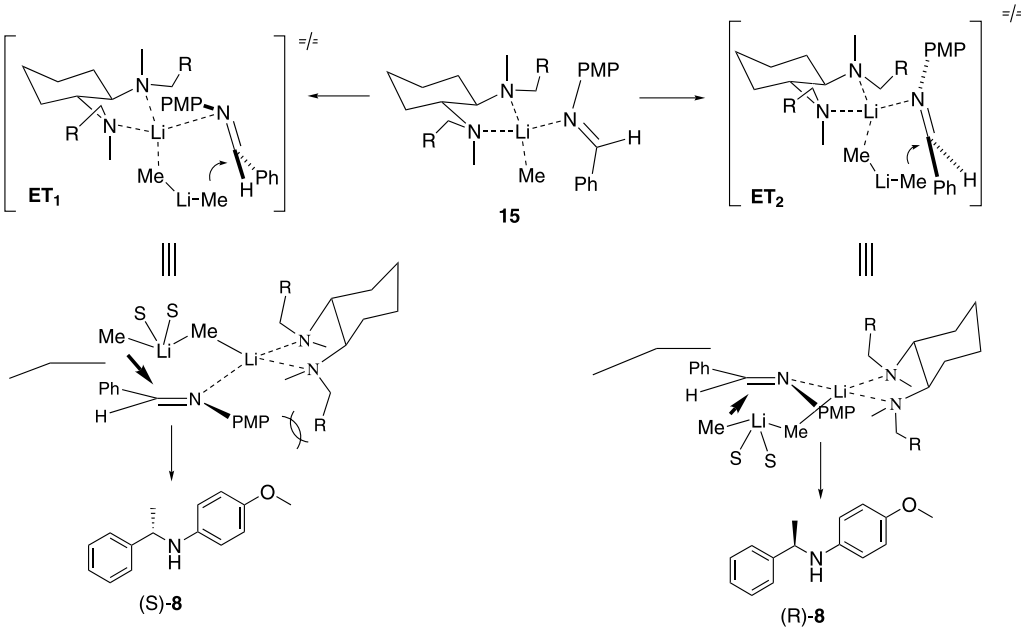
(a) Absolute configuration or sign of the optical rotation. (b) Reaction run at −65 °C. (c) Reaction run at −30 °C.

phenyl derivatives **13a–c** showing quite small electronic effects. On the other hand, the reactivity was found to be very different in some cases: for instance, poor yields were obtained with imines **13g** and **13h**, which reacted at −65 and −30 °C, respectively. Heteroaromatic substituted imines **13d–f** showed as well a very different behavior. Unlike imine **13e**, with the thienyl substitution, both imines **13d** and **13f** were not good substrates (Scheme 7).

This difference can be explained by the availability of the lone pair of the heteroatom in the aromatic ring system. As this lone pair is less engaged in the aromaticity of the heteroaromatic ring for pyridyl and furyl compared to thienyl, the corresponding imines **13d** and **13f** are much more activated than **13e** and react easily in a non-catalyzed process leading to the products with low selectivities.

The absolute configuration of product **8** has been already reported by Tomioka et al.[9] By using Tomioka's ligand, we have been able to assign by comparison the R absolute configuration for amine **8** when we have used our diamines. If the same stereochemical pathway is assumed for all imines employed, compounds **14a–h** should present the same R configuration as for product **8**. To explain these results, we think that a first complexation occurs between the diamine–MeLi complex and the nitrogen lone pair of the imine leading to the intermediate **15** close to the transition state (Scheme 8). The latter is reached after a small rotation of the imine double bond. If the imine turns to react with its Si face (ET_1, Scheme 8), an unfavorable interaction appears between the PMP nitrogen substituent and the R part of the ligand. On the contrary, if the imine turns to react with its Re face (ET_2, Scheme 8), a much more favored transition state is formed without destabilizing interactions. The addition occurs on the Re face of the imine leading to the product of R configuration.

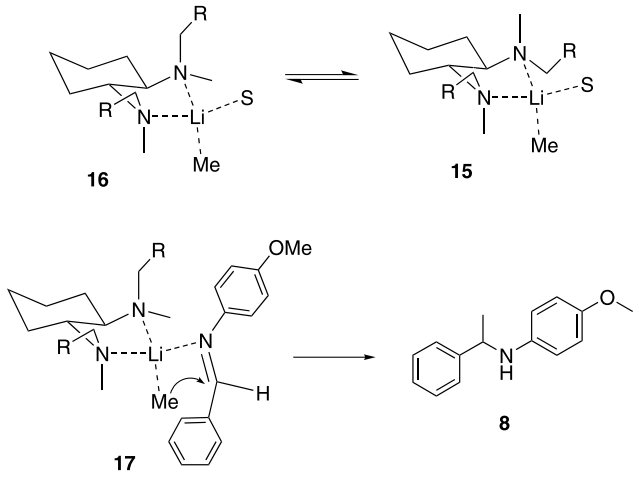
It has been noticed that increasing the amount of methyllithium accelerates the rate of the reaction. One reason for that has already been discussed by Tomioka et al.[9] by considering that the amide product can also be a ligand for the diamine. We envisioned a 6 centers transition state, in which two methyllithium aggregates to reach the most favored transition state ET_2 (Scheme 8). It then follows that with 1 equiv of methyllithium, much lower yields are obtained and it is very difficult for the reaction to



Scheme 8.

go to completion. In the catalytic process, we used only 20% of diamine with respect to substrate and 3 equiv of methyllithium, meaning that the ratio diamine–RLi is actually close to 7%. In that situation, and in a non polar solvent such as toluene, it can be reasonable to consider the methyllithium in a more aggregated state than the dimeric reactive intermediate. The second lithium of this species is likely complexed by diethylether present in the reaction medium (methyllithium is available as solution in diethylether). The theoretical studies dedicated to the aldehyde/alkyllithium condensation problem are relatively scarce[13] while no description of the addition pathway of organolithium reagents onto imines has been reported to our knowledge. Nevertheless, in the case of aldehydes, the formation of an open dimer intermediate, which converts into a six membered cyclic transition state has been preferred by theoretical studies.[13]

For less hindered diamines such as **1b**, **1c**, **1e** or **1h**, ET_1 can explain in part the lower selectivities obtained. But for hindered diamines with bulky substituents α to the nitrogen atom, the adduct was formed without any selectivity. As the chiral discrimination should have been much stronger with these diamines (**1d**, **1j**, **1k**), it seems reasonable to envision, for the diamine/RLi complex, the existence of the equilibrium of complexes **15** and **16** through the free diamine (Scheme 9). When R becomes too bulky, a competitive unfavored interaction between CH_2R and Me of methyllithium could lead to complex **16**, which is close to a *meso* situation. The reactive complex **17** would lead to a non selective process as little spatial discimination exists.



Scheme 9.

Further studies are under progress to obtain spectroscopic evidence about the real reactive species with the aim of establishing a more accurate mechanism.

In conclusion, new ligands have been developed for the catalytic asymmetric addition of methyllithium to imines. We have demonstrated that in this new ligand family, the asymmetric induction can be enhanced by a stereogenic nitrogen atom. These new diamines are of interest because they are easy to prepare on a large scale in enantiomerically pure form, stable on storage and recoverable after use. By choosing the appropriate nitrogen substituents, enantioselectivities up to 74% have been obtained. A model, which could explain the stereochemical outcome of the reaction has been proposed. Further studies are currently in progress in our laboratory to give experimental support to this mechanism and to find more efficient and more general diamines.

4. Experimental

4.1. General

Unless otherwise stated, all reagents were employed as received. Solvent were distilled on CaH_2 or Na/benzophenon. NMR spectrum were made on BRUCKER

400 MHz for proton and BRUCKER 100 MHz for carbon in $CDCl_3$.

4.1.1. *N,N,N′N′*-(1*R*,2*R*)-Tetramethylcyclohexane-1,2-diamine 1a. (*R,R*)-1,2-Diammoniumcyclohexane mono-(+)-tartrate salt **5** (24 g, 0.091 mol) was dissolved in formic acid 85% (36 mL) and formaldehyde 40% (44 mL) was added slowly at room temperature. The mixture was heated at reflux 2 h. After cooling, the reaction mixture was made basic until pH 14 and extracted with ether. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The product was distilled (bp=50 °C/0.1 mmHg) to give a colorless liquid (11.35 g, 74%). ^{1}H NMR: δ(ppm) 1.05–1.15 (m, 4H), 1.68–1.74 (m, 2H), 1.78–1.90 (m, 2H), 2.26 (s, 12H), 2.35–2.40 (m, 2H). ^{13}C NMR: δ(ppm) 22.8, 25.6, 40.1, 63.8. $[\alpha]_D^{20}=-62.9$ (*c* 1.05, $CHCl_3$).

4.2. General procedure for reductive amination of 1b–j

To a solution of *N,N′*-dimethyl cyclohexanediamine (1 g, 7 mmol) in methanol (15 mL) was added aldehyde (21 mmol), sodium cyanoborohydride (28 mmol) and acetic acid (7 mmol). The mixture was stirred 24 h, methanol was then evaporated and the residue was diluted in ether (20 mL). The organic layer was washed with sodium hydroxide 10% (2×15 mL) and with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The product was purified by distillation, acid–base extraction or column chromatography.

4.2.1. (1*R*,2*R*)-*N,N′*-Dipropyl-*N,N′*-dimethyl-cyclohexane-1,2-diamine 1b. The product was obtained according to the general procedure, purified by distillation (bp=80–90 °C/0.1 mmHg) and isolated as a colorless oil in 68% yield. ^{1}H NMR: δ(ppm) 0.86 (t, *J*=7.32 Hz, 6H), 1.0–1.2 (m, 4H), 1.4–1.5 (m, 4H), 1.65–1.71 (m, 2H), 1.73–1.80 (m, 2H), 2.23 (s, 6H), 2.35–2.50 (m, 6H). ^{13}C NMR: δ(ppm) 12.1, 21.6, 25.7, 25.9, 36.6, 56.5, 63.1. $[\alpha]_D^{20}=-27.7$ (*c* 1.0, $CHCl_3$). MS (*m/z*) 226, 211, 183, 154, 152, 126, 112, 86, 70, 57. HRMS calcd for $C_{14}H_{30}N_2$ 226.2409, found 226.2408.

4.2.2. (1*R*,2*R*)-*N,N′*-Dibutyl-*N,N′*-dimethyl-cyclohexane-1,2-diamine 1c. The product was obtained according to the general procedure, purified by distillation (bp=90–100 °C/0.1 mmHg) and isolated as a colorless oil in 89% yield. ^{1}H NMR: δ(ppm) 0.88 (t, *J*=7.2 Hz, 6H), 1.0–1.2 (m, 4H), 1.2–1.48 (m, 8H), 1.63–1.69 (m, 2H), 1.72–1.78 (m, 2H), 2.21 (s, 6H), 2.38–2.52 (m, 6H). ^{13}C NMR: δ(ppm) 14.1, 20.8, 25.6, 25.9, 30.8, 36.6, 54.2, 63.0. $[\alpha]_D^{20}=-21.6$ (*c* 0.99, $CHCl_3$). MS (*m/z*) 254, 239, 211, 197, 166, 139, 126, 100, 84, 56. HRMS calcd for $C_{16}H_{34}N_2$ 254.2722, found 254.2719.

4.2.3. (1*R*,2*R*)-*N,N′*-Diisobutyl-*N,N′*-dimethyl-cyclohexane-1,2-diamine 1d. The product was obtained according to the general procedure, purified by distillation (bp=80–90 °C/0.1 mmHg) and isolated as a colorless oil in 72% yield. ^{1}H NMR: δ(ppm) 0.88 (t, *J*=6.2 Hz, 12H), 1.03–1.25 (m, 4H), 1.63–1.80 (m, 6H), 2.12–2.21 (m, 2H), 2.19 (s, 6H), 2.35–2.45 (m, 4H). ^{13}C NMR: δ(ppm) 20.9, 21.1, 26.0, 26.4, 26.8, 36.1, 63.8, 64.9. $[\alpha]_D^{20}=+23.4$ (*c* 0.99, $CHCl_3$). MS (*m/z*) 254, 239, 211, 197, 166, 139, 126, 100, 84, 57. HRMS calcd for $C_{16}H_{34}N_2$ 254.2722, found 254.2719.

4.2.4. (1*R*,2*R*)-*N,N′*-Bis-(3-methylbutyl)-*N,N′*-dimethyl-cyclohexane-1,2-diamine 1e. The product was obtained according to the general procedure and purified by distillation (bp=130–140 °C/1 mmHg) and isolated as a colorless oil in 82% yield. ^{1}H NMR: δ(ppm) 0.87 (d, *J*=2.0 Hz, 6H), 0.89 (d, *J*=2.0 Hz, 6H), 1.03–1.22 (m, 4H), 1.26–1.40 (m, 4H), 1.57 (s, *J*=6.6 Hz, 2H), 1.65–1.72 (m, 2H), 1.74–1.82 (m, 2H), 2.23 (s, 6H), 2.42–2.56 (m, 6H). ^{13}C NMR: δ(ppm) 22.8, 23.0, 25.5, 25.9, 26.5, 36.7, 37.6, 52.6, 62.9. $[\alpha]_D^{20}=-27.2$ (*c* 1.02, $CHCl_3$). MS (*m/z*) 282, 267, 239, 211, 197, 180, 140, 126, 114, 84, 58. HRMS calcd for $C_{18}H_{38}N_2$ 282.3035, found 282.3026.

4.2.5. (1*R*,2*R*)-*N,N′*-Bis-(3,3-dimethyl-butyl)-*N,N′*-dimethyl-cyclohexane-1,2-diamine 1f. The product was obtained according to the general procedure, purified by distillation (bp=150 °C/1 mmHg) and isolated as a colorless oil in 83% yield. ^{1}H NMR: δ(ppm) 0.90 (s, 18H), 1.05–1.25 (m, 4H), 1.35–1.45 (m, 4H), 1.67–1.74 (m, 2H), 1.76–1.83 (m, 2H), 2.25 (s, 6H), 2.42–2.58 (m, 6H). ^{13}C NMR: δ(ppm) 22.3, 25.9, 29.6, 29.8, 37.0, 42.2, 50.1, 62.7. $[\alpha]_D^{20}=-31.1$ (*c* 1.02, $CHCl_3$). MS (*m/z*) 310, 295, 253, 225, 196, 180, 154, 128, 84, 57. HRMS calcd for $C_{20}H_{42}N_2$ 310.3348, found 310.3342.

4.2.6. (1*R*,2*R*)-*N,N′*-Dibenzyl-*N,N′*-dimethyl-cyclohexane-1,2-diamine 1g. The product was obtained according to the general procedure, purified by distillation (bp=170 °C/1 mmHg) and isolated as a colorless oil in 49% yield. ^{1}H NMR: δ(ppm) 1.05–1.35 (m, 4H), 1.7–1.8 (m, 2H), 1.9–2.0 (m, 2H), 2.24 (s, 6H), 2.6–2.7 (m, 2H), 3.68 (d, *J*=13.4 Hz, 2H), 3.76 (d, *J*=13.1 Hz, 2H), 7.2–7.35 (m, 6H), 7.38–7.45 (m, 4H). ^{13}C NMR: δ(ppm) 25.8, 25.9, 36.2, 58.6, 63.7, 126.5, 127.9, 128.8, 140.9. $[\alpha]_D^{20}=+7.22$ (*c* 1.02, $CHCl_3$). MS (*m/z*) 322, 257, 231, 200, 180, 160, 120, 91, 65. HRMS calcd for $C_{22}H_{30}N_2$ 322.2409, found 322.2418.

4.2.7. (1*R*,2*R*)-*N,N′*-Dimethyl-*N,N′*-diphenethyl-cyclohexane-1,2-diamine 1h. The product was obtained according to the general procedure, purified by distillation (bp=240 °C/1 mmHg) and isolated as a brown oil in 83% yield. ^{1}H NMR: δ(ppm) 1.05–1.30 (m, 4H), 1.65–1.75 (m, 2H), 1.78–1.88 (m, 2H), 2.35 (s, 6H), 2.52–2.62 (m, 2H), 2.70–2.85 (m, 8H), 7.15–7.25 (m, 6H), 7.28–7.40 (m, 4H). ^{13}C NMR: δ(ppm) 25.8, 25.9, 35.5, 36.6, 56.6, 63.5, 125.7, 128.1, 128.8, 141.1. $[\alpha]_D^{20}=-13.84$ (*c* 1.0, $CHCl_3$). MS (*m/z*) 350, 259, 214, 155, 112, 70. HRMS calcd for $C_{24}H_{34}N_2$ 350.2722, found 350.2737.

4.2.8. (1*R*,2*R*)-*N,N′*-Dimethyl-*N,N′*-bis-(3-phenyl-propyl)-cyclohexane-1,2-diamine 1i. The product was obtained according to the general procedure, purified by distillation (bp=190–210 °C/0.1 mmHg) and isolated as a yellow oil in 75% yield. ^{1}H NMR: δ(ppm) 1.05–1.25 (m, 4H), 1.68–1.75 (m, 2H), 1.76–1.90 (m, 6H), 2.28 (s, 6H), 2.50–2.72 (m, 10H), 7.18–7.25 (m, 6H), 7.28–7.33 (m, 4H). ^{13}C NMR: δ(ppm) 25.6, 25.7, 30.1, 33.8, 36.3, 54.1, 63.1, 125.5, 128.1, 128.3, 142.6. $[\alpha]_D^{20}=-6.7$ (*c* 1.44, $CHCl_3$). MS (*m/z*) 378, 287, 230, 188, 149, 91. HRMS calcd for $C_{26}H_{38}N_2$ 378.3035, found 378.3013.

4.2.9. (1*R*,2*R*)-*N*,*N*′-Dimethyl-*N*,*N*′-bis-(2,4,6-trimethyl-benzyl)-cyclohexane-1,2-diamine 1j. The product was obtained according to the general procedure, purified by acid–base treatment and isolated as a solid in 87% yield. ^{1}H NMR: δ(ppm) 1.10–1.26 (m, 4H), 1.75–1.82 (m, 2H), 1.87 (s, 6H), 1.96–2.04 (m, 2H), 2.27 (s, 6H), 2.38 (s, 12H), 2.40–2.47 (m, 2H), 3.48 (d, J=12.6 Hz, 2H), 3.72 (d, J=12.9 Hz, 2H), 6.81 (s, 4H). ^{13}C NMR: δ(ppm) 20.0, 20.9, 24.6, 26.1, 34.0, 52.5, 61.1, 128.7, 133.6, 135.8, 138.2. $[\alpha]_D^{20}$= −16.7 (*c* 0.88, $CHCl_3$). MS (*m*/*z*) 406, 302, 273, 202, 163, 133, 91. HRMS calcd for $C_{28}H_{42}N_2$ 406.3348, found 406.3329.

4.2.10. Bis-(*tert*-butyl)-cyclohexane-1,2-diamide 6. (*R*,*R*)-1,2-Diammoniumcyclohexane mono-(+)-tartrate salt (2 g, 7.6 mmol) was dissolved in water (5 mL) with sodium hydroxide (600 mg, 15.2 mmol) and pivaloyl chloride (9.3 mL, 76 mmol) was added while the mixture was heated at 40 °C. After stirring 3 h at this temperature, the solution was quenched with sodium hydroxide until basic pH and extracted with dichloromethane. The combined organic layer was dried over Na_2SO_4 and solvent are evaporated. The solid was diluted in ether and insoluble impurities were filtered. After evaporation under reduced pressure, the bis-(*tert*-butyl)-cyclohexane diamide was isolated in 70% yield. ^{1}H NMR: δ(ppm) 1.17 (s, 18H), 1.20–1.45 (m, 4H), 1.68–1.82 (m, 2H), 2.0–2.11 (m, 2H), 3.60–6.38 (m, 2H), 6.17 (br s, 2H). ^{13}C NMR: δ(ppm) 24.7, 27.5, 32.4, 38.5, 53.6, 179.1.

4.2.11. (1*R*,2*R*)-*N*,*N*′-Bis-(2,2-dimethyl-propyl)-*N*,*N*′-dimethyl-cyclohexane-1,2-diamine 1k. To a suspension of LAH (4.8 g, 0.13 mmol) in THF (50 mL) was added bis-(*tert*-butyl)-cyclohexane diamide **6** (3.55 g, 0.013 mmol) and the mixture was refluxed 2 weeks. The solution was cooled to room temperature, poured into crushed ice and extracted with ether. The combined organic layer was dried over Na_2CO_3, filtered and concentrated to give the crude diamine as a solid, which was methylated without purification. The diamine (1.2 g, 4.7 mmol) was dissolved in formic acid 85% (2.3 mL), formaldehyde 40% (1.9 mL) was added and the mixture was refluxed 3 h. After basification and extraction with ether, the organic layer was dried over Na_2SO_4, filtered and concentrate. The residue was purified by bulb-to-bulb distillation (120 °C/1 mmHg) to give the product as a colorless oil in 72% yield. ^{1}H NMR: δ(ppm) 0.90 (s, 18H), 1.03–1.12 (m, 2H), 1.17–1.30 (m, 2H), 1.64–1.70 (m, 2H), 1.73–1.80 (m, 2H), 2.25–2.40 (m, 12H). ^{13}C NMR: δ(ppm) 26.1, 27.1, 28.6, 33.3, 38.7, 66.6, 70.0. $[\alpha]_D^{20}$= +6.3 (*c* 1.03, $CHCl_3$). MS (*m*/*z*) 282, 225, 169, 124, 114, 58. HRMS calcd for $C_{18}H_{38}N_2$ 282.3035, found 282.3024.

4.3. Asymmetric addition of MeLi to imines. General procedure

To a cooled (−78 °C) stirred solution of imine (0.48 mmol) and diamine ligand (0.096 or 0.96 mmol), in dry toluene (8 mL) under an inert atmosphere, was added an ether solution of MeLi (low halide, 1.6 M in ether, 1.44 mmol) over a period of 5 min. The mixture was stirred at −78 °C for the time indicated and quenched with methanol (1 mL) at deep temperature and with water (5 mL) at room temperature. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the pure product.

4.3.1. (4-Methoxy-phenyl)-(1-phenyl-ethyl)-amine 8. The mixture was stirred 15 h before the quench. The product was purified by silica gel chromatography to give the product as a yellow oil. ^{1}H NMR: δ(ppm) 1.49 (d, J=6.8 Hz, 3H), 3.68 (s, 3H), 4.39 (q, J=6.8 Hz, 1H), 6.46 (d, J=9.0 Hz, 2H), 6.70 (d, J=9.0 Hz, 2H), 7.1–7.5 (m, 5H). ^{13}C NMR: δ(ppm) 25.2, 54.3, 55.8, 114.6, 114.8, 125.9, 126.9, 128.7, 141.6, 145.6, 151.9. Enantiomeric excess was determined by SFC Chiralcel OD-H, 200 bar, 2 mL min^{-1}, 2% MeOH in CO_2, 30 °C, R t_1=9.26 min, S t_2=9.84 min. Chiralcel OB-H, 200 bar, 2 mL min^{-1}, 15% MeOH in CO_2, 30 °C, S t_1=6.77 min, R t_2=12.31 min.

4.3.2. (2-Methoxy-phenyl)-(1-phenyl-ethyl)-amine 11. The mixture was stirred 2 h before the quench. The residue was purified by silica gel column chromatography (toluene) to give the product as a yellow oil. ^{1}H NMR: δ(ppm) 1.61 (d, J=6.6 Hz, 3H), 3.93 (s, 3H), 4.53 (q, J=6.7 Hz, 1H), 4.6–4.85 (s, 1H), 6.41 (dd, J_1=7.8 Hz, J_2=1.5 Hz, 1H), 6.67 (dt, J_1=7.6 Hz, J_2=1.5 Hz, 1H), 6.76 (dt, J_1=7.6 Hz, J_2=1.5 Hz, 1H), 6.82 (dd, J_1=7.8 Hz, J_2=1.5 Hz, 1H), 7.25–7.30 (m, 1H), 7.37 (t, J=7.8 Hz, 1H), 7.41–7.45 (m, 1H). ^{13}C NMR: δ(ppm) 25.1, 53.3, 55.3, 109.2, 111.0, 116.3, 121.1, 125.8, 126.7, 128.5, 137.1, 145.4, 146.5. Enantiomeric excess was determined by SFC Chiralcel OD-H, 200 bar, 2 mL min^{-1}, 2% MeOH in CO_2, (2%, 6 min, 1% min^{-1}, 15%), 30 °C, t_1=5.58 min, t_2=7.90 min.

4.3.3. (2-Isopropyl-phenyl)-(1-phenyl-ethyl)-amine 12. The mixture was stirred 2 h before the quench. The residue was purified by silica gel column chromatography (toluene) to give the product as a yellow oil. R_F=0.61 (cyclohexane/ether=95:5). ^{1}H NMR: δ(ppm) 1.47 (d, J=6.8 Hz, 6H), 1.69 (d, J=6.8 Hz, 3H), 3.12 (sept, J=6.8 Hz, 1H), 4.0–4.3 (br s, 1H), 4.67 (q, J=6.8 Hz, 1H), 6.55 (dd, J_1=1.0 Hz, J_2=8.3 Hz, 1H), 6.83 (dt, J_1=1.0 Hz, J_2=7.6 Hz, 1H), 7.09 (dt, J_1=1.8 Hz, J_2=7.6 Hz, 1H), 7.26–7.55 (m, 6H). ^{13}C NMR: δ(ppm) 22.2, 22.3, 25.25, 27.3, 53.35, 111.6, 117.1, 124.7, 125.7, 126.5, 126.75, 128.6, 131.6, 143.7, 145.3. Enantiomeric excess was determined by SFC Chiralcel OJ, 200 bar, 2 mL min^{-1}, 2% MeOH in CO_2, (2%, 6 min, 1% min^{-1}, 15%), 30 °C, t_1=5.12 min, t_2=6.00 min. $[\alpha]_D^{20}$= −29.5 (*c* 1.25, $CHCl_3$, ee=20% with **1f**). MS (*m*/*z*) 239, 224, 182, 134, 105, 91, 51. HRMS calcd for $C_{17}H_{21}N$ 239.1674, found 239.1686.

4.3.4. (4-Methoxy-phenyl)-(1-*p*-tolyl-ethyl)-amine 13a. The mixture was stirred 12 h before the quench. The residue was purified by silica gel column chromatography (pentane/ether=10:1) to give the product as a yellow oil. ^{1}H NMR: δ(ppm) 7.17 (d, 2H, J=7.8 Hz), 7.04 (d, 2H, J=7.8 Hz), 6.61 (d, 2H, J=9.0 Hz), 6.39 (d, 2H, J=9.0 Hz), 4.30 (q, 1H, J=6.6 Hz), 3.80–3.62 (br s, 1H), 3.61 (s, 3H), 2.24 (s, 3H), 1.40 (d, 3H, J=6.6 Hz). ^{13}C NMR: δ(ppm) 151.8, 142.5, 141.7, 136.3, 129.3, 125.8, 114.5, 55.7, 53.9, 25.2, 21.1. Enantiomeric excess was determined by SFC Chiralpak AS-H, 200 bar, 2 mL min^{-1}, 5% MeOH in CO_2, (5%, 5 min, 1% min^{-1}, 20%), 30 °C, t_1=3.34 min, t_2=4.47 min. MS (*m*/*z*) 241, 226, 119, 108, 91, 65. HRMS calcd for $C_{16}H_{19}NO$ 241.1466, found 241.1462.

4.3.5. [1-(4-Chloro-phenyl)-ethyl]-(4-methoxy-phenyl)-amine 13b. The mixture was stirred 12 h before the quench. The residue was purified by silica gel column chromatography (pentane/ether=10:1) to give the product as a yellow oil. ^{1}H NMR: δ(ppm) 7.35–7.30 (m, 4H), 6.74 (d, 2H, J=9.1 Hz), 6.48 (d, 2H, J=9.1 Hz), 4.42 (q, 1H, J=6.8 Hz), 3.83–3.74 (br s, 1H), 3.74 (s, 3H), 1.51 (d, 3H, J=6.8 Hz). ^{13}C NMR: δ(ppm) 152.3, 132.5, 129.8, 128.8, 127.4, 114.9, 114.8, 114.0, 55.7, 54.1, 25.0. Enantiomeric excess was determined by SFC Chiralpak AD, 200 bar, 2 mL min^{-1}, 5% MeOH in CO_2, (5%, 5 min, 1% min^{-1}, 20%), 30 °C, t_1=7.56 min, t_2=8.61 min, and Chiralpak AS-H, 200 bar, 2 mL min^{-1}, 5% MeOH in CO_2, (5%, 5 min, 1% min^{-1}, 20%), 30 °C, t_1=4.65 min, t_2=5.86 min. $[\alpha]_D^{20}$= +15.0 (c 0.93, $CHCl_3$, ee=68% with **1h**). MS (m/z) 261, 246, 139, 123, 108, 77, 52. HRMS calcd for $C_{15}H_{16}^{35}ClNO$ 261.0920, found 261.0921. HRMS calcd for $C_{15}H_{16}^{37}ClNO$ 263.0890, found 263.0902.

4.3.6. (4-Methoxy-phenyl)-[1-(4-trifluoromethyl-phenyl)-ethyl]-amine 13c. The mixture was stirred 12 h before the quench. The residue was purified by silica gel column chromatography (pentane/ether=10:1) to give the product as a yellow oil. RF=0.22 (eluent:pentane/ether=10:1). ^{1}H NMR: δ(ppm) 7.49 (d, 2H, J=8.2 Hz), 7.40 (d, 2H, J=8.2 Hz), 6.61 (d, 2H, J=8.8 Hz), 6.34 (d, 2H, J=8.8 Hz), 4.37 (q, 1H, J=6.7 Hz), 3.78–3.75 (br s, 1H), 3.60 (s, 3H), 1.42 (d, 3H, J=6.7 Hz). ^{13}C NMR: δ(ppm) 152.3, 149.8, 141.0, 126.2, 125.7, 125.6, 125.6, 114.9, 114.6, 55.7, 54.1, 25.1. Enantiomeric excess was determined by SFC Chiralpak AD, 200 bar, 2 mL min^{-1}, 5% MeOH in CO_2, (5%, 5 min, 1% min^{-1}, 20%), 30 °C, t_1=3.27 min, t_2=3.64 min. $[\alpha]_D^{20}$= −4.3 (c 0.91, $CHCl_3$, ee=58% with **1h**). MS (m/z) 295, 280, 173, 122, 95, 77. HRMS calcd for $C_{16}H_{16}F_3NO$ 295.1184, found 295.1186.

4.3.7. (1-Furan-2-yl-ethyl)-(4-methoxy-phenyl)-amine 13d. The mixture was stirred 15 h before the quench. The residue was purified by silica gel column chromatography (pentane/ether=10:1) to give the product as a yellow oil. ^{1}H NMR: δ(ppm) 1.54 (d, J=6.6 Hz, 3H), 3.74 (s, 3H), 4.55 (q, J=6.6 Hz, 1H), 6.14 (d, J=3.3 Hz, 1H), 6.28 (dd, J_1=1.7 Hz, J_2=3.0 Hz, 1H), 6.61 (d, J=8.8 Hz, 2H), 6.76 (d, J=8.8 Hz, 2H), 7.33 (dd, J_1=0.8 Hz, J_2=1.8 Hz, 1H). ^{13}C NMR: δ(ppm) 20.9, 48.3, 55.7, 105.0, 110.0, 114.7, 115.1, 141.1, 141.35, 152.4, 157.4. Enantiomeric excess was determined by SFC Chiralcel OD-H, 200 bar, 2 mL min^{-1}, 2% MeOH in CO_2, (2%, 6 min, 1% min^{-1}, 10%), 30 °C, t_1=5.82 min, t_2=6.24 min.

4.3.8. (4-Methoxy-phenyl)-(1-thiophen-2-yl-ethyl)-amine 13e. The mixture was stirred 15 h before the quench. The residue was purified by silica gel column chromatography to give the product as a yellow oil. ^{1}H NMR: δ(ppm) 1.64 (d, J=6.6 Hz, 3H), 3.76 (s, 3H), 4.78 (q, J=6.6 Hz, 1H), 6.63 (d, J=9.1 Hz, 2H), 6.79 (d, J=9.1 Hz, 2H), 6.96–7.02 (m, 2H), 7.20 (dd, J_1=1.4 Hz, J_2=4.9 Hz, 1H). ^{13}C NMR: δ(ppm) 24.6, 50.4, 55.6, 114.7, 115.0, 122.8, 123.5, 126.6, 141.0, 150.4, 152.3. Enantiomeric excess was determined by SFC Chiralcel OD-H, 200 bar, 2 mL min^{-1}, 2% MeOH in CO_2, (2%, 20 min), 30 °C, t_1=9.48 min, t_2=10.06 min.

4.3.9. (4-Methoxy-phenyl)-(1-pyridin-2-yl-ethyl)-amine 13f. The mixture was stirred 15 h before the quench. The residue was purified by silica gel column chromatography to give the product as a yellow oil. ^{1}H NMR: δ(ppm) 1.53 (d, J=6.6 Hz, 3H), 3.69 (s, 3H), 4.1 (br s, 1H), 4.55 (q, J=6.8 Hz, 1H), 6.53 (d, J=8.8 Hz, 2H), 6.71 (d, J=9.1 Hz, 2H), 7.12 (m, 1H), 7.34 (d, J=7.8 Hz, 1H), 7.59 (dt, J_1=1.8 Hz, J_2=7.6 Hz, 1H), 8.57 (d, J=4.8 Hz, 1H). ^{13}C NMR: δ(ppm) 23.3, 55.6, 55.7, 114.75, 114.8, 120.4, 121.9, 136.8, 141.4, 149.3, 152.0, 164.2. Enantiomeric excess was determined by SFC Chiralcel OJ, 200 bar, 2 mL min^{-1}, 5% MeOH in CO_2, (5%, 6 min, 1% min^{-1}, 15%), 30 °C, t_1=8.58 min, t_2=10.45 min.

4.3.10. (4-Methoxy-phenyl)-(2-naphtalen-2-yl-ethyl)-amine 13g. The mixture was stirred 35 h before the quench. The residue was purified by silica gel column chromatography (pentane/ether=10:1) to give the product as a yellow oil. ^{1}H NMR: δ(ppm) 7.73–7.70 (m, 4H), 7.42 (d, J=8.6 Hz, 1H), 7.39–7.32 (m, 2H), 6.59 (d, J=8.8 Hz, 2H), 6.43 (d, J=8.8 Hz, 2H), 4.48 (q, 1H, J=6.6 Hz), 4.35–3.65 (br s, 1H), 3.58 (s, 3H), 1.49 (d, 3H, J=6.6 Hz). ^{13}C NMR: δ(ppm) 152.0, 142.9, 141.4, 133.6, 132.7, 129.1, 128.4, 127.8, 127.7, 126.0, 125.5, 124.5, 124.4, 114.8, 55.7, 54.6, 25.1. Enantiomeric excess was determined by SFC Chiracel OD-H, 175 bar, 2 mL min^{-1}, 2% MeOH in CO_2, (2%, 20 min), 30 °C, t_1=8.36 min, t_2=8.72 min.

4.3.11. (1-Benzo[1,3]dioxol-5-yl-ethyl)-(4-methoxy-phenyl)-amine 13h. The mixture was stirred 14 h at −30 °C before the quench. The residue was purified by silica gel column chromatography (pentane/ether=10:1) to give the product as a yellow oil. ^{1}H NMR: δ(ppm) 6.79–6.74 (m, 2H), 6.68–6.61 (m, 3H), 6.40 (d, J=8.8 Hz, 2H), 5.84 (d, J=5.3 Hz, 2H), 4.25 (q, J=6.6 Hz, 1H), 3.80–3.50 (br s, 1H), 3.62 (s, 3H), 1.38 (d, J=6.6 Hz, 3H). ^{13}C NMR: δ(ppm) 151.9, 147.9, 146.3, 141.5, 139.7, 118.9, 114.7, 114.5, 108.3, 106.3, 100.9, 55.7, 54.1, 25.4. Enantiomeric excess was determined by SFC Chiralcel OD-H, 200 bar, 2 mL min^{-1}, 5% MeOH in CO_2, (5%, 5 min, 1% min^{-1}, 20%), 30 °C, t_1=6.76 min, t_2=7.28 min, and Chiralcel OJ, 200 bar, 2 mL min^{-1}, 5%, 5 min, 1% min^{-1}, 20%), 30 °C, t_1=11.5 min, t_2=12.4 min. $[\alpha]_D^{20}$= +12.7 (c 1.1, $CHCl_3$, ee=48% with **1h**). MS (m/z) 271, 149, 123, 108, 91, 65. HRMS calcd for $C_{16}H_{17}NO_3$ 271.1208, found 271.1202.

Acknowledgements

The authors wish to thank PPG-SIPSY for financial support, Du Pont de Nemours GmbH for a generous gift of cyclohexane diamine, Ségolène Gille and Iain Rudkin for fruitfull discussions.

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Tetrahedron 61 (2005) 8947–8955

New cytotoxic bufadienolides from the biotransformation of resibufogenin by *Mucor polymorphosporus*

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Received 31 January 2005; revised 1 July 2005; accepted 5 July 2005

Available online 26 July 2005

Abstract—Resibufogenin is a cytotoxic steroid isolated from the Chinese drug ChanSu. The biotransformation of resibufogenin by *Mucor polymorphosporus* afforded 22 products, and 15 of them were new. The transformation reactions involved hydroxylations at C-1β, C-5, C-7α, C-7β, C-12α, C-12β and C-16α, as well as epimerization or dehydrogenation of 3-OH. Hydroxylations at C-12α, C-12β and C-16α were the major reactions, each giving products in >5% yields, whereas the other products were obtained in fairly low yields. Some of the products showed decreased but still potent cytotoxicities. This investigation provided a useful approach to prepare new bufadienolides and most of them were difficult to obtain by chemical means.

1. Introduction

Biotransformation is an important tool in the structural modification of organic compounds, especially natural products, due to its significant regio- and stereo-selectivities.[1,2] Filamentous fungi have frequently been used to catalyze selective hydroxylation reactions, which are usually difficult to achieve by chemical means.[3] In the past several years, we have studied the biotransformation of several natural bufadienolides, including bufalin, cinobufagin and resibufogenin, and obtained more than 30 products, 22 of them being new compounds.[4–8] These substrates are the major effective constituents of the Chinese drug ChanSu,[9] and possess significant anticancer activities.[10–13] Our prospective aim is to discover new bufadienolide derivatives with more potent bioactivities and improved physico-chemical properties as drug candidates. Herein we report the biotransformation of resibufogenin by the filamentous fungus *Mucor polymorphosporus*.

2. Results and discussion

In the screening test, resibufogenin (**1**) was completely metabolized in the cultures of *M. polymorphosporus*. A number of new peaks were observed by HPLC in the incubation mixture, whereas no corresponding peak was found in control tests. The peaks showed UV absorption maxima at 294–298 nm, which is characteristic for the α-pyrone ring of bufadienolides. Thus, they should be the biotransformed products of resibufogenin. In the preparative biotransformation, a total amount of 800 mg of resibufogenin was fed to the microbial cultures. After 6 days incubation, the products were recovered by extracting with ethyl acetate. The obtained extract was subjected to ODS column chromatography and preparative HPLC to afford 22 pure compounds, which were structurally characterized by MS and extensive NMR techniques, including ^{1}H NMR, ^{13}C NMR, DEPT, HMQC, HMBC, ^{1}H–^{1}H COSY and NOESY. These products were 7β-hydroxyl resibufogenin (**2**),[14] 3-*epi*-7β-hydroxyl resibufogenin (**3**), marinobufagin (5-hydroxyl resibufogenin, **4**),[15] 5,7β-dihydroxyl resibufogenin (**5**), 7α-hydroxyl resibufogenin (**6**), 12β-hydroxyl resibufogenin (**7**),[15] 3-*epi*-12β-hydroxyl resibufogenin (**8**), 5,12β-dihydroxyl resibufogenin (**9**), 12α-hydroxyl resibufogenin (**10**),[14] 3-*epi*-12α-hydroxyl resibufogenin (**11**), 5,12α-dihydroxyl resibufogenin (**12**), 1β,12α-dihydroxyl resibufogenin (**13**), 3-oxo-12α-hydroxyl resibufogenin (**14**), 12-oxo-resibufogenin (**15**), 16α-hydroxyl resibufogenin (**16**),[16] 3-*epi*-16α-hydroxyl resibufogenin (**17**), 3-oxo-16α-hydroxyl resibufogenin (**18**),[17] 7β,16α-dihydroxyl resibufogenin (**19**), 12β,16α-dihydroxyl resibufogenin (**20**), 1β,16α-dihydroxyl resibufogenin (**21**), 12α,16α-dihydroxyl resibufogenin (**22**), and 3-oxo-Δ^{4}-resibufogenin (**23**).[18]

Keywords: Biotransformation; Bufadienolide; Cytotoxicity; *Mucor polymorphosporus*; Resibufogenin.
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doi:10.1016/j.tet.2005.07.012

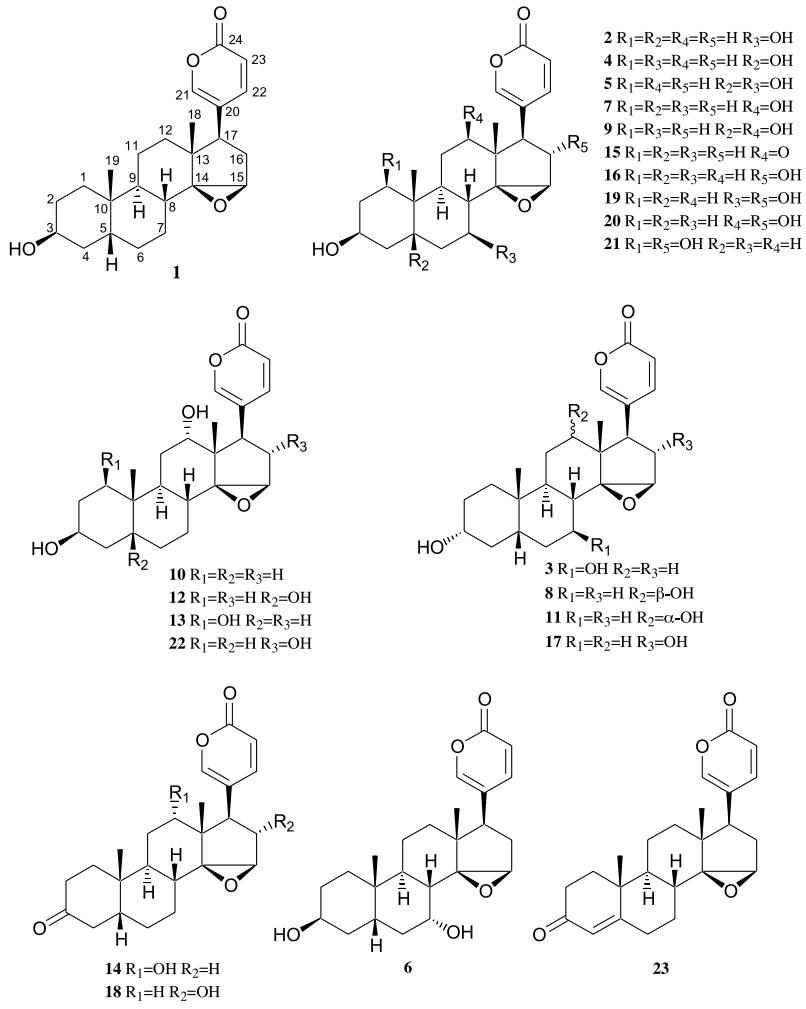

Figure 1. Chemical structures of compounds **1**–**23**.

Table 1. ^{13}C NMR spectral data for compounds **1**–**12** (125 MHz, DMSO-d_6)

C	1	2	3	4	5	6	7	8	9	10	11	12
1	29.4t	29.0t	30.2t	24.8t	24.6t	29.4t	29.4t	30.3t	24.9t	29.3t	30.3t	24.7t
2	27.5t	27.4t	34.3t	27.3t	27.1t	27.6t	27.4t	34.7t	27.2t	27.5t	34.8t	27.3t
3	64.5d	64.2d	69.6d	66.4d	66.2d	64.4d	64.5d	69.7d	66.4d	64.5d	69.8d	66.4d
4	33.0t	34.0t	37.0t	36.5t	37.7t	34.4t	32.9t	36.0t	36.5t	33.1t	36.1t	36.7t
5	35.5d	36.2d	41.5d	74.1s	73.2s	35.4d	35.6d	41.1d	73.4s	35.6d	41.2d	73.5s
6	25.6t	34.1t	34.5t	34.0t	42.2t	36.5t	25.5t	26.0t	34.0t	25.7t	26.2t	34.1t
7	20.2t	66.2d	66.3d	22.4t	67.1d	63.6d	20.4t	20.6t	22.4t	20.2t	20.5t	22.5t
8	33.1d	40.3d	40.4d	32.2d	39.8d	36.5d	32.4d	32.6d	31.5d	33.0d	33.2d	32.1d
9	38.5d	37.3d	38.0d	41.6d	40.0d	31.8d	33.8d	34.6d	36.9d	30.8d	31.6d	34.2d
10	35.0s	34.6s	34.1s	40.4s	40.4s	35.9s	34.7s	34.2s	40.0s	34.6s	34.1s	40.0s
11	20.6t	21.0t	20.7t	21.1t	21.3t	20.2t	29.4t	29.2t	29.9t	28.4t	28.3t	29.0t
12	38.2t	38.2t	38.2t	38.3t	38.1t	37.4t	72.9d	72.8d	72.8d	74.0d	74.0d	74.2d
13	44.6s	45.1s	45.1s	44.4s	44.9s	45.2s	50.7s	50.7s	50.5s	48.9s	48.8s	48.7s
14	74.0s	76.2s	76.2s	73.4s	76.1s	72.2s	73.5s	73.5s	73.5s	72.5s	72.5s	72.6s
15	59.3d	61.1d	61.1d	59.4d	61.1d	63.3d	59.2d	59.3d	59.3d	61.2d	61.2d	61.3d
16	31.6t	31.2t	31.2t	31.5t	31.1t	31.7t	31.6t	31.6t	31.6t	34.8t	34.8t	34.8t
17	46.1d	45.8d	45.7d	46.0d	45.6d	45.3d	41.7d	41.7d	41.6d	42.7d	42.7d	42.7d
18	16.5q	16.3q	16.3q	16.4q	16.3q	16.3q	11.4q	11.4q	11.3q	17.3q	17.3q	17.2q
19	23.7q	23.6q	23.1q	16.7q	16.7q	23.3q	23.6q	23.0q	16.7q	23.4q	22.8q	16.5q
20	122.0s	121.5s	121.5s	121.9s	121.5s	122.2s	122.1s	122.1s	122.1s	122.8s	122.7s	122.7s
21	150.5d	150.8d	150.8d	150.5d	150.8d	150.4d	150.5d	150.5d	150.5d	150.1d	150.1d	150.1d
22	147.4d	147.2d	147.2d	147.3d	147.2d	147.4d	147.5d	147.5d	147.4d	147.7d	147.7d	147.7d
23	114.1d	114.2d	114.2d	114.1d	114.2d	114.1d	114.2d	114.2d	114.2d	114.3d	114.3d	114.3d
24	161.0s	161.0s	161.0s	161.0s	161.0s	161.0s	161.0s	161.0s	161.0s	161.1s	161.1s	161.1s

Table 2. ^{13}C NMR spectral data for compounds **13–23** (125 MHz, DMSO-d_6)

C	13	14	15	16	17	18	19	20	21	22	23
1	71.8d	35.9t	29.1t	29.4t	30.4t	35.9t	29.0t	29.4t	71.8d	29.3t	34.9t
2	32.0t	36.7t	27.3t	27.6t	34.8t	36.7t	27.5t	27.5t	32.1t	27.5t	33.5t
3	66.7d	211.6s	64.4d	64.4d	69.7d	211.5s	64.1d	64.4d	66.6d	64.4d	197.9s
4	33.0t	41.6t	32.8t	33.0t	36.0t	41.6t	34.0t	33.0t	32.9t	33.1t	123.2d
5	30.1d	43.0d	35.7d	35.4d	41.0d	42.9d	36.1d	35.6d	29.9d	35.6d	170.1s
6	25.2t	25.5t	25.2t	25.6t	26.0t	25.4t	34.2t	25.5t	25.0t	25.6t	31.3t
7	19.9t	19.7t	20.4t	20.2t	20.4t	19.6t	66.2d	20.4t	19.9t	20.1t	26.3t
8	33.2d	32.8d	32.5d	33.1d	33.3d	32.9d	40.4d	32.4d	33.3d	32.8d	33.0d
9	32.6d	31.6d	34.4d	38.5d	39.2d	38.5d	37.5d	33.8d	40.2d	30.9d	51.9d
10	40.0s	34.3s	35.0s	35.0s	34.5s	34.8s	34.5s	34.7s	39.5s	34.6s	38.3s
11	28.3t	28.4t	37.3t	20.6t	20.4t	20.7t	21.1t	29.4t	20.5t	28.1t	20.6t
12	73.9d	74.0d	210.5s	39.0t	39.0t	39.0t	39.7t	73.8d	38.9t	73.1d	37.8t
13	48.7s	48.9s	59.8s	43.9s	43.9s	43.9s	44.6s	50.0s	43.8s	48.0s	44.4s
14	72.4s	72.3s	73.4s	74.9s	74.9s	74.8s	77.0s	74.4s	74.8s	72.7s	73.4s
15	61.2d	61.2d	59.6d	61.4d	61.4d	61.4d	62.3d	61.2d	61.3d	61.9d	59.4d
16	34.8t	34.8t	32.1t	75.6d	75.6d	75.6d	75.2d	75.6d	75.5d	75.3d	31.4t
17	42.7d	42.7d	37.6d	58.4d	58.3d	58.5d	58.0d	54.5d	58.3d	53.8d	45.9d
18	17.3q	17.2q	17.5q	17.0q	17.0q	17.0q	16.9q	11.7q	17.0q	17.5q	16.4q
19	18.4q	21.8q	23.0q	23.7q	23.1q	22.1q	23.7q	23.6q	18.7q	23.5q	17.1q
20	122.7s	122.7s	120.8s	120.2s	120.2s	120.2s	119.8s	120.4s	120.1s	120.2s	121.8s
21	150.1d	150.1d	151.1d	150.4d	150.4d	150.5d	150.7d	150.3d	150.5d	150.5d	150.6d
22	147.7d	147.7d	114.5d	147.1d	147.1d	147.1d	147.0d	147.3d	147.1d	147.1d	147.3d
23	114.3d	114.3d	147.3d	114.3d	114.3d	114.3d	114.4d	114.3d	114.3d	114.4d	114.2d
24	161.1s	161.1s	160.9s	160.9s	160.9s	160.9s	160.8s	160.9s	160.9s	160.8s	161.0s

Fifteen of them (**3**, **5**, **6**, **8**, **9**, **11–15**, **17**, **19–22**) were new compounds. The chemical structures were illustrated in Figure 1. Compounds **2**, **7**, **10**, **16** and **23** had been obtained previously by chemical or enzymatic means, and **18** had been reported as a metabolite of cinobufagin in rat liver microsomes. However, their NMR data were determined and assigned here for the first time.

The products were fully characterized by comparing their NMR spectra with the substrate or related compounds. The general NMR rules of bufadienolides we previously reported were extensively utilized in this procedure.[7] The NMR data are given in Tables 1–5.

All the bufadienolides gave significant $[M+H]^+$ ions in the

Table 3. ^{1}H NMR spectral data for compounds **2–9** (500 MHz, DMSO-d_6, J in Hz)

H	2	3	4	5	6	7	8	9
1	1.42m	1.50m	1.68m	1.70m	1.42m	1.43m	1.52m	1.70m
	1.35m	1.18m	1.25m	1.25m	1.38m	1.35m	1.22m	1.24m
2	1.42m	1.68m	1.52m	1.50m	1.44m	1.45m	1.69m	1.54m
	1.32m	0.97m	1.46m	1.42m	1.31m	1.35m	0.97m	1.45m
3	3.83br s	3.31br s	3.99br s	3.95br s	3.78br s	3.87br s	3.38br s	3.99br s
4	1.82m	1.43m	2.08br d (11.5)	1.92m	1.76m	1.78m	1.57m	2.07m
	1.28m	1.41m	1.29m	1.40m	1.25m	1.16m	1.33m	1.28m
5	1.77m	1.38m			1.65m	1.72m	1.32m	
6	1.60m	1.58m	1.52m	1.42m	2.42m	1.72m	1.71m	1.52m
	1.25m	1.37m	1.14m	1.38m	1.29m	1.08m	1.16m	1.16m
7	3.54br s	3.57br s	1.42m	3.40br s	3.78br s	1.27m	1.29m	1.38m
			0.92m			0.94m	0.96m	0.90m
8	1.82m	1.82t (10.0)	1.86m	1.80t (10.0)	1.94br d (11.5)	1.80m	1.81m	1.80m
9	1.68m	1.72m	1.54m	1.45m	2.05m	1.65m	1.63m	1.55m
11	1.46m	1.45m	1.48m	1.51m	1.50m	1.58m	1.57m	1.60m
	1.18m	1.20m	1.24m	1.22m	1.28m	1.18m	1.17m	1.25m
12	1.63m	1.64m	1.60m	1.62m	1.57m	3.28m	3.28br s	3.30m
	1.44m	1.42m	1.41m	1.45m	1.40m			
15	4.08s	4.11s	3.58s	4.06s	4.17s	3.55s	3.58s	3.55s
16	2.44dd	2.46m	2.33dd	2.41dd	2.35dd	2.30dd	2.32dd	2.29dd
	(15.0,10.5)		(15.0,10.5)	(15.0,10.0)	(15.0,11.5)	(15.0,10.5)	(15.0,10.5)	(15.0,11.0)
	1.90d (15.0)	1.92d (15.5)	1.80m	1.90m	1.77m	1.82m	1.84m	1.82m
17	2.55d (10.0)	2.56d (10.0)	2.52d (10.5)	2.56d (10.0)	2.45d (11.5)	3.02d (10.5)	3.03d (10.5)	3.02d (10.5)
18	0.71 (3H,s)	0.71 (3H,s)	0.66 (3H,s)	0.70 (3H,s)	0.65 (3H,s)	0.56 (3H,s)	0.56 (3H,s)	0.56 (3H,s)
19	0.90 (3H,s)	0.88 (3H,s)	0.85 (3H,s)	0.86 (3H,s)	0.88 (3H,s)	0.88 (3H,s)	0.87 (3H,s)	0.84 (3H,s)
21	7.54d (2.0)	7.54d (2.5)	7.52d (2.5)	7.54d (2.5)	7.52d (2.5)	7.43s	7.44d (2.0)	7.43s
22	7.70dd	7.70dd	7.75dd	7.70dd	7.77dd	7.67d	7.67dd	7.66d
	(10.0,2.0)	(9.5,2.5)	(10.0,2.5)	(9.5,2.5)	(9.5,2.5)	(10.0)	(10.0,2.0)	(10.0)
23	6.26d (10.0)	6.26d (9.5)	6.25d (10.0)	6.26d (9.5)	6.25d (9.5)	6.25d (10.0)	6.25d (10.0)	6.25d (10.0)
3-OH	4.22d (2.5)	4.53d (4.0)	5.21d (3.5)	5.25br s	4.03d (3.0)	4.21br s	4.50d (4.0)	5.22d (3.5)
5-OH			4.80s	4.91s				4.82s
7-OH	3.64s	3.67s		3.62s	4.26d (2.5)			
12-OH						4.77d (4.5)	4.79d (5.0)	4.80d (5.0)

Table 4. ^{1}H NMR spectral data for compounds **10–15** and **23** (500 MHz, DMSO-d_6, J in Hz)

H	**10**	**11**	**12**	**13**	**14**	**15**	**23**
1	1.39m	1.47m	1.70m	3.58br s	1.94m	1.44m	2.02m
	1.32m	1.28m	1.22m		1.35m	1.27m	1.62m
2	1.50m	1.66m	1.55m	1.72m	2.42m	1.42m	2.38m
	1.31m	0.94m	1.36m	1.68m	1.98m	1.34m	2.16m
3	3.88br s	3.37br s	4.01br s	4.01br s		3.86br s	
4	1.85m	1.62m	2.10m	1.88m	2.70t (14.0)	1.78m	5.63s
	1.17m	1.33m	1.32m	1.29m	1.85br d (15.0)	1.16m	
5	1.68m	1.31m		1.94m	1.74m	1.75m	
6	1.75m	1.72m	1.55m	1.70m	1.76m	1.82m	2.35m
	1.06m	1.17m	1.12m	1.16m	1.18m	1.11m	2.23m
7	1.36m	1.34m	1.42m	1.42m	1.38m	1.40m	1.74m
	1.02m	1.03m	0.95m	1.35m	1.12m	1.07m	0.93m
8	1.80m	1.85brt (12.0)	1.84m	1.90m	1.96m	2.30dt (3.5,13.5)	2.06m
9	2.18dt (2.0,11.0)	2.26brt (11.0)	2.08m	2.04m	2.36m	1.92dt (3.5,13.5)	1.24m
11	1.55m	1.55m	1.58m	1.45m	1.62m	2.52t (14.0)	1.57m
	1.42m	1.40m	1.40m	1.42m	1.56m	2.19dd (14.0,3.5)	1.39m
12	3.57br s	3.57br s	3.54m	3.54br s	3.60br s		1.67dt (3.0,11.5)
							1.42m
15	3.57s	3.58s	3.57s	3.57s	3.63s	3.64s	3.54s
16	2.32dd	2.34brt (14.5)	2.32m	2.32m	2.32m	2.08dd	2.30dd
	(14.0,11.0)					(15.0,10.0)	(15.0,10.0)
	1.68m	1.70m	1.71m	1.68m	1.71m	1.84m	1.82d (15.0)
17	3.08d (10.0)	3.07d (10.0)	3.08d (10.0)	3.07d (10.5)	3.11br d (10.5)	3.80d (10.0)	2.55d (9.5)
18	0.61 (3H,s)	0.61 (3H,s)	0.61 (3H,s)	0.61 (3H,s)	0.64 (3H,s)	0.90 (3H,s)	0.73 (3H,s)
19	0.88 (3H,s)	0.86 (3H,s)	0.84 (3H,s)	0.97 (3H,s)	0.96 (3H,s)	0.95 (3H,s)	1.16 (3H,s)
21	7.47d (2.0)	7.48s	7.48s	7.47s	7.50s	7.54d (2.5)	7.54d (2.5)
22	7.74dd (10.0,2.0)	7.74d (10.0)	7.74d (10.0)	7.54d (9.5)	7.75d (9.5)	7.65dd (10.0,2.5)	7.75dd (9.5,2.5)
23	6.25d (10.0)	6.25d (10.0)	6.25d (10.0)	6.25d (9.5)	6.26d (9.5)	6.27d (10.0)	6.26d (9.5)
1-OH				4.82d (7.5)			
3-OH	4.18d (2.5)	4.49d (4.0)	5.19d (3.5)	5.17d (3.5)		4.23d (3.0)	
5-OH			4.79s				
12-OH	4.72d (4.5)	4.79d (3.5)	4.75d (4.0)	4.72d(3.0)	4.74d (4.5)		

Table 5. ^{1}H NMR spectral data for compounds **16–22** (500 MHz, DMSO-d_6, J in Hz)

H	**16**	**17**	**18**	**19**	**20**	**21**	**22**
1	1.42m	1.70m	1.98m	1.44m	1.44m	3.62br d (7.5)	1.43m
	1.42m	1.52m	1.34m	1.38m	1.35m		1.35m
2	1.44m	1.72m	2.34m	1.42m	1.43m	1.72m	1.52m
	1.35m	0.96m	2.01m	1.36m	1.32m	1.66m	1.34m
3	3.89br s	3.39br s		3.86br s	3.89br s	4.01br s	3.90br s
4	1.80m	1.65m	2.71m	1.65m	1.75m	1.88m	1.80m
	1.18m	1.32m	1.84m	1.32m	1.18m	1.32m	1.18m
5	1.70m	1.80m	1.72m	1.78m	1.72m	1.93m	1.69m
6	1.75m	1.74m	1.78m	1.60m	1.72m	1.70m	1.76m
	1.08m	1.21m	1.21m	1.28m	1.08m	1.18m	1.07m
7	1.32m	1.30m	1.38m	3.55br s	1.30m	1.38m	1.32m
	1.02m	1.02m	1.12m		0.92m	1.04m	1.02m
8	1.91m	1.88m	1.96m	1.88t (10.0)	1.82m	1.96m	1.91m
9	1.68m	1.68m	1.80m	1.72m	1.65m	1.54m	2.17m
11	1.46m	1.46m	1.54m	1.42m	1.58m	1.30m	1.65m
	1.15m	1.15m	1.31m	1.20m	1.18m	1.21m	1.47m
12	1.76m	1.74m	1.84m	1.79m	3.62br s	1.72m	3.65br s
	1.65m	1.62m	1.65m	1.68m		1.63m	
15	3.44s	3.46s	3.52s	3.92s	3.42s	3.45s	3.41s
16	4.00d (3.5)	4.01br s	4.01br s	4.08br s	4.03d (4.5)	3.99d (4.5)	3.87d (11.0)
17	2.43s	2.44s	2.46 (overlapped)	2.49s	2.92s	2.42s	2.89s
18	0.63 (3H,s)	0.63 (3H,s)	0.67 (3H,s)	0.69 (3H,s)	0.53 (3H,s)	0.63 (3H,s)	0.64 (3H,s)
19	0.90 (3H,s)	0.88 (3H,s)	0.98 (3H,s)	0.92 (3H,s)	0.89 (3H,s)	0.99 (3H,s)	0.90 (3H,s)
21	7.54d (2.0)	7.55d (2.0)	7.56s	7.57s	7.44s	7.54d (2.5)	7.56d (2.5)
22	7.60dd	7.60dd	7.61d (10.0)	7.59d (10.0)	7.51d (10.0)	7.60dd	7.61dd
	(10.0,2.0)	(10.0,2.0)				(10.0, 2.5)	(10.0,2.5)
23	6.25d (10.0)	6.25d (10.0)	6.26d (10.0)	6.27d (10.0)	6.26d (10.0)	6.25d (10.0)	6.26d (10.0)
1-OH						4.82d (7.5)	
3-OH	4.22d (2.5)	4.54d (4.5)		4.25d (2.5)	4.24d (3.0)	5.18d (4.5)	4.21d (3.0)
7-OH				3.53s			
12-OH					4.75d (5.0)		5.94d (2.5)
16-OH	5.47d (4.0)	5.53d (3.0)	5.46d (4.5)	5.62br s	5.46d (4.5)	5.45d (4.5)	5.21d (11.0)

atmospheric pressure chemical ionization (APCI) mass spectra. The mono- or dihydroxylation of resibufogenin could be deduced by the $[M+H]^+$ ions at *m/z* 401 or *m/z* 417, respectively. And a 2-unit decrease of $[M+H]^+$ indicated the presence of a carbonyl group. The exact position and stereo-configuration of the hydroxyl or carbonyl groups, however, were determined by NMR spectroscopy. Any substitution led to stable and characteristic changes in the ^1H and ^{13}C NMR spectra, as we had summarized.[7] Upon the introduction of 1β-OH, C-19 was shifted upfield to δ 17–18 due to γ-*gauche* effect, and C-10 was shifted downfield by 4–5 ppm. The 1β- and 3β-hydroxyl groups were both resonated at much lower fields (δ 4.8–5.2) because of their intra-molecular hydrogen bonding. The W-type long-range coupling between H-1α and H-3α could be observed in the ^1H–^1H COSY spectrum. When 3-OH was epimerized into α-configuration, H-3β appeared as a very broad peak ($W_{1/2}$ = 20–25 Hz) due to its $^3J_{aa}$ couplings with H-2α and H-4α. Similar to 1β-OH, the introduction of 5-OH led to the upfield shift of C-19 to δ 16–17, and the downfield shift of 3-OH. When a 7β-OH was present, H-7α appeared as a very broad peak due to its $^3J_{aa}$ couplings with H-6β and H-8. When 7-OH was in the α-configuration, however, the H-7β signal was much narrower, and C-9 was shifted upfield to δ 31–33 due to the γ-*gauche* effect of 7α-OH. Both 12α-OH and 12β-OH resulted in 3–4 ppm upfield shift of C-17, 4–6 ppm downfield shift of C-13, and 0.5 ppm downfield shift of H-17. The epimers could be differentiated by the chemical shift of C-18, which was shifted upfield to δ 10–12 at the presence of a 12β-OH due to γ-*gauche* effect, while was hardly affected by 12α-OH. The 16α-OH could be characterized by the 12–14 ppm downfield shift of C-17. In addition, H-17 appeared as a sharp singlet since the dihedral angle of H-17 and H-16β was approximately 90°. This feature was different from 16β-OH bufadienolides, where H-17 appeared as a doublet (J = 8.5–9.5 Hz). The above rules were applied to the structural elucidation of biotransformation products.

The ^{13}C NMR spectrum of **2** showed an additional oxygenated methine signal at δ 66.2, and C-8 appeared at a lower field at δ 40.3, suggesting the hydroxylation at C-7. According to the HMQC spectrum, the signal of H-7 (δ 3.54) appeared as a very broad peak, indicating the β-configuration of 7-OH. Thus, compound **2** was identified as 7β-hydroxyl resibufogenin. The presence of 7β-OH in compound **3** was established by comparing its NMR data with **2**. In addition, the HMBC spectrum showed a long-range coupling of H-8 (δ 1.82) with C-7 (δ 66.3). However, the A-ring carbon signals were very different from those of **2**, but similar to 3-*epi*-7β-hydroxyl bufalin,[7] indicating that the 3-OH should be in the α-configuration. Thus, compound **3** was identified as 3-*epi*-7β-hydroxyl resibufogenin. A comparison of the NMR data of **5** with those of **2** and 5-hydroxyl resibufogenin (**4**) suggested that compound **5** was hydroxylated both at C-5 and C-7β. The quaternary carbon signal at δ 73.2 should thus be assigned to C-5. In accordance, C-19 was shifted upfield to δ 16.7, and 3-OH was shifted downfield to δ 5.25 because of its hydrogen bonding with 5-OH. The signal of H-8 (δ 1.80) appeared as a triplet (J = 10.0 Hz) due to its $^3J_{aa}$ couplings with H-7α and H-9, and confirmed the β-configuration of 7-OH. Therefore, compound **5** was identified as 5,7β-dihydroxyl resibufogenin.

The ^{13}C NMR spectrum of **6** showed an extra oxygenated methine signal at δ 63.6. Its HMBC correlation with H-8 (δ 1.94) indicated that C-7 was hydroxylated. When compared to **2**, the signal of H-7 (δ 3.78) appeared as a much narrower peak, suggesting the α-configuration of 7-OH. In accordance, C-9 (δ 31.8) resonated at a much higher field than that of **2** ($\delta_{C\text{-}9}$37.3), due to the γ-*gauche* effect of 7α-OH. The NOE enhancement of 7-OH (δ 4.26) with H-4α (δ 1.25) was also observed. Therefore, compound **6** was identified as 7α-hydroxyl resibufogenin.

Compounds **7** and **8** were both hydroxylated at 12β-position since their C-18 signals shifted upfield to δ 11.4, and C-13 downfield to δ 50.7. In addition, the NOESY spectra showed NOE enhancements of H-12 (δ 3.28) with H-16α (δ 2.32) and H-17 (δ 3.03). However, their A-ring carbon signals were very different. The H-3 signal (δ 3.38) of **8** appeared as a very broad peak, suggesting that its 3-OH should be in α-configuration. Thus, they were identified as 12β-hydroxyl resibufogenin (**7**) and 3-*epi*-12β-hydroxyl resibufogenin (**8**), respectively. The presence of 12β-OH in compound **9** was established in a similar manner, and was confirmed by the HMBC correlation of H-18 (δ 0.56) with C-12 (δ 72.8). In addition, a new quaternary carbon appeared at δ 73.4, and C-10 was shifted downfield to δ 40.0, while C-19 upfield to δ 16.7. All evidences supported the presence of 5-OH. The HMBC correlation of H-19 (δ 0.84) with C-5 was also observed. Therefore, compound **9** was identified as 5,12β-dihydroxyl resibufogenin.

Compounds **10**–**14** were identified as 12α-hydroxylated derivatives of resibufogenin, since their C-13 signals were shifted downfield to δ 48.7–48.9, C-17 shifted upfield to δ 42.7, and C-18 appeared at δ 17.2–17.3. The chemical shift of C-18 could hardly be affected by 12α-OH since they were in γ-*trans* positions. In the HMBC spectrum of compound **11**, H-18 (δ 0.61) had a long-range coupling with C-12 (δ 74.0). The signal of C-9 was shifted upfield by 6.9 ppm when compared to resibufogenin, due to the γ-*gauche* effect of 12α-OH. The NOE enhancement of 12α-OH (δ 4.79) with H-17 (δ 3.07) was also observed in the NOESY spectrum (Fig. 2). The 3α-OH of **11** was established by the broad peak of H-3 (δ 3.37). Therefore, compound **11** was identified as 3-*epi*-12α-hydroxyl resibufogenin. A carbonyl signal appeared at δ 211.6 in the ^{13}C NMR spectrum of **14**, and C-2 (δ 36.7) and C-4 (δ 41.6) were significantly shifted downfield, suggesting the presence of C-3 carbonyl group. The HMBC spectrum showed long-range couplings of C-3 with H-2 and H-4. Thus, compound **14** was identified as 3-oxo-12α-hydroxyl resibufogenin. It could be considered as an intermediate in the epimerization from 12α-hydroxyl resibufogenin (**10**) to **11**. For compound **12**, a quaternary carbon appeared at δ 73.5, C-10 shifted downfield to δ 40.0, and C-19 upfield to δ 16.5, suggesting the hydroxylation at C-5. Both 3-OH (δ 5.19) and 5-OH (δ 4.79) shifted remarkably downfield due to intra-molecular hydrogen bonding effect. Thus, the structure of **12** should be 5,12α-dihydroxyl resibufogenin. The NMR spectra of **13** was very similar to that of **12**, with C-10 shifted downfield to δ 40.0, C-19 upfield to δ 18.4, and 3-OH downfield to δ 5.17.

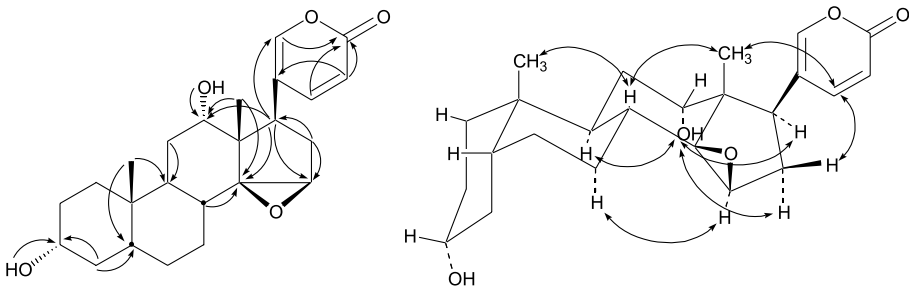

Figure 2. Key HMBC (left) and NOESY (right) correlations of compound **11**.

However, the additional oxygenated signal at δ 71.8 was assigned as a methine carbon by the DEPT experiment, and suggested the hydroxylation at C-1β. Compound **13** was thus identified as 1β,12α-dihydroxyl resibufogenin. In the ^{13}C NMR spectrum of **15**, a carbonyl signal appeared at δ 210.5. It showed HMBC correlations with H-18 (δ 0.90) and H-17 (δ 3.80), which allowed its assignment to C-12. In accordance, C-11 (δ 37.3) and C-13 (δ 59.8) were shifted downfield remarkably by 16.7 and 15.2 ppm, respectively, when compared to resibufogenin. Thus, compound **15** was identified as 12-oxo-resibufogenin. It could be considered as the transformation intermediate from **7** to **10**.

Compounds **16**–**22** were characterized as 16α-hydroxylated derivatives of resibufogenin. In the ^{13}C NMR spectrum of compound **17**, a new oxygenated methine signal appeared at δ 75.6, and C-17 shifted downfield to δ 58.3, suggesting the presence of 16-OH. It was confirmed by the HMBC correlations of H-16 (δ 4.01) with C-20 (δ 120.2) and C-13 (δ 43.9). The signal of H-17 appeared as a singlet, consistent with the α-configuration of 16-OH. In the NOESY spectrum, the NOE enhancements of H-16β (δ 4.01) with H-21 (δ 7.55) and H-22 (δ 7.60) were observed. The 3α-OH of **17** was established by the broad peak of H-3 (δ 3.39). Thus, compound **17** was identified as 3-*epi*-16α-hydroxyl resibufogenin. In the ^{13}C NMR spectrum of **18**, a carbonyl signal appeared at δ 211.5, and C-2 (δ 36.7) and C-4 (δ 41.6) resonated at relatively lower fields. Its structure was established as 3-oxo-16α-hydroxyl resibufogenin. Compounds **19**–**22** were dihydroxylated derivatives of resibufogenin, giving $[M+H]^+$ ions at *m/z* 417. Their structures were identified by comparing with structurally related compounds and confirmed by 2D NMR spectra. The additional carbon signal of **19** at δ 66.2 showed long-range correlation with H-8 (δ 1.88), and its corresponding proton signal at δ 3.55 appeared as a very broad peak, suggesting the hydroxylation at C-7β. Thus, compound **19** was identified as 7β,16α-dihydroxyl resibufogenin. The signal of C-18 in compound **20** was shifted upfield to δ 11.7, and C-13 shifted downfield to δ 50.0, suggesting the hydroxylation at C-12β, and allowed the identification of compound **20** as 12β,16α-dihydroxyl resibufogenin. In the ^{13}C NMR spectrum of **21**, an additional methine signal appeared at δ 71.8, the signal of C-10 was shifted downfield to δ 39.5 and C-19 upfield to δ 18.7, suggesting that C-1 was hydroxylated. The ^{1}H–^{1}H COSY spectrum showed a W-type long-range coupling of H-1 (δ 3.62) with H-3 (δ 4.01), suggesting the β-configuration of 1-OH. Both 1-OH (δ 4.82) and 3-OH (δ 5.18) were resonated at much lower fields due to intra-molecular hydrogen bonding, and each showed NOE enhancements with H-5 (δ 1.93). In addition, the signal of C-17 was shifted downfield to δ 58.3, suggesting the presence of 16-OH. The NOESY spectrum showed NOE enhancements of H-16 (δ 3.99) with H-22 (δ 7.60), and 16-OH (δ 5.45) with H-17 (δ 2.42), and established the α-configuration of 16-OH. In accordance, the signal of H-17 appeared as a sharp singlet. Thus, compound **21** was identified as 1β,16α-dihydroxyl resibufogenin. The C-13 signal of **22** was shifted downfield to δ 48.0 and C-18 appeared at δ 17.5, suggesting the hydroxylation at C-12α. In addition, downfielded C-17 (δ 53.8) and a sharp singlet for H-17 (δ 2.89) suggested the presence of 16α-OH. They were confirmed by the HMBC correlations of H-18 (δ 0.64) with C-12 (δ 73.1), and H-16 (δ 3.87) with C-20 (δ 120.2), respectively. Thus, compound **22** was identified as 12α,16α-dihydroxyl resibufogenin.

Most of the biotransformed products of resibufogenin by *M. polymorphosporus* were mono- or dihydroxylated derivatives at various positions, including C-1β, C-5, C-7α, C-7β, C-12α, C-12β and C-16α. The epimerization and dehydrogenation at C-3 were also observed. Hydroxylations at C-12α, C-12β and C-16α were found to be the major reactions, each giving products in $>5\%$ isolated yields, while the other products were only obtained in fairly low yields. Previously, we had reported the biotransformation of bufalin by *M. spinosus*, where the major reactions involved hydroxylations at C-7β, C-12β and C-16α, and 7β-hydroxylated products constituted more than 50% of the total metabolites.[7] Since the structures of resibufogenin and bufalin only differed in the 14β,15β-epoxy ring, this ring appeared to inhibit 7β-hydroxylation of bufadienolides due to its close spatial position with C-7. This assumption could be evidenced by our latest results from the substrate specificity investigation of bufadienolide 12β-hydroxylation by *Alternaria alternata*.[19]

For natural bufadienolides, hydroxyl substitutions usually occur at C-5, C-11α or C-12β, and bufadienolides with a hydroxyl group at C-1β, C-7α, C-7β or C-16α obtained in this study are rarely seen.[20] Our result obviously indicated the great potential of biotransformation in the synthesis of natural product-derived new compounds.

Mucor species are of the most frequently used microbial catalysts, and have been reported to catalyze the hydroxylation of a variety of natural products, including steroids, diterpenes and sesquiterpenes, leading to arrays of hydroxylated products in most of the reactions.[21–27] The potent hydroxylation capabilities of *Mucor* species should

Table 6. Cytotoxic activities of the biotransformed products against human cancer cell lines ($n=3$)

Compound	IC_{50} (μmol/L)		
	Bel-7402	BGC-823	HeLa
Taxol	3.4×10^{-1}	1.0	1.5×10^{-2}
1	1.3×10^{-1}	1.1×10^{-1}	1.0×10^{-2}
2	2.7	2.2	5.0×10^{-1}
3	28.7	14.0	3.9
4	4.3	10.8	1.4
6	4.9	3.7	2.1
7	3.5	3.9	8.0×10^{-1}
8	45.9	33.4	37.0
9	16.8	17.8	7.2
10	14.4	8.2	3.4
11	35.7	57.0	19.1
12	97.3	53.8	78.4
13	41.8	30.5	5.8
14	17.3	30.5	4.2
15	5.4	19.0	4.6
16	>100	>100	44.6
17	38.1	>100	34.7
19	>100	>100	55.4
20	25.2	10.0	6.7
21	18.5	20.3	2.9
22	14.4	4.3	4.5
23	5.8	5.6	3.6

be due to their cytochrome P450 enzyme systems.[1,2] Obviously, there could be a family of monohydroxylases responsible for the hydroxylation reactions. Their properties warrant further investigation so that the biotransformation process could be monitored more directly and efficiently.

The cytotoxic activities of the biotransformed products were evaluated by the MTT method (Table 6). The results were in agreement with the structure-activity relationships previously proposed by Ye et al. and Kamano et al.[7,28,29] Resibufogenin showed strong inhibitory activities against human hepatoma Bel-7402 cells, human gastric cancer BGC-823 cells and human cervical carcinoma HeLa cells, with IC_{50} values of 0.13, 0.11, and 0.01 μmol/L, respectively. All the products showed less potent activities. The 7β- or 12β-hydroxylations reduced the activities by less than 10 folds, and the resultant products still showed significant cytotoxic effects. However, the epimerization or dehydrogenation of 3-OH, and 12α-hydroxylation remarkably reduced the activities. All the 16α-hydroxylated products showed very weak cytotoxic activities, with IC_{50} values >10 μmol/L. These results provided guidance for the future directed synthesis of bufadienolides of pharmaceutical interest.

3. Experimental

3.1. General

Melting points were determined with an XT4A apparatus (uncorrected). Optical rotations were measured with a Perkin-Elmer 243B polarimeter. UV spectra were detected with a TU-1901 UV–vis spectrophotometer. IR spectra were recorded in KBr with an Avatar 360 FT-IR spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX-500 spectrometer (500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR) in DMSO-d_6 at ambient temperature with tetramethylsilane (TMS) as the internal standard. The chemical shifts (δ values) were given in parts per million (ppm) relative to TMS at 0 ppm. The coupling constants (J values) were reported in hertz (Hz). Mass spectra were measured on a Finnigan LCQ Advantage mass spectrometer equipped with an APCI source. High-resolution mass spectra (HR-MS) were obtained on a Bruker APEX II Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer.

3.2. Chemicals

Resibufogenin (**1**) was isolated from the Chinese drug ChanSu, and unambiguously identified by NMR and MS techniques. The purity was determined to be >99.5% by HPLC analysis. Chromatorex ODS (100–200 mesh) for column chromatography was purchased from Fuji Silysia Chemical Ltd., Japan. All chemical solvents used for products isolation were of analytical grade or higher.

3.3. Microorganism and culture media

The fungal strain of *Mucor polymorphosporus* AS 3.3443 was purchased from China General Microbiological Culture Collection Center (Beijing, China) and maintained on potato agar slants at 4 °C. Fermentations of fungi were carried out in a potato medium consisting of 20 g of potato extract, 20 g of glucose, and 1000 mL of distilled H_2O. The media were sterilized in an autoclave at 121 °C and 1.06 kg/cm^2 for 30 min.

3.4. Preparative HPLC conditions

For the isolation of biotransformation products, a Spectra-SERIES HPLC apparatus (Thermo Quest) with a 100 μL loop was used. Samples were separated on a YMC ODS-A column (5 μm, ∅10×250 mm). The flow rate was 2.0 mL/min, and the detection wavelength was 296 nm.

3.5. Biotransformation procedure

Mycelia of *M. polymorphosporus* from agar slants were aseptically transferred to 250 mL Erlenmeyer flasks containing 80 mL of liquid potato medium. The fungus was incubated at 25 °C on a rotary shaker (180 rpm) in the dark for 24 h to make a stock inoculum. An amount of 8 mL of the inoculum was then added to each of the 40 1-L flasks containing 350 mL of potato medium. After 24 h incubation, a total amount of 800 mg of resibufogenin dissolved in 40 mL of ethanol was distributed equally among the 40 flasks. The incubation was allowed to continue for additional 6 days on the shaker. The cultures were then pooled and filtered in vacuo. The filtrate was extracted with 10 L of ethyl acetate for three times. The organic extract was evaporated to dryness in a rotary evaporator under reduced pressure at 60 °C to yield 2.7 g of a brownish solid.

3.6. Isolation and purification of biotransformation products

The sample was separated on an ODS open column (∅3 cm by 30 cm) and eluted with MeOH–H_2O (10:90–80:20, v/v) to afford five fractions (I–V). Fraction I was subjected to preparative HPLC and eluted with MeOH–H_2O (45:55, v/v) to yield **9** (7.5 mg). Fraction II was separated by preparative HPLC and eluted with MeOH–H_2O (47.5:52.5, v/v) to give **12** (28.9 mg), **19** (8.4 mg), and **20** (25.8 mg). Fraction III was subjected to preparative HPLC and eluted with MeOH–H_2O (50:50, v/v) to yield **5** (1.6 mg), **7** (48.0 mg), and **13** (14.0 mg). Fraction IV was separated by preparative HPLC and eluted with MeOH–H_2O (58:42, v/v) to yield **2** (10.7 mg), **3** (3.4 mg), **4** (2.1 mg), **6** (2.0 mg), **8** (43.7 mg), **10** (39.7 mg), **11** (5.9 mg), **14** (3.0 mg), **15** (2.8 mg), **16** (58.6 mg), **21** (3.2 mg), **22** (3.8 mg), and **23** (2.8 mg). Fraction V was subjected to preparative HPLC and eluted with MeOH–H_2O (75:25, v/v) to yield **17** (7.1 mg) and **18** (1.0 mg). Purities of the above products were >95%, determined by HPLC-UV means.

3.6.1. 3-*epi*-7β-Hydroxyl resibufogenin (3). White powder; $C_{24}H_{32}O_5$; mp 249–251 °C; $[\alpha]_D^{25}$ +5.2 (*c* 0.2, MeOH); UVλ_{max}(MeOH): 204.0, 298.0 nm; APCI-MS (*m/z*): 401 $[M+H]^+$; HR-FT-ICRMS *m/z* calcd for $C_{24}H_{33}O_5$ $[M+H]^+$, 401.2328, found 401.2319; ^{13}C and ^1H NMR data, see Tables 1 and 3.

3.6.2. 5,7β-Dihydroxyl resibufogenin (5). White powder; $C_{24}H_{32}O_6$; mp 224–227 °C; $[\alpha]_D^{25}$ +32.1 (*c* 0.1, MeOH); UVλ_{max}(MeOH): 206.0, 299.0 nm; APCI-MS (*m/z*): 417 $[M+H]^+$; ^{13}C and ^1H NMR data, see Tables 1 and 3.

3.6.3. 7α-Hydroxyl resibufogenin (6). White powder; $C_{24}H_{32}O_5$; mp 138–140 °C; $[\alpha]_D^{25}$ +11.7 (*c* 0.1, MeOH); UVλ_{max}(MeOH): 204.0, 299.0 nm; IRν_{max}(KBr): 3437, 2928, 1713, 1634, 1538, 1453, 1125, 1037 cm^{-1}; APCI-MS (*m/z*): 401 $[M+H]^+$; HR-FT-ICRMS *m/z* calcd for $C_{24}H_{33}O_5$ $[M+H]^+$, 401.2328, found 401.2322; ^{13}C and ^1H NMR data, see Tables 1 and 3.

3.6.4. 3-*epi*-12β-Hydroxyl resibufogenin (8). White powder; $C_{24}H_{32}O_5$; mp 233–235 °C; $[\alpha]_D^{25}$ +12.1 (*c* 1.0, MeOH); UVλ_{max}(MeOH): 206.0, 299.0 nm; IRν_{max}(KBr): 3379, 2934, 2868, 1705, 1632, 1538, 1454, 1130, 1033 cm^{-1}; APCI-MS (*m/z*): 401 $[M+H]^+$; HR-FT-ICRMS *m/z* calcd for $C_{24}H_{33}O_5$ $[M+H]^+$, 401.2328, found 401.2322; ^{13}C and ^1H NMR data, see Tables 1 and 3.

3.6.5. 5,12β-Dihydroxyl resibufogenin (9). White powder; $C_{24}H_{32}O_6$; mp 149–152 °C; $[\alpha]_D^{25}$ +7.9 (*c* 0.4, MeOH); UVλ_{max}(MeOH): 205.0, 297.0 nm; IRν_{max}(KBr): 3408, 2938, 1712, 1631, 1537, 1452, 1040 cm^{-1}; APCI-MS (*m/z*): 417 $[M+H]^+$; HR-FT-ICRMS *m/z* calcd for $C_{24}H_{33}O_6$ $[M+H]^+$, 417.2276, found 417.2268; ^{13}C and ^1H NMR data, see Tables 1 and 3.

3.6.6. 3-*epi*-12α-Hydroxyl resibufogenin (11). White powder; $C_{24}H_{32}O_5$; mp 139–140 °C; $[\alpha]_D^{25}$ +3.7 (*c* 0.4, MeOH); UVλ_{max}(MeOH): 204.0, 300.0 nm; IRν_{max}(KBr): 3427, 2936, 2867, 1712, 1631, 1537, 1453, 1127, 1046 cm^{-1}; APCI-MS (*m/z*): 401 $[M+H]^+$; HR-FT-ICRMS *m/z* calcd for $C_{24}H_{33}O_5$ $[M+H]^+$, 401.2328, found 401.2323; ^{13}C and ^1H NMR data, see Tables 1 and 4.

3.6.7. 5,12α-Dihydroxyl resibufogenin (12). White powder; $C_{24}H_{32}O_6$; mp 146–148 °C; $[\alpha]_D^{25}$ +23.4 (*c* 1.2, MeOH); UVλ_{max}(MeOH): 204.0, 300.0 nm; IRν_{max}(KBr): 3420, 2936, 1712, 1632, 1538, 1452, 1377, 1223, 1128, 1040 cm^{-1}; APCI-MS (*m/z*): 417 $[M+H]^+$; HR-FT-ICRMS *m/z* calcd for $C_{24}H_{33}O_6$ $[M+H]^+$, 417.2276, found 417.2268; ^{13}C and ^1H NMR data, see Tables 1 and 4.

3.6.8. 1β,12α-Dihydroxyl resibufogenin (13). White powder; $C_{24}H_{32}O_6$; mp 164–166 °C; $[\alpha]_D^{25}$ +5.2 (*c* 0.6, MeOH); UVλ_{max}(MeOH): 205.0, 299.0 nm; IRν_{max}(KBr): 3410, 2935, 1714, 1633, 1537, 1451, 1377, 1228, 1134, 1051 cm^{-1}; APCI-MS (*m/z*): 417 $[M+H]^+$; HR-FT-ICRMS *m/z* calcd for $C_{24}H_{33}O_6$ $[M+H]^+$, 417.2276, found 417.2267; ^{13}C and ^1H NMR data, see Tables 2 and 4.

3.6.9. 3-Oxo-12α-hydroxyl resibufogenin (14). White powder; $C_{24}H_{30}O_5$; mp 131–133 °C; $[\alpha]_D^{25}$ +6.2 (*c* 0.2, MeOH); UVλ_{max}(MeOH): 206.0, 298.0 nm; IRν_{max}(KBr): 3444, 2937, 1707, 1635, 1538, 1454, 1378, 1119, 1051 cm^{-1}; APCI-MS (*m/z*): 399 $[M+H]^+$; HR-FT-ICRMS *m/z* calcd for $C_{24}H_{31}O_5$ $[M+H]^+$, 399.2171, found 399.2164; ^{13}C and ^1H NMR data, see Tables 2 and 4.

3.6.10. 12-Oxo-resibufogenin (15). White powder; $C_{24}H_{30}O_5$; mp 209–210 °C; $[\alpha]_D^{25}$ +35.1 (*c* 0.2, MeOH); UVλ_{max}(MeOH): 204.0, 298.0 nm; IRν_{max}(KBr): 3492, 2930, 1713, 1539, 1453, 1373, 1126, 1038 cm^{-1}; APCI-MS (*m/z*): 399 $[M+H]^+$; HR-FT-ICRMS *m/z* calcd for $C_{24}H_{31}O_5$ $[M+H]^+$, 399.2171, found 399.2163; ^{13}C and ^1H NMR data, see Tables 2 and 4.

3.6.11. 3-*epi*-16α-Hydroxyl resibufogenin (17). White powder; $C_{24}H_{32}O_5$; mp 140–142 °C; $[\alpha]_D^{25}$ −24.1 (*c* 0.4, MeOH); UVλ_{max}(MeOH): 205.0, 299.0 nm; IRν_{max}(KBr): 3417, 2934, 2867, 1712, 1631, 1536, 1454, 1374, 1241, 1128, 1040 cm^{-1}; APCI-MS (*m/z*): 401 $[M+H]^+$; HR-FT-ICRMS *m/z* calcd for $C_{24}H_{33}O_5$ $[M+H]^+$, 401.2328, found 401.2321; ^{13}C and ^1H NMR data, see Tables 2 and 5.

3.6.12. 7β,16α-Dihydroxyl resibufogenin (19). White powder; $C_{24}H_{32}O_6$; mp 127–130 °C; $[\alpha]_D^{25}$ −33.4 (*c* 0.5,

MeOH); UVλ_{max}(MeOH): 206.0, 298.0 nm; IRν_{max}(KBr): 3492, 3284, 2932, 1708, 1630, 1539, 1453, 1261, 1141, 1036 cm^{-1}; APCI-MS (*m/z*): 417 $[M+H]^+$; HR-FT-ICRMS *m/z* calcd for $C_{24}H_{33}O_6$ $[M+H]^+$, 417.2276, found 417.2265; ^{13}C and ^1H NMR data, see Tables 2 and 5.

3.6.13. 12β,16α-Dihydroxyl resibufogenin (20). White powder; $C_{24}H_{32}O_6$; mp 159–160 °C; $[\alpha]_D^{25}$ −3.2 (*c* 1.1, MeOH); UVλ_{max}(MeOH): 203.0, 299.0 nm; IRν_{max}(KBr): 3419, 2936, 2879, 1711, 1632, 1537, 1453, 1237, 1134, 1035 cm^{-1}; APCI-MS (*m/z*): 417 $[M+H]^+$; HR-FT-ICRMS *m/z* calcd for $C_{24}H_{33}O_6$ $[M+H]^+$, 417.2276, found 417.2267; ^{13}C and ^1H NMR data, see Tables 2 and 5.

3.6.14. 1β,16α-Dihydroxyl resibufogenin (21). White powder; $C_{24}H_{32}O_6$; mp 188–190 °C; $[\alpha]_D^{25}$ −20.7 (*c* 0.2, MeOH); UVλ_{max}(MeOH): 205.0, 296.0 nm; APCI-MS (*m/z*): 417 $[M+H]^+$; HR-FT-ICRMS *m/z* calcd for $C_{24}H_{33}O_6$ $[M+H]^+$, 417.2276, found 417.2269; ^{13}C and ^1H NMR data, see Tables 2 and 5.

3.6.15. 12α,16α-Dihydroxyl resibufogenin (22). White powder; $C_{24}H_{32}O_6$; mp 136–140 °C; $[\alpha]_D^{25}$ −42.1 (*c* 0.2, MeOH); UVλ_{max}(MeOH): 205.0, 298.0 nm; IRν_{max}(KBr): 3400, 2934, 1714, 1631, 1536, 1452, 1237, 1132, 1034 cm^{-1}; APCI-MS (*m/z*): 417 $[M+H]^+$; HR-FT-ICRMS *m/z* calcd for $C_{24}H_{33}O_6$ $[M+H]^+$, 417.2276, found 417.2269; ^{13}C and ^1H NMR data, see Tables 2 and 5.

3.7. Bioassay

Human hepatoma Bel-7402 cells, human gastric cancer BGC-823 cells and human cervical carcinoma HeLa cells were maintained in RPMI 1640 medium (GIBCO/BRL, Maryland, USA) supplemented with 10% (v/v) fetal bovine serum and cultured in 96-well microtiter plates for the assay. Appropriate dilutions (10^{-3}–10^2 μmol/L) of the test compounds were added to the cultures. After incubation at 37 °C, 5% CO_2 for 72 h, the survival rates of the cancer cells were evaluated by the MTT method.[30] The activity was shown as the IC_{50} value, which is the concentration (μmol/L) of test compound to give 50% inhibition of cell growth. Results were expressed as the mean value of triplicate determinations. Taxol™ was selected as the positive control.

Acknowledgements

We thank the National Outstanding Youth Foundation by NSF of China (Grant No. 39925040) and Trans-Century Training Program Foundation for the Talents by the Ministry of Education for financial support.

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Transformations of lignans. Part 10: Acid-catalysed rearrangements of arboreol and wodeshiol and conversion of gmelanone oxime into a dihydropyranone derivative

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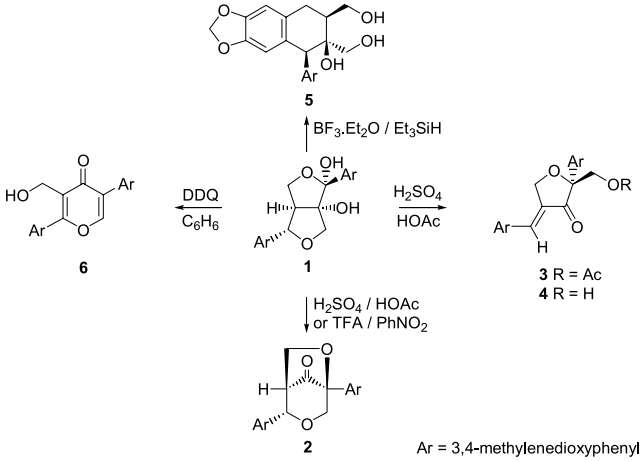
Received 8 April 2005; revised 14 June 2005; accepted 30 June 2005

Abstract—The synthesis of gmelanone **2** by a pinacol-type rearrangement of arboreol **1** supports its biogenesis and confirms its relative and absolute configuration. The further transformation of gmelanone oxime **12** into the dihydropyranone oxime **13** supports the intermediacy of gmelanone like intermediates in the rearrangements of furofuran lignans to pyran derivatives. In contrast, acid-catalysed rearrangement of wodeshiol **7** affords the dihydropyranone **8**.

1. Introduction

As part of our studies of lignan transformations,[1–9] we reported that when arboreol **1**, a diol isolated from *Gmelina arborea*, was treated with H_2SO_4 and acetic acid (1:1) in ethyl acetate it produced gmelanone **2** but, when treated with H_2SO_4 in acetic acid (1:10), it gave the acetoxyfuranone **3**, which is an acetylated cleavage product of gmelanone.[1] Furthermore, the synthetic sample of **2** was identical with natural gmelanone, thus confirming the configuration of the natural product.[10]

Since **1** and **2** are both acid-sensitive, the selection of an acid catalyst for the rearrangement of arboreol to gmelanone is important. Thus, treatment of **1** with methanolic HCl in acetone resulted only in epimerisation, while treatment with H_2SO_4 in acetic acid gave the acetoxyfuranone **3**. Treatment with BF_3–etherate in dichloromethane in the presence of triethylsilane resulted in formation of the aryltetralin **5** (Scheme 1). Furthermore, treatment of **1** with DDQ in benzene or dichloromethane produced the pyrone **6**, presumably via a gmelanone like intermediate (see below).



Scheme 1.

Keywords: Lignans; Pinacol rearrangement; Biomimetic synthesis; Oxime rearrangement.

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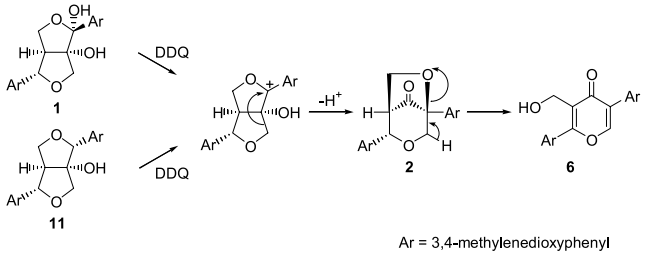
0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2005.06.106

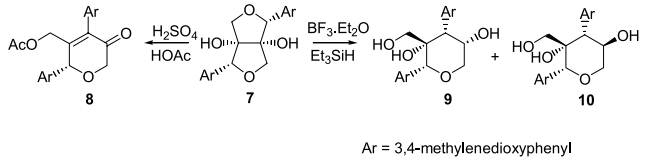
The present investigation has further revealed that when arboreol **1** is refluxed with trifluoroacetic acid in solvents such as benzene, toluene and anisole, gmelanone **2** is obtained in moderate yields (30–40%), along with unreacted arboreol. However, in nitrobenzene gmelanone is produced in 80% yield.

Although it has long been assumed[11,12] that rearrangement of **1** and other furofuran lignans such as paulownin **11** with DDQ in benzene or dichloromethane proceeds through a gmelanone like intermediate to form the pyrone **6** (Scheme 2), no conclusive evidence for this pathway has been provided. Gmelanone **2** was not isolated as an intermediate during the reaction of **1** with DDQ nor was gmelanone itself converted into a pyrone derivative. In the present investigation, although gmelanone could not be converted into a pyran derivative by the action of DDQ, it was observed that when gmelanone oxime **12** was treated with *p*-toluenesulphonyl chloride in pyridine, 3,6-bis-(3′,4′-methylenedioxyphenyl)-5-hydroxymethyl-5,6-dihydropyranone oxime **13** was obtained in 80% yield. This finding supports the view that a gmelanone like intermediate is involved in the transformation of arboreol and paulownin to pyrone derivatives.



Scheme 2.

In contrast, wodeshiol **7**, another lignan diol isolated from *Cleistanthus collinus*, did not undergo a pinacol rearrangement with trifluoroacetic acid in benzene, but on treatment with H_2SO_4 in acetic acid it produced a compound that was originally assumed to be a 1,2-dehydropaulownin derivative.[1] Further investigation has shown that this product is in fact the dihydropyrone **8**, closely related in structure to the epimeric pyran derivatives **9** and **10** produced by treatment of **7** with BF_3–etherate and triethylsilane in dichloromethane (Scheme 3).[4,12]



Scheme 3.

2. Results and discussions

2.1. Synthesis of gmelanone 2 and gmelanone oxime 12

With a view to improving the yield of gmelanone **2**, several experiments were carried out on arboreol **1** under different reaction conditions. These involved the use of H_2SO_4 in formic acid, BF_3–etherate in THF, $POCl_3$ in pyridine, and TFA in benzene, toluene, anisole and nitrobenzene. The combination of trifluoroacetic acid and nitrobenzene was found to be the optimal system giving gmelanone in 80% yield.

Thus treatment of arboreol **1** in nitrobenzene with trifluoroacetic acid under reflux yielded a major product, along with remaining starting material. This product was a crystalline solid, mp 202 °C, which had a molecular ion at *m/z* 368 in its mass spectrum and showed a band at 1765 cm^{-1} in its IR spectrum. Its ^{13}C NMR spectrum showed a quaternary carbon signal at δ 204.5 ppm. Based on its ^1H and ^{13}C NMR spectra (Tables 1 and 2), and by comparison with the literature data,[10] this product was identified as **2** and its structure was confirmed by X-ray analysis (Fig. 1). From consideration of the X-ray structure of arboreol (Fig. 2) it appears that the C_1–C_8 bond migrates from the *endo* side to the stabilized carbocation formed by the dissociation of protonated arboreol (Scheme 4).

Table 1. ^1H NMR of gmelanone **2** and its oxime **12**

	2	**12**
H-3	4.49dd (4.9, 7.9), 4.74d (7.9)	4.28m, 4.60d (6.6)
H-4	3.40dd (3.0, 4.9)	4.28m
H-5	5.40d (3.0)	5.19br s
H-7	3.93d (11.5), 4.10d (11.5)	3.85d (11.0), 4.16d (11.0)
OCH_2O	6.00s, 5.94 ABq (1.4)	5.99s, 5.95 ABq (1.4)
Arom.	6.75–7.02m	6.77–7.14m

All spectra run in $CDCl_3$, coupling constant (Hz) in brackets.

Table 2. ^{13}C NMR of gmelanone **2** and its oxime **12**

	2	**12**
C-1	81.2	80.2
C-3	67.3	69.4
C-4	49.7	39.9
C-5	85.0	81.4
C-7	76.9	73.5
C-8	204.5	158.7
C-1′	130.4	131.8
C-1″	129.1	129.1
C-2′	108.4	108.1
C-2″	108.1	108.0
C-3′	148.2	147.6
C-3″	147.6	147.3
C-4′	147.4	
C-4″	147.4	
C-5′	106.8	107.0
C-5″	106.7	107.0
C-6′	119.9	119.7
C-6″	119.3	119.3
OCH_2O	101.2	101.1
	101.1	

All spectra run in $CDCl_3$.

Treatment of gmelanone **2** with one equivalent of hydroxylamine hydrochloride and sodium carbonate in ethanol under reflux yielded gmelanone oxime **12** as a crystalline solid (Scheme 5). This product had molecular formula $C_{20}H_{17}O_7N$ and its IR spectrum contained bands at 3501 (OH), and 1630 (C=N) cm^{-1}. The ^1H NMR spectrum of **12** displayed a similar pattern to that of **2** except for changes due to the effect of the oximino function. Thus, the

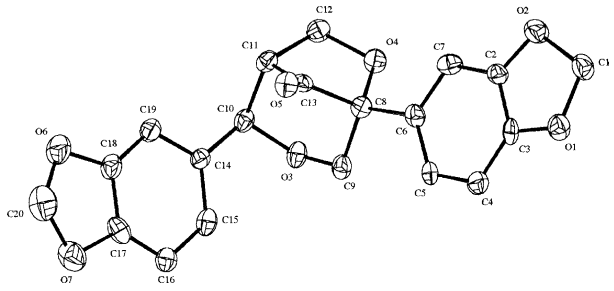


Figure 1.

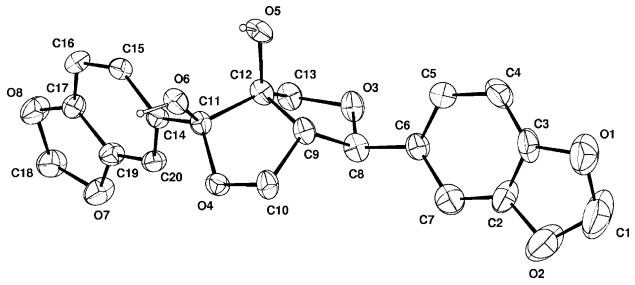
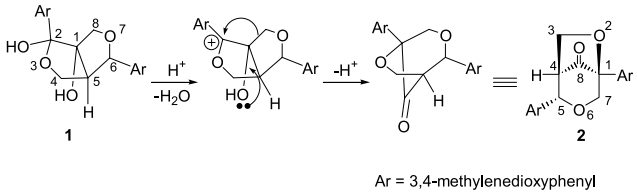
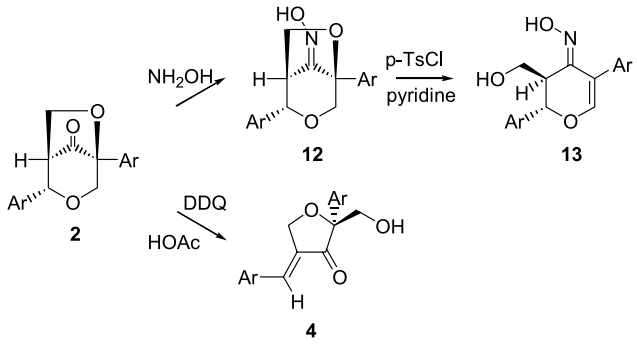


Figure 2.



Scheme 4.



Scheme 5.

proton at C-4, which appeared as a double doublet at δ 3.40 in **2**, moved downfield and appeared as a multiplet at δ 4.28 in **12**, due to the anisotropic effect of the oximino function. The benzylic proton at C-5, which appeared as a doublet at δ 5.40 in **2**, moved slightly upfield and appeared as a broad singlet at δ 5.19 in **12**, due to the shielding effect of the axial aryl group at C-5. Its structure was further confirmed by X-ray analysis (Fig. 3).

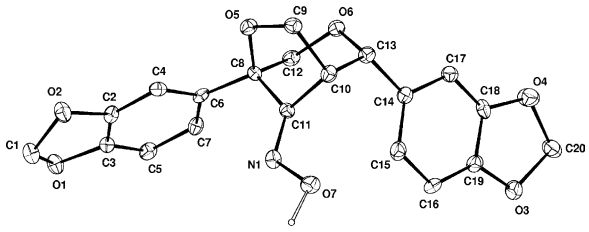


Figure 3.

2.2. Reactions of gmelanone 2

In order to test whether a gmelanone like intermediate was involved in the rearrangement of furofuran lignans such as arboreol **1** and paulownin **11** to form 4-pyrone derivatives, gmelanone **2** was treated with one equivalent of DDQ in benzene to see whether any pyrone derivative could be obtained. Unfortunately, a pyrone derivative was not obtained, but instead an intractable gum was produced. On the other hand, when **2** was treated with one equivalent of DDQ in acetic acid at room temperature, a major product **4** was obtained (Scheme 5). It had molecular formula $C_{20}H_{16}O_7$ and its IR spectrum indicated the presence of a hydroxyl group (3460 cm^{-1}) and a five membered ketone (1748 cm^{-1}). Its ^1H NMR spectrum (Table 3) differed significantly from that of **2**. Besides the two methylenedioxy groups at δ 6.05 and 5.96 and aromatic protons between δ 6.75 and 7.10, the spectrum contained a singlet at δ 7.40, assigned to the β-hydrogen of an α,β-unsaturated ketone. Further, the ^1H NMR spectrum of **4** contained signals due to two methylene protons as double doublets at δ 5.07 (J= 14.0, 2.6 Hz) and 5.21 (J=14.0, 2.4 Hz), due to the furanone portion of **4**, and two methylene protons as doublets at δ 3.76 and 4.11, assigned to the CH_2OH group.

Table 3. ^1H NMR of **4** and **3**

	4	**3**
H-5	5.21dd (2.4, 14.0), 5.07dd (2.6, 14.0)	4.46dd (3.0, 14.0), 5.06dd (3.0, 14.0)
H-6	4.11d (11.9), 3.76d (11.9)	5.38d (12.0), 5.22 d (12.0)
H-7	7.40t (2.5)	7.5t (3.0)
Arom.	6.75–7.10m	6.8–7.2m
OCH_2O	6.05s, 5.96 ABq (1.3)	6.03s, 5.99s
OAc	—	2.00s

All spectra run in $CDCl_3$, coupling constant (Hz) in brackets.

Compound **4** gave a monoacetate on treatment with acetic anhydride in pyridine. The ^1H NMR spectrum of the acetate showed that the two methylene protons of the CH_2OH group had moved downfield to δ 5.22 and 5.38, thus confirming that the hydroxyl group of **4** is primary in nature. The ^{13}C NMR spectrum of the product **4** (Table 4) contained a signal at δ 201.2 (C=O) and two signals due to benzylidene carbons at δ 134.7 and 127.3, in addition to signals due to oxymethylene carbons at δ 68.0 and 67.7 ppm. By comparison of the spectra of **4** and its acetate with the acetoxyfuranone **3** formed directly by reaction of arboreol with H_2SO_4–HOAc,[1] compound **3** was identified as the acetate of **4**. The formation of compound **4** in the above reaction shows that under acidic conditions, gmelanone **2** undergoes a simple cleavage of the pyran ring system.

Table 4. ^{13}C NMR of **4** and **3**

	4		**3**	
C-2	86.8		84.5	
C-3	201.2		199.9	
C-4	127.3		127.3	
C-5	68.0		68.0	
C-6	67.7		67.7	
C-7	134.7		134.8	
OCH_2O	101.8		101.9	
	101.2		101.3	
OAc	—		174.5	
			20.2	
Arom.	106.1	128.7	108.4	128.6
	108.4	129.5	109.0	129.2
	109.0	147.6	109.7	147.9
	109.6	148.1	119.3	148.2
	119.0	148.5	128.3	148.6
	128.5	149.8	128.4	150.0

All spectra run in $CDCl_3$.

2.3. Reactions of gmelanone oxime 12

When gmelanone oxime **12** was treated with *p*-toluenesulphonyl chloride in pyridine, it produced a crystalline solid **13**, which had molecular formula $C_{20}H_{17}O_7N$. Its IR spectrum showed bands at 3450 (OH) and 1630 (C=N) cm^{-1}. The ^{1}H NMR spectrum of **13** (Table 5) contained a highly deshielded olefinic proton as a singlet at δ 6.66, which may be assigned to H-6 and a benzylic proton as a broad singlet at δ 5.52 (H-2). Another proton that appeared as a double doublet at δ 4.10 (J=6.4, 8.3 Hz) is assigned to H-3. The ^{1}H NMR spectrum also showed two methylene protons as double doublets at δ 3.99 (J=10.4, 6.4 Hz) and 3.94 (J=10.4, 8.3 Hz) indicating the presence of a hydroxymethyl group. Its ^{13}C NMR spectrum (Table 6) showed the absence of the quaternary carbon at δ 80.2 and the bridgehead carbon at δ 39.9, which were present in **12**, but instead showed two olefinic carbons at δ 146.4 and 114.2 in addition to the oximino carbon at δ 147.9 ppm, indicating that **12** had not undergone a Beckmann rearrangement but had instead suffered cleavage of the five-membered ring to give the dihydropyranone oxime **13** (Scheme 5). Structure **13** was confirmed by X-ray analysis (Fig. 4). The formation of the dihydropyranone oxime **13** from **12** provides indirect support for the proposal that a gmelanone like intermediate is involved in the conversion of arboreal and paulownin to pyrone derivatives.

Table 5. ^{1}H NMR of **13** and **6**

	13	**6**[11]
H-2	5.52br s	—
H-3	4.10dd (6.4, 8.3)	—
H-6	6.66s	7.72s
H-7	3.99dd (6.4, 10.4), 3.94dd (8.3, 10.4)	4.64d (7.0)
OCH_2O	5.97s, 5.94s	6.00s, 5.94s
Arom.	6.67–6.91 m	6.74–6.9 m
		7.0–7.6 m
OH	1.6br s	4.11t (7.0)
N–OH	1.2br s	

All spectra run in $CDCl_3$, coupling constant (Hz) in brackets.

Table 6. ^{13}C NMR of **13** and **6**

	13		**6**[11]	
C-2	146.4		148.2	
C-3	114.2		123.4	
C-4 (C=N)	147.9		189.8	
C-5	38.2		120.0	
C-6	75.7		147.8	
C-7	61.6		55.4	
OCH_2O	101.0		101.5	
	101.2		101.5	
Arom.	107.3	127.9	107.8	127.5
	108.1	132.4	108.0	133.1
	108.1	146.9	108.7	148.1
	110.2	147.2	109.1	148.1
	120.0	150.9	121.8	152.1
	122.6		125.6	153.1

All spectra run in $CDCl_3$.

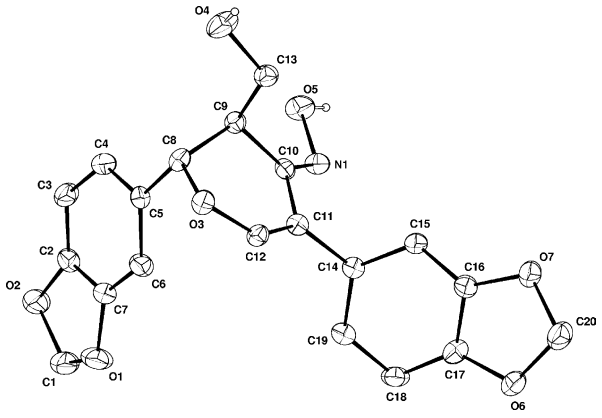


Figure 4.

2.4. Reactions of wodeshiol 7

Treatment of wodeshiol **7** with H_2SO_4 in acetic acid produced a product having molecular formula $C_{22}H_{18}O_8$. Its IR spectrum showed bands at 1730, 1680 and 1600 cm^{-1}. The ^{13}C spectrum (Table 8) confirmed the presence of a carbonyl group with a signal at 193.7 ppm (in addition to an acetate carbonyl at 170.4). The ^{1}H spectrum (Table 7) showed the presence of two isolated methylene groups as pairs of doublets at 4.15 and 4.20 (J=16.8 Hz), and 4.39 and 4.54 (J=13.7 Hz), in addition to the two methylenedioxy groups (at 5.92 and 5.93), and a further singlet at 5.45, in addition to the acetoxy methyl at 1.82 ppm. The ^{13}C NMR confirmed the presence of the two methylene groups at 62.5 and 68.9, the methylenedioxy groups at 101.8 and 101.9, and the acetoxy methyl at 20.9 ppm. In addition it revealed two quaternary carbon atoms at 138.2 and 151.8 assigned to an α,β-unsaturated ketone, and the methine carbon at 77.3 ppm assigned to C-6. The dihydropyranone structure **8** formed by ring expansion of one of the five-membered rings is assigned to this product (Scheme 6).

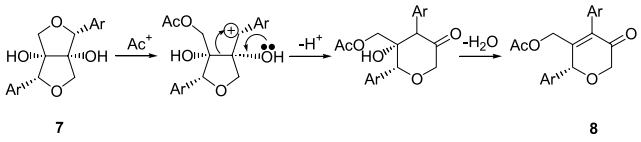
3. Conclusion

The above investigation reports the synthesis of gmelanone **2** from arboreol **1** by an acid-catalysed, pinacol-type rearrangement in the presence of trifluoroacetic acid in nitrobenzene and supports the proposed mechanism for the biogenesis of **2**. The conversion of gmelanone oxime **12** into the dihydropyranone oxime **13** supports the proposed

Table 7. ^{1}H NMR of **8**, **9a** and **10a**

	8	**10a**[4]	**10b**[4]
H-2	5.45s	4.52s	4.56s
H-4	—	3.27d (3.11)	3.15d (11.5)
H-5	—	5.13m	5.60m
H-6	4.15d (16.78), 4.20d (16.78)	4.21dd (1.25, 13.06), 3.94dd (1.47, 13.06)	4.33dd (5.3, 10.7), 3.48 t (10.7)
H-7	4.39d (13.74), 4.54d (13.74)	3.63d (11.15), 3.34d (11.15)	3.51d (11.2), 3.28d (11.2)
OCH_2O	5.92s, 5.93s	5.92s, 5.94s	5.94s, 5.95s
Arom.	6.56–6.81m	6.70–7.07m	6.7–6.9m
OAc	1.82s	2.06s, 2.16s	1.83s, 2.10s

All spectra run in $CDCl_3$, coupling constant (Hz) in brackets.



Scheme 6.

Table 8. ^{13}C NMR of **8**, **9a** and **10a**

	8		**10a**[4]		**10b**[4]	
C-2	77.33		84.81		148.2	
C-3	151.82		74.01		123.4	
C-4	138.21		48.99		189.8	
C-5	193.75		73.34		120.0	
C-6	62.46		71.55		147.8	
C-7	68.95		64.43		55.4	
OAc	20.90		20.94		20.67	
	170.43		21.49		20.76	
			169.58		169.53	
					169.97	
OCH_2O	101.77		101.19		101.01	
	101.94		101.24		101.22	
Arom.	108.84	108.84	107.95	108.36	108.07	108.07
	109.39	110.61	108.68	110.21	108.25	108.25
	122.89	123.86	121.65	123.33	121.03	121.03
	125.40	129.83	130.29	130.38	129.24	129.90
	148.16	148.39	147.21	147.74	147.08	147.83
	148.76	148.90	147.79		147.95	

All spectra run in $CDCl_3$.

mechanism for the rearrangement of furofuran lignans such as arboreol and paulownin into 4-pyrone derivatives through gmelanone like intermediates. In contrast, the acid-catalysed reactions of wodeshiol **7** involve cleavage of one five-membered ring followed by ring expansion, rather than pinacol-type rearrangement.

4. Experimental

4.1. General procedure

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AC 400 instrument at 400 and 100 MHz, respectively. All spectra used tretramethylsilane as internal standard and were run in $CDCl_3$. Mass spectra were recorded either on a VG 12-250 quadrupole instrument or on a VG Micromass Quattro II instrument. Accurate mass measurements were made using either a ZAB-E high-resolution double focussing instrument or a Finnigan Mat 900 instrument. Infra-red spectra were recorded either as a nujol mull or as films on NaCl plates using a Perkin-Elmer Fourier transform 1725X spectrophotometer. Dichloromethane was purified by passing it down on alumina column followed by distillation over calcium hydride. Silica gel-G was used for column chromatography and for TLC. Melting points were recorded on an Electrothermal 9100 melting point apparatus and are uncorrected.

Suitable crystals of **1**, **2**, **12** and **13** were selected for single crystal X-ray diffraction. Cell dimensions and intensity data were recorded at 150K, using either a Bruker Nonius Kappa CCD (**1**, **12**, **13**) or an Enraf Nonius FAST (**2**) equipped with a rotating anode; standard procedures were followed. Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC 217991 (**1**), 241178 (**2**), 241177 (**12**), 217988 (**13**). Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 IEZ, UK (fax: +44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).

4.1.1. Reaction of arboreol 1 with trifluoroacetic acid in nitrobenzene: isolation of gmelanone 2. To a solution of arboreol **1** (0.2 g, 0.5 mmol) in nitrobenzene (10 ml) was added trifluoroacetic acid (0.5 ml) and the mixture was refluxed for 4 h. It was then poured onto crushed ice and, after removal of nitrobenzene by steam distillation, it was extracted with ethyl acetate (3×10 ml). The combined ethyl acetate extracts were washed successively with $NaHCO_3$ solution (3×10 ml) and brine (3×10 ml), then dried ($MgSO_4$) and filtered. After removal of the solvent under reduced pressure a pale yellow residue (0.2 g) was obtained. Column chromatography on silica (eluent: hexane/EtOAc 9:1) yielded gmelanone **2** (0.18 g, 80%) as a gum, which crystallised from benzene as colourless crystals, mp 201–2 °C (lit.[10] 190 °C). $[\alpha]_D^{25}$ −72 (*c* 0.5, $CHCl_3$, lit.[10] −78), *m/z* (EI) 368 (M^+, 1%), 338 (20), 164 (10), 161 (30), 149 (100), 131 (50), 103 (50), *m/z* (CI) 386 ($M+NH_4^+$, 30%), 369 ($M+H^+$, 100), 259 (20), 242 (100), 161 (60), 149 (40). ν_{max} (KBr) 1765 (C=O), 1610 (arom.) and 935 (OCH_2O) cm^{-1}. See Tables 1 and 2 for NMR data.

4.1.2. Reaction of gmelanone 2 with DDQ in acetic acid: isolation of 4. To a solution of **2** (0.2 g. 0.54 mmol) in acetic acid was added DDQ (0.124 g, 0.54 mmol) and the mixture was stirred for 1 h. The reaction mixture was then poured into ice-water and extracted with ethyl acetate (3×10 ml). The combined ethyl acetate extracts were washed successively with sodium metabisulphite solution (3×20 ml), sodium carbonate solution (3×20 ml), and brine (3×20 ml), then dried ($MgSO_4$) and filtered. Removal of the

solvent under reduced pressure gave a light brown residue (0.18 g), which on column chromatography on silica (eluent: hexane/EtOAc 7:3) yielded **4** (0.162 g, 90%) as a gum, *m/z* (EI) 368 (M^+,2%), 338 (40), 216 (10), 203 (35), 188 (20), 160 (95), 149 (100), 135 (40), 121 (30), 102 (75), *m/z* (CI) 386 ($M+NH_4^+$, 40%), 369 ($M+H^+$, 60), 358 (100), 356 (40), 339 (45). λ_{max} 226, 289 nm. ν_{max} 3460 (OH), 1748 (C=O), 1610 (arom.), 930 (OCH_2O) cm^{-1}. [Found: $M+H^+$369.0967, $C_{20}H_{16}O_7$ requires 369.0974]. See Tables 3 and 4 for NMR data.

4.1.3. Preparation of acetate 3 from 4. To a solution of **4** (0.1 g) in dry pyridine (1 ml) was added acetic anhydride (1 ml) with stirring and the mixture was heated under reflux for 1 h. The reaction mixture was then poured into cold water and extracted with ethyl acetate (3×10 ml). The combined ethyl acetate extracts were washed successively with dil HCl (3×20 ml) and brine (3×20 ml), then dried ($MgSO_4$) and filtered. Removal of the solvent under reduced pressure gave a light brown residue (0.1 g), which on column chromatography on silica (eluent: hexane/EtOAc 7:3) yielded the acetate **3**[1] as a gum (0.09 g, 90%), *m/z* (EI) 410 (M^+, 50%). λ_{max} 240, 290 nm. ν_{max}^{KBr} 1760 (C=O), 1615 (arom.), 935 (OCH_2O) cm^{-1}. Anal. Calcd ($C_{22}H_{18}O_8$) C, 64.39; H, 4.42, found C, 64.20; H, 4.39. See Tables 3 and 4 for NMR data.

4.1.4. Reaction of gmelanone 2 with hydroxylamine: isolation of gmelanone oxime 12. To a solution of gmelanone (**2**) (0.2 g, 0.54 mmol) in ethanol was added hydroxylamine hydrochloride (0.04 g, 0.54 mmol) and sodium bicarbonate (50 mg). The mixture was heated under reflux for 4 h and then poured into ice-water and extracted with ethyl acetate (3×10 ml). The combined ethyl acetate extracts were washed successively with dil HCl (3×20 ml) and brine (3×10 ml), then dried ($MgSO_4$) and filtered. Removal of the solvent under reduced pressure gave a light brown residue (0.2 g), which on column chromatography on silica (eluent: hexane/EtOAc 7:3) followed by crystallisation from methanol yielded gmelanone oxime **12** (0.2 g, 96%) as colourless shining crystals, mp 180 °C. $[\alpha]_D^{25}-30$ (*c* 0.02, $CHCl_3$), *m/z* (CI) 401 ($M+NH_4^+$, 2%), 384 ($M+H^+$, 30), 368 (15), 205 (35), 182 (100), 165 (65). λ_{max} 226, 289 nm. ν_{max}^{KBr} 3501 (OH), 2360 (N–H), 1630 (C=N) and 935 (OCH_2O) cm^{-1}. [Found: $M+H^+$384.1083, $C_{20}H_{17}O_7N$ requires 384.1083]. See Tables 1 and 2 for NMR data.

4.1.5. Reaction of gmelanone oxime 12 with *p*-toluenesulphonyl chloride in pyridine: isolation of 3,6-bis-(3,4-methylenedioxyphenyl)-5-hydroxymethyl-5,6-dihydropyranone oxime 13. To a solution of gmelanone oxime **12** (0.2 g, 0.52 mmol) in dry pyridine was added *p*-toluenesulphonyl chloride (0.05 g) and the mixture was refluxed for 2 h. After completion of the reaction, the mixture was poured onto crushed ice, extracted with ethyl acetate (3×10 ml) and combined ethyl acetate extracts were washed successively with dil HCl (3×10 ml) and brine (3×10 ml), then dried ($MgSO_4$) and filtered. After removal of the solvent under reduced pressure a dark brown residue (0.2 g) was obtained. Column chromatography on silica (eluent: hexane/EtOAc 3:7) yielded the dihydropyranone oxime **13** (0.2 g, 100%) as a gum, which crystallised from methanol into shining crystals, mp 122 °C. IR (KBr) 3450 (OH), 1630 (C=N) 1615 (arom.) and 935 (OCH_2O) cm^{-1}. λ_{max} 226, 289, 305 nm, *m/z* (CI) 384 ($M+H^+$, 3%), 356 (45), 279 (15), 236 (30), 220 (50), 203 (65), 148 (100). [Found: $M+H^+$384.1087, $C_{20}H_{17}O_7N$ requires 384.1083]. See Tables 5 and 6 for NMR data.

4.1.6. Action of H_2SO_4 on wodeshiol in glacial acetic acid: isolation of 3-acetoxymethyl-2,4-bis-(3,4-methylene-dioxyphenyl)-2,4-dihydropyranone 8. Wodeshiol **7** (0.2 g, 0.52 mmol) in glacial acetic acid (10 ml) was treated with concd H_2SO_4 (0.1 ml). On warming for 5 min at 90 °C the solution changed from pink to dark red. The mixture was poured into ice-water and extracted with $CHCl_3$ (3×20 ml). The combined extracts were washed with brine (2×10 ml), then dried ($MgSO_4$) and filtered. Removal of the solvent under reduced pressure yielded a red gum, which on chromatography on silica yielded the dihydropyranone **8** (0.18 g, 84%). ν_{max} ($CHCl_3$): 1730, 1680, 1600, 1040 and 910 cm^{-1}. Anal. Calcd ($C_{22}H_{18}O_8$) C, 64.39; H, 4.42, found C, 63.52; H, 4.58. See Tables 7 and 8 for NMR data.

Acknowledgements

The authors express gratefully their thanks to SERC (DST), New Delhi for their financial assistance to R.V. and C.K.

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