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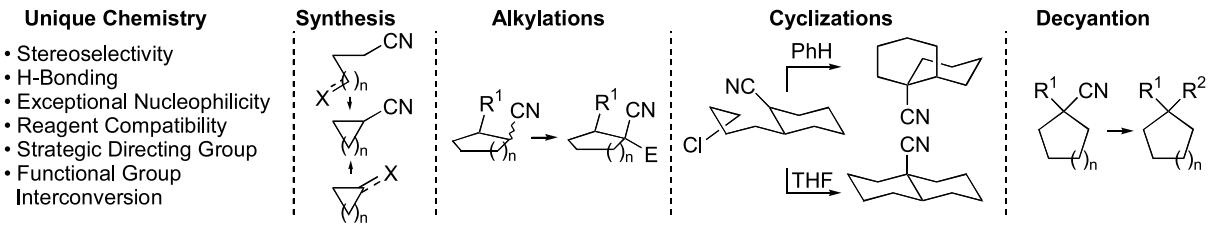
Tetrahedron

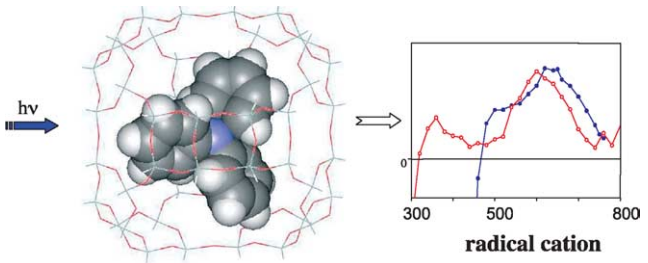
Tetrahedron Vol. 61, No. 4, 2005

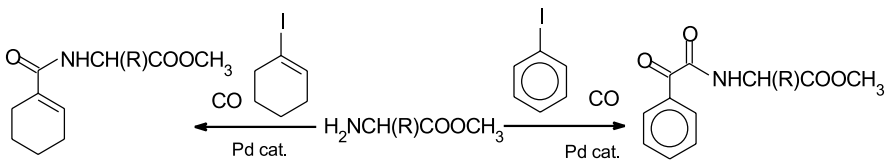
Contents

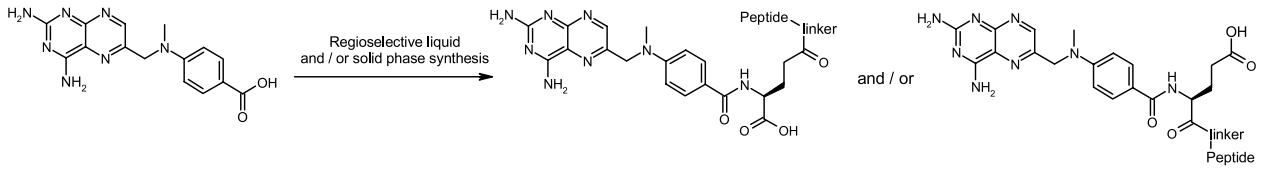
REPORT

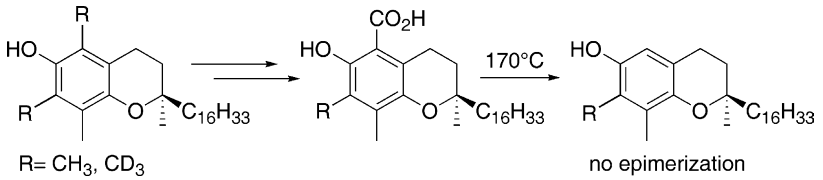


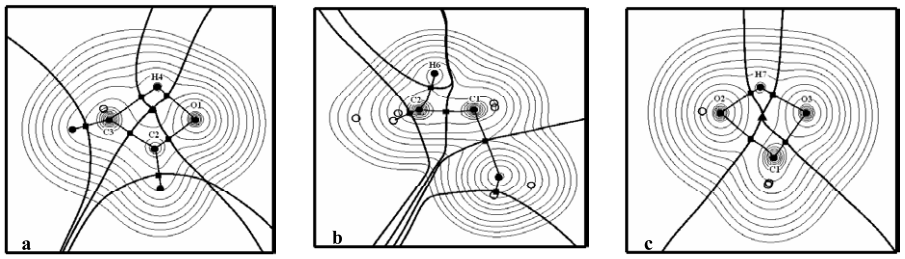
ARTICLES



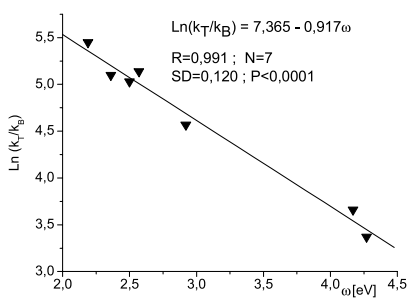


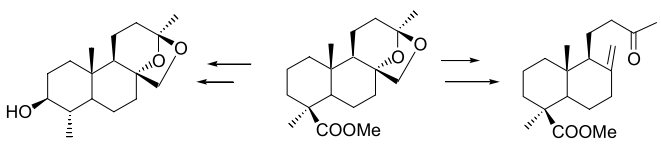


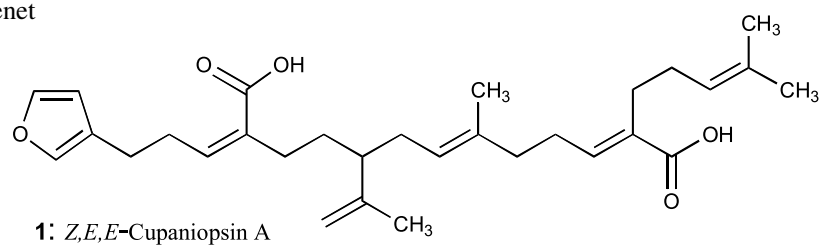




The mechanisms of: (a) keto–enol tautomerism, (b) pinacol rearrangement, and (c) methanediol decomposition were studied by means of AIM and Hirshfeld atomic partitionings. The three processes follow concerted mechanisms, but a hydrogen-bridged structure is never formed along the reaction path of the pinacol rearrangement.

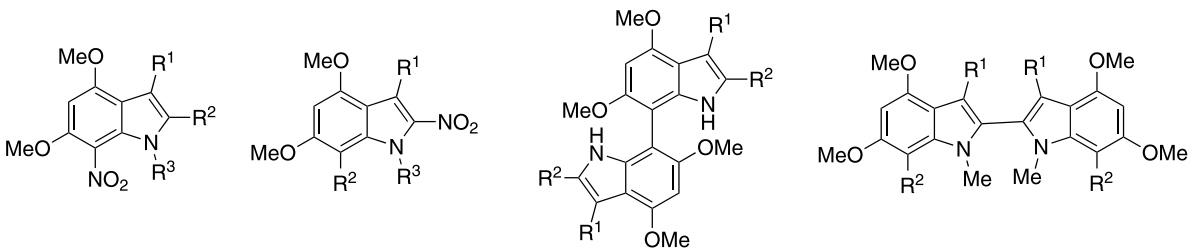




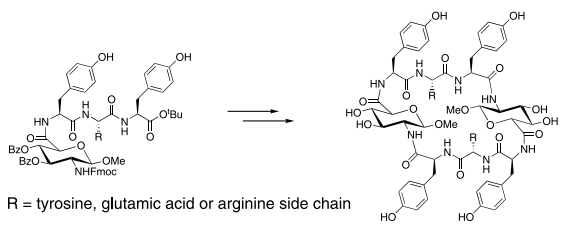


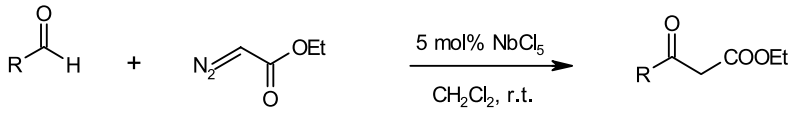
1: *Z,E,E*-Cupaniopsin A

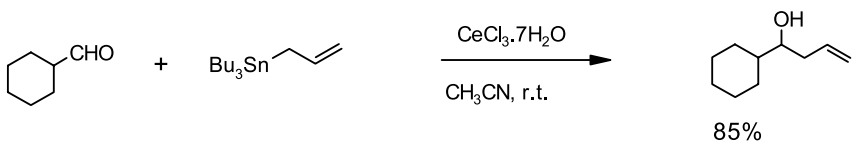
Ten new linear triterpenes named cupaniopsins A–E and analogues (**1**–**10**) have been isolated from the bark of three neocaledonian *Cupaniopsis* species (Sapindaceae). The structures were elucidated by extensive mono- and bi-dimensional spectroscopy and mass spectrometry. Five compounds exhibited potent binding affinity for peroxisome proliferator-activated receptor (PPAR-γ).

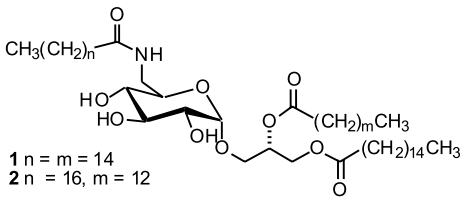


Some 3-substituted-4,6-dimethoxyindoles can be nitrated using nitric acid in acetonitrile, to give 7-nitro and 2-nitro-indoles. Some undergo oxidative dimerisation at C7, if 2,3-disubstituted, and at C2, if *N*-methylated and unsubstituted at C2.

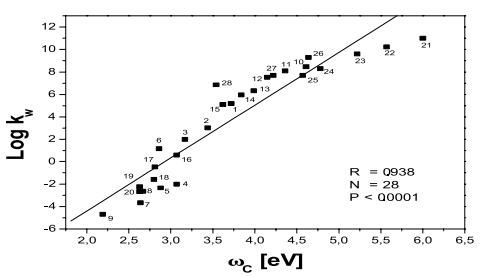


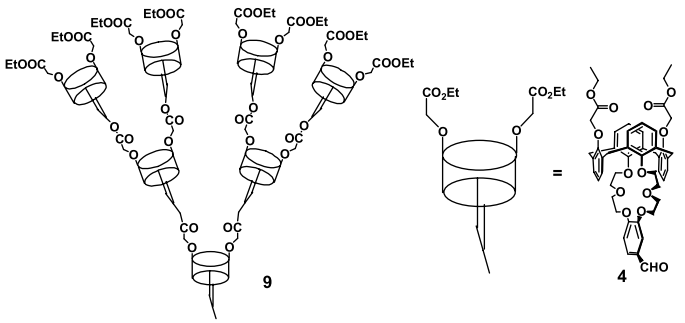


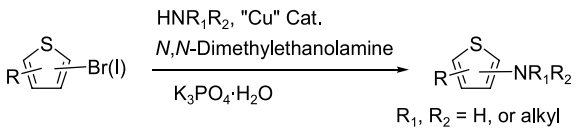




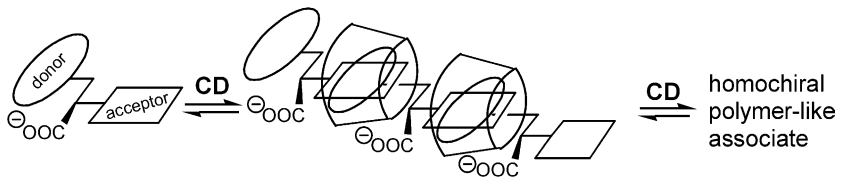
Bioassay-directed fractionation of a marine algal species using a bioassay for inhibitors of the enzyme Myt1 kinase yielded the novel glycoglycerolipids **1** and **2** as the major bioactive constituents. The structures of **1** and **2** were assigned by NMR spectra together with chemical and enzymatic degradations and by analyses of mass spectroscopic data.



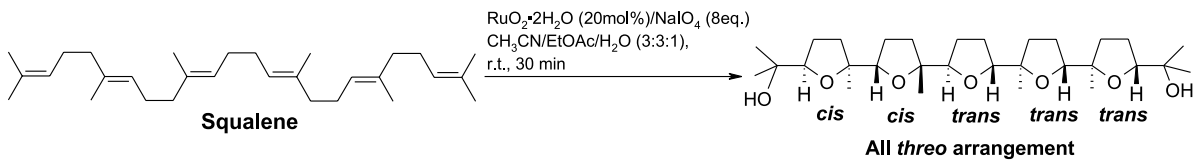


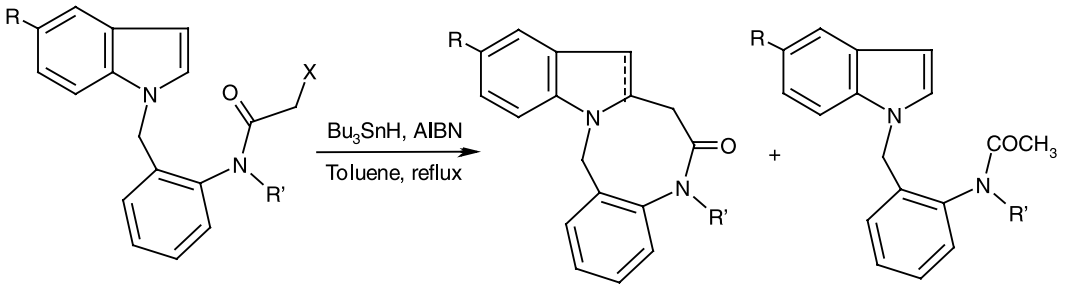


We have found that *N*,*N*-dimethylethanolamine (deanol) is a useful solvent and ligand for copper catalyzed amination of a variety of unactivated and activated 2- or 3-halothiophenes. Primary amines, acyclic secondary amines, cyclic secondary amines and acyclic secondary amines with 2-hydroxyethyl functionality react with halothiophenes providing the corresponding aminated thiophenes in moderate to excellent yields. The mildly basic conditions utilized are compatible with many functional groups. The amination of halobithiophenes has also been examined. The aminothiophenes produced by this method are important intermediates in a variety of electronic and optoelectronic materials.

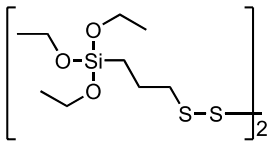


Spectroscopic evidence for enantiomeric recognition through formation of cyclodextrin assisted molecular associates was presented.

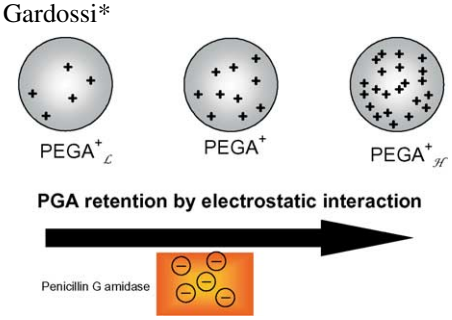


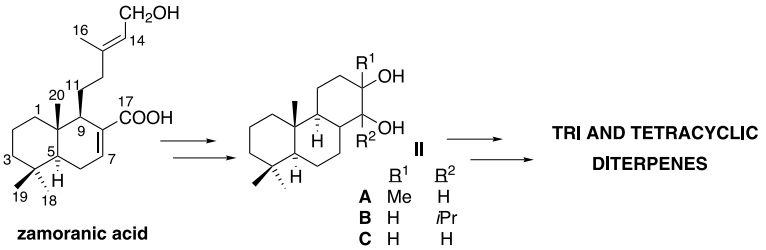


Seven new polychlorinated derivatives have been isolated in addition to the known dysidamide, dysidamide B and dysidamide C from the Red Sea marine sponge *Lamellodysidea herbacea*. Their structures were established by extensive spectroscopic methods. Dysidamide exhibited neurotoxic effects towards both mesencephalic and cortical murine neurones at 0.8 μg/ml.



bis[3-(triethoxysilyl)propyl] tetrasulfide





Several tri- and tetracyclic diterpenes have been synthesised from zamoranic acid. The key step is the cyclization of a dicarbonyl 13,14-secoderivative by SmI_2.

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) 747–789

Tetrahedron report number 701

Cyclic nitriles: tactical advantages in synthesis

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Received 10 September 2004

Available online 8 December 2004

Contents

1. Introduction

Cyclic nitriles have a long and distinguished history as versatile synthetic intermediates.[1] The versatility stems from the nitrile's extremely high polar-inductive effect,[2] their excellent hydrogen bond acceptor properties,[3] and the

Keywords: Cyclic nitriles; Stereoselectivity; Cyanation.
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0040–4020/$ - see front matter
doi:10.1016/j.tet.2004.11.012

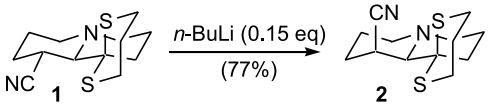
minimal steric demand of the extremely small nitrile group—the *A*-value is only 0.2 kcal mol^{-1} [4] Nitriles are highly unusual among functional groups in stabilizing adjacent radicals,[5] cations,[6] and anions[7] with the latter often exhibiting unique reactivity in providing chemo-, regio-, and stereo-selectivities that are practically inaccessible with carbonyl compounds.

This review surveys the reactions and syntheses of cyclic, aliphatic nitriles in which the nitrile is directly bound to the ring. Among the myriad reactions of nitriles, the focus is on anionic reactions of nitriles that are significantly different from those of related electron-withdrawing groups. The review is partitioned into five main sections beginning with an overview of the structural features of nitriles as a prelude to understanding their unique properties (Section 2). Syntheses of cyclic nitriles are then surveyed (Section 3) followed by stereoselective alkylations (Section 4) and cyclizations (Section 5) of cyclic nitriles, and concluding with methods for removing the nitrile group (Section 6). The broad utility of nitriles in synthesis means that some sections are necessarily selective whereas some of the lesser known reactions are comprehensively surveyed. Highlighting the unique reactions of cyclic, aliphatic nitriles is anticipated to enhance their use as synthetic intermediates, particularly in the total synthesis of nitrile-containing natural products.[8]

2. Unique chemistry of cyclic nitriles

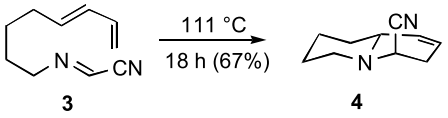
2.1. Stereoselectivity

Nitriles exhibit unique stereoselectivity because of the exceptionally small size of the C≡N group. The linear, rod-like geometry localizes the strong dipole more than the triangular carbonyl electron-withdrawing groups which, through bond rotation, spread the effective charge over a larger surface area. The compact, cylindrical nature of the C≡N unit (3.6 Å cylindrical diameter of the π-system)[9] creates one of the smallest electron-withdrawing groups with an A value of 0.2 kcal mol^{-1} which compares to 0.6–2.0 kcal mol^{-1} for carbonyl functionalities.[4] This difference gives rise to some surprising stereochemical preferences. For example, equilibration of the quinolizidine **1** favors positioning the nitrile in an axial orientation in direct contrast to most electron-withdrawing groups where the equatorial orientation[10] is favored (Eq. (1)).[11]



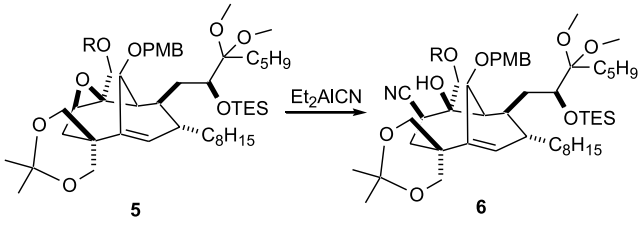
(1)

Similarly, intramolecular Diels–Alder cycloadditions generate cycloadducts that equilibrate in situ to install an axial nitrile[12] as a consequence of anomeric stabilization[13] (Eq. (2)).



(2)

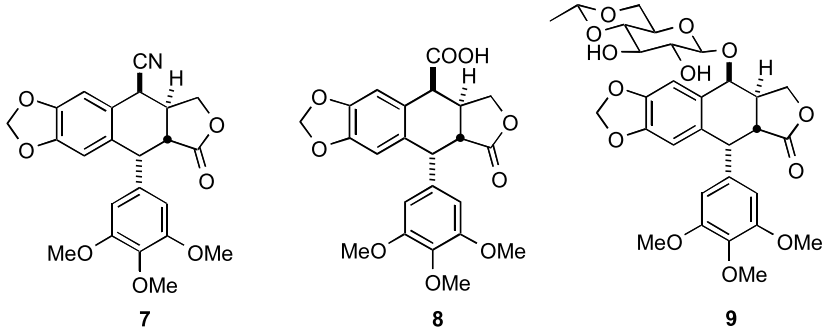
The small steric demand makes nitriles ideal for installing a one-carbon unit in sterically crowded environments. Historically, the classical Nagata cyanation of enones has served as a particularly efficient introduction of nitriles into sterically congested enones.[14] Cyanation continues to serve as an incomparably efficient one-carbon installation as illustrated in the synthesis of CP-263,114 (Eq. (3)) where formation of the hindered hydroxy nitrile **6** was crucial for assembling the highly hindered and sensitive molecular architecture of CP-263,114.[15]



(3)

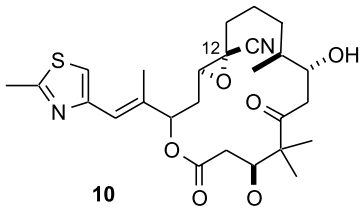
2.2. Hydrogen bonding

Nitriles are Lewis basic by virtue of the lone pair on nitrogen and readily participate in hydrogen bonding.[3] An extensive survey of X-ray structures reveals that electron donating substituents accentuate the basicity of the CN unit,



	7	8	9
Inhibition of L_{1210} IC_{50}(ng/mL)	3.48	164	155
Inhibition of KB IC_{50}(ng/mL)	155	10,000	308

Figure 1. Podophylotoxin analogs.



10

Figure 2. 12-Cyano-epothilone A.

effectively increasing hydrogen bonding. The hydrogen bond acceptor properties[3] of nitriles, combined with the extremely high polarization[2] and minimal steric demand,[4] conspire to make the nitrile a particularly useful hydroxyl surrogate. An indication of the nitrile's efficacy is exemplified by the structure-activity refinement of the nitrile and acid podophylotoxin analogs **7** and **8** (Fig. 1). Substituting a nitrile for the hydroxyl group of podophylotoxin gave **7** with a higher potency than the anti-cancer drug etoposide (**9**).[16]

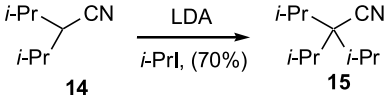
Nitrile-substitution in potential drug leads has also been used to increase drug stability through inductive polarization. Nitrile substitution increases the stability of epothilone at low pH, which is posited as a critical issue for establishing oral dosing regimens for this potent anticancer agent.[17] Installing a nitrile substituent at C-12 of epothilone A (Fig. 2) not only retains excellent activity in in vitro assays but markedly improves the pH stability [pH 1 buffer (aq.), $T_{95}=11$ h].

2.3. Exceptional nucleophilicity

Metallated nitriles are powerful nucleophiles with an exceptional propensity for C-alkylation. Nitrile anions alkylate almost exclusively on carbon with silyl[18] and acetyl[19] chlorides being virtually the only electrophiles with a propensity for reaction on nitrogen. The exceptional nucleophilicity stems primarily from the powerful inductive stabilization of metallated nitriles rather than resonance stabilization as is the case with enolates.[20]

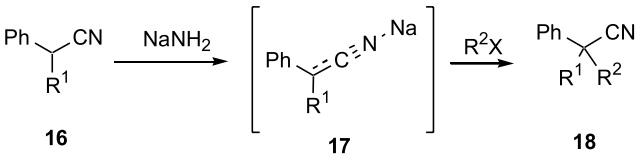
Pioneering X-ray structures of metallated nitriles have been instrumental in developing a coherent understanding of the reactivity and structure of metallated nitriles.[21] Surprisingly, metallated nitriles exhibit minimal elongation of the C≡N bond (1.15–1.20 Å, Fig. 3) when compared to the C≡N bond length of neutral nitriles (1.14 Å),[3] whereas the bond length between the nitrile carbon and the formal anionic carbon is typically intermediate between that of C–C double and single bonds (1.36–1.40 Å, **11**). Essentially, the powerful electron withdrawal of the CN group[20,22] stabilizes the adjacent negative charge in a manner akin to an ylide. Consistent with this inductive stabilization is the range of geometries for the metallated carbon which spans a continuum varying from planar in **12**[23] to pyramidal in the cyclopropanecarbonitrile **13**.[24]

Localization of negative charge on the carbon atom of metallated nitriles correlates with the high propensity for C-alkylation, even for sterically demanding alkylations. Comparative alkylations of sterically hindered nitriles, acids, and esters demonstrate the superior nucleophilicity of metallated nitriles.[25] Deprotonation and alkylation of diisopropylacetonitrile (**14**) with *i*-PrI proceeds in 70% yield whereas the corresponding acid (diisopropylacetic acid) or ester, are not alkylated by MeI (Eq. (4)).



14 15 (4)

Consistent with the high nucleophilicity of sterically hindered metallated nitriles are a series of alkylations of sodiated α-substituted phenylacetonitriles (Eq. (5)). Surprisingly, comparative methylations are increasingly efficient for α-substituents in the order butyl-ethyl > methyl > isopropyl > phenyl, consistent with a greater nucleophilicity being imparted from stronger electron donating substituents. Presumably the large steric demand of the large isopropyl substituent offsets the σ-donor character.[26]

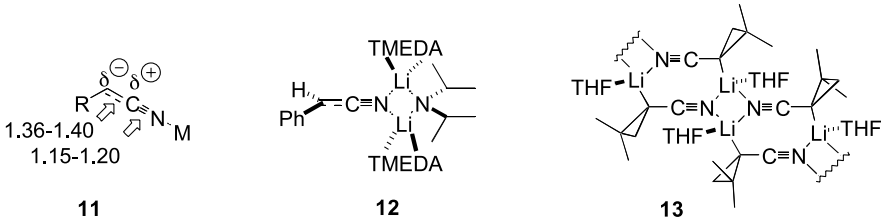


16 17 18 (5)

Comparative alkylations of malonates and malononitriles reiterate the exceptional nucleophilicity of nitriles.[27] Deprotonated malononitrile exhibits a particularly low intrinsic barrier, allowing facile hybridization changes during alkylation, consistent with the minimal resonance stabilization of nitriles.

2.4. High reagent compatibility

Unlike most carbonyl compounds, the nitrile group is inert to many nucleophilic reagents at low temperatures and has even been incorporated as a substituent within alkyllithium[28] and Grignard reagents.[29] Significantly fewer cyclic nitrile-substituted organometallics have been prepared despite the early incorporation within lithiated benzonitriles (**19**, Fig. 4)[30] and organozincs (**20**, Fig. 4).[31]



11 12 13

Figure 3. X-ray structures of metallated nitriles.

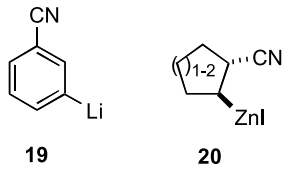
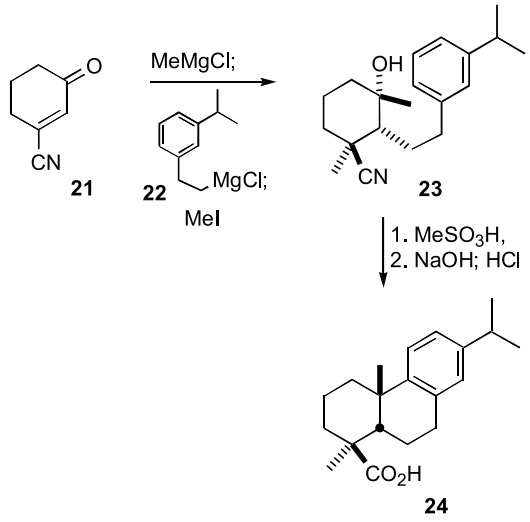


Figure 4. Cyclic metallated nitriles.

A direct benefit of the low electrophilicity of nitriles is that nitrile-containing intermediates are inert to many reagents and may be taken through many synthetic steps without the use of protecting groups.[32] Reduction of carbonyl groups in the presence of nitriles has long exploited the modest electrophilicity of the C≡N functionality.[33] Similarly, despite a perception to the contrary, nitriles react slowly with Grignard reagents as illustrated in the preferential 1,2-addition, 1,4-addition of Grignard reagents to the carbonyl and alkenenitrile functionalities in **21** (Scheme 1), with no observable addition to the nitrile.[34] Furthermore, the cyclic nitrile **23** readily cyclizes to the abietane whereas the corresponding ester[35] prematurely intercepts the intermediate carbocation, generating a lactone, which precludes Friedel–Crafts cyclization.

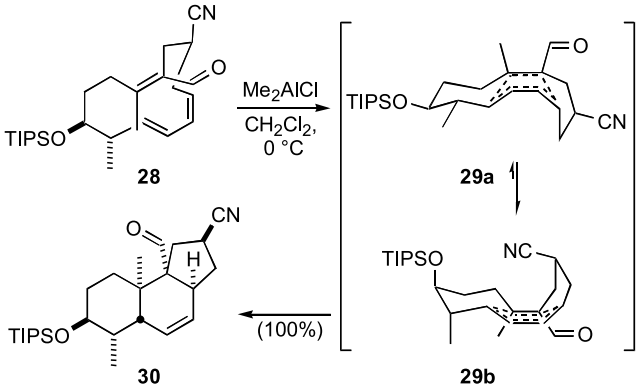


Scheme 1. Multi-component addition-cyclization of an oxonitrile.

2.5. Strategic directing group

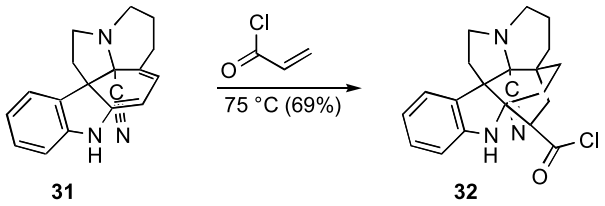
Nitriles exhibit dramatic stereocontrol in cycloaddition, electrocyclization, radical, and oxomercuration reactions despite having a minimal steric demand. Pioneering [6+4] cycloadditions[36] demonstrate that a nitrile substituent causes higher yields and improved selectivity (Scheme 2) relative to a methyl substituent, analogous to the 100-fold rate acceleration in Claisen rearrangements.[37] Thermolysis of **25a** initiates ring opening to the *o*-xylylene **26** triggering a [6+4] cycloaddition to afford **27**, whereas the analogous hydrocarbon **25b** affords a 1.7:1 ratio of [6+4] to [4+2] regioisomers.

Remarkably, the nitrile substituent within the cyclic triene **28** completely controls the stereoselectivity of the transannular Diels–Alder cycloaddition (Scheme 3).[38] The preferred transition state **29b** places the nitrile on the sterically more demanding concave face with concomitant positioning of two axial substituents on the developing cyclohexane ring, suggesting an intriguing stabilization of the cycloaddition by the nitrile group. Excellent use of the nitrile to control the cycloaddition features in an analogous transannular Diels–Alder route to the stemodane diterpenoid maritimol.[39]

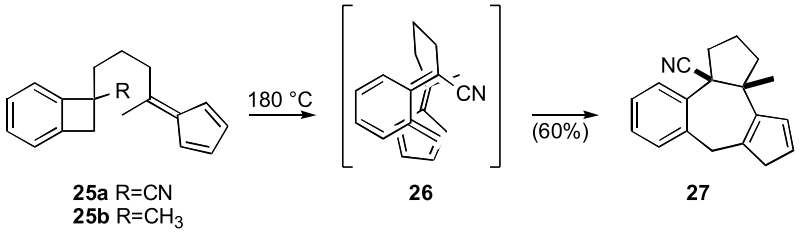


Scheme 3. Nitrile-directed intramolecular [4+2] cycloaddition.

A related nitrile-substituted diene was critical for the successful intermolecular Diels–Alder synthesis of *Kopsia* alkaloids (Scheme 4).[40] The nitrile-substituted diene **31** efficiently participates in Diels–Alder cycloadditions with a range of dienophiles, affording the key intermediate **32** on



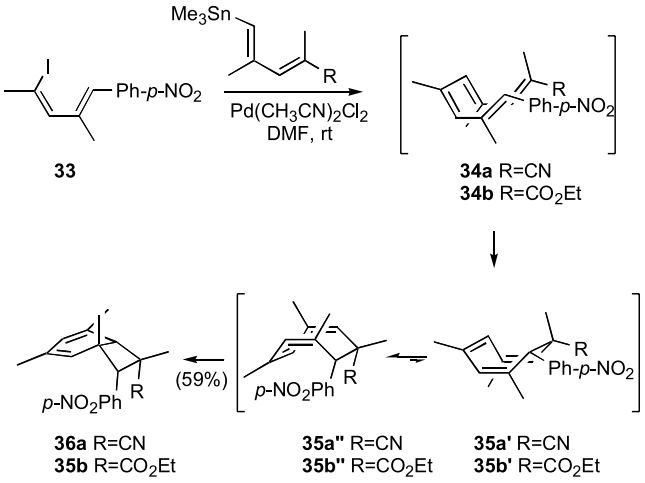
Scheme 4. Nitrile-directed intermolecular [4+2] cycloaddition.



Scheme 2. Nitrile-directed [6+4] cycloaddition.

thermolysis with acryloyl chloride. Analogous cycloadditions in which the nitrile group of **31** was replaced by methoxy or thiophenyl groups, were unsuccessful.[41]

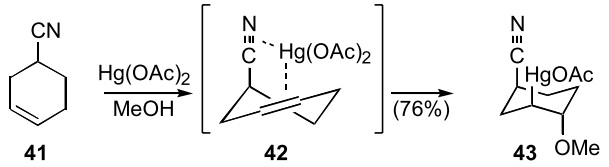
A further example in which nitrile-controlled stereoinduction overrides steric compression occurs in a 6π electrocyclization (Scheme 5). Palladium-catalyzed coupling of **33** generates **34a**, triggering an initial 8π electrocyclization followed by a 6π electrocyclization through the sterically more-crowded conformation **35a″** where the nitrile is proximal to the π-system, analogous to the [4+2] cycloadditions.[42] For comparison, the nitrile exhibits a 9:1 preference for cyclization through **35a″** over **35a′** whereas the stereoselectivity is reversed for the corresponding ester **35b**.



Scheme 5. Nitrile-directed 8π-electrocyclization.

Nitrile-substitution significantly enhances the radical polyene cyclization of **37** to **40** (Scheme 6).[43] Incorporating the electron deficient alkenenitrile acceptor promotes the second ring closure of the nucleophilic radical **38** and generates the long-lived electrophilic radical **39**, which is not oxidized by Mn(III) or Cu(II), and ideally suited for attack by a nucleophilic olefin. For comparison, cyclization of the nitrile **37** is 20% more efficient than with the analog in which the nitrile is replaced by a methyl group.

Few nitrile-directed reactions initiated by transition metal complexation have been explored[44] despite the propensity of nitriles to complex transition metals.[45] The remarkably stereoselective oxymercuration of cyclohexenecarbonitrile **41** is proposed to arise through two simultaneous π-interactions of mercuric acetate with the nitrile and alkene groups **42**, directing mercuration syn to the nitrile. The rationale effectively explains the superb stereoselectivity for what is otherwise a small, remote nitrile substituent (Scheme 7).[46]



Scheme 7. Nitrile-directed oxymercuration.

2.6. Functional group interconversion

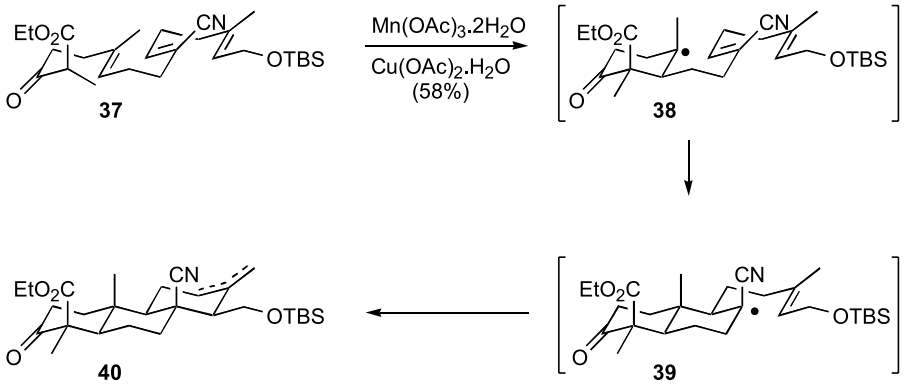
Nitriles are exquisitely positioned in terms of their reactivity; at low temperatures the nitrile can be incorporated within reactive intermediates such as organolithium reagents[28] whereas higher temperatures induce nucleophilic additions to the nitrile group.[1] The classical addition of Grignard reagents to afford ketones is probably the best-known example, but the nitrile group is readily converted to a plethora of functionalities,[47] providing an excellent entry to carbocycles[21a] and heterocycles.[48]

3. Syntheses of cyclic nitriles

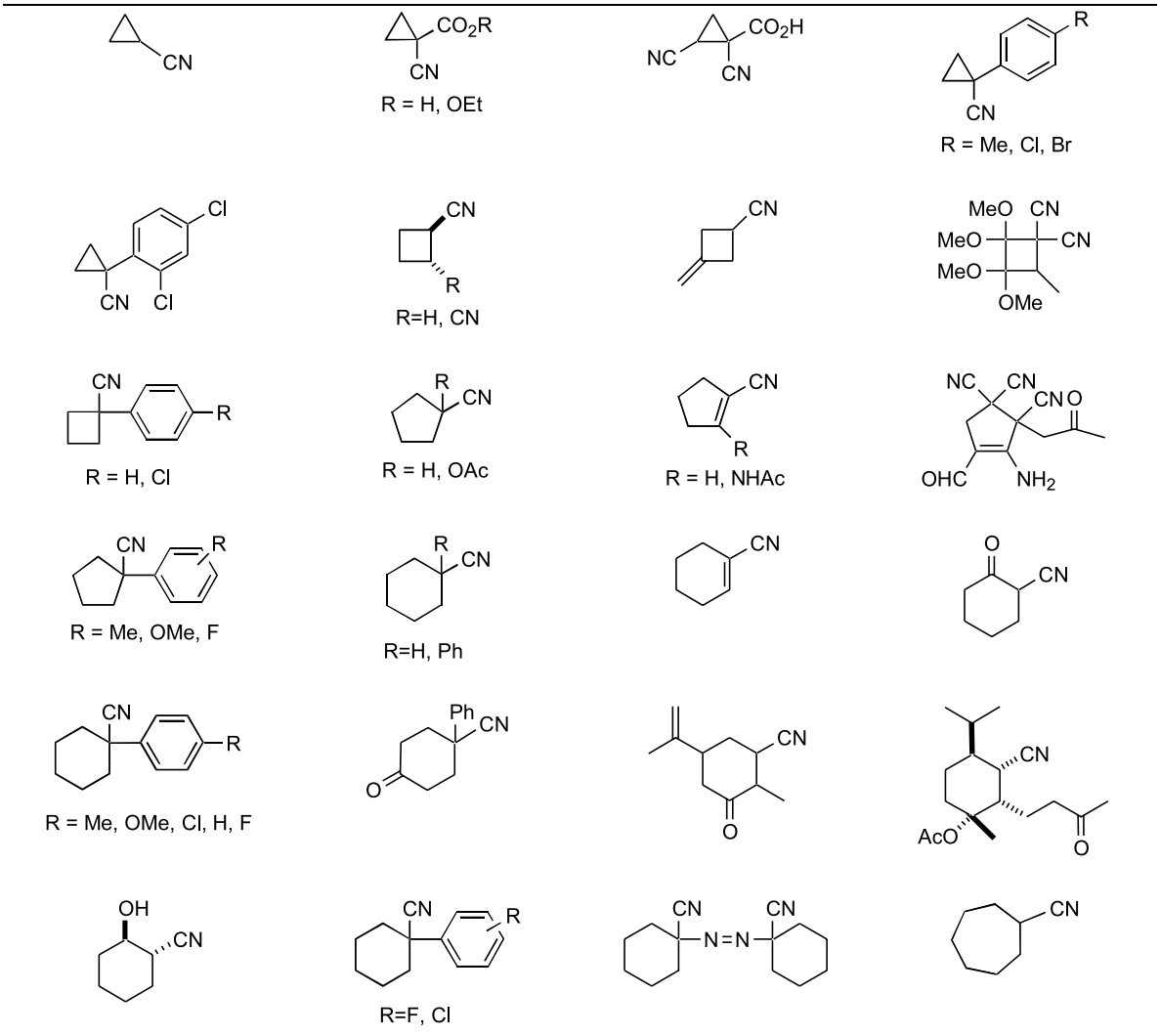
A surprising number of cyclic, aliphatic nitriles are commercially available (Chart 1) The parent 3- through 7-membered carbonitriles, and several substituted derivatives, are available in 1 g quantities or larger. In all instances except for the terpeneol derivative, the nitriles are achiral or racemic.

3.1. Alkanenitrile syntheses

3.1.1. Cyclization. A recent review[21a] summarizes the two most prevalent intramolecular alkylation routes to cyclic alkanenitriles: cyclization of a metallated nitrile bearing a pendant electrophile (Scheme 8, **44**→**46**) or, less

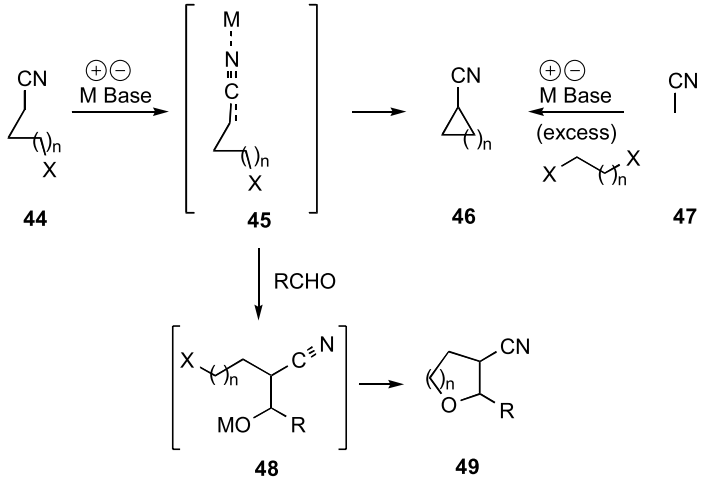


Scheme 6. Nitrile-directed radical cyclization cascade.



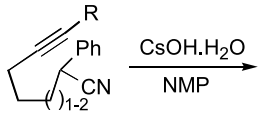
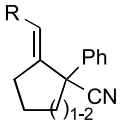
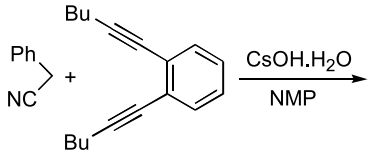
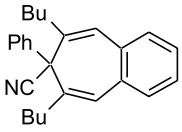
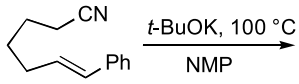
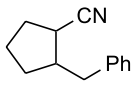
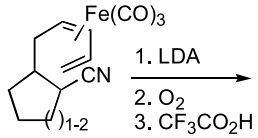
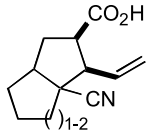
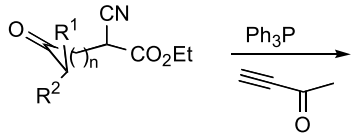
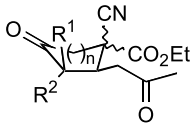
[a] The commercial availability, in sample sizes ≥1g, was determined by a SciFinder structure search (June 2004).

Chart 1. Commercially available cyclic aliphatic nitriles.[a]



Scheme 8. Cyclic nitrile synthesis.

Table 1. Syntheses of cyclic nitriles by cyclization

Entry	Reaction	Cyclic nitrile	Yield (%)
1			60–80[53]
2			75[53]
3			61[a54]
4			80–100[b 55]
5			55–82[56]

[a] A 1:1 ratio of isomers.
[b] A mixture of diastereomers at the ring junction.

commonly, through two sequential deprotonation alkylation reactions performed in one synthetic operation **47**→**46**. The direct dialkylation is particularly well suited to the formation of cyclopropanes using slightly greater than two equivalents of BuLi and epibromohydrin.[49]

An expedient variation for synthesizing nitrile-substituted furans[50] and pyrans[51] is to intercept the metallated nitrile **45** in situ with aldehyde electrophiles to generate an alkoxide **48** that internally alkylates a pendent electrophile to deliver the corresponding nitrile-substituted furan or pyran **49**.

The high nucleophilicity of metallated nitriles allows an unusual cyclization onto unactivated alkenes[52] and alkynes to generate cyclic nitriles (Table 1). The key to this unusual cyclization is the use of a relatively naked nitrile anion that is achieved through the use of CsOH or *t*-BuOK in highly polar solvents. Michael additions of metallated nitriles to activated alkenes provides cyclic nitriles through a clever double addition strategy with the cyclic nitriles providing the possibility for further cyclization through an intramolecular Claisen condensation (Table 1, entry 5).

3.1.2. Syntheses from cyclic precursors. Historically cyclic nitriles were commonly accessed through the conjugate addition of cyanide to cyclic enones which has been reviewed and is therefore not included.[14] The current survey of cyclic nitrile syntheses focuses on stereochemically complex transformations, and is necessarily selective rather than comprehensive

Cyclic 5- and 6-membered nitriles are most commonly synthesized by S_N2 displacement of the corresponding cyclic halide or sulfonate (Table 2, entries 1–10).[57] The small, nucleophilic cyanide unit allows an efficient displacement with predictable stereochemical inversion[58] although neighboring group participation may redirect the usual displacement to net retention (Table 2, entries 9 and 10). Cyanide nucleophilicity can be enhanced with tetraethylammonium cyanide[59] (Table 2, entry 1) or with hypervalent silicates (Table 2, entry 2) which allows preferential displacement of a secondary triflate in the presence of an epoxide (Table 2, entry 4). A mechanistically distinct alternative displacement of allylic acetates is possible through palladium catalysis (Table 2, entry 12). In cases where cyanide displacement is unsuccessful the corresponding iodide can be converted to a radical and cyanated with *tert*-butyl isocyanide (Table 2, entry 11). Direct cyanation of secondary alcohols can be performed in situ by a modified Mitsunobu inversion, avoiding sequential activation and cyanide displacement (Table 2, entry 13).

Benzylic and tertiary alcohols are conveniently converted to cyclic nitriles by intercepting the corresponding carbocation with cyanide (Table 2, entries 14–17, respectively) in a reaction that has been extended to the cyanation of tertiary halides and formally[60] ethers (Table 2, entries 18 and 19). Carbocation formation through oxidation with in situ cyanide attack similarly provides access to cyclic nitriles (Table 2, entries 20–22).

Nucleophilic cyanide cleanly opens epoxides to generate hydroxy nitriles (Table 2, entries 23–29). Diethylaluminum cyanide is the reagent of choice since coordination of the Lewis acid facilitates the ring opening,[121] allowing installation of quaternary nitriles even in the presence of

Table 2. Alkanenitrile syntheses from cyclic precursors

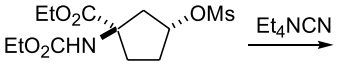
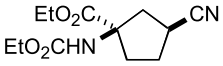
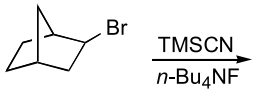
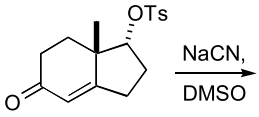
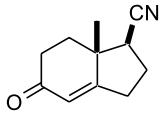
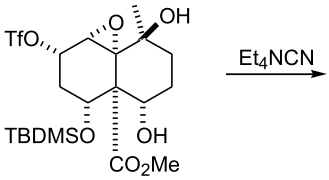
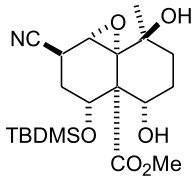
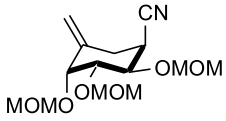
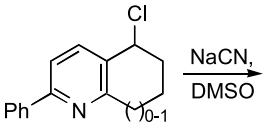
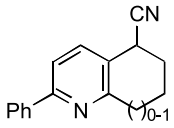
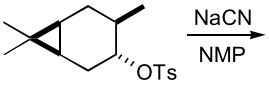
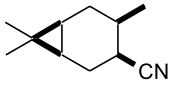
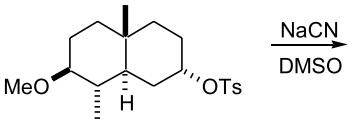
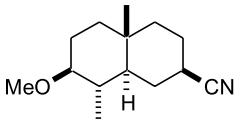
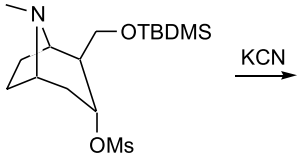
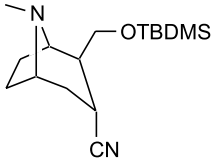
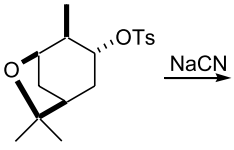
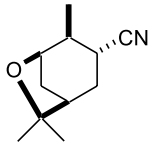
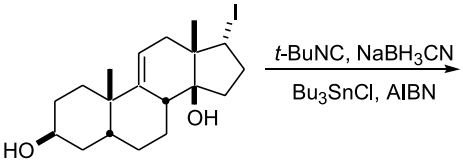
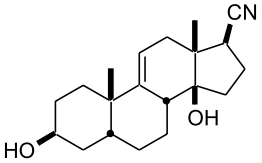
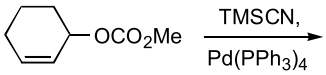
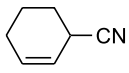
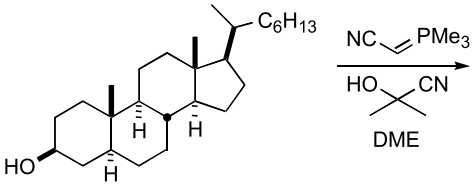
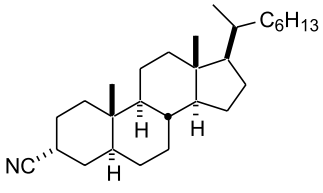
Entry	Reaction	Cyclic nitrile	Yield (%)
1			63[a,61]
2			70[b,62]
3			85[63]
4			74[64]
5			90[65]
6			78[66]
7			100[67]
8			50[68]
9			74[69]
10			50[70]
11			82[71]
12			88[72]
13			73[73]

Table 2 (*continued*)

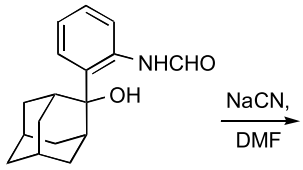
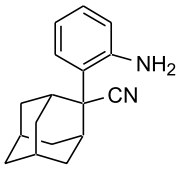
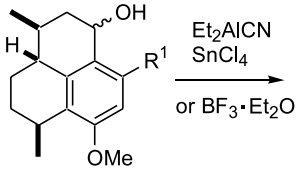
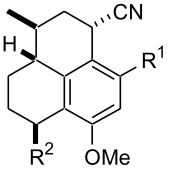
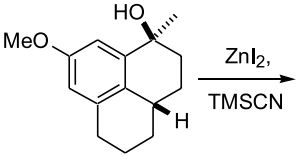
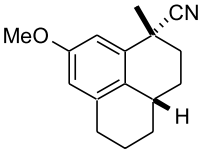
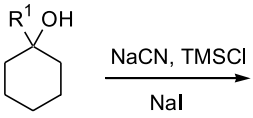
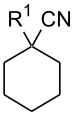
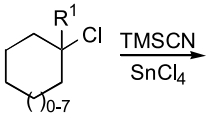
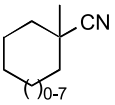
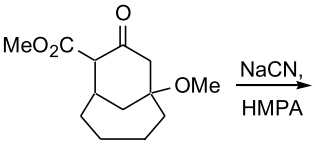
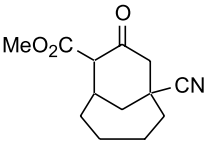
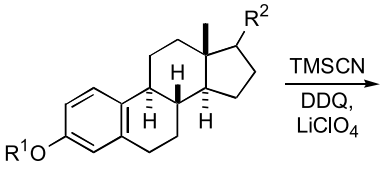
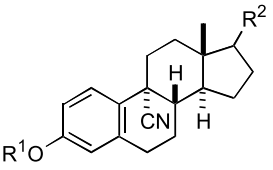
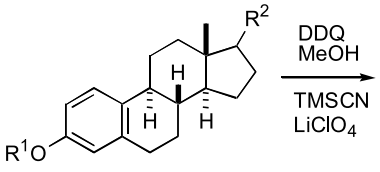
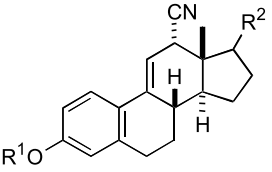
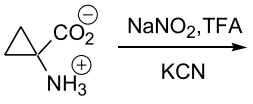
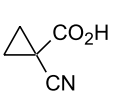
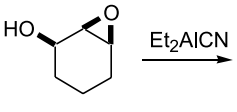
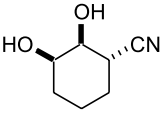
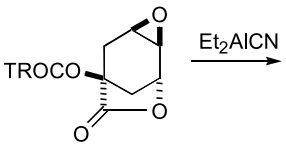
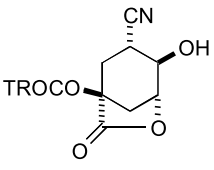
Entry	Reaction	Cyclic nitrile	Yield (%)
14			82[74]
15			60–70[75]
16			70[76]
17			64–91[77]
18			75–90[78]
19			45[79]
20			60–75[80]
21			36–52[80]
22			64[81]
23			65[82]
24			45[83]

Table 2 (*continued*)

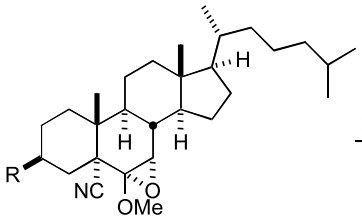
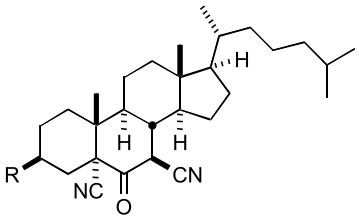
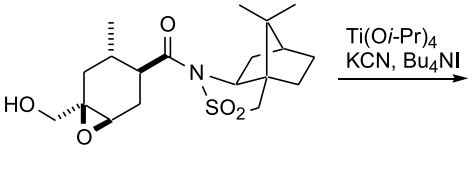
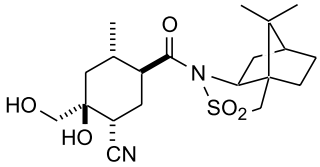
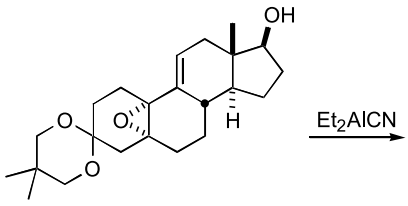
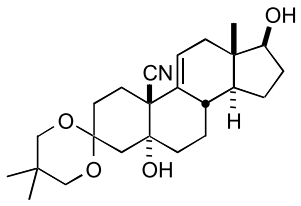
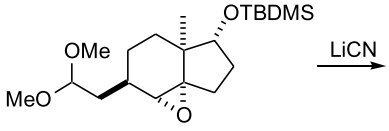
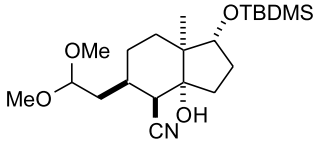
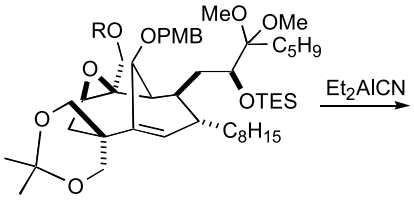
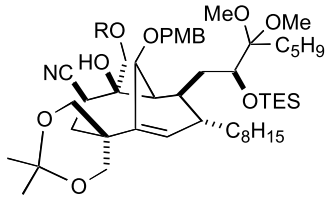
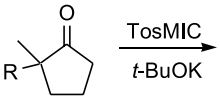
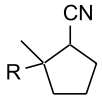
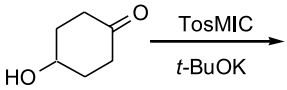
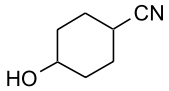
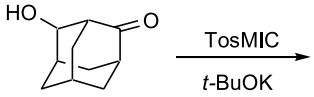
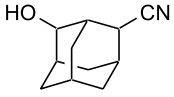
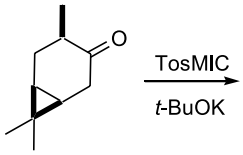
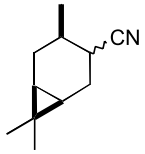
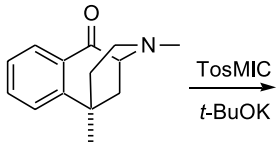
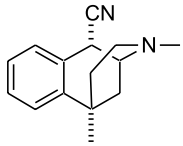
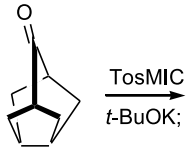
Entry	Reaction	Cyclic nitrile	Yield (%)
25			70–80[84]
26			83[85]
27			84[86]
28			84[87]
29			73[15]
30			50[88]
31			75[89]
32			88[90]
33			86[67]
34			76[91]
35			68[92]

Table 2 (*continued*)

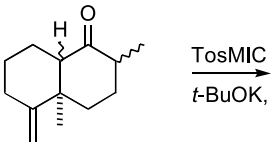
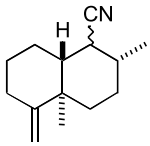
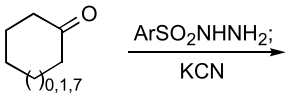
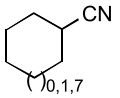
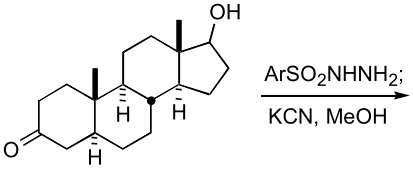
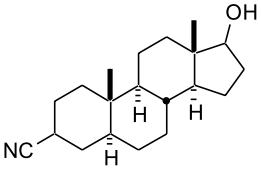
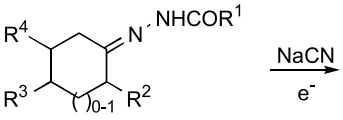
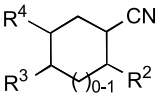
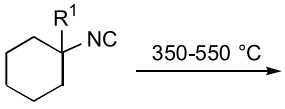
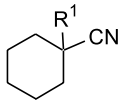
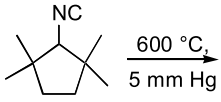
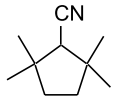
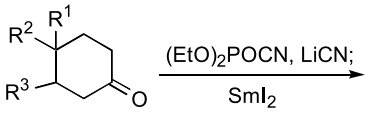
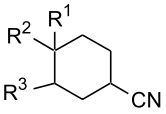
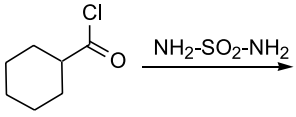
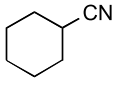
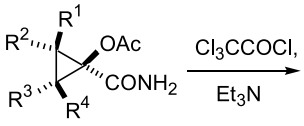
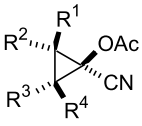
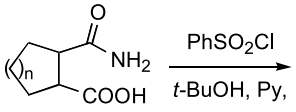
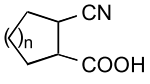
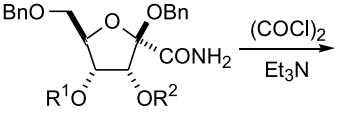
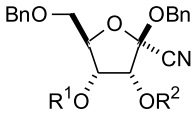
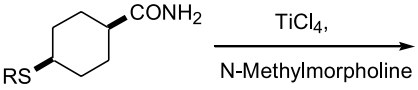
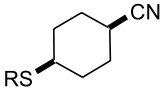
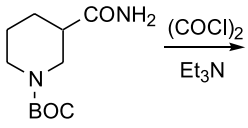
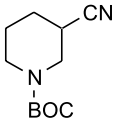
Entry	Reaction	Cyclic nitrile	Yield (%)
36			68[93]
37			67–77[94]
38			70[95]
39			43–82[96]
40			80–100[97]
41			90[98]
42			83–100[99]
43			95[100]
44			81–94[101]
45			90[102]
46			94–95[103]
47			30–52[104]
48			89[103]

Table 2 (*continued*)

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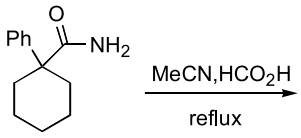
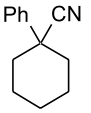
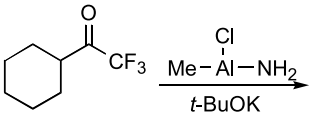
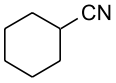
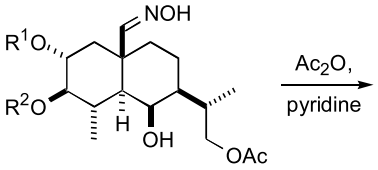
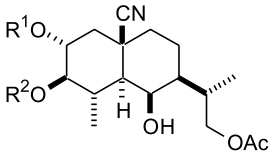
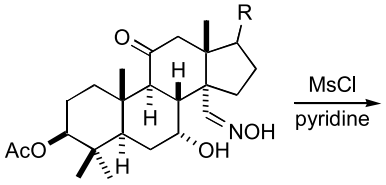
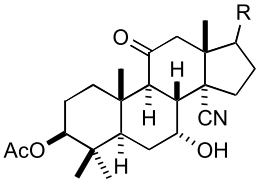
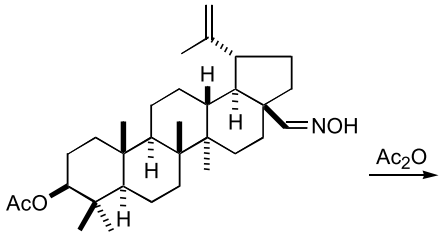
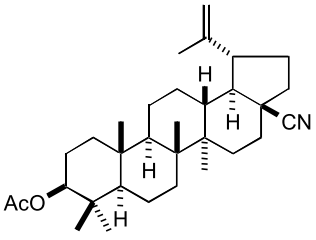
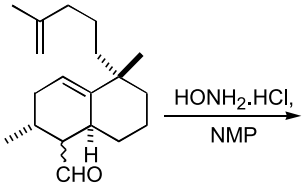
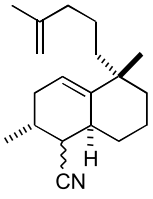
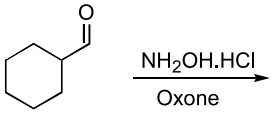
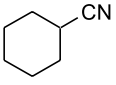
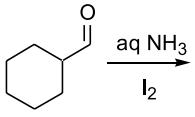
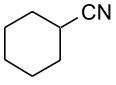
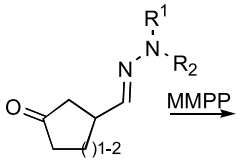
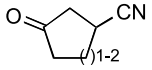
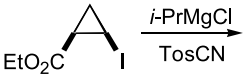
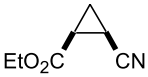
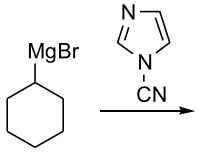
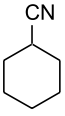
49			92[105]
50			35[106]
51			89[107]
52			85[108]
53			66[109]
54			96[110]
55			70[111]
56			94[112]
57			83–89[113]
58			67[114]
59			90[112]

Table 2 (*continued*)

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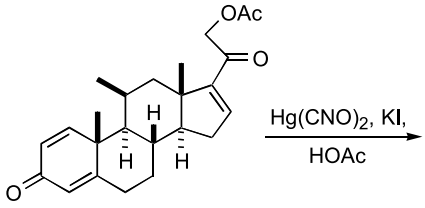
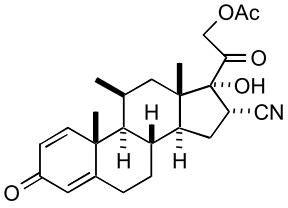
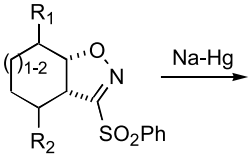
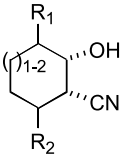
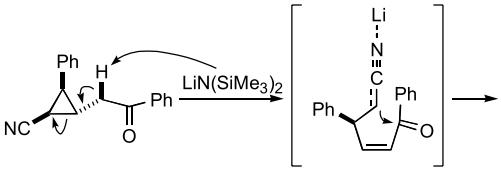
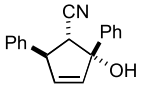
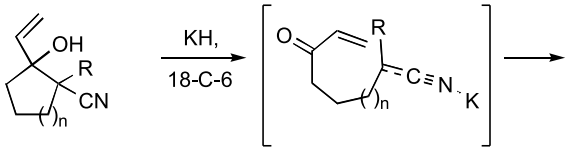
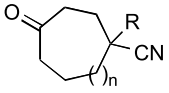
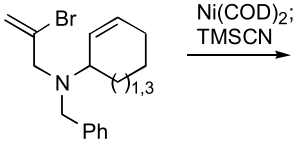
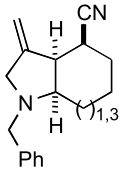
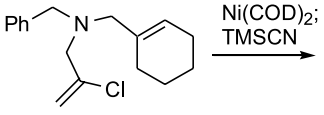
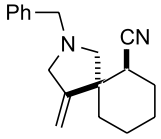
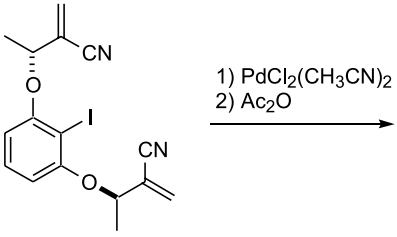
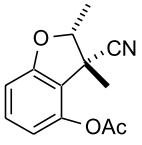
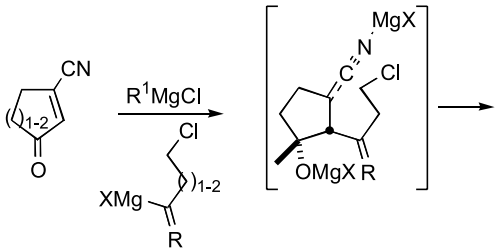
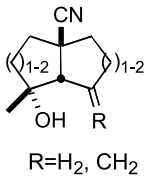
60			55[115]
61			81–94[116]
62			45[117]
63			51–86[118]
64			44–99[119]
65			84[119]
66			81[120]
67		 R=H, CH	50–58[34]

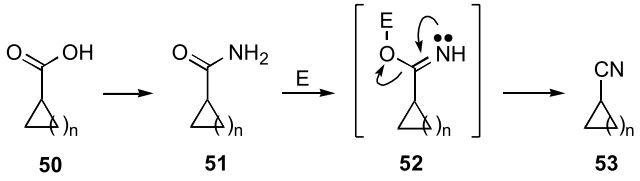
[a] A 7:3 ratio of isomers.
[b] 7:1 mixture of *endo*/*exo* isomers.

acetals (Table 2, entries 27–29), with LiCN being an excellent alternative via lithium-assisted epoxide ring opening (Table 2, entry 28). Nucleophilic cyanide additions generate *trans*-diaxial hydroxy nitriles in all cases (Table 2, entries 23–28) except for a sterically congested system (Table 2, entry 29) where equilibration is likely, possibly through a retro-aldol sequence[122] or prototropic transfer.[123]

Cyclic ketones are readily homologated to nitriles with *t*-BuOK and *p*-toluenesulphonylmethyl isocyanide (TosMIC).[124] The reaction is particularly effective with hindered ketones (Table 2, entries 30–36) although the presence of *t*-BuOK causes epimerization of the ketone precursor as well as the resulting nitrile (Table 2, entry 36). The efficacy of TosMIC has largely superseded conversion of ketones to nitriles through the addition of KCN to arylsulfonyl hydrazones, generating an intermediate cyanohydrazine that fragments to the nitrile via the diazo species (Table 2, entries 37–39).[125]

Thermolytic rearrangement of isonitriles similarly constitutes an effective method for accessing hindered nitriles (Table 2, entries 40 and 41), although synthesizing the precursor isonitrile can require several steps. Formation of a cyanohydrin derivative followed by deoxygenation represents a more efficient strategy (Table 2, entry 42).

A variety of cyclic carboxylic acid derivatives are readily converted into the corresponding nitriles by dehydrating amide intermediates in a reaction that tolerates numerous functional groups (Table 2, entries 43–49). The methods require conversion to the cyclic primary amide **51** and subsequent dehydration by sequential *O*-activation and elimination (Scheme 9, **51**→**53**).[126] An analogous sequence occurs on addition of methylchloroaluminum amide to trifluoromethyl ketones with the intermediate imine (**52**, CF_3 replacing EO) ejecting CF_3 to form the nitrile (Table 2, entry 50).



Scheme 9. Dehydration of amides to nitriles.

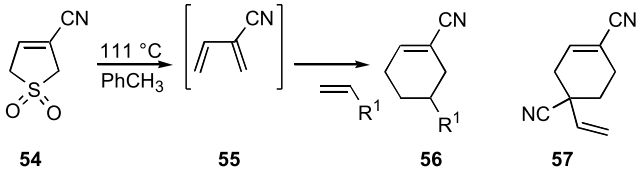
Dehydration of oximes, with a variety of mild reagents, provides a robust route to cyclic nitriles (Table 2, entries 51–55). Direct conversion of aldehydes to nitriles is possible with ammonia through an in situ oxidation-dehydration (Table 2, entry 56). The oxidation-elimination of dialkylhydrazones provides a useful ketone alkylation–cyanation which, for chiral dialkylhydrazone alkylations, provides an excellent route to chiral nitriles (Table 2, entry 57).

Alkyllithium and Grignard reagents react with a variety of electrophilic cyanide sources, although only a few cyclic nitriles have been synthesized in this way (Table 2, entries 58 and 59). Several cyclic β-hydroxy nitriles are efficiently accessed through a [3+2] cycloaddition followed by N–O cleavage of the resulting oxazole (Table 2, entries 60 and 61) or alternatively through a ring expansion triggered by cyclopropane fragmentation (Table 2, entry 62). A related two-carbon ring expansion, with considerable generality, is initiated by a retroaldol-type reaction that generates a metallated nitrile ideally aligned for an intramolecular Michael addition (Table 2, entry 63).

A series of nickel-induced carbometallation–cyanations are particularly effective at generating an array of bicyclic nitriles (Table 2, entries 64 and 65). Alkylations to form bicyclic nitriles are uncommon because synthesizing the cyclization precursors often requires multiple steps. An exception is the chelation-controlled conjugate addition of ω-chloro Grignard reagents to oxoalkenenitriles that accesses a variety of ring sizes (Table 2, entry 67).

3.1.3. Diels–Alder cycloaddition. The venerable Diels–Alder reaction is ideal for assembling an array of cyclic nitriles (Chart 2). Acrylonitrile is a reactive dienophile in Diels–Alder reactions, typically affording mixtures of *endo*/*exo* isomers with cyclopentadienes, cyclohexadienes, and cycloheptadienes,[154] with slightly lower *endo*/*exo* selectivities than comparable carbonyl-containing dienophiles.[127] Despite the high reactivity of acrylonitrile, the Diels–Alder cycloaddition with furan is particularly challenging, requiring 5 weeks for a synthetically acceptable yield,[128] or acceleration by high pressure[157] or Lewis acids.[129] Electron donating substituents sufficiently activate furan for relatively facile cycloadditions.[130]

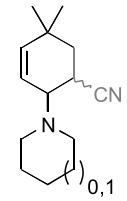
Few Diels–Alder reactions of cyanobutadiene have been pursued because of the propensity toward dimerization.[159] A particularly expedient synthesis of cyanobutadiene is through thermal extrusion of sulfur dioxide from the thiophene dioxide **54** (Scheme 10) with in situ trapping with reactive dienophiles.[160] Cycloaddition of **55** with nonsymmetrical dienophiles is modestly selective (~3:1 ratio) except for the cyanobutadiene dimer **57** that is isolated as a single regioisomer.



Scheme 10. Diels–Alder reaction of cyanobutadiene.

α-Chloroacrylonitrile Diels–Alder cycloadditions provides a versatile ketene equivalent (Chart 3) since ketenes are poor dienophiles.[161] A pioneering study[172g] revealed 2-chloroacrylonitrile cycloadditions to be more efficient, and more regioselective, than 2-acetoxyacrylonitrile, with the added advantage of being commercially available. Generally 2-chloroacrylonitrile adds regioselectively (>20:1) to dienes, placing the nitrile adjacent to the electron donating substituent as mixtures of *endo*/*exo* isomers. The modest stereoselectivity is inconsequential for subsequent hydrolyses to the corresponding ketone.

Vinyl furan is a particularly demanding diene requiring 1 day at 1.9 Gpa for an acceptable yield,[162] with a lower yield, and diminished regioselectivity at lower pressures,[163] or with 1-acetoxyacrylonitrile. The cycloaddition of α-chloroacrylonitrile with furan similarly requires either high pressures,[164] copper catalysts[165] or, more effectively, ZnI_2 catalysis,[170] with complete conversion often requiring several weeks.



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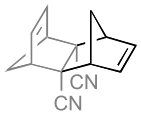

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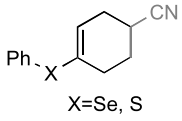
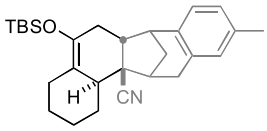
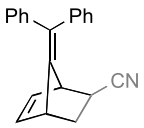


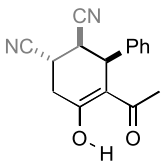
94-95%[133]



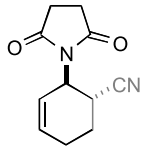
87%[134]



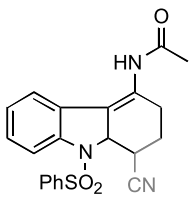
91%[135b]



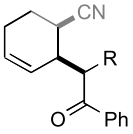
64%[136c]



76%[137d]



38%[138]



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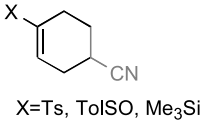
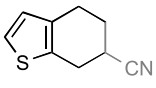
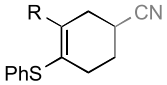


67-89%[140]



92%[141e]



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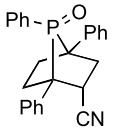

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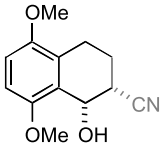

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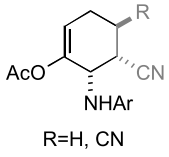

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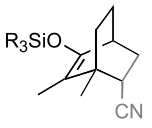

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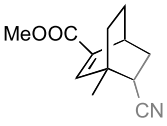

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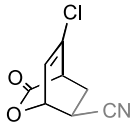

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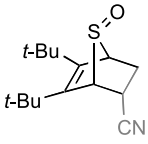

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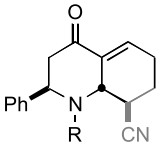

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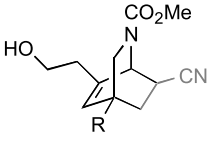

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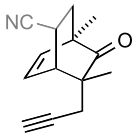

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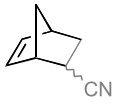

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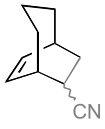

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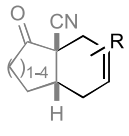

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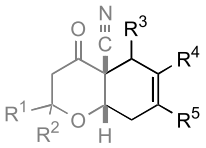

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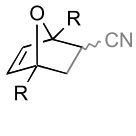
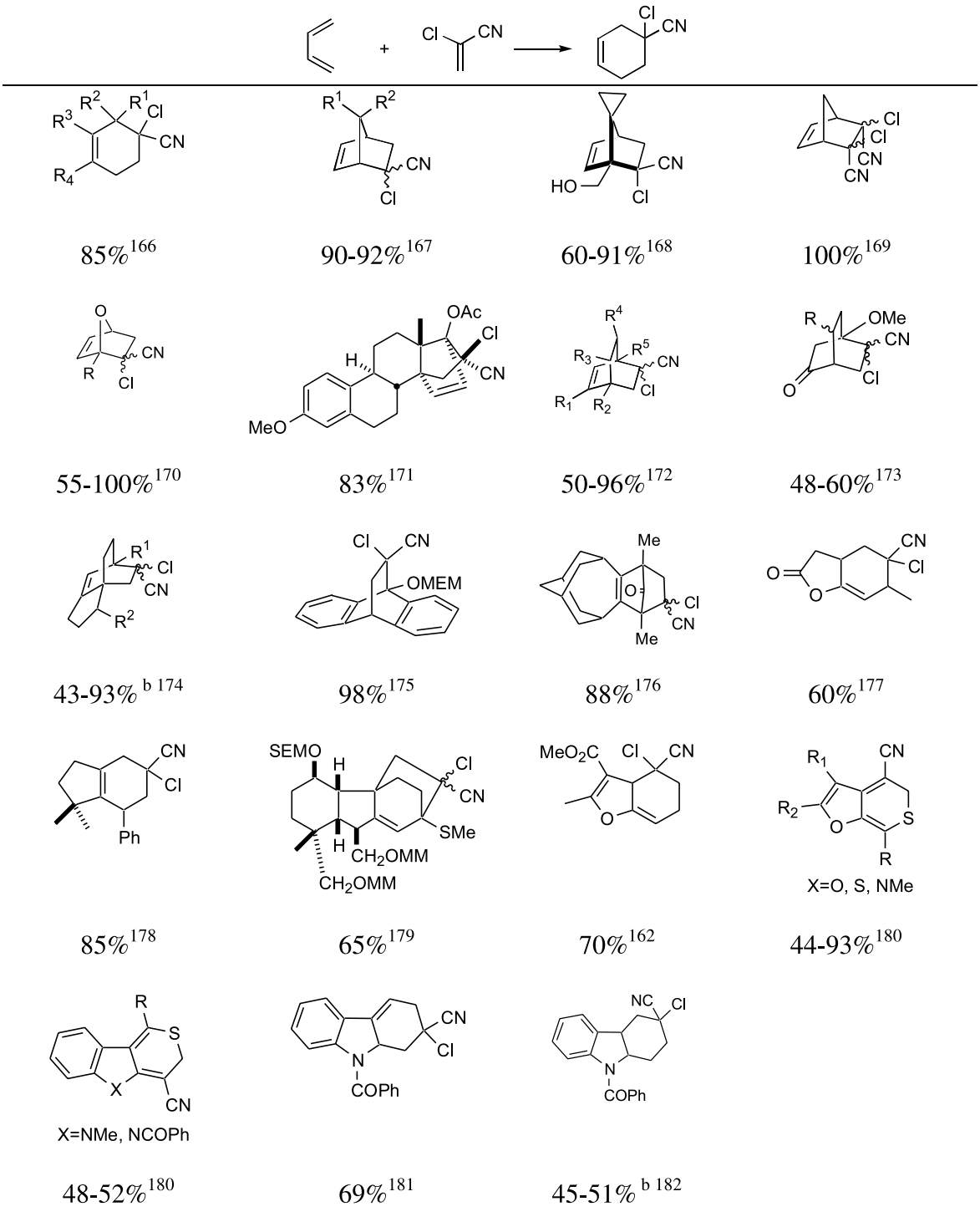


55-65%[157]

95%[158]

[a] The alkenenitrile component is shown in grey. [b] A mixture of regioisomers: 1.6:1.[c] A mixture of regioisomers: 5.5 :1. A mixture of regioisomers: 3.5:1. [d] A mixture of regioisomers: 2.4:1. [e] A mixture of regioisomers: 3:1. [f] A mixture of epimers: 2:1.

Chart 2. Cyclic nitriles generated by Diels–Alder reactions.[a]



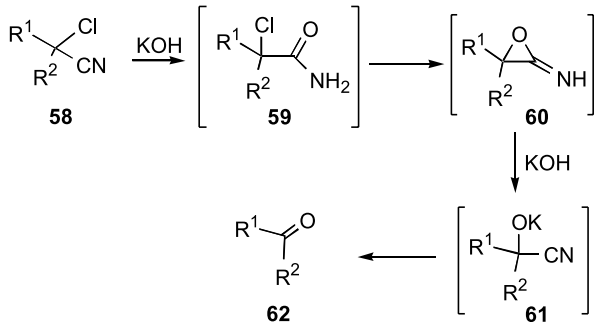
[a] A 3:1 ratio of regioisomers. [b] A mixture of regioisomers.

Chart 3. Diels–Alder reactions of α-chloroacrylonitrile.

Numerous cyclohexadienes react with α-chloroacrylonitrile to afford bicyclo-[2.2.2]-octenes (Chart 3). The attraction of this transformation stems from the prevalence of the bicyclo-[2.2.2]-octene unit in terpenoids and the rapid synthesis of alkoxycyclodienes through the Birch reduction of substituted anisoles. Unconjugated cyclohexadienes are rapidly isomerized to the conjugated dienes by base[183] and acid,[184] including the acidic silica sites on glass surfaces,[183d] which permits the sequential Birch reduction–Diels–Alder–cycloaddition without prior isomerization.[185] Not surprisingly, Birch reduction-cycloaddition has been employed in total syntheses of several natural products: modhephene, antheridic acid[174] and hinesol.[179]

Cyclic α-chloronitriles are usually hydrolyzed to the corresponding ketone with base,[172d] although multistep

sequences are sometimes required.[186] The KOH hydrolysis mechanism[187] is highly unusual in proceeding through the α-chloroamide **59** (Scheme 11) that most likely cyclizes to the imino oxirane **60**,[188] fragments to the deprotonated cyanohydrin **61**, and ejects cyanide to form the ketone.[187] In some instances the α-chloroamide[165,189]**59** and the corresponding chloroacid have been isolated.[164,186]



Scheme 11. Hydrolysis mechanism of α-chloronitriles.

Cyclic α-halonitriles are generally precursors to ketones but also provide access to a range of functionalized nitriles (Table 3). Base- or CO-induced elimination affords the corresponding unsaturated nitriles (Table 3, entries 1 and 2) whereas reduction with zinc-copper couple gives the parent nitrile that may subsequently be deprotonated and exposed to oxygen in an indirect route to the corresponding ketone (entry 3). Alternatively metal-halogen exchange of α-chloro- or α-bromonitriles with BuLi, or more effectively, *i*-PrMgBr, affords metallated nitriles that alkylate a range of electrophiles (entry 4),[190] explaining the previously perplexing conversion of α-chloronitriles to ketones upon sequential exposure to BuLi,[175] or LDA,[191] and oxygen (Table 3, entries 5 and 6).

Intramolecular Diels–Alder reactions of alkenenitriles have a long and distinguished history beginning with the thermolysis of nitrile-substituted benzocyclobutanes (Table 4, entries 1–3).[194] Thermolysis triggers a torquoselective ring opening[195] which positions the nitrile group on the inside of a reactive *o*-quinodimethane with an ideal alignment for cycloaddition onto a tethered alkene (Table 4, entry 1). The benzocyclobutane strategy accommodates a variety of geminal substituents in the tether and on the terminus of the alkene, providing predominantly *cis*-fused tricyclic nitriles that have been employed in terpenoid syntheses. The most demanding example of this strategy is the convergent synthesis of a *trans*-fused, pentacyclic system (Table 4, entry 3).

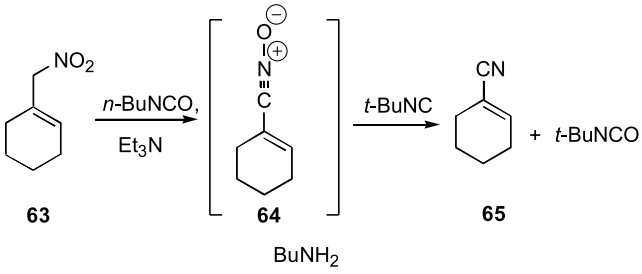
More recent intramolecular Diels–Alder reactions continue to focus on assembling multicyclic ring systems (Table 4, entries 4–10). Intramolecular cycloadditions with nitrile-substituted imine- and oximino-dienes provide bicyclic heterocycles (Table 4, 8-10) which, in the latter case, undergo base-induced fragmentation to substituted pyridines (Table 4, entry 10). Alkenenitriles are particularly well suited for dipolar cycloadditions in providing excellent stereo- and regioselective control in the intramolecular cycloaddition (Table 4, entry 11).

3.2. Alkenenitrile syntheses

Most cyclic alkenenitriles are synthesized from cyclic ketone precursors by conversion to the corresponding cyanohydrin followed by dehydration (Table 5, entries 1–12). In some instances cyanide addition to cyclopentenones and cyclohexenones directly generates the corresponding cyanohydrin whereas less reactive ketones require the use of TMSCN in the presence of a Lewis acid. The resulting TMS-protected cyanohydrins eliminate on heating with $POCl_3$ to afford the corresponding alkenenitrile, typically as a mixture of regioisomers (Table 5, entries 6–9). Related eliminations of phosphate, and an epoxide ring opening, similarly afford cyclic alkenenitriles (Table 5, entries 13 and 14) with the opportunity for further substitution in the case of phosphorylated cyanohydrins derived from unsaturated ketones. Complementing these α-eliminations is the less common β-elimination of selenoxides and bromide (Table 5, entries 15–16).

A distinct drawback of many elimination-based syntheses of cyclic alkenenitriles is the formation of regioisomers. Regioselective conversion of ketones to the corresponding alkenenitrile is effectively achieved by regioselective conversion to the corresponding enol triflate, and coupling with LiCN (Table 5, entry 18). Analogous coupling of vinyl chlorides are similarly regioselective although fewer methods exist for synthesizing the vinyl chloride precursors (Table 5, entry 19).

A diverse array of cyclic nitriles are generated from allylic nitro alkanes (Table 5, entry 20), that are prepared through a Knoevenagel condensation between nitromethane and a cyclic ketone. Mechanistically, deprotonation of the allylic nitro alkane **63**, *O*-acylation by *n*-BuNCO, and elimination, generates the nitrile oxide **64** in situ, which is reduced with *t*-BuNC to the unsaturated nitrile **65** (Scheme 12).



Scheme 12. Conversion of allylic nitro alkanes to cyclic nitriles.

Intramolecular aldol-type reactions provide one of the few direct routes to cyclic alkenenitriles from acyclic precursors (Table 5, entries 21–25).[206] A less used, but equally effective method, is via an intramolecular Wittig condensation either by unmasking an aldehyde, or Wittig formation in situ (Table 5, entries 26–28). Several isolated syntheses of cyclic alkenenitriles have emerged during method development (Table 5, entries 29–36) although the generality of these methods remains unexplored.

Table 3. Transformations of α-chloronitriles

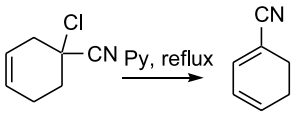
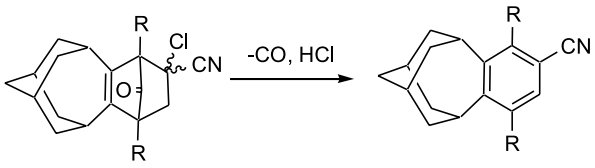
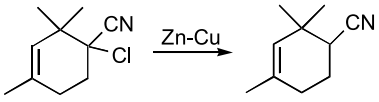
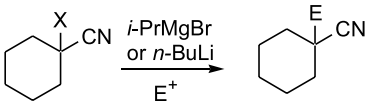
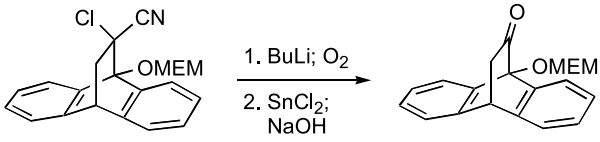
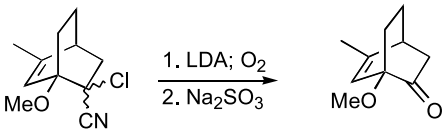
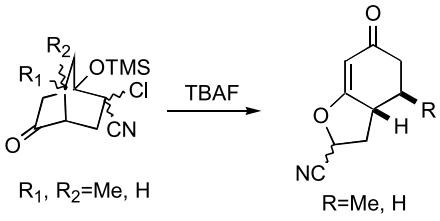
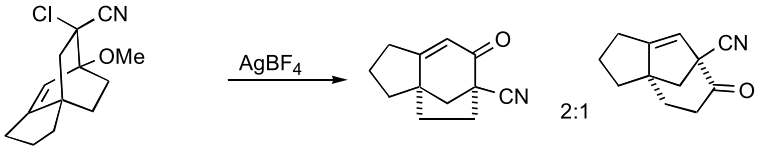
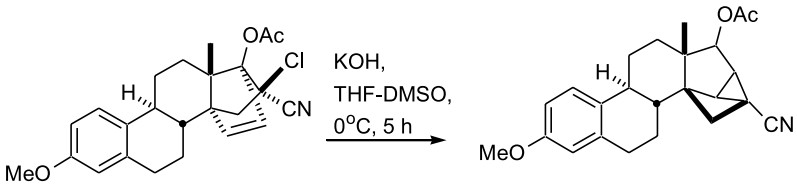
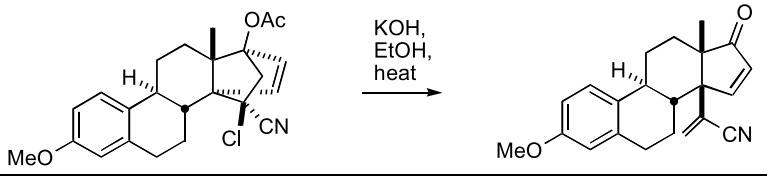
Entry	Reaction	Yield (%)
1		72[166d,e]
2		76–86[176]
3		98[166b]
4		52–82[190]
5		58[175]
6		70[191]
7		42–89[192]
8		95[193]
9		90[171]
10		69[171]

Table 4. Intramolecular cycloaddition syntheses of multicyclic nitriles

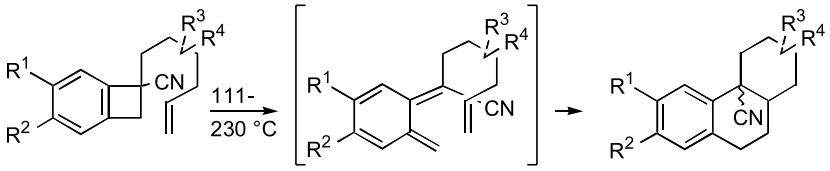
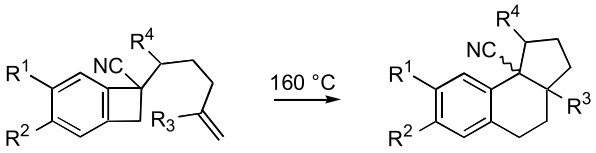
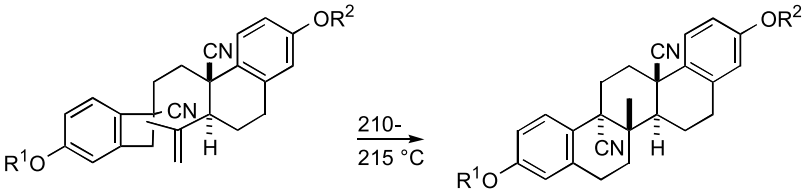
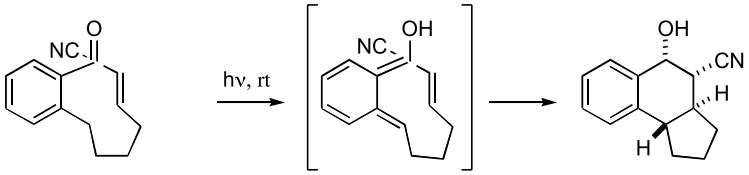
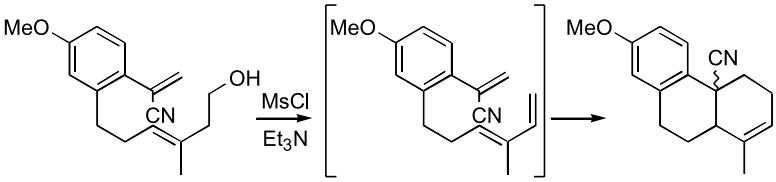
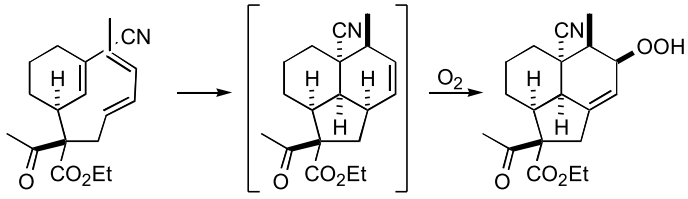
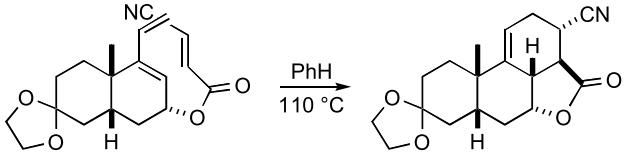
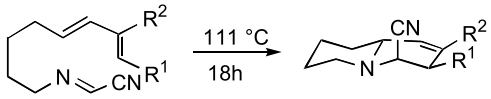
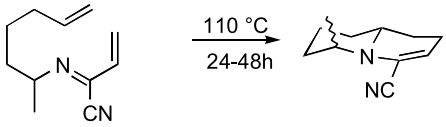
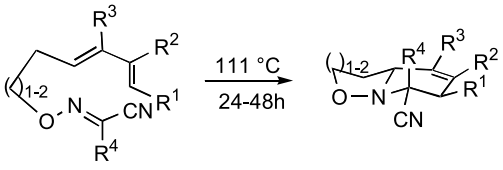
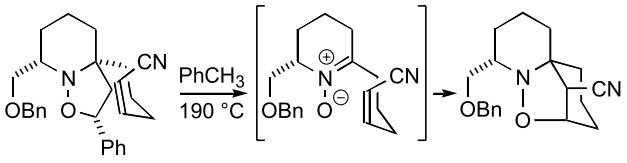
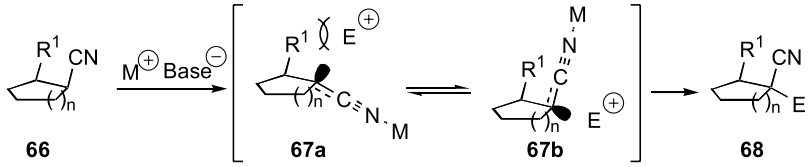
Entry	Reaction	Yield (%)
1		43–90[196]
2		70–92[197]
3		58–60[198]
4		78[199]
5		61[200]
6		—[201]
7		55[202]
8		67[12]
9		86–94[203]
10		35–74[204]

Table 4 (*continued*)

Entry	Reaction	Yield (%)
11		80[205]



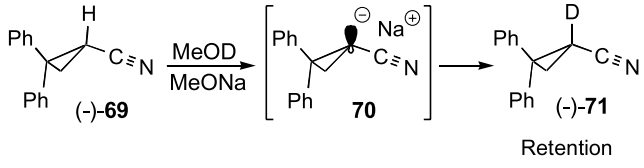
Scheme 13. Stereoselective alkylations of cyclic nitriles.

4. Alkylations of cyclic nitriles

4.1. Stereoselective alkylations of alkanenitriles

Metallated nitriles are powerful nucleophiles capable of forming new C–C bonds in sterically demanding alkylations. In fact, alkylation of cyclic metallated nitriles necessarily installs a new quaternary center. Installation of hindered stereocenters is facilitated by the small size of the nitrile group that readily occupies the sterically most hindered position, eclipsing adjacent substituents (Scheme 13, **67b**), to provide the sterically most accessible trajectory for the incoming electrophile. The focus of this section is the highly unusual stereoselectivity of cyclic, metallated nitrile alkylations attending formation of quaternary stereocenters.

Intriguing stereoselectivity with cyclic metalated nitriles was first demonstrated in pioneering deuterations of cyclopropanecarbonitrile **69** (Scheme 14).[241] The remarkable 99.9% stereochemical retention demonstrates the intermediacy of a chiral metallated nitrile **70** which is most likely a contact ion pair that rapidly abstracts deuterium from the adjacent solvent shell upon formation.



Scheme 14. Retentive deuteration of a metallated nitrile.

Despite the pioneering alkylation of **69** only one diastereoselective alkylation of a cyclopropanecarbonitrile has appeared (Table 6, entry 1). Stereochemical inversion of the nitrile-bearing carbon reflects the facile inversion[242] of the metalated nitrile, allowing installation of the electrophile opposite to the aromatic substituent. Few stereoselective alkylations of cyclic 5-membered nitriles are known (Table 6, entries 2 and 3). In each instance the electrophile approaches from the more accessible convex face, concomitantly installing the small nitrile group on the more sterically demanding concave face (cf. Scheme 13). Forming the particularly hindered, contiguous quaternary–quaternary stereocenters in the triquinane nitrile (Table 6, entry 3) was a strategic step in the synthesis of (±) laurenene.[209]

Deprotonating sterically hindered nitriles is often challenging with LDA.[134] For example, during the synthesis of palauolide,[93,256] LDA was found to deprotonate the β–nitrile but not the α-epimer unless HMPA was added (Table 6, entry 14). Nitriles that are resistant to deprotonation[254] with LDA–HMPA combinations are often successfully deprotonated with the base prepared from *t*-BuOK, *i*-Pr_2NH, and BuLi (KDA)[257] (Table 6, entry 14).

Pioneering alkylations with the conformationally constrained nitrile **72**[245] established a modest preference for equatorial alkylation in 6-membered carbonitriles (Scheme 15, **72**→**74**). Deprotonating **72** with $LiNEt_2$ most probably generates the planar, *N*-lithiated[258] nitrile **73**[259] that modestly disfavors an electrophilic approach from the axial direction by virtue of the steric interactions with the axial protons. Alkylation of a planar, *N*-lithiated nitrile directly correlates with very similar methylation selectivities of the corresponding planar enolate (5.7:1).[245] Analogous levels of stereoselectivity are obtained in alkylations of constrained, unhindered nitriles (Table 6, entries 4–6) although the stereoselectivity is increased with sterically more demanding electrophiles (Table 6, entry 7),

Alkylations of cyclohexanecarbonitriles with increased steric demand on the β-face, often from axial methyl substituents, occur almost exclusively from the equatorial direction, a stereoselectivity preference that has been extensively employed in terpenoid syntheses (Table 6, entries 8–14). A single example of a conformationally locked cyclohexenecarbonitrile with an axial α-substituent alkylates exclusively from the axial direction (Table 6, entry 15), implying an increased steric interaction between the axial methine group and the incoming electrophile in an equatorial trajectory.

Preferential equatorial alkylations of sterically unbiased cyclohexanecarbonitriles, by metal amide deprotonation–alkylation, are consistent with planar, *N*-metallated nitrile intermediates. In direct contrast, sequential halogen–

Table 5. Alkenenitrile syntheses

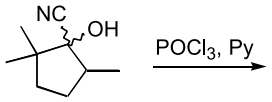
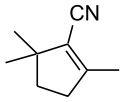
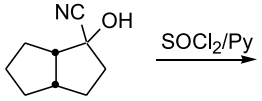
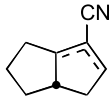
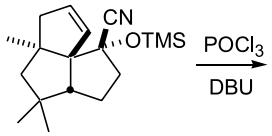
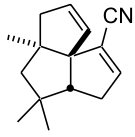
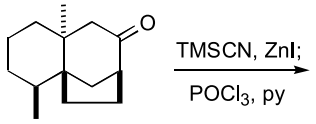
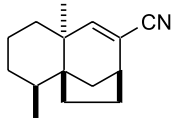
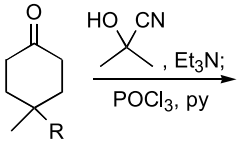
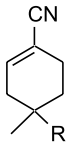
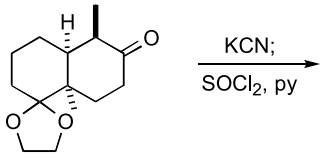
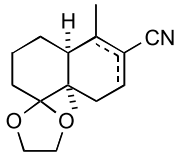
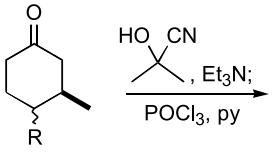
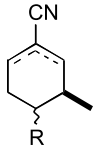
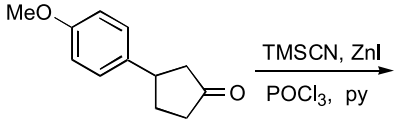
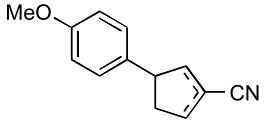
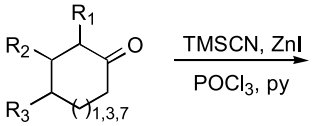
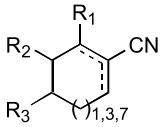
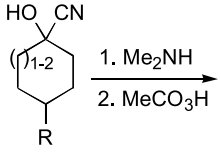
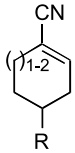
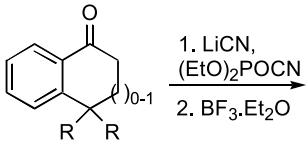
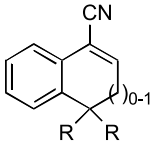
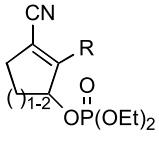
Entry	Reaction	Cyclic alkenenitrile	Yield (%)
1			57–76[207]
2			56[208]
3			92[209]
4			88[210]
5			80[211]
6			70[212]
7			52–89[213]
8			96[214]
9			79–93[215]
10			38–56[216]
11			61–94[217]
12			51–81[218]

Table 5 (*continued*)

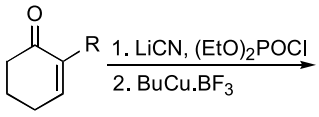
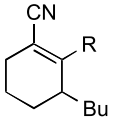
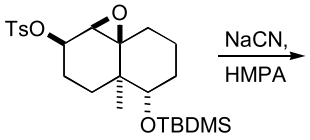
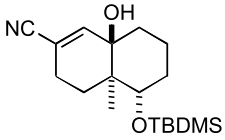
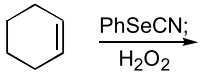
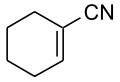
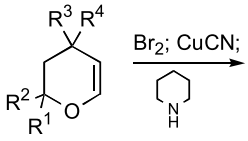
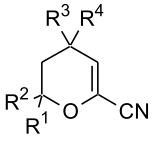
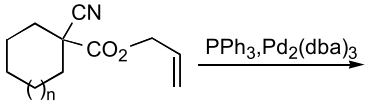
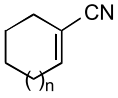
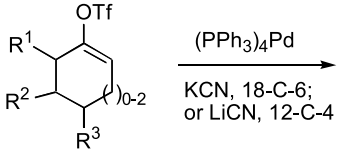
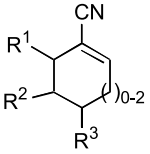
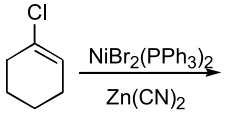
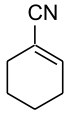
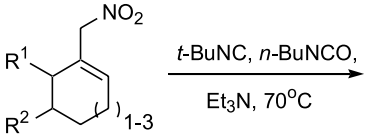
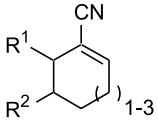
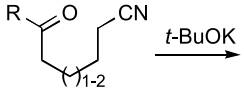
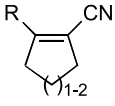
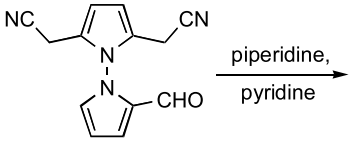
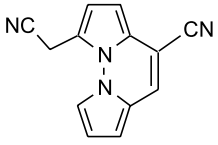
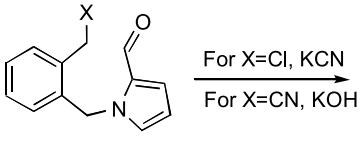
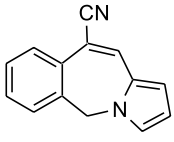
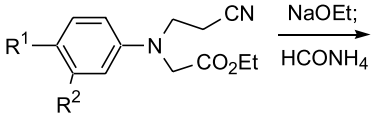
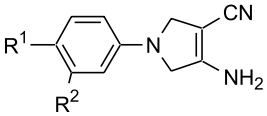
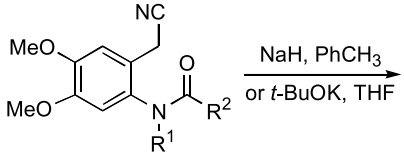
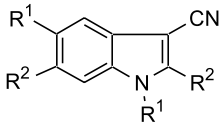
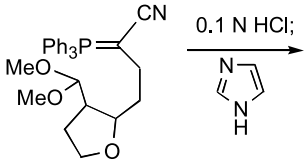
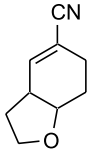
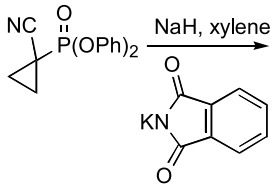
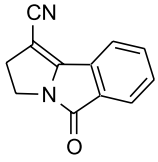
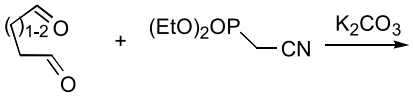
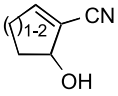
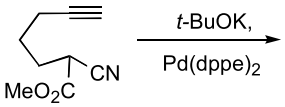
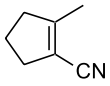
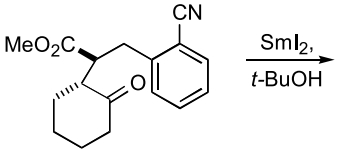
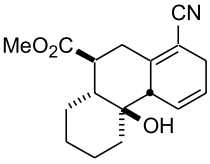
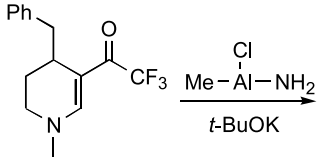
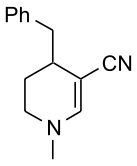
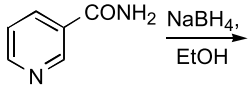
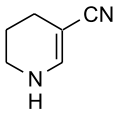
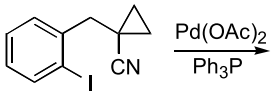
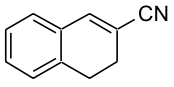
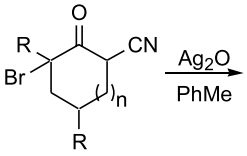
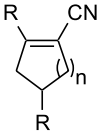
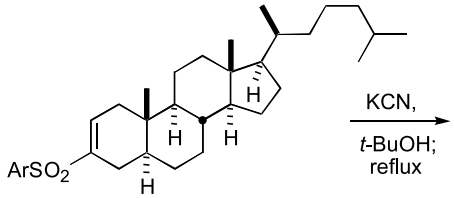
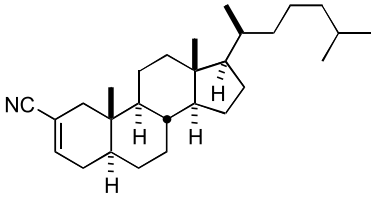
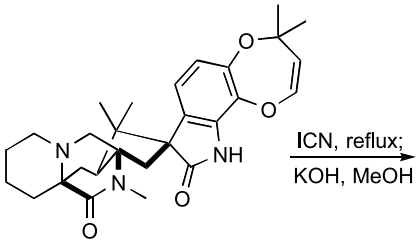
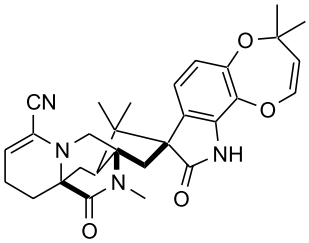
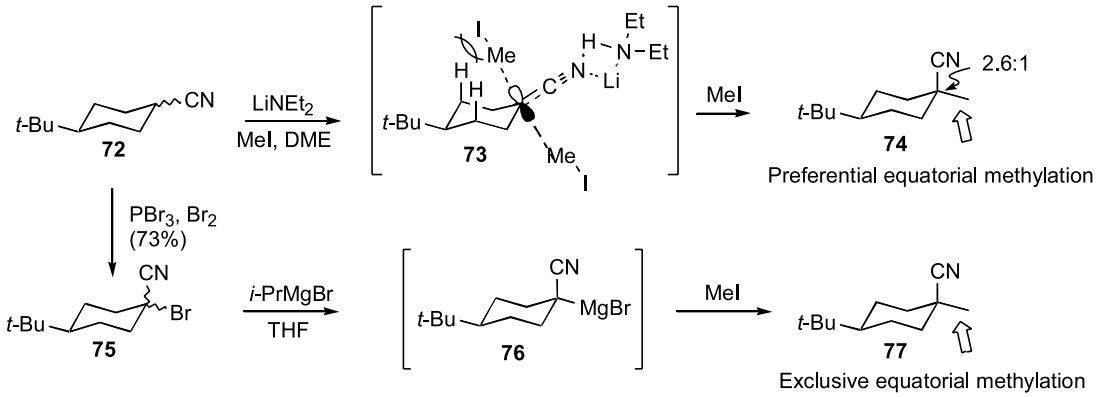
Entry	Reaction	Cyclic alkenenitrile	Yield (%)
13			73–78[219]
14			79[220]
15			89[221]
16			63–93[222]
17			78–81[223]
18			59–89[224]
19			92[225]
20			57–82[226]
21			60–79[123]
22			80[227]
23			44–88[228]
24			50[229]
25			61–90[230]

Table 5 (*continued*)

Entry	Reaction	Cyclic alkenenitrile	Yield (%)
26			71[231]
27			87[232]
28			68–81[233]
29			86[234]
30			60[235]
31			91[106]
32			42[236]
33			58[237]
34			34–69[238]
35			60[239]
36			81[240]

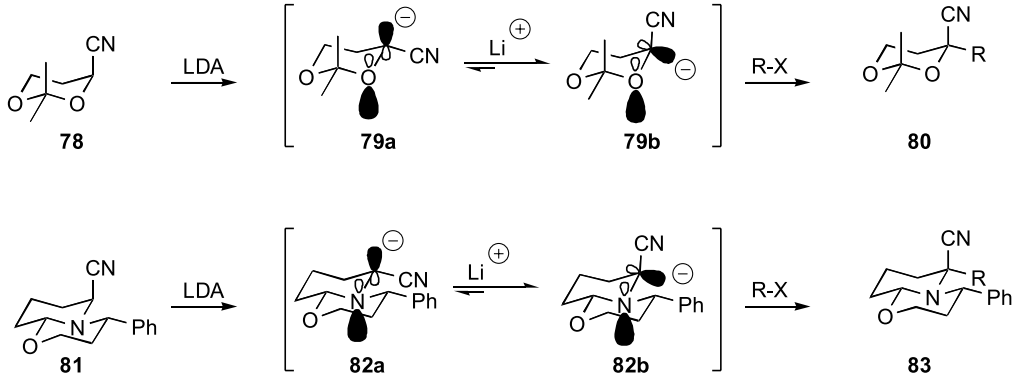


Scheme 15. Stereoselective alkylation of *C*- and *N*-metallated nitriles.

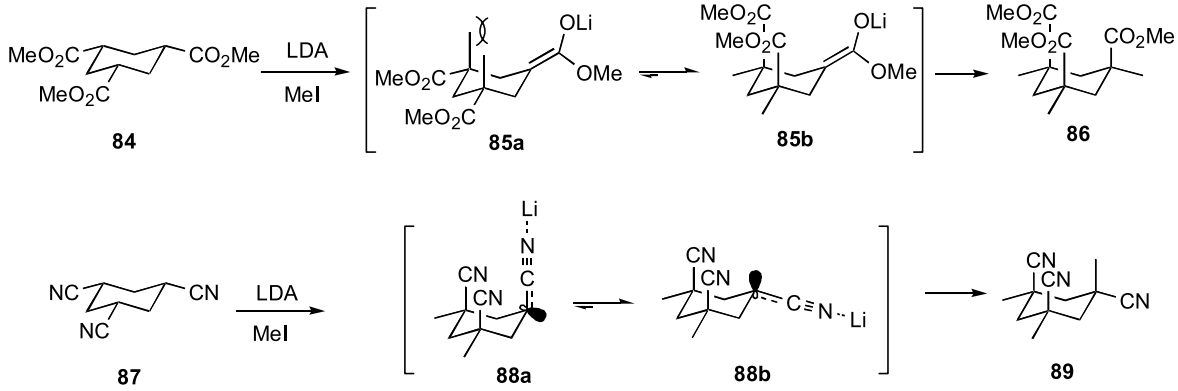
magnesium exchange and methylation (Scheme 15, **75**→**77**)[190] affords exclusively the equatorially alkylated nitrile **77**, which requires a distinctly different metallated nitrile. Halogen–magnesium exchange most likely generates the *C*-metallated nitrile **76** which rapidly equilibrates since a mixture of bromonitriles converge to a single methylated nitrile **77**.[242]

Alkylations of sterically unbiased cyano-1,3-dioxanes **78**[260] and α-aminonitriles **81**[261] are extremely stereoselective (Scheme 16). Cyano-1,3-dioxanes selectively alkylate a broad range of electrophiles from the equatorial direction (**78**→**80**) which could arise from steric interactions between the incoming electrophile and the axial methyl group in the acetal or by stereoelectronic control. Collectively, the stereoselective alkylations of **78** and **81**, with minimal steric bias, correlate with stereoelectronically controlled alkylations of the pyramidal nitrile anions **79b** and **82b** which avoid the anti-anomeric destabilization present in **79a** and **82a**.

The disparate alkylation stereoselectivities of nitriles and carbonyl compounds are encapsulated in the comparative alkylations of trisubstituted cyclohexanes (Scheme 17).[262] Exhaustive methylation of the tri-ester **84** affords the all *cis*-ester **86** through three consecutive equatorial alkylations whereas the corresponding nitrile **87** affords **89** in which the final methylation occurs exclusively from the axial



Scheme 16. Stereoelectronically controlled nitrile anion alkylations.



Scheme 17. Stereodivergent nitrile and ester alkylations.

Table 6. Stereoselective alkylation of cyclic alkanenitriles

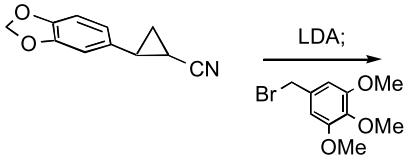
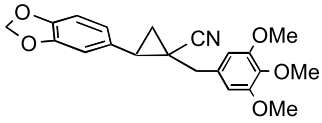
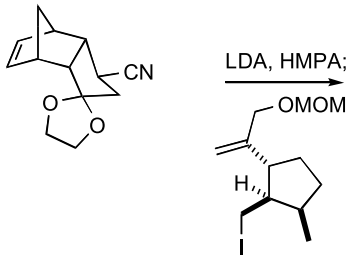
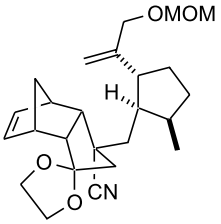
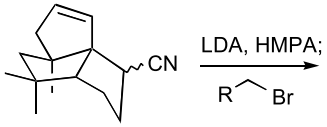
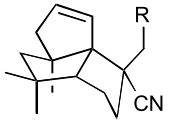
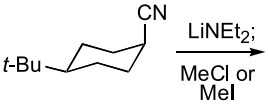
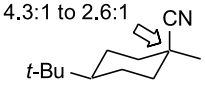
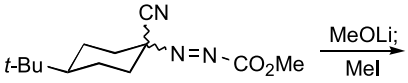
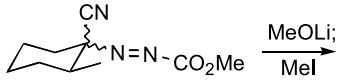
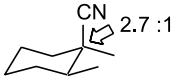
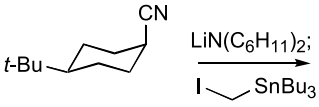
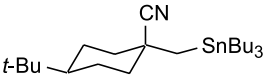
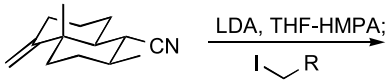
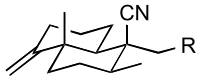
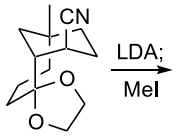
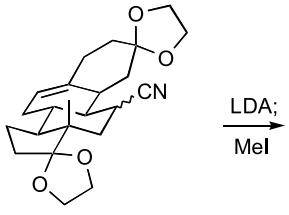
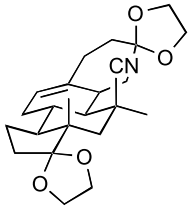
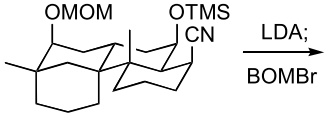
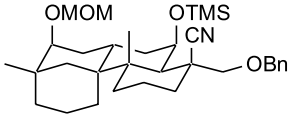
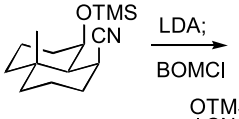
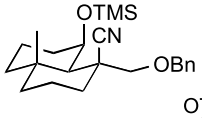
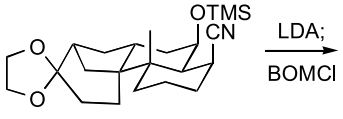
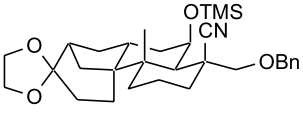
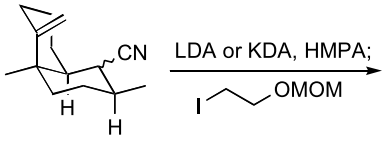
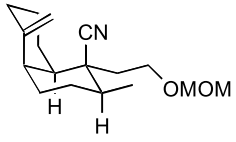
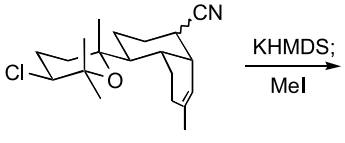
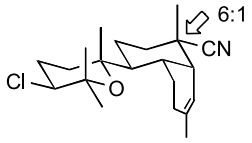
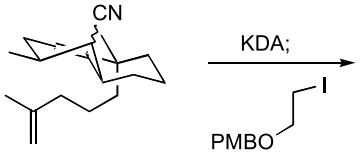
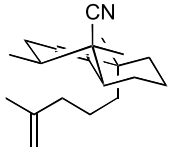
Entry	Reaction	Product	Yield (%)
1			74[243]
2			55–67[244]
3			62–83[209]
4			82–96[245]
5			84[246]
6			63[246]
7			83[247]
8			97[248,93]
9			80[249]
10			90[250]
11			88[251]
12			91[252]
13			89[253]

Table 6 (*continued*)

Entry	Reaction	Product	Yield (%)
14			79–93[254]
15			83[255]
16			88[110]

direction. Alkylation of the most stable ester enolate conformer **85b** preferentially occurs from an equatorial trajectory whereas the pyramidal, metallated nitrile **88** can readily access the two conformations **88a** and **88b**, because of the small size of the nitrile group. Alkylation with small electrophiles, such as MeI, occurs from the axial direction to afford **89** whereas addition of a large electrophile, such as Ph_2PCl, experiences a severe steric interaction with the axial nitriles and therefore alkylates exclusively from the equatorial direction of **88a**.

4.2. Alkylations of dianionic nitriles

Nitriles containing additional protic functionality are smoothly deprotonated with excess base to afford formal dianions. Double deprotonation of this type was first demonstrated with LDA and β-ketonitriles,[265] at −70 °C, which allows enolate formation without expulsion of cyanide prior to a second deprotonation adjacent to the nitrile (Scheme 18). Monoalkylation of these dimetallated nitriles is highly selective, with no double alkylation even in the presence of excess electrophile (Table 7). The greater reactivity of the metallated nitrile correlates with the significantly higher pK_a of nitriles as compared to ketones ($\Delta pK_a = 5–10$).[263] The remarkable regioselectivity in this alkylation has been harnessed in a route to bruceantin in which the metallated nitrile controls formation of two stereocenters[264] during installation of contiguous quaternary–tertiary stereocenters (Table 7, entries 3 and 4).

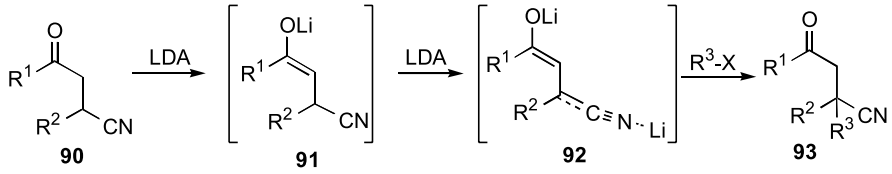
The double deprotonation of hydroxynitriles generates particularly reactive metallated nitriles that selective cyclize to *trans*-decalins or reprotonate to provide a means for equilibrating the nitrile-bearing carbon (Table 7, entries 6 and 7).

4.3. Alkylations with sp^2-hybridized electrophiles

Nucleophilic additions of enolates to aryl- or vinyl halides are particularly challenging.[269] Metallated nitriles are particularly well-suited for alkylation with sp^2-hybridized electrophiles because nitriles tolerate alkyllithium and Grignard reagents (Section 2.4) and are not prone to self-condensation, traits that have been exploited in benzyne-based α-arylations.[196] Recently, a mechanistically distinct α-arylation of cyclic nitriles was achieved with fluorine-substituted aromatic electrophiles (Table 8, entries 1 and 2). The high nucleophilicity of metallated nitriles has been exploited in the direct addition to activated alkenes (Table 8, entries 3 and 4, see also Table 1, Section 3.1) and to aryl halides with palladium catalysis (Table 8, entries 5–7).

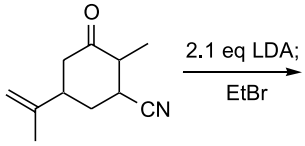
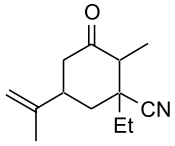
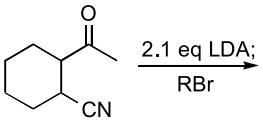
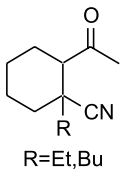
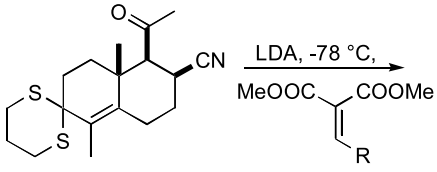
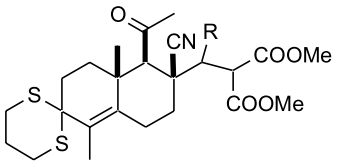
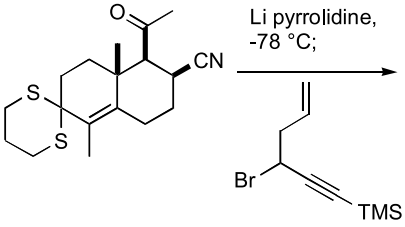
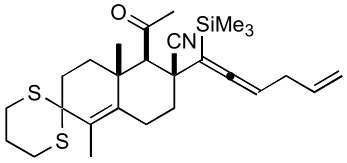
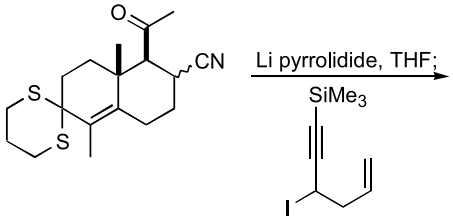
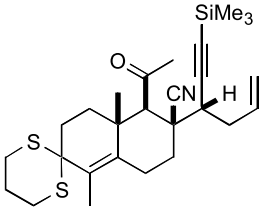
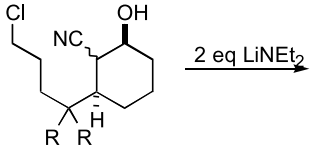
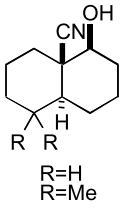
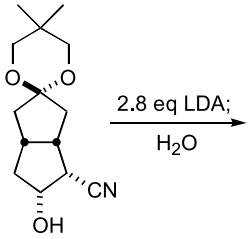
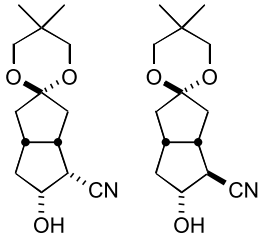
4.4. Alkylations of oxonitriles

Oxonitrile alkylations were first developed during the emergence of modern synthesis. The high acidity of cyclic oxonitriles allows deprotonation with metal hydroxides,[276] although many alkylations have employed stronger bases, presumably for convenience (Table 9). Soft alkyl halides



Scheme 18. Alkylations of doubly metallated ketonitriles.

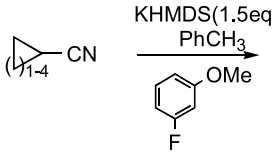
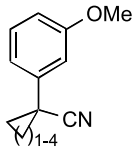
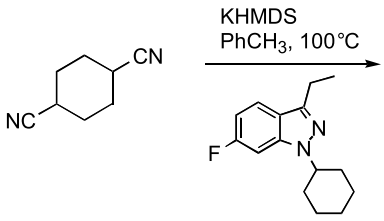
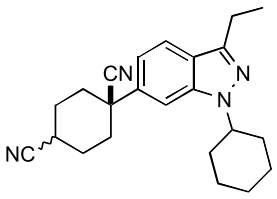
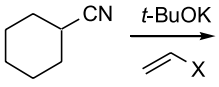
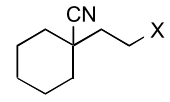
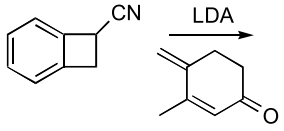
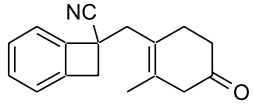
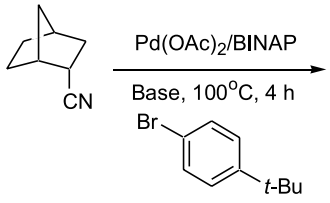
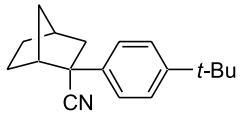
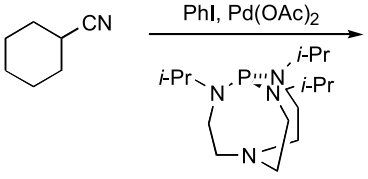
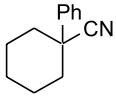
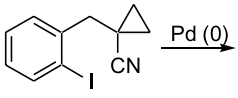
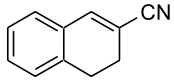
Table 7. Alkylations of dianionic nitriles

Entry	Reaction	Product	Yield (%)
1			74[265]
2			85–86[265]
3			49–77[264]
4			80[264]
5			93[266]
6			78–85[267]
7			90 (1:1)[268]

selectively alkylate on carbon even in the presence of electrophilic esters (Table 9, entry 3). Allylic halides alkylate at the sterically less congested carbon through direct S_N2 displacement (Table 9, entries 4 and 5) although the regioselectivity can be reversed by ruthenium catalysis (Table 9, entry 6).

Alkylation of the ambident nucleophile may occur on oxygen or carbon, though for most endeavors *C*-alkylation is preferable. A clever exception was *O*-alkylation of an allylic electrophile to generate an alkyl vinyl ether en route to a stereoselective Claisen rearrangement (Table 9, entry 7). An analogous *O*-alkylation-Claisen rearrangement is likely in the high-temperature alkylations with allyl and allenyl ethers, although no detailed mechanistic studies have been undertaken (Table 9, entries 8–10).

Table 8. Nitrile alkylations with sp^2-hybridized electrophiles

Entry	Reaction	Nitrile	Yield (%)
1			47–90[270]
2			77[271]
3		 X=Ph, $SiPh_3$, SPh	75–91[272]
4			81[273]
5			69[274]
6			81[275]
7			58[237]

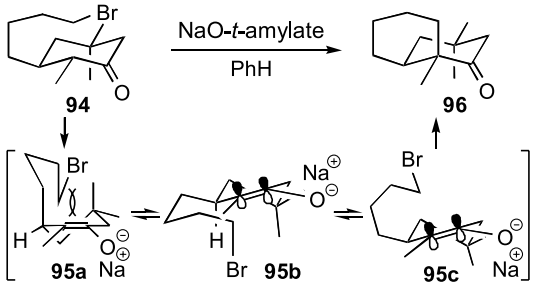
Enantioselective alkylation can be achieved by converting oxonitriles to a proline-derived hydrazone that alkylates with exceptional diastereoselectivity (Table 9, entry 11).

Oxonitriles are excellent nucleophiles for conjugate additions and have been used extensively for Robinson annulations (Table 9, entries 12–18). Detailed mechanistic studies indicate that the initial oxonitrile alkylation occurs from the axial direction, followed by intramolecular aldol cyclization.[290]

5. Stereoselective cyclizations

5.1. Alkanenitrile cyclizations

Cyclic nitriles and ketones cyclize with very different stereoselectivities, reflecting the fundamental stabilization differences between metallated nitriles and enolates.[21a] Cyclization of the ketone **94** to the *cis*-decalin **96** cleverly identified a requirement for orienting of the nucleophilic enolate π orbital[292] directly toward the electrophilic



Scheme 19. *cis*-Selective enolate cyclizations.

Table 9. Oxonitrile alkylations

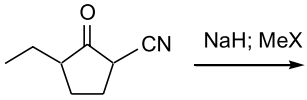
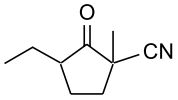
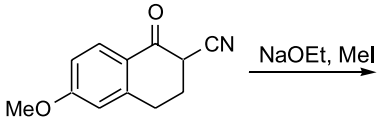
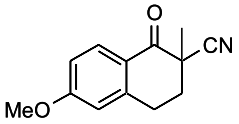
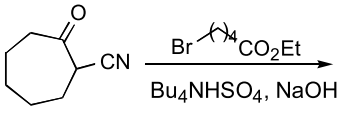
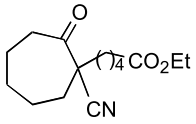
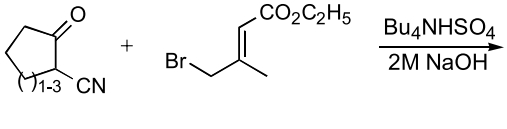
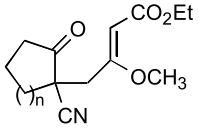
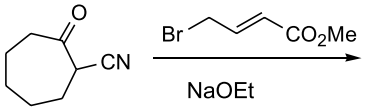
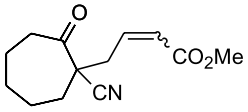
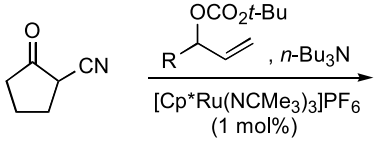
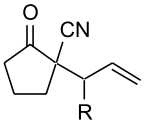
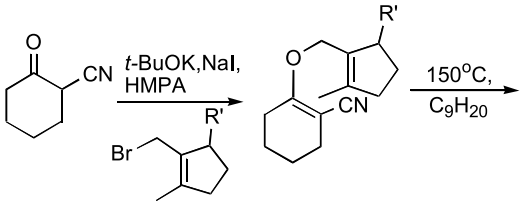
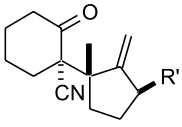
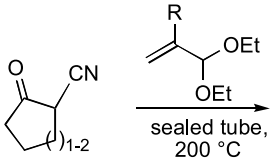
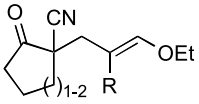
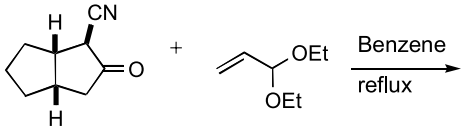
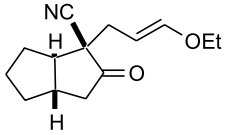
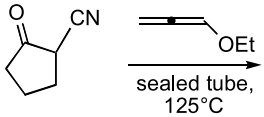
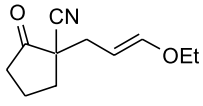
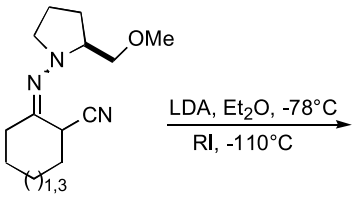
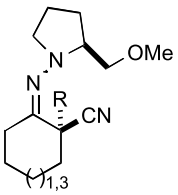
Entry	Reaction	Product	Yield (%)
1			74[277]
2			80–85[278]
3			62[279]
4			80–82[280]
5			87[279]
6			85–98[281]
7			44–79[282]
8			71–81[283]
9			68–71[284]
10			70[283]
11			52–80[285]

Table 9 (*continued*)

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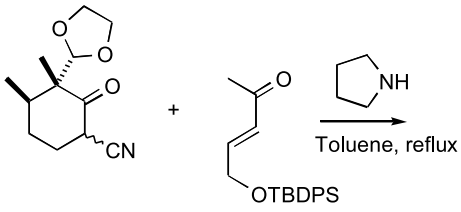
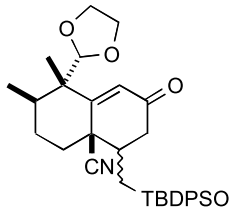
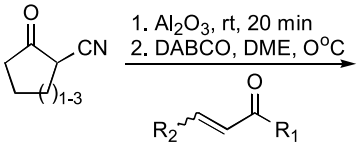
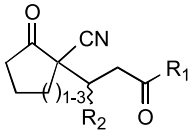
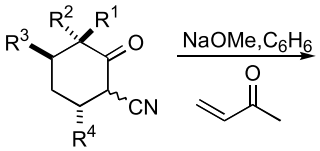
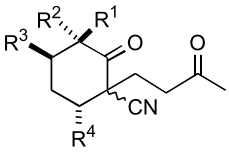
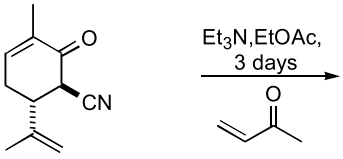
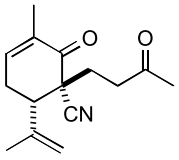
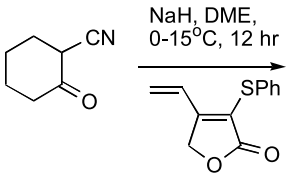
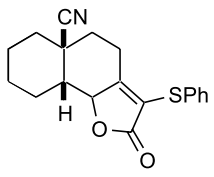
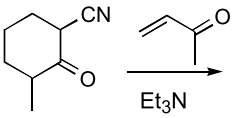
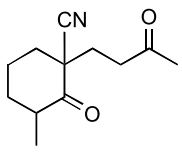
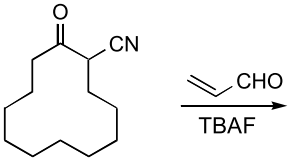
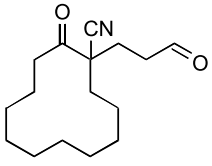
12			65[286]
13			74–98[287]
14			76–95[288]
15			78[287]
16			53[289]
17			94 (5:1)[290]
18			39–72[291]

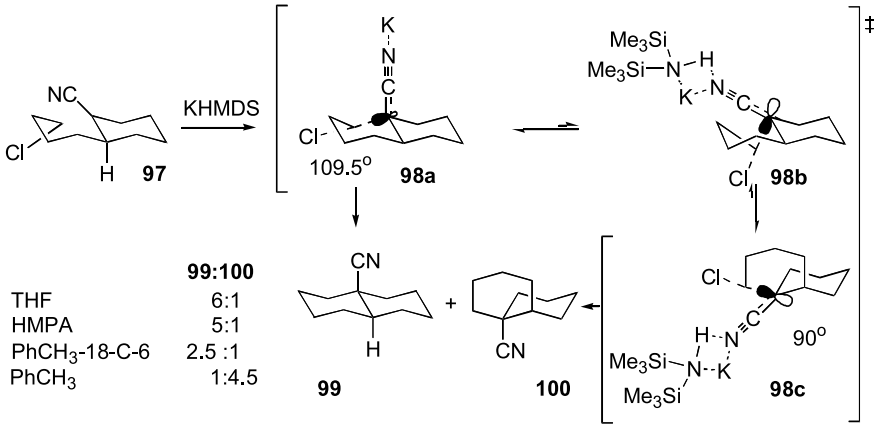
carbon–bromine bond for an S_N2 displacement through conformer **95c** (Scheme 19).

The contrasting preference of metallated nitriles to cyclize to *trans*-decalins was first identified more than 30 years ago[293] and has been analyzed in detail for the parent decalin **97** (Scheme 20). The stereoselectivity in THF correlates with cyclization through a pyramidal transition state **98a** in which the metallated nitrile directly orients the pyramidal, nucleophilic orbital toward the electrophilic chloromethylene carbon. Changing the solvent from THF to toluene reverses the stereoselectivity by generating a planar, amine-complexed, metallated nitrile. Subsequent cyclization through transition state **98c** occurs in order to relieve twisting of the electrophilic tether induced in **98b** that accompanies the change in geometry of the metallated nitrile from pyramidal to planar. Disrupting the complexation through the addition of 18-crown-6 restores the preference for the *trans*-decalin **99**, though the selectivity is not as high as in the better donor solvent THF (Scheme 20).

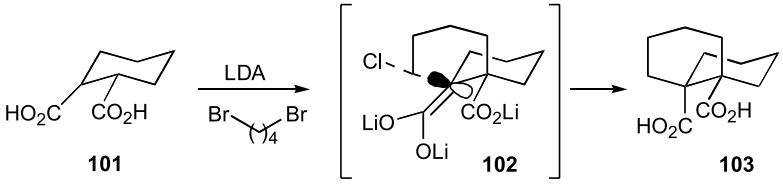
Preferential cyclization of the planar metallated nitrile **98c** to *cis*-decalin **99**, occurs with the same stereochemical sense as the cyclization of the planar enolate **102** (Scheme 21).[294] Deprotonating the diacid **101**, in THF, affords exclusively the *cis*-decalin **103** through an exocyclic enolate cyclization that closely parallels cyclization of the exocyclic metallated nitrile **98c** to *cis*-decalin **100** (Scheme 20).

5.2. Oxonitrile cyclizations

Oxonitrile cyclizations are particularly attractive since the opportunity exists for selective cyclization to *cis*- or *trans*-



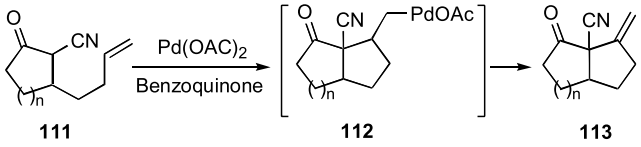
Scheme 20. Stereodivergent cyclizations of metallated nitriles.



Scheme 21. Cyclization of diacids to *cis*-decalins.

decalins from the same precursor. Cyclization of the oxonitrile enolate **105** generates exclusively the *cis*-decalin **106** (Scheme 22). Reduction of **104** to the corresponding β-hydroxy nitrile **107**, and lithium diethylamide-induced cyclization, gives exclusively the *trans*-decalin **109** that was oxidized to the *trans*-decalone **110**, diastereomeric to **106**. The stereodivergent cyclizations stem from trajectory differences enforced by the planar enolate **105** and the pyramidal, metallated nitrile **108**. The selective cyclization of the same nitrile precursor **104** to both *cis*- and *trans*-decalins highlights the complementarity of oxonitrile enolate and metallated nitrile cyclizations.

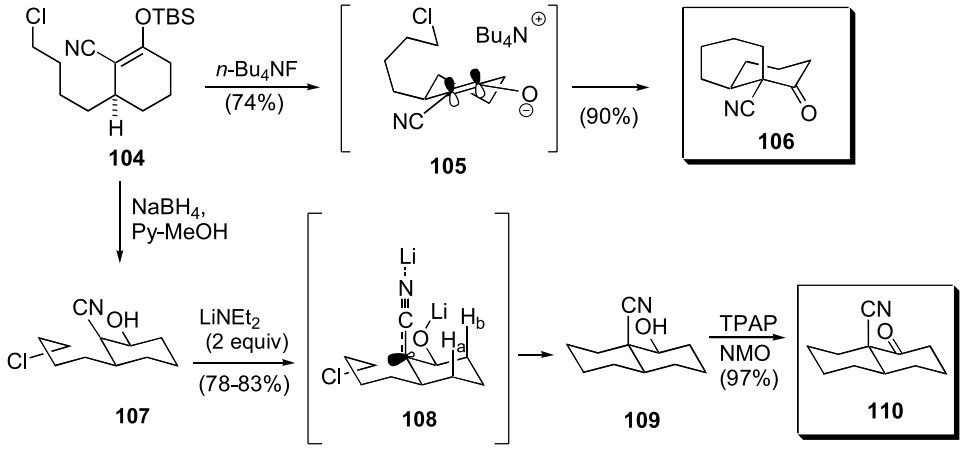
Oxonitriles bearing a pendant δ-alkene are readily cyclized in an efficient oxidative cyclization (Scheme 23).[295] Facile enolization followed by an intramolecular Heck reaction generates **112** that β-eliminates hydride to afford the bicyclic oxonitrile **113**.



Scheme 23. Oxonitrile cyclization.

6. Decyanation of cyclic nitriles

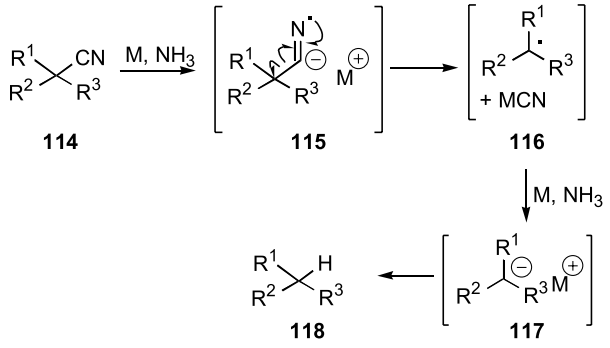
The versatility of nitrile-based alkylations is enhanced through the facile removal of the nitrile group by several complementary methods. Decyanation can be tuned to selectively give alkanes, alkenes, or, in instances where the nitrile is flanked by an adjacent electron-withdrawing group, a reactive nucleophile for subsequent alkylation.



Scheme 22. Stereodivergent oxonitrile cyclizations.

6.1. Decyanation to alkanes

Decyanation is most frequently achieved by dissolving metal reduction. The reaction is usually most efficient in the absence of a proton source, exhibiting a modest dependence on the reducing ability of the metal–ammonia combination, generally with increasing yields in the series Li, Na, K (Chart 4, methods A–E). Nitrile reduction proceeds through a series of electron transfers initially generating the radical anion intermediate **115** that ejects cyanide with concomitant formation of radical **116**. Further reduction of **116** affords the carbanion **117** which is ultimately protonated to give **118** (Scheme 24).



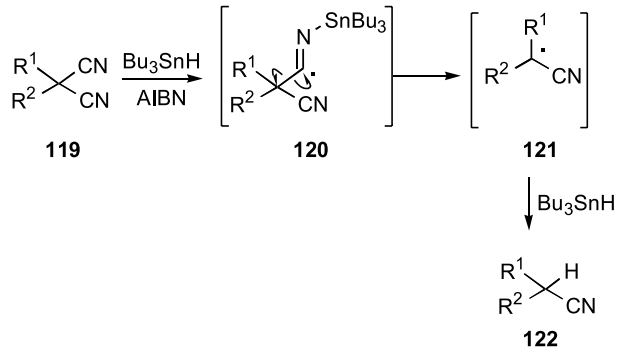
Scheme 24. Dissolving metal decyanation mechanism.

Decyanation by electrochemical reduction has the attendant advantage of affording hydrocarbons in 60–80% yield, even with secondary nitriles that generally react poorly with metal–ammonia combinations (Chart 4, method F). Alternatively, the use of K in HMPA or potassium and dicyclohexyl-18-C-6 in toluene (Chart 4, methods D and E, respectively) is effective for the decyanation of primary and secondary nitriles. Mechanistically distinct from the metal reduction and electrolysis reactions is the reduction with $Fe(acac)_3$–Na that is postulated to involve oxidative addition of reduced iron into the C–CN bond followed by proton abstraction from the ligand (Chart 4, method G). Despite the attractiveness of decyanation, decyanation of cyclic nitriles has only once been used in synthesis: electrolysis was used to trigger reduction and decyanation in 45% yield en route to hirsutene (Chart 4, line 1, third entry).

Cyclic dinitriles are readily reduced under mild conditions with Bu_3SnH (Chart 4, method I). Mechanistically the reaction is similar to the dissolving metal decyanation. Bu_3SnH is proposed to generate a metalloimine radical **120** (Scheme 25) that fragments to a nitrile stabilized radical **121** that is further reduced to the cyclic nitrile **122**. No further reduction occurs, providing a selective method for removing one nitrile group in cyclic dinitriles.

6.2. Decyanation of cyclic nitriles to alkenes

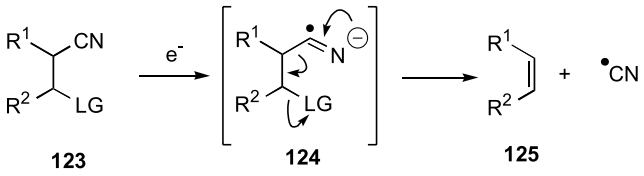
Nitriles exhibit pseudohalide reactivity in being eliminated through loss of HCN. The elimination of cyanide is commonly encountered in the reversible formation of ketones from cyanohydrins (Table 10, entry 1). Elimination of HCN to give alkenes generally requires a carbonyl or



Scheme 25. Bu_3SnH decyanation mechanism.

olefinic group adjacent to the proton being abstracted, allowing elimination with modest bases *t*-BuOK, KOH, triethylamine, and DBU (Table 10, entries 2–5). A related elimination of β-trimethylstannyl nitriles is triggered by addition of MeLi. Formation of a stannate intermediate, upon addition of MeLi, likely triggers subsequent cyanide expulsion and simultaneously installation of a methylene group (Table 10, entry 6).

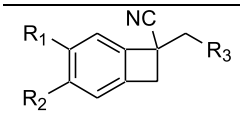
The reduction of nitriles containing β-leaving groups constitutes a mechanistically distinct decyanation to alkenes. Addition of an electron to the nitrile generates a radical anion **124** that ejects an adjacent leaving group to form an alkene and a CN radical that is further reduced (Scheme 26). Strong reducing agents such as lithium or sodium efficiently reduce a variety of nitriles to the corresponding alkenes (Table 10, entries 8–10). The reaction has been comprehensively examined for the elimination of β-mesyloxy nitriles to afford an array of alkenes (Table 10, entry 8).



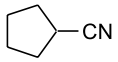
Scheme 26. Reductive decyanation to alkenes.

6.3. Sequential decyanation–alkylation

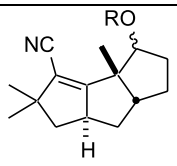
Reductive decyanation generates an anion for potential interception by electrophiles. Direct alkylation of the organolithium intermediates is rare (Table 11, entry 1), although intramolecular cyclization of the organolithium species or nascent radical is very efficient and demonstrates the potential for this chemistry (Table 11, entry 2). The decyanation of ketonitriles readily generates reactive enolates (Table 11, entries 3–7) and dienolates (Table 11, entries 8 and 9) that are stereoselectively alkylated by a range of electrophiles. Historically, most decyanations have employed lithium naphthalenide. The recent use of SmI_2 (Table 11, entry 6) represents a significant advance in being particularly mild and ideally suited to use in total synthesis where the decyanation of highly functionalized intermediates is required.



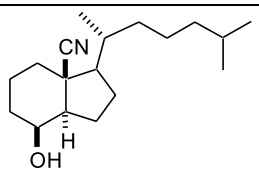
A. 80-96%[296]



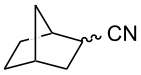
G. 77%[297]



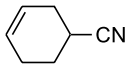
B.[b] 28%[298] F. 45%[299]



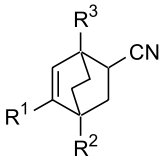
D. 76%[300]



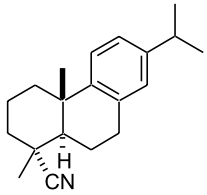
F. 72%[299]



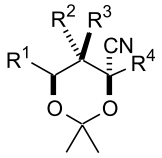
G. 80%[297]



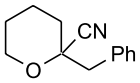
B. 26-97%[301]



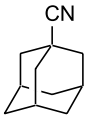
A.95%[302] F. 60-80%[303]



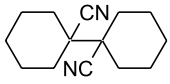
A. 63-95%[301]



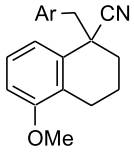
F. 57%[299]



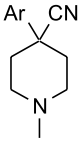
E. quant[301], F.73%[304]



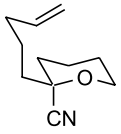
A.95%,[303] C. 80%,[297,303] F.70%,[303] G.76%[297]



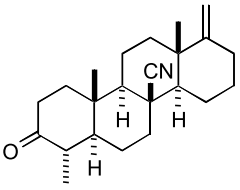
I. 33-100%[305]



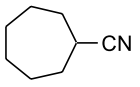
H. 75%[306]



B. 76%[307]

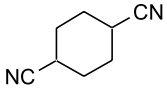


B. 91%[43]

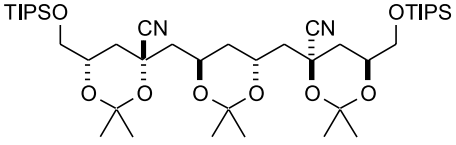


E.60-80%[303]

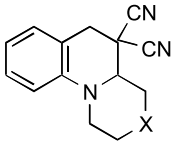
D. 70%[308]



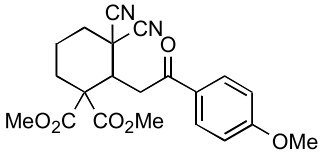
C. 49%[309]



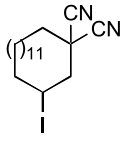
B. 69%[310]



I. 75-99%[311]



I. 59%[312]



I. 54%[313]

[a]A. Na, NH_3; B. Li, NH_3; C. Li, $EtNH_2$; D. K, HMPA; E. K, PhMe, Dicyclohexyl-18-c-6; F. electrolysis; G. $Fe(acac)_3$, Na; H. Na, ROH; I. Bu_3SnH, AIBN. [b]Conjugate reduction accompanies decyanation.

Chart 4. Decyanation of cyclic nitriles.[a]

Table 10. Decyanation generating cyclic alkenes

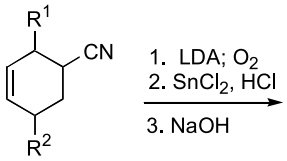
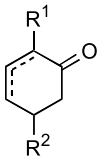
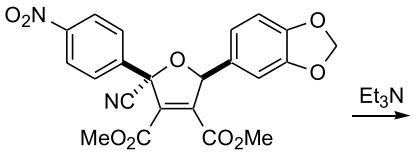
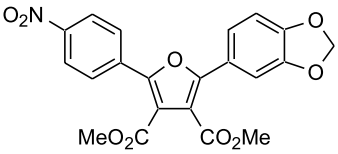
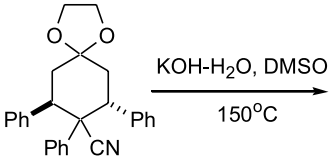
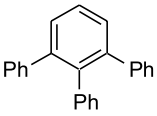
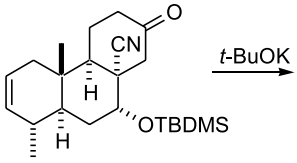
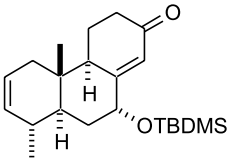
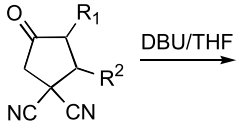
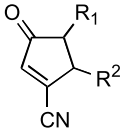
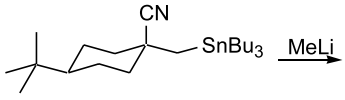
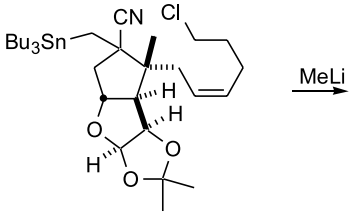
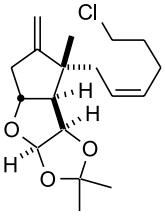
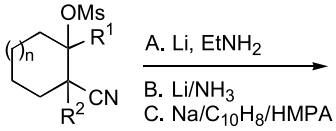
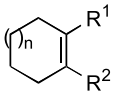
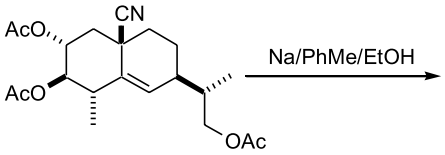
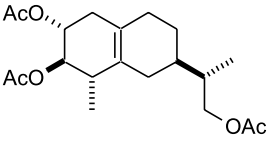
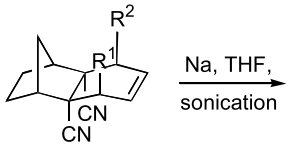
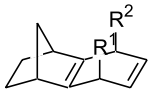
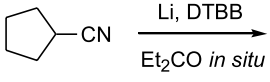
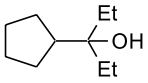
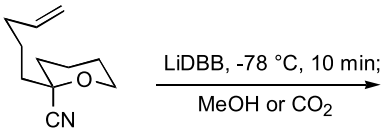
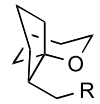
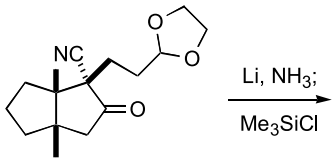
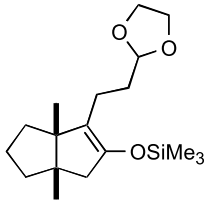
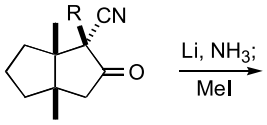
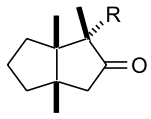
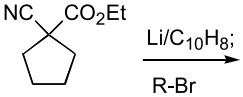
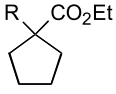
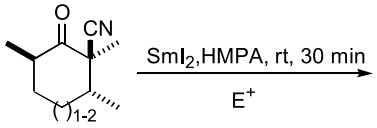
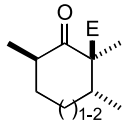
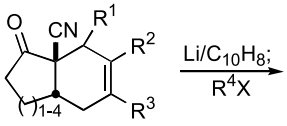
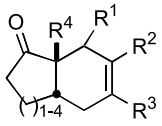
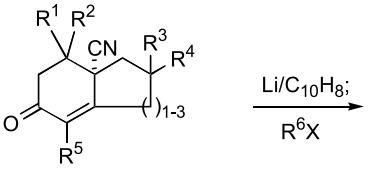
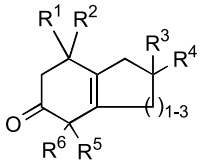
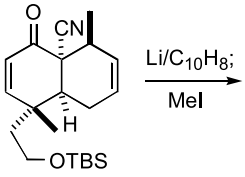
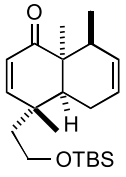
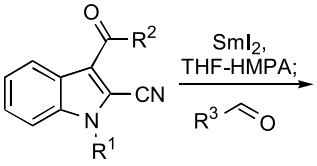
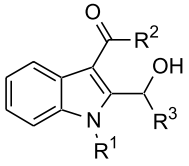
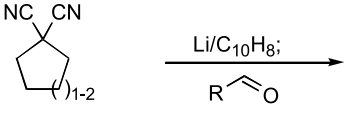
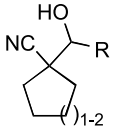
Entry	Reaction	Alkene	Yield (%)
1			56–65[314]
2			81[315]
3			44[316]
4			57[317]
5			82–87[318]
6			64[247]
7			98[247]
8			30–99[319]
9			71[107]
10			30[320]

Table 11. Decyanation–alkylation of cyclic nitriles

Entry	Reaction	Products	Yield (%)
1			47[321]
2			65–78[307]
3			86[322]
4			40–86[323]
5			67–71[324]
6			60–80[325]
7			60–80[155c]
8			41–99[287]
9			76[155a]
10			51–85[326]
11			65–78[327]

7. New directions

Cyclic nitriles are versatile chameleons that tolerate a diverse array of organometallic reagents and yet, under more forcing conditions, are readily converted to a plethora of functional derivatives. Complementing the nitrile's modest electrophilicity is the powerful nucleophilicity upon deprotonation. Alkylations of cyclic nitriles efficiently install highly hindered quaternary stereocenters with excellent stereoselectivity with distinct, and tunable, reactivity differences emerging between C-metallated nitriles and the more common *N*-metallated nitriles. By controlling the metal coordination site, the alkylation stereoselectivity of a single cyclic nitrile can be controlled, providing diastereomeric *R* and *S* quaternary nitriles, even at the crucial ring junction.

The compact electron density of the rod-like CN unit confers unique properties to cyclic nitriles. As the smallest carbon-based electron-withdrawing group, nitriles have a minimal steric demand, resulting in stereoselectivity preferences that are complementary to those of the significantly larger carbonyl-based electron-withdrawing groups. The combined steric and electronic features of nitriles mimic the properties of hydroxyl groups, providing a robust hydroxyl surrogate for drug design. In fact, the small steric demand and high electron withdrawal have historically made nitriles ideal mechanistic probes. Recent cycloaddition and electrocyclization reactions of nitriles suggest that their unique directing effects can be harnessed in fundamentally new ways. The combination of high stereocontrol, complementary reactivity to carbonyl compounds, and post-alkylation versatility bodes well for the continued development of cyclic nitriles as versatile synthetic intermediates.

Acknowledgements

Drs. Paul Carlier, Robert Grossman, and Gary Posner are gratefully acknowledged for insightful comments on a draft manuscript.

References and notes

1. Rappoport, Z. *The Chemistry of Cyano Group*; Wiley-Interscience: New York, 1970.
2. Bradamante, S.; Pagani, G. A. *Adv. Carbanion Chem.* **1996**, *2*, 189.
3. le Questel, J.-Y.; Berthelot, M.; Laurence, C. *J. Phys. Org. Chem.* **2000**, *13*, 347.
4. Eliel, E. L.; Wilen, S. H.; Mander, L. N. *Stereochemistry of Organic Compounds*; Wiley: New York, 1994; pp 696–697.
5. (a) Curran, D. P.; Shu, M. *Bull. Soc. Chim. Fr.* **1993**, *130*, 314. (b) Curran, D. P.; Thoma, G. *J. Am. Chem. Soc.* **1992**, *114*, 4436. (c) Curran, D. P.; Seong, C. M. *Tetrahedron* **1992**, *48*, 2175.
6. (a) Creary, X. *Chem. Rev.* **1991**, *91*, 1625. (b) Tekeuchi, K.; Kitagawa, T.; Ohga, Y.; Nakakimura, A.; Munakata, M. *Tetrahedron* **1997**, *53*, 8155.
7. Arseniyadis, S.; Kyler, K. S.; Watt, D. S. *Org. React.* **1984**, *32*, 1.
8. Fleming, F. F. *Nat. Prod. Rep.* **1999**, *16*, 597.
9. Sheppard, W. A. *The Chemistry of the Cyano Group*; Rappoport, Z., Ed.; 1970. Chapter 5.
10. For an excellent survey of the difficulties inherent in obtaining axially substituted quinolizidines see: Morley, C.; Knight, D. W.; Share, A. C. *J. Chem. Soc. Perkin Trans. 1* **1994**, 2903.
11. Fleming, F. F.; Hussain, Z.; Weaver, D.; Norman, R. E. *J. Org. Chem.* **1997**, *62*, 1305.
12. Amos, D. T.; Renslo, A. R.; Danheiser, R. L. *J. Am. Chem. Soc.* **2003**, *125*, 4970.
13. Bonin, M.; Romero, J. R.; Grierson, D. S.; Husson, H.-P. *J. Org. Chem.* **1984**, *49*, 2392.
14. Nagata, W.; Yoshikoshi, M. *Org. React.* **1977**, *25*, 255.
15. Nicolaou, K. C.; Baran, P. S.; Zhong, Y.-L.; Choi, H.-S.; Yoon, W. H.; He, Y.; Fong, K. C. *Angew. Chem., Int. Ed.* **1999**, *38*, 1669.
16. Chen, Z. X.; Ma, W. Y.; Zhang, C. N. *Chin. Chem. Lett.* **2000**, *11*, 505.
17. Regueiro-Ren, A.; Leavitt, K.; Kim, S.-H.; Höfle, G.; Kiffe, M.; Gougoutas, J. Z.; DiMarco, J. D.; Lee, F. Y. F.; Fairchild, C. R.; Long, B. H.; Vite, G. D. *Org. Lett.* **2002**, *4*, 3815.
18. (a) Kawakami, Y.; Hisada, H.; Yamashita, Y. *Tetrahedron Lett.* **1985**, *26*, 5835. (b) Watt, D. S. *Synth. Commun.* **1974**, *4*, 127. (c) Kruger, C. R.; Rochow, E. G. *Angew. Chem., Int. Ed. Engl.* **1963**, *2*, 617. (d) Prober, M. *J. Am. Chem. Soc.* **1956**, *78*, 2274.
19. Enders, D.; Kirchhoff, J.; Gerdes, P.; Mannes, D.; Raabe, G.; Runsink, J.; Boche, G.; Marsch, M.; Ahlbrecht, H.; Sommer, H. *Eur. J. Org. Chem.* **1998**, 63.
20. Bradamante, S.; Pagani, G. A. *J. Chem. Soc. Perkin Trans. 2* **1986**, 1035.
21. For reviews see: (a) Fleming, F. F.; Shook, B. C. *Tetrahedron* **2002**, *58*, 1. (b) Boche, G. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 277.
22. Nitriles are powerful inductive stabilizing groups with weak delocalizing effects: (a) Richard, J. P.; Williams, G.; Gao, J. *J. Am. Chem. Soc.* **1999**, *121*, 715. (b) Dayal, S. K.; Ehrenson, S.; Taft, R. W. *J. Am. Chem. Soc.* **1972**, *94*, 9113.
23. Zarges, W.; Marsch, M.; Harms, K.; Boche, G. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 1392.
24. Boche, G.; Harms, K.; Marsch, M. *J. Am. Chem. Soc.* **1988**, *110*, 6925.
25. MacPhee, J.-A.; DuBois, J.-E. *Tetrahedron* **1980**, *36*, 775.
26. Smith, H. A.; Bissell, R. L.; Kenyon, W. G.; MacClarence, J. W.; Hauser, C. R. *J. Org. Chem.* **1971**, *36*, 2132.
27. Bug, T.; Mayr, H. *J. Am. Chem. Soc.* **2003**, *125*, 12980.
28. (a) Tucker, C. E.; Majid, T. N.; Knochel, P. *J. Am. Chem. Soc.* **1992**, *114*, 3983. (b) Parham, W. E.; Bradsher, C. K. *Acc. Chem. Res.* **1982**, *15*, 300.
29. For leading references see: (a) Lee, J.-s.; Velarde-Ortiz, R.; Guijarro, A.; Wurst, J. R.; Rieke, R. D. *J. Org. Chem.* **2000**, *65*, 5428. (b) Boudier, A.; Bromm, L. O.; Lotz, M.; Knochel, P. *Angew. Chem.* **2000**, *39*, 4415. (c) Abarbri, M.; Thibonnet, J.; Bérillon, L.; Dehmel, F.; Rottländer, M.; Knochel, P. *J. Org. Chem.* **2000**, *65*, 4618. (d) Inoue, A.; Kitagawa, K.; Shinokubo, H.; Oshima, K. *J. Org. Chem.* **2001**, *66*, 4333.
30. (a) Cheung, E.; Rademacher, K.; Scheffer, J. R.; Trotter, J. *Tetrahedron* **2000**, *56*, 6739. (b) Parham, W. E.; Jones, L. D. *J. Org. Chem.* **1976**, *41*, 1187. (c) Parham, W. E.; Piccirilli, R. M. *J. Org. Chem.* **1976**, *41*, 1268.

31. Majid, T. N.; Yeh, M. C. P.; Knochel, P. *Tetrahedron Lett.* **1989**, *30*, 5069.
32. Kuehne, M. E.; Nelson, J. A. *J. Org. Chem.* **1970**, *35*, 161.
33. (a) Collins, C. J.; Fisher, G. B.; Reem, A.; Goralski, C. T.; Singaram, B. *Tetrahedron Lett.* **1997**, *38*, 529. (b) Kamitori, Y.; Hojo, M.; Masuda, R.; Inoue, T.; Izumi, T. *Tetrahedron Lett.* **1983**, *24*, 2575. (c) Deiters, A.; Chen, K.; Todd Eary, C.; Martin, S. F. *J. Am. Chem. Soc.* **2003**, *125*, 4541.
34. Fleming, F. F.; Zhang, Z.; Wang, Q.; Steward, O. W. *Angew. Chem., Int. Ed.* **2004**, *43*, 1126.
35. Fuji, K.; Zheng, S.-Z.; Node, M.; Hao, X.-J. *Chem. Pharm. Bull.* **1991**, *39*, 202.
36. Gupta, Y. N.; Doa, M. J.; Houk, K. N. *J. Am. Chem. Soc.* **1982**, *104*, 7336.
37. (a) Burrows, C. J.; Carpenter, B. K. *J. Am. Chem. Soc.* **1981**, *103*, 6983. (b) Burrows, C. J.; Carpenter, B. K. *J. Am. Chem. Soc.* **1981**, *103*, 6984.
38. Toró, A.; Lemelin, C.-A.; Préville, P.; Bélanger, G.; Deslongchamps, P. *Tetrahedron* **1999**, *55*, 4655.
39. Toró, A.; Nowak, P.; Deslongchamps, P. *J. Am. Chem. Soc.* **2000**, *122*, 4526.
40. Magnus, P.; Gazzard, L.; Hobson, L.; Payne, A. H.; Rainey, T. J.; Westlund, N.; Lynch, V. *Tetrahedron* **2002**, *58*, 3423.
41. The stereochemical induction caused by the nitrile-substituent is unclear since analogous substitution in an intermolecular Diels–Alder cycloaddition does not appear to control the *endo*/*exo* selectivity: Grigg, R.; Scott, R.; Stevenson, P. *J. Chem. Soc. Perkin Trans. 1* **1988**, 1365.
42. Parker, K. A.; Lim, Y.-H. *Org. Lett.* **2004**, *6*, 161.
43. (a) Zoretic, P. A.; Zhang, Y.; Fang, H.; Ribeiro, A. A.; Dubay, G. *J. Org. Chem.* **1998**, *63*, 1162. (b) Zoretic, P. A.; Zhang, Y.; Ribeiro, A. A. *Tetrahedron Lett.* **1996**, *37*, 1751. For another example see: (c) Zoretic, P. A.; Fang, H.; Ribeiro, A. A. *J. Org. Chem.* **1998**, *63*, 7213. (d) Zoretic, P. A.; Chen, Z.; Zhang, Y.; Ribeiro, A. A. *Tetrahedron Lett.* **1996**, *37*, 7909.
44. For a recent transition metal directed reaction of an aromatic nitrile see: Kakiuchi, F.; Sonoda, M.; Tsujimoto, T.; Chatani, N.; Murai, S. *Chem. Lett.* **1999**, 1083.
45. Michelin, R. A.; Mozzon, M.; Bertani, R. *Coord. Chem. Rev.* **1996**, *147*, 299.
46. Henbest, H. B.; Nicholls, B. *J. Chem. Soc.* **1959**, 227.
47. Fatiadi, A. J. In *The Chemistry of Functional Groups, Supplement C*; Patai, S., Rappoport, Z., Eds.; Wiley: New York, 1983; pp 1057–1303.
48. Sharanin, Y. A.; Goncharenko, M. P.; Litvinov, V. P. *Russ. Chem. Rev.* **1998**, *67*, 393–422.
49. Langer, P.; Freifeld, I. *Org. Lett.* **2001**, *3*, 3903.
50. Makosza, M.; Przyborowski, J.; Klajn, R.; Kwast, A. *Synlett* **2000**, 1773.
51. (a) Makosza, M.; Judka, M. *Chem. Eur. J.* **2002**, *8*, 4234. (b) Fleming, F. F.; Gudipati, V.; Steward, O. W. *J. Org. Chem.* **2003**, *68*, 3943.
52. The reaction appears to have been first discovered during mechanistic work on chrysanthemic nitrile: Guittet, E.; Julia, S. A.; Linstrumelle, G.; Lorne, R. *Tetrahedron* **1984**, *40*, 4267.
53. Koradin, C.; Rodriguez, A.; Knochel, P. *Synlett* **2000**, 1452.
54. Rodriguez, A.; Bunlaksananusorn, T.; Knochel, P. *Org. Lett.* **2000**, *2*, 3285.
55. (a) Yeh, M.-C.P.; Chuang, L.-W. *J. Chin. Chem. Soc.* **1997**, *44*, 291. (b) Yeh, M.-C.P.; Chuang, L.-W.; Ueng, C.-H. *J. Org. Chem.* **1996**, *61*, 3874.
56. Grossman, R. B. *Synlett* **2001**, 13.
57. Cyanation of α-alkoxy and α-amino nitriles are not surveyed, see: Enders, D.; Shilvock, J. P. *Chem. Soc. Rev.* **2000**, *29*, 359.
58. Grashey, R. In Trost, B. M., Fleming, I., Eds.; Comprehensive Organic Synthesis; Pergamon: Oxford, 1991; Vol. 3, p 225.
59. Simchen, G.; Kobler, H. *Synthesis* **1975**, 605.
60. The reaction is likely an E1cb elimination–conjugate addition.
61. Ma, D.; Ma, J.; Dai, L. *Tetrahedron: Asymmetry* **1997**, *6*, 1452.
62. Soli, E. D.; Manoso, A. S.; Patterson, M. C.; DeShong, P.; Favor, D. A.; Hirschmann, R.; Smith, A. B., III *J. Org. Chem.* **1999**, *64*, 3171.
63. Yang, Z.; Shannon, D.; Truong, V.-L.; Deslongchamps, P. *Org. Lett.* **2002**, *4*, 4693.
64. Boyer, F.-D.; Descoins, C.; Ducrot, P.-H. *Eur. J. Org. Chem.* **2003**, 1184.
65. Ogawa, S.; Maruyama, A.; Odagiri, T.; Yuasa, H.; Hashimoto, H. *Eur. J. Org. Chem.* **2001**, *66*, 967.
66. Schroder, E.; Lehmann, M.; Bottcher, I. *Eur. J. Med. Chem.* **1979**, *4*, 309.
67. Fringuelli, F.; Minuti, L.; Taticchi, A. *Synth. Commun.* **1990**, *20*, 1497.
68. Benerjee, A. K.; Canudas-Gonzalez, N.; Cabrera-Nieto, G.; Pena-Matheud, C. A. *J. Chem. Res.* **1990**, 266.
69. Deng, S. X.; Huang, D. W.; Landry, D. W. *Tetrahedron Lett.* **2001**, *42*, 6259.
70. Szalkowska-Pagowska, H.; Piatkowski, K. *Pol. J. Chem.* **1985**, *59*, 1121.
71. Daniewski, A. R.; Kabat, M. M.; Masnyk, M.; Wicha, J.; Wojciechowska, W. *J. Org. Chem.* **1988**, *53*, 4855.
72. Tsuji, Y.; Kusui, T.; Kojima, T.; Sugiura, Y.; Yamada, N.; Tanaka, S.; Ebihara, M.; Kawamura, T. *Organometallics* **1998**, *17*, 4835.
73. Tsunoda, T.; Uemoto, K.; Nagino, C.; Kawamura, M.; Kaku, H.; Ito, S. *Tetrahedron Lett.* **1999**, *40*, 7355.
74. (a) Fleming, I.; Loreto, M. A.; Michael, J. P.; Wallace, I. H. M. *Tetrahedron Lett.* **1982**, *23*, 2053. (b) Fleming, I.; Loreto, M. A.; Wallace, I. H. M. *J. Chem. Soc., Perkin Trans. 1* **1986**, 349.
75. McCombie, S. W.; Cox, B.; Ganguly, A. K. *Tetrahedron Lett.* **1991**, *32*, 2087.
76. Schwarz, O.; Brun, R.; Bats, J. W.; Schmalz, H.-G. *Tetrahedron Lett.* **2002**, *43*, 1009.
77. (a) Davis, R.; Untch, K. G. *J. Org. Chem.* **1981**, *46*, 2985. (b) Kraus, G. A.; Kirihara, M. *Tetrahedron Lett.* **1992**, *33*, 7727.
78. Reetz, M. T.; Chatziiosifidis, I.; Kuenzer, H.; Mueller-Starke, H. *Tetrahedron* **1983**, *39*, 961.
79. House, H. O.; Lee, T. V. *J. Org. Chem.* **1979**, *44*, 2819.
80. Guy, A.; Doussot, J.; Guette, J. P.; Garreau, R.; Lemaire, M. *Synlett* **1992**, 821.
81. Pirrung, M. C.; Brown, W. L. *J. Am. Chem. Soc.* **1990**, *112*, 6388.
82. Benedetti, F.; Berti, F.; Norbedo, S. *Tetrahedron Lett.* **1999**, *40*, 1041.
83. Nikolaides, N.; Ganem, B. *Tetrahedron Lett.* **1989**, *30*, 1461.
84. Habib, R.; Husain, M.; Fazal, A.; Husain, M. *Indian J. Chem., Sect. B* **1988**, *27B*, 584.
85. Boulet, S. L.; Paquette, L. A. *Synthesis* **2002**, 895.
86. Neef, G.; Eckle, E.; Müller-Fahrnow, A. *Tetrahedron* **1993**, *49*, 833.

87. Renneberg, D.; Pfander, H.; Leumann, C. J. *J. Org. Chem.* **2000**, *65*, 9069.
88. Peterson, E. M.; Xu, K.; Holland, K. D.; McKeon, A. C.; Rothman, S. M.; Ferrendelli, J. A.; Covey, D. F. *J. Med. Chem.* **1994**, *37*, 275.
89. Praefcke, K.; Schmidt, D. *Z. Naturforsch. B Chem. Sci.* **1980**, *35B*, 1451.
90. Grob, C. A.; Wittwer, G.; Rao, K. R. *Helv. Chim. Acta* **1985**, *68*, 760.
91. Hirani, S. K.; Parafitt, R. T.; Chowdhury, B. K. *Indian J. Chem. Sect. B* **1987**, *26B*, 104.
92. Nakazaki, M.; Naemura, K.; Hashimoto, M. *J. Org. Chem.* **1983**, *48*, 2289.
93. Piers, E.; Wai, J. S. M.; *Can. J. Chem.* **1994**, *72*, 146.
94. Jiricny, J.; Orere, D. M.; Reese, C. B. *J. Chem. Soc., Perkin Trans. 1* **1980**, 1487.
95. Orere, D. M.; Reese, C. B. *J. Chem. Soc., Chem. Commun.* **1977**, 280.
96. Okimoto, M.; Chiba, T. *J. Org. Chem.* **1990**, *55*, 1070.
97. Meier, M.; Ruechardt, C. *Chem. Ber.* **1987**, *120*, 1.
98. Luening, U.; Mueller, M.; Gelbert, M.; Ruechardt, C. *Chem. Ber.* **1991**, *124*, 2555.
99. Yoneda, R.; Harusawa, S.; Kurihara, T. *J. Org. Chem.* **1991**, *56*, 1827.
100. Hulkenberg, A.; Troost, J. J. *Tetrahedron Lett.* **1982**, *23*, 1505.
101. Hartmann, W. *Synthesis* **1989**, 272.
102. Portevin, B.; Tordjman, C.; Pastoureau, P.; Bonnet, J.; de Nanteuil, G. *J. Med. Chem.* **2000**, *43*, 4582.
103. Nakajima, N.; Ubukata, M. *Tetrahedron Lett.* **1997**, *38*, 2099.
104. Ueda, Y.; Vinet, V. *Can. J. Chem.* **1986**, *64*, 2184.
105. Heck, M.-P.; Wagner, A.; Mioskowski, C. *J. Org. Chem.* **1996**, *61*, 6486.
106. Kende, A. S.; Liu, K. *Tetrahedron Lett.* **1995**, *36*, 4035.
107. Murai, A.; Nishizakura, K.; Katsui, N.; Masamune, T. *Bull. Chem. Soc. Jpn* **1977**, *50*, 1206.
108. Solaja, B.; Dermanovic, M.; Lim, D.-M.; Paik, Y.-K. *J. Serb. Chem. Soc.* **1996**, *61*, 1113.
109. Klinot, J.; Svetly, J.; Klinotova, E.; Vystrcil, A. *Coll. Czech. Chem. Commun.* **1985**, *50*, 2918.
110. Piers, E.; Caille, S.; Chen, G. *Org. Lett.* **2000**, *2*, 2483.
111. Bose, D. S.; Narsaiah, A. V. *Tetrahedron Lett.* **1998**, *39*, 6533.
112. Wu, Y.-Q.; Limburg, D. C.; Wilkinson, D. E.; Hamilton, G. S. *Org. Lett.* **2000**, *2*, 795.
113. Diez, E.; Fernandez, R.; Gasch, C.; Lassaletta, J. M.; Llera, J. M.; Martin-Zamora, E.; Vazquez, J. *J. Org. Chem.* **1997**, *62*, 5144.
114. Vu, V. A.; Marek, I.; Polborn, K.; Knochel, P. *Angew. Chem. Int. Ed.* **2002**, *41*, 351.
115. You, Z.; Lee, H. J. *Tetrahedron Lett.* **1996**, *37*, 1165.
116. Wade, P. A.; Hinney, H. R. *J. Am. Chem. Soc.* **1979**, *101*, 1319.
117. Greatrex, B. W.; Taylor, D. K.; Tiekink, E. R. T. *Org. Lett.* **2002**, *4*, 221.
118. Shia, K.-S.; Jan, N.-W.; Zhu, J.-L.; Ly, T. W.; Liu, H.-J. *Tetrahedron Lett.* **1999**, *40*, 6753.
119. (a) Sole, D.; Cancho, Y.; Llebaria, A.; Moreto, J.; Delgado, A. *J. Am. Chem. Soc.* **1994**, *116*, 12133. (b) Sole, D.; Cancho, Y.; Llebaria, A.; Moreto, J.; Delgado, A. *J. Org. Chem.* **1996**, *61*, 5895.
120. Trost, B. M.; Thiel, O. R.; Tsui, H.-C. *J. Am. Chem. Soc.* **2003**, *125*, 13155.
121. Numerous Lewis acids facilitate ring opening: Chini, M.; Crotti, P.; Favero, L.; Macchia, F. *Tetrahedron Lett.* **1991**, *32*, 4775.
122. (a) Carlier, P. R.; Lo, C. W.-S.; Lo, M. M.-C.; Wan, N. C.; Williams, I. D. *Org. Lett.* **2000**, *2*, 2443. (b) Wade, P. A.; Bereznak, J. F. *J. Org. Chem.* **1987**, *52*, 2973.
123. Fleming, F. F.; Funk, L. A.; Altundas, R.; Sharief, V. *J. Org. Chem.* **2002**, *67*, 9414.
124. Oldenziel, O. H.; Van Leusen, D.; Van Leusen, A. M. *J. Org. Chem.* **1977**, *42*, 3114.
125. Cacchi, S.; Caglioti, L.; Paolucci, G. *Chem. Ind.* **1972**, 213.
126. North, M.; Grashey, R. In Trost, B. M., Fleming, I., Eds.; Comprehensive Organic Synthesis; Pergamon: Oxford, 1991; Vol. 3.
127. Güner, O. F.; Ottenbrite, R. M.; Shillady, D. D.; Alston, P. V. *J. Org. Chem.* **1988**, *53*, 5348.
128. Kienzle, F. *Helv. Chem. Acta* **1975**, 58.
129. Brion, F. *Tetrahedron Lett.* **1982**, *23*, 5299.
130. Rai, U. K.; Shanker, B.; Singh, S.; Rao, R. B. *Ind. J. Chem.* **1989**, 870.
131. Kienzle, F.; Mergelsberg, I.; Stadlwieser, J.; Arnold, W. *Helv. Chim. Acta* **1985**, *68*, 1133.
132. de Lucchi, O.; Piccolrovazzi, N.; Modena, G. *Tetrahedron Lett.* **1986**, *27*, 4347.
133. Liotta, C. L.; Verbicky, J. W., Jr. *Tetrahedron Lett.* **1985**, *26*, 1395.
134. Mander, L. N.; McLachlan, M. W. *J. Am. Chem. Soc.* **2003**, *125*, 2400.
135. Siegel, H. *Synthesis* **1985**, 798.
136. Tsuge, O.; Wada, E.; Kanemasa, S.; Sakoh, H. *Bull. Chem. Soc. Jpn* **1984**, *57*, 3221.
137. Defacqz, N.; Touillaux, R.; Marchand-Brynaert, J. *J. Chem. Res., Synopses* **1998**, 512.
138. Goetz, P. H.; Bats, J. W.; Fritz, H. *Liebigs Ann. Chem.* **1986**, 2065.
139. Oppolzer, W.; Bieber, L.; Francotte, E. *Tetrahedron Lett.* **1979**, *19*, 4537.
140. (a) Trost, B. M.; Mignani, S. *J. Org. Chem.* **1986**, *51*, 3435. (b) Inomata, K.; Kinoshita, H.; Takemoto, H.; Murata, Y.; Kotake, H. *Bull. Chem. Soc. Jpn* **1978**, *51*, 3341.
141. Van Leusen, A. M.; Van den Berg, K. J. *Tetrahedron Lett.* **1988**, *29*, 2689.
142. (a) Proteau, P. J.; Hopkins, P. B. *J. Org. Chem.* **1985**, *50*, 141. (b) Trost, B. M.; Bridges, A. J. *J. Am. Chem. Soc.* **1976**, *98*, 5017.
143. Hocking, M. B.; Bushnell, G. W. *Can. J. Chem.* **1990**, *68*, 1020.
144. Azadi-Ardakani, M.; Wallace, T. W. *Tetrahedron* **1988**, *44*, 5939.
145. Kvita, V.; Sauter, H.; Rihs, G. *Helv. Chim. Acta* **1983**, *66*, 2769.
146. White, K. B.; Reusch, W. *Tetrahedron* **1978**, *34*, 2439.
147. Rao, G.S.R. Subba; Vijaybhaskar, K.; Srikrishna, A. *Indian J. Chem., Sect. B* **1986**, *25B*, 785.
148. Afarinkia, K.; Bearpark, M. J.; Ndibwami, A. *J. Org. Chem.* **2003**, *68*, 7158.
149. Otani, T.; Takayama, J.; Sugihara, Y.; Ishii, A.; Nakayama, J. *J. Am. Chem. Soc.* **2003**, *125*, 8255.
150. Kuethe, J. T.; Brooks, C. A.; Comins, D. L. *Org. Lett.* **2003**, *5*, 321.
151. MaGee, D. I.; Leach, J. D.; Setiadji, S. *Tetrahedron* **2001**, *42*, 7177.

152. Srikrishna, A.; Vijaykumar, D.; Sharma, G. V. R. *Tetrahedron Lett.* **1997**, *38*, 2003.
153. (a) Avalos, M.; Babiano, R.; Bravo, J. L.; Cintas, P.; Jimenez, J. L.; Palacios, J. C.; Ranu, B. C. *Tetrahedron Lett.* **1998**, *39*, 2013. (b) Maruoka, K.; Imoto, H.; Yamamoto, H. *J. Am. Chem. Soc.* **1994**, *116*, 12115. (c) Muller, J.-C.; Fleury, J.-P. *Bull. Chim. Soc. Fr.* **1970**, 738.
154. Takakis, I. M.; Agosta, W. C. *J. Org. Chem.* **1978**, *43*, 1952.
155. (a) Wu, J.-D.; Shia, K.-S.; Liu, H.-J. *Tetrahedron Lett.* **2001**, *42*, 4207. (b) Liu, H.-J.; Ho, Y.-L.; Wu, J.-D.; Shia, K.-S. *Synlett* **2001**, 1805. (c) Zhu, J.-L.; Shia, K.-S.; Liu, H.-J. *Chem. Commun.* **2000**, 1599.
156. (a) Seth, P. P.; Totah, N. I. *Org. Lett.* **1999**, *1*, 1411. (b) Seth, P. P.; Totah, N. I. *J. Org. Chem.* **1999**, *64*, 8750. (c) Chen, D.; Wang, J.; Totah, N. I. *J. Org. Chem.* **1999**, *64*, 1776. (d) Hsung, R. P.; Zificsak, C. A.; Wei, L.-L.; Zehnder, L. R.; Park, F.; Kim, M.; Tran, T.-T.T. *J. Org. Chem.* **1999**, *64*, 8736. (e) Hsung, R. P. *J. Org. Chem.* **1997**, *62*, 7904.
157. (a) Dauben, W. G.; Krabbenhoft, H. O. *J. Am. Chem. Soc.* **1992**, *1976*, 98. (b) Kienzle, F. *Helv. Chim. Acta* **1975**, *58*, 1180. (c) Lekhok, K. C.; Kandoli, J. C.; Prajapati, D.; Sandhu, J. S. *Indian J. Chem.* **1987**, *26B*, 374.
158. Rodrigo, R.; Knabe, S. M.; Taylor, N. J.; Rajapaksa, D.; Chernishenko, M. J. *J. Org. Chem.* **1986**, *51*, 3973.
159. Sato, M.; Suzuki, T.; Morisawa, H.; Fujita, S.; Inukai, N.; Kaneko, C. *Chem. Pharm. Bull.* **1987**, *35*, 3647.
160. Baraldi, P. G.; Barco, A.; Benetti, S.; Manfredini, S.; Pollini, G. P.; Simoni, D.; Zanirato, V. *Tetrahedron* **1988**, *44*, 6451.
161. (a) Aggarwal, V. K.; Ali, A.; Coogan, M. P. *Tetrahedron* **1999**, *55*, 293. (b) Ranganathan, S.; Ranganathan, D.; Mehrotra, A. K. *Synthesis* **1977**, 289.
162. Drew, M. G. B.; Jahans, A.; Harwood, L. M.; Apoux, S. A. B. H.; *Eur. J. Org. Chem.* **2002**, 3589.
163. Schuda, P. F.; Bennett, J. M. *Tetrahedron Lett.* **1982**, *23*, 5525.
164. Kotsuki, H.; Nishizawa, H. *Heterocycles* **1981**, *16*, 1287.
165. Vieria, E.; Vogel, P. *Helv. Chim. Acta* **1982**, *65*, 1700.
166. (a) Nicolaou, K. C.; Hwang, C. K.; Sorensen, E. J.; Clairborne, C. F. *J. Chem. Soc., Chem. Commun.* **1992**, 1117. (b) Masamune, T.; Fukuzawa, A.; Furusaki, A.; Ikura, M.; Matsue, H.; Kaneko, T.; Abiko, A.; Sakamoto, N. *Bull. Chem. Soc. Jpn* **1987**, *60*, 1001. (c) Bhat, L.; Steinig, A. G.; Appelbe, R.; de Meijere, A. *Eur. J. Org. Chem.* **2001**, 1673. (d) Baldwin, J. E.; Otsuka, M.; Wallace, P. M. *J. Chem. Soc., Chem. Commun.* **1985**, 1549. (e) Baldwin, J. E.; Otsuka, M.; Wallace, P. M. *Tetrahedron* **1986**, *42*, 3097.
167. (a) Marchand, A. P.; Wu, A. *J. Org. Chem.* **1985**, *50*, 396. (b) Brown, E. D.; Clarkson, R.; Leeney, T. J.; Robinson, G. E. *J. Chem. Soc., Perkin Trans. 1* **1978**, 1507. (c) Corey, E. J.; Shiner, C. S.; Volante, R. P.; Cyr, C. R. *Tetrahedron Lett.* **1975**, 1161. (d) Brown, E. D.; Clarkson, R.; Leeney, T. J.; Robinson, G. E. *J. Chem. Soc., Chem. Commun.* **1974**, 642. (e) Corey, E. J.; Weinshenker, N. M.; Schaaf, T. K.; Huber, W. *J. Am. Chem. Soc.* **1969**, *99*, 5675. (f) Ref. 135
168. Foehlisch, B.; Bakr, D. A.; Fischer, P. *J. Org. Chem.* **2002**, *67*, 3682.
169. Jung, M. E.; Blum, R. B.; Gaede, B. J.; Gisler, M. R. *Heterocycles* **1989**, *28*, 93.
170. (a) DiFazio, M. P.; Wallace, W. A.; Sneden, A. T. *Heterocycles* **1989**, *29*, 2391. (b) Ref. 129.
171. Bull, J. R.; Grundler, C.; Niven, M. L. *J. Chem. Soc., Chem. Commun.* **1993**, 271.
172. (a) Weyerstahl, P.; Gansau, C.; Marschall, H. *Flavour Fragrance J.* **1993**, *8*, 297. (b) Clark, R. S. J.; Holmes, A. B.; Matassa, V. G. *J. Chem. Soc., Perkin Trans. 1* **1990**, 1401. (c) Holmes, A. B.; Madge, N. C. *Tetrahedron* **1989**, *45*, 789. (d) Snowden, R. L.; Brauchli, R.; Sonnay, P. *Helv. Chim. Acta* **1989**, *72*, 570. (e) Hua, D. H.; Gung, W. Y.; Ostrander, R. A.; Takusagawa, F. *J. Org. Chem.* **1987**, *52*, 2509. (f) Claret, F.; Carrupt, P.-A.; Vogel, P. *Helv. Chim. Acta* **1987**, *70*, 1886. (g) Evans, D. A.; Scott, W. L.; Truesdale, L. K. *Tetrahedron Lett.* **1972**, 121. (h) Ref. 147.
173. Clark, R. S. J.; Holmes, A. B.; Matassa, V. G. *J. Chem. Soc., Perkin Trans. 1* **1990**, 1389.
174. (a) Mehta, G.; Subrahmanyam, D. *J. Chem. Soc., Chem. Commun.* **1985**, 768. (b) Pramod, K.; Rao, G.S.R. Subba *Indian J. Chem.* **1982**, *21B*, 984.
175. Banwell, M. G.; Clark, G. R.; Hockless, D. C. R.; Pallich, S. *Aust. J. Chem.* **2001**, *54*, 691.
176. Eguchi, S.; Ishiura, K.; Noda, T.; Sasaki, T. *J. Org. Chem.* **1987**, *52*, 496.
177. Alexandre, C.; Rouessac, F.; Tabti, B. *Tetrahedron Lett.* **1985**, *26*, 5453.
178. Bailey, W. F.; Wachter-Jurcsak, N. M.; Pineau, M. R.; Ovaska, T. V.; Warren, R. R.; Lewis, C. E. *J. Org. Chem.* **1996**, *61*, 8216.
179. Shimano, M.; Nagaoka, H.; Yamada, Y. *Tetrahedron Lett.* **1995**, *36*, 8227.
180. Saito, T.; Shizuta, T.; Kikuchi, H.; Nakagawa, J.; Hirotsu, K.; Ohmura, H.; Motoki, S. *Synthesis* **1994**, 727.
181. Pindur, U.; Rogge, M. *Heterocycles* **1995**, *41*, 2785.
182. Pindur, U.; Haber, M. *Heterocycles* **1991**, *32*, 1463.
183. (a) Birch, A. J.; Stronkry, E. M. A.; Stansfield, F. *J. Chem. Soc., Chem. Commun.* **1961**, 5376. (b) Birch, A. J.; McKague, B. *Aust. J. Chem.* **1970**, *23*, 341. (c) Birch, A. J.; Graves, J. M. H.; Siddall, J. B. *Aust. J. Chem.* **1963**, 4234. (d) Alfaro, I.; Ashton, W.; McManus, L. D.; Newstead, R. C.; Rathbone, K. L.; Rogers, N. A. J.; Kerrick, W. *Tetrahedron* **1970**, *26*, 201.
184. Birch, A. J.; Dastur, K. P. *J. Chem. Soc., Perkin Trans. 1* **1973**, 1650.
185. (a) Colvin, E. W.; Malchenko, S.; Raphael, R. A.; Roberts, J. S. *J. Chem. Soc., Perkin Trans. 1* **1989**, 1973. (b) Demuth, M. *Helv. Chim. Acta* **1987**, *70*, 1886. (c) Ref. 172g.
186. Moursounidis, J.; Wege, D. *Aust. J. Chem.* **1983**, *36*, 2473.
187. Shiner, C. S.; Fisher, A. M.; Yacoby, F. *Tetrahedron Lett.* **1983**, *24*, 5687.
188. Knittel, P.; Warkentin, J. *Can. J. Chem.* **1972**, *50*, 4066.
189. Weyerstahl, P.; Brendel, J. *Liebigs Ann. Chem.* **1990**, 1029.
190. Fleming, F. F.; Zhang, Z.; Knochel, P. *Org. Lett.* **2004**, *6*, 501.
191. Monti, S. A.; Chen, S.-C.; Yang, Y.-L.; Yuan, S.-S.; Bourgeois, O. P. *J. Org. Chem.* **1978**, *43*, 4062.
192. Clark, R. S. J.; Holmes, A. B.; Matassa, V. G. *Tetrahedron Lett.* **1989**, *30*, 3224.
193. Yamada, Y.; Kimura, M.; Nagaoka, H.; Ohnishi, K. *Tetrahedron Lett.* **1977**, 2379.
194. (a) Kametani, T. *Pure Appl. Chem.* **1979**, *51*, 747. (b) Kametani, T.; Fukumoto, K. *Heterocycles* **1977**, *8*, 465.
195. For leading references see: (a) Lee, P. S.; Zhang, X.; Houk, K. N. *J. Am. Chem. Soc.* **2003**, *125*, 5072. (b) Jefford, C. W.; Bernardinelli, G.; Wang, Y.; Spellmeyer, D. C.; Buda, A.; Houk, K. N. *J. Am. Chem. Soc.* **1992**, *114*, 1157. (c) Kametani, T.; Tsubuki, M.; Shiratori, Y.; Kato, Y.; Nemoto, H.; Ihara, M.; Fukumoto, K.; Satoh, F.; Inoue, H. *J. Org. Chem.* **1977**, *42*, 2672.

196. (a) Bush, E. J.; Jones, D. W. *J. Chem. Soc. Perkin Trans. 1* **1997**, 3531. (b) Kametani, T.; Kondoh, H.; Tsubuki, M.; Honda, T. *J. Chem. Soc., Perkin Trans. 1* **1990**, 5. (c) Kametani, T.; Toya, T.; Tsubuki, M.; Kawai, K.-I.; Honda, T. *Chem. Pharm. Bull.* **1986**, *34*, 3169. (d) Kametani, T.; Suzuki, Y.; Honda, T. *J. Chem. Soc. Perkin Trans. 1* **1986**, 1373. (e) Kametani, T.; Suzuki, Y.; Honda, T. *Chem. Pharm. Bull.* **1986**, *34*, 4971. (f) Kametani, T.; Suzuki, Y.; Honda, T. *Heterocycles* **1985**, *23*, 305. (g) Kametani, T.; Tsubuki, M.; Nemoto, H.; Fukumoto, K. *Chem. Pharm. Bull.* **1979**, *27*, 152. (h) Kametani, T.; Kato, Y.; Honda, T.; Fukumoto, K. *J. Am. Chem. Soc.* **1976**, *98*, 8185. (i) Kametani, T.; Kato, Y.; Honda, T.; Fukumoto, K. *Heterocycles* **1976**, *4*, 241.
197. (a) Shishido, K.; Shimada, S.-I.; Fukumoto, K.; Kametani, T. *Chem. Pharm. Bull.* **1984**, *32*, 922. (b) Ref. 159.
198. Kametani, T.; Hirai, Y.; Shiratori, Y.; Fukumoto, K.; Satoh, F. *J. Am. Chem. Soc.* **1978**, *100*, 554.
199. Nicolaou, K. C.; Gray, D. L. F.; Tae, J. *J. Am. Chem. Soc.* **2004**, *126*, 613.
200. Shishido, K.; Hiroya, K.; Fukumoto, K.; Kametani, T.; Kabuto, C. *J. Chem. Soc., Perkin Trans. 1* **1989**, 1443.
201. Yang, Z. Q.; Chen, S. Z.; Huang, L. *Chin. Chem. Lett.* **1998**, *9*, 261.
202. Magnus, P.; Leapheart, T.; Walker, C. *J. Chem. Soc. Chem. Commun.* **1985**, 1185.
203. Sisti, N. J.; Zeller, E.; Grierson, D. S.; Fowler, F. W. *J. Org. Chem.* **1997**, *62*, 2093.
204. Bland, D. C.; Raudenbush, B. C.; Weinreb, S. M. *Org. Lett.* **2000**, *2*, 4007.
205. Williams, G. M.; Roughly, S. D.; Davies, J. E.; Holmes, A. B. *J. Am. Chem. Soc.* **1999**, *121*, 4900.
206. In some instances the dehydration requires activation in a separate step: Nakashima, K.; Inoue, K.; Sono, M.; Tori, M. *J. Org. Chem.* **2002**, *67*, 6034.
207. Solaja, B.; Huguet, J.; Karpf, M.; Dreiding, A. S. *Helv. Chim. Acta* **1986**, *69*, 1163.
208. Tabushi, I.; Fujita, K.; Oda, R. *J. Org. Chem.* **1970**, *35*, 2376.
209. Paquette, L. A.; Okazaki, M. E.; Caille, J. C. *J. Org. Chem.* **1988**, *53*, 477.
210. Ho, T.-L.; Su, C.-Y. *J. Org. Chem.* **2000**, *65*, 3566.
211. Isoda, S.; Hirata, M. *Chem. Pharm. Bull.* **1979**, 2735.
212. Kawano, H.; Itoh, M.; Katoh, T.; Terashima, S. *Tetrahedron Lett.* **1997**, *38*, 7769.
213. Isoda, S. *Chem. Pharm. Bull.* **1979**, 3039.
214. Galemmo, R. A., Jr.; Johnson, W. H., Jr.; Learn, K. S.; Lee, T. D. Y.; Huang, F. C.; Campbell, H. F.; Youssefyeh, R.; O'Rourke, S. V.; Schuessler, G.; Sweeny, D. M.; Travis, J. J.; Sutherland, C. A.; Nuss, G. W.; Carnathan, G. W.; Van Inwegen, R. G. *J. Med. Chem.* **1990**, *33*, 2828.
215. Oda, M.; Yamamuro, A.; Watabe, T. *Chem. Lett.* **1979**, 1427.
216. Blinn, D. A.; Button, R. S.; Farazi, V.; Neeb, M. K.; Tapley, C. L.; Trehearne, T. E.; West, S. D.; Kruger, T. L.; Storhoff, B. N. *J. Organomet. Chem.* **1990**, *393*, 143.
217. Harusawa, S.; Yoneda, R.; Kurihara, T.; Hamada, Y.; Shioiri, T. *Tetrahedron Lett.* **1984**, *25*, 427.
218. Kurihara, T.; Miki, M.; Yoneda, R.; Harusawa, S. *Chem. Pharm. Bull.* **1986**, *34*, 2747.
219. Yoneda, R.; Harusawa, S.; Kurihara, T. *J. Chem. Soc., Perkin Trans. 1* **1988**, 3163.
220. (a) Boyer, F.-D.; Ducrot, P.-H. *Synthesis* **2000**, 1868. (b) Boyer, F.-D.; Descoins, C. L.; Descoins, C.; Prange, T.; Ducrot, P.-H. *Tetrahedron Lett.* **2002**, *43*, 8277.
221. Tomoda, S.; Takeuchi, Y.; Nomura, Y. *J. Chem. Soc., Chem. Commun.* **1982**, 871.
222. Hoffmann, H. M. R.; Giesel, K.; Lies, R.; Ismail, Z. M. *Synthesis* **1986**, 548.
223. Minami, I.; Yuhara, M.; Shimizu, I.; Tsuji, J. *J. Chem. Soc., Chem. Commun.* **1986**, 118.
224. Piers, E.; Fleming, F. F. *Can. J. Chem.* **1993**, *71*, 1867.
225. Sakakibara, Y.; Yadani, N.; Ibuki, I.; Sakai, M.; Uchino, N. *Chem. Lett.* **1982**, 1565.
226. el Kaim, L.; Gacon, A. *Tetrahedron Lett.* **1997**, *38*, 3391.
227. Flitsch, W.; Lerner, H.; Zimmermann, H. *Chem. Ber.* **1977**, *110*, 2765.
228. Stefancich, G.; Artico, M.; Massa, S.; Vomero, S. *J. Heterocycl. Chem.* **1979**, *16*, 1443.
229. Merchant, J. R.; Dike, S. Y. *Proc. Indian Acad. Sci., Sect. A* **1978**, *87A*, 229.
230. (a) Verboom, W.; Orlemans, E. O. M.; Berga, H. J.; Scheltinga, M. W.; Reinhoudt, D. N. *Tetrahedron* **1986**, *42*, 5053. (b) Verboom, W.; Berga, H. J.; Trompenaars, W. P.; Reinhoudt, D. N. *Tetrahedron Lett.* **1985**, *26*, 685. (c) Orlemans, E. O. M.; Schreuder, A. H.; Conti, P. G. M.; Verboom, W.; Reinhoudt, D. N. *Tetrahedron* **1987**, *43*, 3817.
231. Bestmann, H. J.; Schmidt, M. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 79.
232. Zhang, R.-Y.; Zhao, C.-G. *Chem. Commun.* **1996**, 511.
233. Graff, M.; al Dilaimi, A.; Seguineau, P.; Rambaud, M.; Villieras, J. *Tetrahedron Lett.* **1986**, *27*, 1577.
234. Monteiro, N.; Gore, J.; Balme, G. *Tetrahedron* **1992**, *48*, 10103.
235. Dinesh, C. U.; Reissig, H.-U. *Angew. Chem., Int. Ed.* **1999**, *38*, 789.
236. Kikugawa, Y.; Ikegami, S.; Yamada, S. *Chem. Pharm. Bull.* **1969**, *17*, 98.
237. Pletnev, A. A.; Larock, R. C. *J. Org. Chem.* **2002**, *67*, 9428.
238. Herter, R.; Foehlisch, B. *Chem. Ber.* **1982**, *115*, 381.
239. Taber, D. F.; Saleh, S. A. *J. Org. Chem.* **1981**, *46*, 4817.
240. Lee, B. H.; Clothier, M. F. *J. Org. Chem.* **1997**, *62*, 1863.
241. Walborsky, H. M.; Motes, J. M. *J. Am. Chem. Soc.* **1970**, *92*, 2445.
242. For a discussion of inversion barrier and mechanism see: Carlier, P. R. *Chirality* **2003**, *15*, 340.
243. Magnus, P.; Schultz, J.; Gallagher, T. *J. Am. Chem. Soc.* **1985**, *107*, 4984.
244. Mander, L. N.; Thomson, R. J. *Org. Lett.* **2003**, *5*, 1321.
245. Bare, T. M.; Hershey, N. D.; House, H. O.; Swain, C. G. *J. Org. Chem.* **1972**, *37*, 997.
246. Ziegler, F. E.; Wender, P. A. *J. Org. Chem.* **1977**, *42*, 2001.
247. Pearlman, B. A.; Putt, S. R.; Fleming, J. A. *J. Org. Chem.* **1985**, *50*, 3625.
248. (a) Piers, E.; Roberge, J. Y. *Tetrahedron Lett.* **1992**, *33*, 6923. (b) Piers, E.; Wai, J. S. M. *J. Chem. Soc. Chem. Commun.* **1988**, 1245. (c) Piers, E.; Wai, J. S. M. *J. Chem. Soc. Chem. Commun.* **1987**, 1342.
249. (a) Ahmad, Z.; Goswami, P.; Venkateswaran, R. V. *Tetrahedron* **1989**, *45*, 6833. (b) Ahmad, Z.; Goswami, P.; Venkateswaran, R. V. *Tetrahedron Lett.* **1987**, *28*, 4329.
250. Schwede, W.; Cleve, A.; Ottow, E.; Wiechert, R. *Tetrahedron Lett.* **1993**, *33*, 5257.
251. Kucera, D. J.; O'Connor, S. J.; Overman, L. E. *J. Org. Chem.* **1993**, *58*, 5304.
252. (a) Rahman, S. M. A.; Ohno, H.; Maezaki, N.; Iwata, C.; Tanaka, T. *Org. Lett.* **2000**, *2*, 2893. (b) Rahman, S. M. A.; Ohno, H.; Yoshino, H.; Satoh, N.; Tsukaguchi, M.;

Murakami, K.; Iwata, C.; Maezaki, N.; Tanaka, T. *Tetrahedron* **2001**, *57*, 127.
253. (a) Rahman, S. M. A.; Ohno, H.; Murata, T.; Yoshino, H.; Satoh, N.; Murakami, K.; Patra, D.; Iwata, C.; Maezaki, N.; Tanaka, T. *Org. Lett.* **2001**, *3*, 619. (b) Rahman, S. M. A.; Ohno, H.; Murata, T.; Yoshino, H.; Satoh, N.; Murakami, K.; Patra, D.; Iwata, C.; Maezaki, N.; Tanaka, T. *J. Org. Chem.* **2001**, *66*, 4831.
254. Piers, E.; Breau, M. L.; Han, Y.; Plourde, G. L.; Yeh, W.-L. *J. Chem. Soc., Perkin Trans. 1* **1995**, 963.
255. Miyaoka, H.; Shida, H.; Yamada, N.; Mitome, H.; Yamada, Y. *Tetrahedron Lett.* **2003**, *43*, 2227.
256. Optimized conditions have also been used to alkylate ICH_2CHM_{OMOM}.[248a]
257. Raucher, S.; Koolpe, G. A. *J. Org. Chem.* **1978**, *43*, 3794.
258. For leading references see: (a) Corset, J.; Castellà-Ventura, M.; Froment, F.; Strzalko, T.; Wartski, L. *J. Org. Chem.* **2003**, *68*, 3902. (b) Carlier, P. R.; Lo, C. W.-S. *J. Am. Chem. Soc.* **2000**, *122*, 12819. (c) Carlier, P. R.; Lucht, B. L.; Collum, D. B. *J. Am. Chem. Soc.* **1994**, *116*, 11602.
259. For a discussion of the structure of metallated nitriles see Ref. 21a.
260. Sinz, C. J.; Rychnoysky, S. D. *Top. Curr. Chem.* **2001**, *216*, 52.
261. Husson, H.-P.; Royer, J. *Chem. Soc. Rev.* **1999**, *28*, 383.
262. Chuang, T.-H.; Fang, J.-M. *J. Chin. Chem. Soc.* **2001**, *48*, 193.
263. The pK_a of acetone and acetonitrile are 20 and 25,a respectively, with the pK_a of acetonitrile being recently revised upward to ~30.[22b] Bordwell, F. G.; Matthews, W. S. *J. Am. Chem. Soc.* **1974**, *96*, 1214.
264. Darvesh, S.; Grant, A. S.; MaGee, D. I.; Valenta, Z. *Can. J. Chem.* **1991**, *69*, 712.
265. Debal, A.; Cuvigny, T.; Larcheveque, M. *Tetrahedron Lett.* **1977**, *36*, 3187.
266. Darvesh, S.; Grant, A. S.; MaGee, D. I.; Valenta, Z. *Can. J. Chem.* **1989**, *67*, 2237.
267. Fleming, F. F.; Shook, B. C.; Jiang, T.; Steward, O. W. *Org. Lett.* **1999**, *1*, 1547.
268. Lerm, M.; Gais, H.-J.; Cheng, K.; Vermeeren, C. *J. Am. Chem. Soc.* **2003**, *125*, 9653.
269. For an excellent survey see: Culkin, D. A.; Hartwig, J. F. *Acc. Chem. Res.* **2003**, *36*, 234.
270. (a) Caron, S.; Vazquez, E.; Wojcik, J. M. *J. Am. Chem. Soc.* **2000**, *122*, 712. (b) Krishnamurthy, D.; Han, Z.; Wald, S. A.; Senanayake, C. H. *Tetrahedron Lett.* **2002**, *43*, 2331.
271. Caron, S.; Vazquez, E. *Org. Process. Res. Dev.* **2000**, *5*, 587.
272. (a) Bunlaksananusorn, T.; Rodriguez, A. L.; Knochel, P. *Chem. Commun.* **2001**, *8*, 745. (b) Ref. 54.
273. Nemoto, H.; Hashimoto, M.; Kurobe, H.; Fukumoto, K.; Kametani, T. *J. Chem. Soc., Perkin Trans. 1* **1985**, 927.
274. Culkin, D. A.; Hartwig, J. F. *J. Am. Chem. Soc.* **2002**, *124*, 9330.
275. You, J.; Verkade, J. G. *Angew. Chem., Int. Ed.* **2003**, *42*, 5051.
276. Elnagdi, M. H.; Elmoghayar, M. R. H.; Elgemeie, G. E. H. *Synthesis* **1984**, 1.
277. Larcheveque, M.; Mulot, P. *Can. J. Chem.* **1979**, *57*, 17.
278. Mandal, A. N.; Raychaudhuri, S. R.; Chatterjee, A. *Synthesis* **1983**, 727.
279. Fuson, R. C.; Wolf, D. E. *J. Am. Chem. Soc.* **1939**, *61*, 1940.
280. Süsse, M.; Hájicek, J.; Hesse, M. *Helv. Chim. Acta* **1985**, *68*, 1986.
281. Trost, B. M.; Fraisse, P. L.; Ball, Z. T. *Angew. Chem., Int. Ed.* **2002**, *41*, 1059.
282. (a) Ziegler, F. E.; Nangia, A.; Schulte, G. *J. Am. Chem. Soc.* **1987**, *109*, 3987. (b) Ziegler, F. E.; Nangia, A.; Schulte, G. *Tetrahedron Lett.* **1988**, *29*, 1669.
283. Coates, R. M.; Hobbs, S. J. *J. Org. Chem.* **1984**, *49*, 140.
284. Ikeda, M.; Uchino, T.; Maruyama, K.; Sato, A. *Heterocycles* **1988**, *27*, 2349.
285. Enders, D.; Zamponi, A.; Raabe, G.; Runsink, J. *Synthesis* **1993**, 725.
286. Olkhovik, V.; Masalov, N.; Jansen, B. J. M.; de Groot, A. *Tetrahedron Lett.* **2001**, *42*, 4903.
287. Liu, H.-J.; Ly, T. W.; Tai, C.-L.; Wu, J.-D.; Liang, J.-K.; Guo, J.-C.; Tseng, N.-W.; Shia, K.-S. *Tetrahedron* **2003**, *59*, 1209.
288. Jansen, B. J. M.; Hendrikx, C. C. J.; Masalov, N.; Stork, G. A.; Meulemans, T. M.; Macaev, F. Z.; de Groot, A. *Tetrahedron* **2000**, *56*, 2075.
289. Kido, F.; Noda, Y.; Yoshikoshi, A. *J. Am. Chem. Soc.* **1982**, *104*, 5509.
290. Nubaummer, C. *Helv. Chim. Acta* **1990**, *73*, 1621.
291. Milenkov, B.; Süsse, M.; Hesse, M. *Helv. Chim. Acta* **1985**, *68*, 2115.
292. (a) Vite, G. D.; Spencer, T. A. *J. Org. Chem.* **1988**, *53*, 2560. (b) Piers, E.; Yeung, B. W. A. *J. Org. Chem.* **1984**, *23*, 4567. (c) Posner, G. H.; Sterling, J. J.; Whitten, C. E.; Lentz, C. M.; Brunelle, D. J. *J. Am. Chem. Soc.* **1975**, *97*, 107.
293. Stork, G.; Gardner, J. O.; Boeckman, R. K., Jr.; Parker, K. A. *J. Am. Chem. Soc.* **1973**, *95*, 2014.
294. (a) Bilyard, K. G.; Garratt, P. J.; Zahler, R. *Synthesis* **1980**, 389. (b) Garratt, P. J.; Pielke, M.; Porter, J. R. *Tetrahedron Lett.* **1987**, *28*, 589.
295. Kung, L.-R.; Tu, C.-H.; Shia, K.-S.; Liu, H.-J. *Chem. Commun.* **2003**, 2490.
296. (a) Nemoto, H.; Nagai, M.; Abe, Y.; Moizumi, M.; Fukumoto, K.; Kametani, T. *J. Chem. Soc., Perkin Trans. 1* **1987**, 1727. (b) Ref. 273.
297. Van Tamelen, E. E.; Rudler, H.; Bjorklund, C. *J. Am. Chem. Soc.* **1971**, *93*, 7113.
298. Ohsawa, T.; Kobayashi, T.; Mizuguchi, Y.; Saitoh, T.; Oishi, T. *Tetrahedron Lett.* **1985**, *26*, 6103.
299. Franck-Neumann, M.; Miesch, M. l.; Lacroix, E.; Metz, B.; Kern, J. M. *Tetrahedron* **1992**, *48*, 1911.
300. Sicinski, R. R.; Perlman, K. L.; Prahl, J.; Smith, C.; DeLuca, H. F. *J. Med. Chem.* **1996**, *39*, 4497.
301. (a) Birch, A. J.; Hutchinson, E. G. *J. Chem. Soc., Perkin Trans. 1* **1972**, 1546. (b) Kraus, G. A.; Hon, Y. S. *J. Org. Chem.* **1985**, *50*, 4605.
302. Arapakos, P. G. *J. Am. Chem. Soc.* **1967**, *89*, 6794.
303. Arapakos, P. G.; Scott, M. K. *Tetrahedron Lett.* **1968**, *16*, 1975.
304. Shono, T.; Terauchi, J.; Kitayama, K.; Takeshima, Y.; Matsumura, Y. *Tetrahedron* **1992**, *48*, 8253.
305. Gregory, G. B.; Johnson, A. L.; Ripka, W. C. *J. Org. Chem.* **1990**, *55*, 1479.
306. Haworth, B. J. W.; Morrison, A. L.; Rinderknecht, H. *J. Chem. Soc.* **1944**, 261.
307. Rychnovsky, S. D.; Hata, T.; Kim, A. I.; Buckmelter, A. J. *Org. Lett.* **2001**, *3*, 807.
308. Guvigny, T.; Larcheveque, M.; Normant, H. *Bull. Soc. Chem. Fr.* **1973**, *3*, 1174.
309. Arapakos, P. G.; Scott, M. K.; Huber, F. E., Jr. *J. Am. Chem. Soc.* **1969**, *91*, 2059.

310. Rychnovsky, S. D.; Zeller, S.; Skalitzky, D. J.; Griesgraber, G. *J. Org. Chem.* **1990**, *55*, 5550.
311. Gerlach, U. *Tetrahedron Lett.* **1995**, *36*, 5159.
312. Hattori, K.; Grossman, R. B. *J. Org. Chem.* **2003**, *68*, 1409.
313. Curran, D. P.; Seong, C. M. *Synlett* **1991**, 107.
314. Freerksen, R. W.; Selikson, S. J.; Wroble, R. R.; Kyler, K. S.; Watt, D. S. *J. Org. Chem.* **1983**, *48*, 4087.
315. (a) Clawson, P.; Whiting, D. A. *Tetrahedron Lett.* **1987**, *28*, 3155. (b) Clawson, P.; Whiting, D. A. *J. Chem. Soc., Perkin Trans. 1* **1990**, 1193.
316. Rowland, A. T.; Filla, S. A.; Coutlangus, M. L.; Winemiller, M. D.; Chamberlin, M. J.; Czulada, G.; Johnson, S. D.; Sabat, M. *J. Org. Chem.* **1998**, *63*, 4359.
317. Chiu, C. K.-F.; Govindan, S. V.; Fuchs, P. L. *J. Org. Chem.* **1994**, *59*, 311.
318. Curran, D. P.; Seong, C. M. *Tetrahedron* **1992**, *48*, 2157.
319. (a) Marshall, J. A.; Karas, L. J. *Synth. Commun.* **1978**, *8*, 65. (b) Marshall, J. A.; Karas, L. J. *J. Am. Chem. Soc.* **1978**, *100*, 3615. (c) Marshall, J. A.; Karas, L. J.; Royce, R. D., Jr. *J. Org. Chem.* **1979**, *44*, 2994.
320. (a) de Lucchi, O.; Piccolrovazzi, N.; Licini, G.; Modena, G.; Valle, G. *Gazz. Chim. Ital.* **1987**, *117*, 401. (b) Ref. 132.
321. Guijarro, D.; Yus, M. *Tetrahedron* **1994**, *50*, 3447.
322. Coates, R. M.; Shah, S. K.; Mason, R. W. *J. Am. Chem. Soc.* **1979**, *101*, 6765.
323. Coates, R. M.; Shah, S. K.; Mason, R. W. *J. Am. Chem. Soc.* **1982**, *104*, 2198.
324. Shia, K.-S.; Chang, N.-Y.; Yip, J.; Liu, H.-J. *Tetrahedron Lett.* **1997**, *38*, 7713.
325. Zhu, J.-L.; Shia, K.-S.; Liu, H.-J. *Tetrahedron Lett.* **1999**, *40*, 7055.
326. Lin, S.-C.; Yang, F.-D.; Shiue, J.-S.; Yang, S.-M.; Fang, J.-M. *J. Org. Chem.* **1998**, *63*, 2909.
327. Tsai, T.-Y.; Shia, K.-S.; Liu, H.-J. *Synlett* **2003**, 97.

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Tetrahedron
Tetrahedron 61 (2005) 791–796

Ship-in-a-bottle synthesis of triphenylamine inside faujasite supercages and generation of the triphenylamminium radical ion

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Received 17 September 2004; revised 17 November 2004; accepted 25 November 2004

Available online 8 December 2004

Abstract—The ship-in-a-bottle synthesis of triphenylamine encapsulated within basic X zeolite has been accomplished by reacting sodium diphenylamide with bromobenzene in the presence of a bifunctional palladium (Hartwig–Buchwald conditions). The presence of incarcerated triphenylamine was demonstrated by dissolving the zeolite with concentrated HF and analyzing the organic material in the dichloromethane extract. Laser flash photolysis (266 nm) gives rise to the generation of triphenylamminium radical cation detected as a transient species decaying in hundreds of microseconds. Upon repetitive cyclic voltammograms, zeolite encapsulated triphenylamine shows a reversible oxidation–reduction process. In contrast, in solution triphenylamine undergoes irreversible oxidation with the formation of coupling dimers.

1. Introduction

Nowadays, the ability of acid zeolites to generate organic radical cations is very well established,[1,2] and there is only certain controversy on the nature of the zeolite electron accepting sites. However, it turned out that the initial report of the ability of zeolites to generate radical cations was not very fortunate since none of the two selected probes, 1,1 diphenylethylene and triphenylamine (**Ph_3N**), do really generate radical cation within the internal space of the zeolite micropores. The blue species generated upon adsorption of 1,1 diphenylethylene is in reality a dimeric carbocation[3,4] and on the other hand, it was demonstrated that triphenylamine is not able to penetrate inside the micropores of zeolites, since its molecular size is too large to cross zeolite micropore apertures.[5]

For tridirectional, large pore zeolites whose internal micropore system is formed by larger cavities interconnected by smaller openings, a situation can be envisioned in which a large guest can be accommodated inside the cages, but cannot be adsorbed from the exterior. In these cases, it is sometimes possible to devise a so-called 'ship-in-a-bottle' synthesis in which the final molecule is prepared inside the cages by reacting smaller precursors.[6,7,8–11] After the synthesis, the large molecule remains 'mechanically entrapped' and incarcerated inside the cavity with some restricted conformational mobility. Herein, we report a ship-in-a-bottle synthesis of triphenylamine inside the cavities of zeolite Y (**Ph_3N@Y**) and the photoinduced generation of its amminium radical cation. Given the historical importance of **Ph_3N** in zeolite science as well as the use of triarylamminium radical cations as initiators for radical cation chain reactions,[12] particularly Diels–Alder cycloadditions, the feasibility to prepare **Ph_3N** encapsulated inside zeolites could open future applications of these solids in radical ion chemistry.

2. Results and discussion

The ship-in-a-bottle synthesis of **Ph_3N** inside zeolite Y was initially attempted by mimicking the reported Ullmann reaction of diphenylamine and iodobenzene in the presence of Cu-containing Y zeolite using potassium carbonate as a base.[13] However, although different conditions were studied in which the copper oxidation state, temperature, solvent and reaction time were varied, the percentage of **Ph_3N** formed as detected in the organic solvent was low and significant amounts of unreacted diphenylamine remained. The best results have been obtained using Cu^{2+}–Y (70% Na^{+}-to-Cu^{++} exchange) in nitrobenzene at 200 °C, in

Keywords: Zeolites; Host–guest chemistry; Palladium catalysis; Laser flash photolysis; Photoinduced electron transfer.
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0040–4020/$ - see front matter © 2004 Published by Elsevier Ltd.
doi:10.1016/j.tet.2004.11.062

where analysis of the solvent demonstrates the presence in the solution of **Ph_3N** with 30% of yield. Probably some in situ reduction of Cu^{2+} to Cu^{+} occurs during the reaction conditions and this Cu^{+} is presumably the active species. IR spectra of the zeolite wafers after the synthesis were complicated due to the presence of residual nitrobenzene or derived products, but anyway these IR spectra conclusively ruled out the presence of **Ph_3N** inside the zeolite.

Recently, it has been reported a novel Pd-catalyzed procedure for the arylation of amines known as the Hartwig–Buchwald reaction.[14,15] The main advantages of this methodology are the milder reaction conditions and the excellent yields that are generally obtained. In order to test if this reaction can serve to effect the ship-in-a-bottle synthesis of **Ph_3N**, we prepared a Pd-containing basic X faujasite by adsorbing $PdCl_2$ on KX zeolite from aqueous solution according to the so-called incipient wetness procedure. For precious, noble metals impregnation with their salts is a common less costly alternative to conventional ion exchange. This bifunctional catalyst (noble metal and basic sites) has been earlier found to be an efficient catalyst for the heterogeneous Suzuki reaction,[16] mechanistically related to the amine arylation needed here to synthesize **Ph_3N**.

Thus, treatment of diphenylamine with sodium *tert*-butoxide renders the corresponding diphenylamide anion. By reacting a suspension of this anion with bromobenzene as solvent at 156 °C using $PdCl_2$–KX as catalyst, the presence of **Ph_3N** was observed in the organic solution as the only detectable product accompanied by the complete disappearance of diphenylamine. Solid–liquid extraction of the solid also leads to the recovery of extra amounts of **Ph_3N** in the absence of any diphenylamine. In a blank control without performing any reaction it was determined that over 70% of the adsorbed diphenylamine is recoverable from $PdCl_2$–KX by simple solid–liquid extraction, thus, it is reasonable to assume that unreacted diphenylamine could have been detected by our reaction procedure.

Normally in the ship-in-a-bottle methodology, analysis of the liquid phase leads to a product distribution that does not differ much or is compatible with the organic material entrapped inside the solid.[7] Therefore, analysis of the supernatant constitutes a rational approach towards the understanding of the organic adsorbate encapsulated inside the zeolite pores.[17] Once the product distribution in the liquid phase is compatible with the formation of the **Ph_3N** and the complete disappearance of diphenylamine is observed, we proceeded to study the solid by diffuse reflectance UV–Vis and IR spectroscopies. In the diffuse-reflectance UV–Vis spectrum, the **Ph_3N**@$PdCl_2$–KX solid exhibits absorption bands at 255 and 315 nm (Fig. 1). The bands are, however, much broader than those recorded of the same compounds in solution, probably indicating the influence of steric constraints, interaction with the zeolite host and/or inhomogeneity in the distribution of **Ph_3N**. The IR spectrum of **Ph_3N**@$PdCl_2$–KX exhibits the vibration bands characteristic of the phenyl rings at 1600 and 1495 cm^{-1} (Fig. 2). None of these features appear in the UV–VIS or IR spectra of $PdCl_2$–KX blank zeolite and they

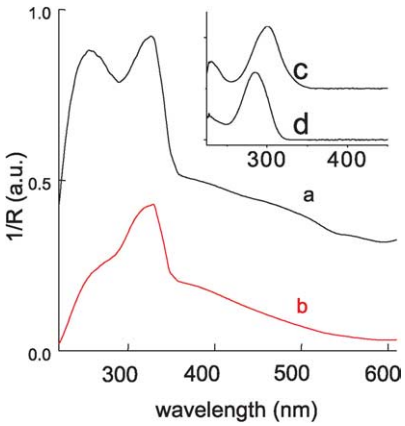


Figure 1. Diffuse-reflectance UV–Vis spectra of **Ph_3N**@$PdCl_2$–KX (spectrum a) and Ph_2NH@$PdCl_2$–KX (spectrum b). For the sake of comparison the inset shows the UV–Vis spectra of **Ph_3N** (spectrum c) and Ph_2NH (spectrum d) recorded in dichloromethane.

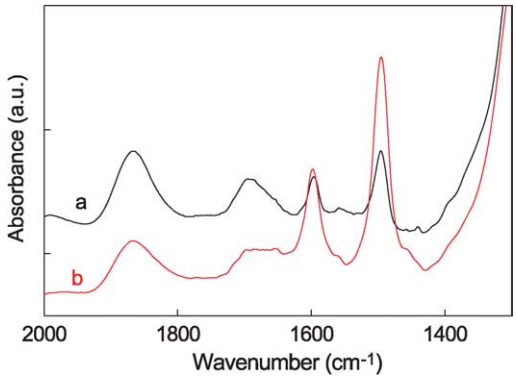


Figure 2. IR spectra of the samples Ph_2NH@$PdCl_2$–KX (a) and **Ph_3N**@$PdCl_2$–KX (b) recorded at room temperature in a sealed cell after outgassing at 100 °C during 1 h at 10^{-2} Pa.

are attributable exclusively to the organic guest formed during the Hartwig–Buchwald reaction.

However, given the similarity between the spectra of diphenylamine and **Ph_3N**, firm spectroscopic evidence for the formation of **Ph_3N** and the absence of diphenylamine could not be obtained, since the spectra are compatible with any of the two compounds or a mixture of both. For the sake of comparison, Figures 1 and 2 also contain the spectra of blank controls in which diphenylamine was adsorbed on $PdCl_2$–KX. Nevertheless, the above spectra, and particularly the IR spectrum of **Ph_3N**@$PdCl_2$–KX, exclude the presence of some adventitious by-products.

To obtain a conclusive evidence for the presence of **Ph_3N** inside the X zeolite, we proceeded to dissolve the aluminosilicate host with concentrated HF and extracted the resulting organic material with CH_2Cl_2 after neutralization with Na_2CO_3. Faujasite X is a suitable zeolite for this type of study, since its aluminosilicate framework easily dissolves in aqueous acids. This experiment served to demonstrate definitely the presence of **Ph_3N** in the solid without being able to detect diphenylamine in the extract. This result is compatible with the indirect analytical and spectroscopical data obtained for the supernatant during

reaction, agrees with the good results of the Hartwig–Buchwald reaction in homogeneous phase[18] and is compatible with the in situ spectroscopic data. Controls using a mixture of Ph_2NH and bromobenzene showed that no **Ph_3N** is formed in the acid treatment. A molecular modeling at the semiempirical level using AM1 of the best docking of **Ph_3N** inside the faujasite α-cages is presented in Figure 3 giving a visual indication of the relative sizes of the organic guest, the zeolite cavity and the connecting cavity windows. To obtain this model the faujasite structure was considered rigid at the crystallographic positions and devoided of aluminium, while the geometry and location of **Ph_3N** was optimized to minimize unfavourable van der Waals interactions.

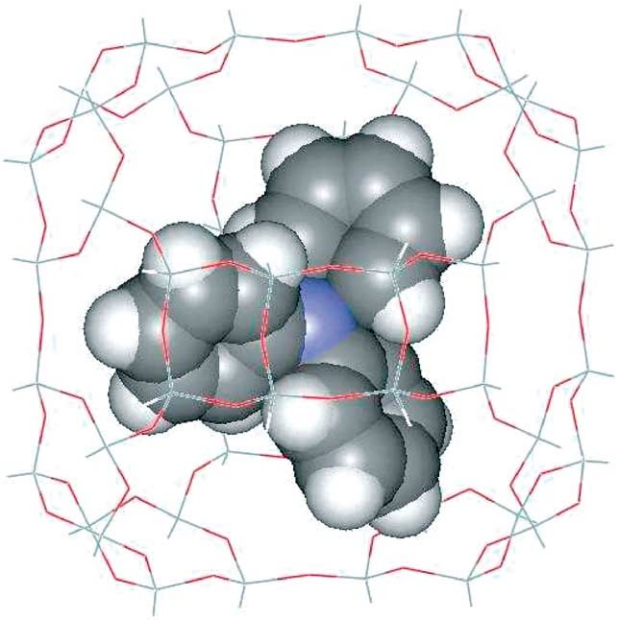


Figure 3. Visualization of the best docking of **Ph_3N** encapsulated inside the supercage of an idealized all-silica faujasite.

Once the identity and purity of the organic material encapsulated within zeolite has been demonstrated, we have used combustion chemical analysis of the extracted **Ph_3N**@$PdCl_2$–KX sample to determine the loading of **Ph_3N**. The experimental data of the C/N ratio for the extracted **Ph_3N**@$PdCl_2$–KX also agrees with the molecular formula $C_{18}H_{15}N$ for **Ph_3N** (C/N 18) and is significantly different from that of the diphenylamine $C_{12}H_{11}N$ (C/N 12). The loading of **Ph_3N** estimated for the weight percent of C or N is of one molecule every 12.5 supercages in average.

After having demonstrated the successful ship-in-a-bottle synthesis of **Ph_3N** inside the cavities of zeolite X, we submitted the **Ph_3N**@$PdCl_2$–KX sample to laser flash photolysis using the forth harmonic of a nanosecond Nd YAG laser system at 266 nm. As can be seen in Figure 4, upon laser excitation a transient species is generated whose diffuse reflectance UV–Vis spectrum recorded 35 μs after the laser flash is characterized by an absorption band at 630 nm having a shoulder in the low wavelength side. This transient decays in hundreds of microseconds and its

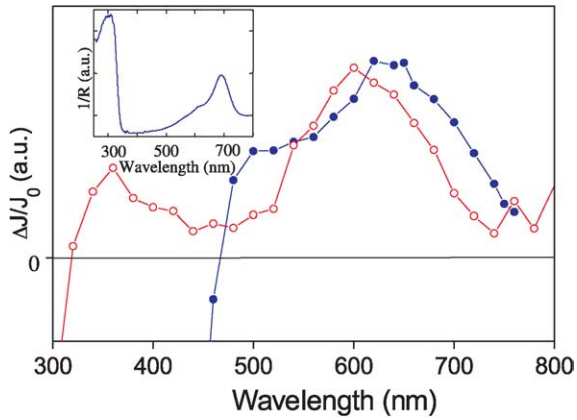


Figure 4. Time resolved diffuse reflectance UV–Vis spectrum of the sample **Ph_3N**@$PdCl_2$–KX recorded 35 μs after 266 nm laser excitation (•) and transient UV–Vis spectrum of **Ph_3N** in aerated MeCN recorded 1 μs after 308 nm laser excitation (○). The latter transient spectrum corresponds to the **$Ph_3N^{\bullet+}$** radical cation. The inset shows the diffuse reflectance UV–Vis spectrum of the persistent diphenylamine radical cation incorporated in a medium pore ZSM-5.

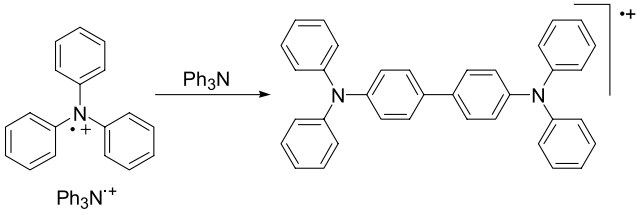
disappearance is not complete 1 ms after the laser pulse that is the longest time scale available in our ns laser system.

This transient was assigned to **Ph_3N** radical cation based on the λ_{max} of the UV–Vis spectrum recorded for **Ph_3N**@$PdCl_2$–KX compared with that of an authentic spectrum of **Ph_3N** radical cation generated by photolyzing **Ph_3N** in acetonitrile solution in the presence of oxygen (Figure 4, λ_{max} 600 nm) and also with the reported spectrum of triphenylamminium radical cation.[19–21] The minor differences in the transient spectra of **$Ph_3N^{\bullet+}$** recorded in solution and from the **Ph_3N**@$PdCl_2$–KX sample can be easily explained by the absorption of the $PdCl_2$–KX matrix as compared with the transparent solvent. Moreover, this transient spectrum is also similar in shape, although significantly shifted, with that recorded for diphenylamine in acid zeolites, particularly ZSM-5 zeolite.[22] It has been shown that diphenylamine radical cation can be generated spontaneously upon adsorption of neutral diphenylamine into the channels of dehydrated ZSM-5.[22–24] Under these circumstances, the resulting radical cation entrapped in ZSM-5 is persistent enough to be characterized by conventional spectroscopy. For the sake of comparison, Figure 4 also includes the diffuse reflectance UV–Vis spectrum of persistent diphenylamine radical cation incorporated in ZSM-5. The absorption maxima wavelength appears at 690 nm, about 60 nm red-shifted as compared to λ_{max} for **Ph_3N**@$PdCl_2$–KX.

The fact that the lifetime of the **Ph_3N** radical cation generated photochemically in faujasite X is dramatically shorter than that of diphenylamine radical cation in ZSM-5 can be attributed to the stabilization introduced by the steric restrictions imposed by the rigid ZSM-5 framework (5.4×5.6 Å) thwarting reaction pathways otherwise accessible to diphenylamminium radical cation. In fact, it has been reported that the radical cation of diphenylamine and other aromatic amines undergoes different reactions when adsorbed on large pore acid zeolites.[25,26] Moreover, as mentioned above our KX zeolite is not acidic but basic. It is known that the persistence of radical cations increases with

the acid strength of the zeolite framework.[1] It has been established that the lifetime of organic radical cations generated by electron abstraction of electron rich molecules from the zeolite framework increases considerably (over six orders of magnitude!) with acidity of the zeolite framework.[27]

Encapsulation of **Ph_3N** inside the cavities of zeolite X cavities should isolate this molecule. Immobilization of **Ph_3N** should impede dimerization of **$Ph_3N^{\cdot+}$** radical cation through ion–molecule reaction (Scheme 1), a process that is known to occur in solution and that leads to benzidine derivatives. Dimerization through **$Ph_3N^{\cdot+}$** radical cation is the process responsible of the lack of reversibility observed in the cyclic voltammetry generation of **$Ph_3N^{\cdot+}$** in solution. To demonstrate the positive influence of encapsulation thwarting ion–molecule reactions of **$Ph_3N^{\cdot+}$** such as that indicated in Scheme 1, we proceeded to record the cyclic voltammetry of the **Ph_3N**@$PdCl_2$–KX sample and compared to results with those recorded for the same molecule in solution.[27–30] It has been agreed that electrochemical techniques only probe those guests located in the outermost layers of the zeolite particles, those others that are located deep inside the particle cannot be probed.[9,31–36] Nevertheless this limitation, cyclic voltammetry is very useful to study the behaviour of zeolite-bound organic radical cations. Figure 5 shows the cyclic voltammogram of **Ph_3N** recorded in acetonitrile solution, compared to the response recorded for a zeolite-modified electrode containing **Ph_3N**@$PdCl_2$–KX.



Scheme 1.

Upon anodic scan at positive potentials, the response of **Ph_3N**@$PdCl_2$–KX shows two anodic oxidation peaks at +0.6 and +0.9 V vs SCE corresponding to palladium oxidation and the radical cation generation of **Ph_3N**. The oxidation of **Ph_3N** in solution takes place at +1.0 V vs. SCE, thus indicating that **Ph_3N** oxidation is facilitated by 0.1 V inside the zeolite pores, a fact that agrees with the general behavior of zeolites stabilizing radical cations.[1] In our case, interaction with co-adsorbed Pd could also be responsible for this shift. The reversibility of the oxidation–reduction cycles of **Ph_3N** incorporated inside zeolite X was clearly demonstrated by the observation of a cathodic peak at +0.8 V vs SCE corresponding to the reduction of **$Ph_3N^{\cdot+}$** to the neutral **Ph_3N** molecule. Although some gradual variations in the cyclic voltammetric profile are apparent during the initial four scans, upon multiple repetitive scans from the fifth to 20th a stationary response was recorded in which the reversibility of the oxidation–reduction for **Ph_3N** in **Ph_3N**@$PdCl_2$–KX is clearly observed. The initial voltammetric scans also contained

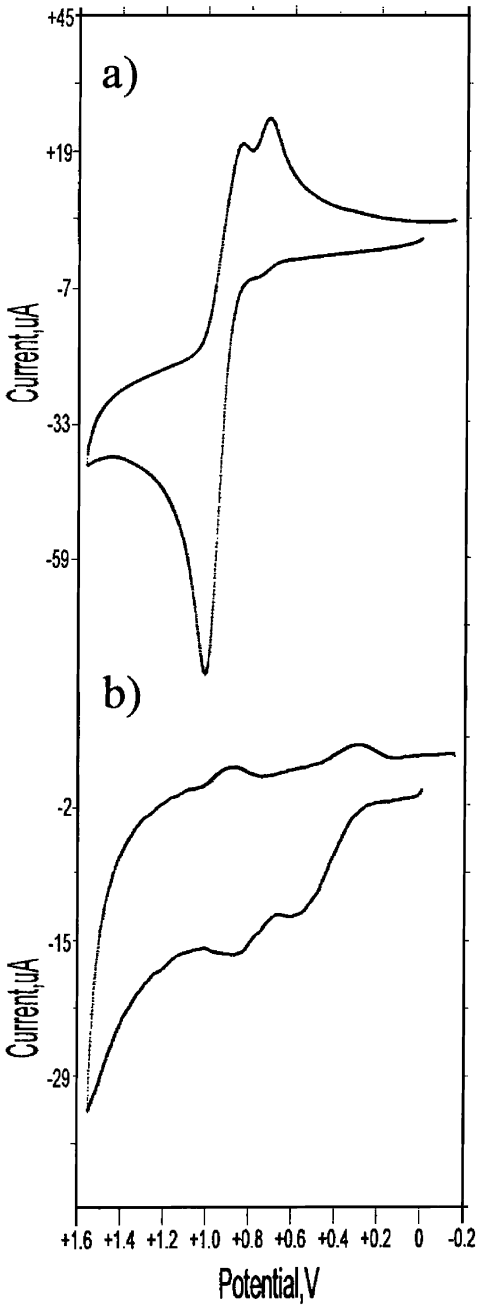


Figure 5. (a) Cyclic voltammogram of **Ph_3N** in acetonitrile using 0.10 M $LiClO_4$ as electrolyte; (b) cyclic voltammogram recorded after 10 previous cycles of a zeolite-modified electrode containing **Ph_3N**@$PdCl_2$–KX in contact with an acetonitrile solution using 0.10 M $LiClO_4$ as electrolyte.

the +0.6 and +0.9 V peaks as in Figure 5, but these waves were considerably less defined appearing on top of a broad background. The initial variations in the profile are most likely due to surface-bound spurious species that become progressively degraded in the first voltammometric cycles.

In conclusion, by using the Hartwig–Buchwald arylation of amines it has been possible to prepare a sample in which bulky **Ph_3N** is encapsulated inside the spherical supercages of faujasites, something that was claimed in the early reports on synthetic zeolites. The corresponding radical cation has been generated and detected in the submillisecond time scale as a transient by laser flash photolysis. By means of

cyclic voltammetry we have shown the reversibility of the oxidation–reduction, a fact that is not observed for **Ph_3N** in solution and that is directly related to the immobilization and site isolation of **Ph_3N** within the zeolite supercages.

3. Experimental

NaX and NaY zeolites were commercial samples (Aldrich). The faujasites in their Na^+-form were ion exchanged by stirring at room temperature a suspension of the solid in a 0.4 M aqueous solution of $Cu(NO_3)_2$ or KOAc using a solid/liquid ratio of 10 for 5 h. The ion exchange was repeated a second time using, in this case a 1 M solution of $Cu(NO_3)_2$ or KOAc under the same conditions. The resulting CuY and KX zeolites were collected and dehydrated by heating at 300 °C overnight. The Cu^{++}, K^+ and Na^+ content was determined by quantitative atomic absorption spectroscopy after dissolving known weight of the zeolites with aqueous HF (20 wt %). $PdCl_2$–KX was obtained by adding dropwise a solution of $PdCl_2$ (16.5 mg) in water (0.7 ml) to dry KX under stirring to afford the $PdCl_2$–KX. The solid was dehydrated before using for the ship-in-a-bottle synthesis of **Ph_3N**.

Diphenylamine (50 mg, 0.3 mmol) was reacted with Na ^{t}BuO (1.2 equiv) in bromobenzene (20 ml) at room temperature for 1 h. After this time, dehydrated $PdCl_2$–KX (1.0 g) was added to the solution, and the suspension stirred magnetically at 156 °C for 48 h. The course of the reaction was periodically followed by GC (25 m, 5% crosslinked phenylmethylsilicone capillary column). After the reaction, the solid was filtered, washed with CH_2Cl_2 and submitted to continuous solid–liquid extraction with CH_2Cl_2. Analysis of the adsorbates was accomplished by dissolving the aluminosilicate (1.00 g) with concentrated aqueous HF (20 ml). Once the solid has been dissolved, the solution was neutralized with HCO_3^- and extracted with CH_2Cl_2. The organic extract was analyzed by GC and the retention time compared to those of authentic samples of diphenylamine and **Ph_3N**.

Diffuse reflectance UV–Vis spectra were recorded under a N_2 flow ($0.5\ ml \times min^{-1}$) on a Cary 5G adapted with a praying mantis accessory. UV–Vis spectra were recorded on a Shimadzu UV-scanning spectrophotometer using dichloromethane as solvent. IR spectra were recorded on a Nicolet 710 spectrophotometer using sealed greaseless quartz cells provided with CaF_2 windows. Self-supported wafers (10 mg) were obtained by pressing the zeolite at $1\ Torr \times cm^{-2}$ for 1 min. The IR spectra were recorded at room temperature under vacuum after outgassing at 300 °C under 10^{-2} Pa. Laser flash photolysis experiments were carried out using the fourth (266 nm, 20 mJ $pulse^{-1}$) harmonic of a Surelite Nd:YAG laser for excitation (pulse $\leq$10 ns). The signal from the monochromator/photomultiplier detection system was captured by a Tektronix TDS640A digitizer and transferred to a PC computer that controlled the experiment and provided suitable processing and data storage capabilities. Fundamentals[37] and details[38] of similar time-resolved laser setup has been published elsewhere.

Zeolite modified electrodes were prepared by transferring a few microlitres (typically 50 μL) of a dispersion of the zeolite (10 mg) in acetone (5 mL) to the surface of a freshly polished glassy carbon electrode and allowing the suspension coating the electrode to dry in air. Then, one drop of a solution of commercially available Paraloid B72 [ethyl acrylate (70%)–methyl acrylate (30%) co-polymer] (1%) in acetone was added and the modified electrode was again air dried. The coatings examined contained 0.2–1.5 mg/cm^2 of the zeolite. Cyclic voltammograms of **Ph_3N** acetonitrile solution or **Ph_3N**@$PdCl_2$–KX deposited on glassy carbon electrodes were performed with a BAS CV50W equipment using a standard three-electrode arrangement with a platinum auxiliary electrode and a AgCl (3 M NaCl)/Ag reference electrode (SCE) in a thermostated cell. All electrochemical measurements were performed in well-deaerated solutions under an atmosphere of argon. To improve peak resolution, background subtraction using the voltammograms recorded at the bare electrode and semi-derivative convolution of the voltammograms were routinely performed.

Acknowledgements

Financial support of the Spanish DGES (Project MAT2003-1226) is gratefully acknowledged. The Spanish ministry of education is also thanked for two postgraduate scholarships to B.F. and A.L.

References and notes

1. Garcia, H.; Roth, H. D. *Chem. Rev.* **2002**, *102*, 3947–4008.
2. Stamires, D. N.; Turkevich, J. *J. Am. Chem. Soc.* **1964**, *86*, 749–757.
3. Fornés, V.; García, H.; Jovanovic, S.; Martí, V. *Tetrahedron* **1997**, *53*, 4715–4726.
4. Fornés, V.; García, H.; Martí, V.; Fernández, L. *Tetrahedron* **1998**, *54*, 3832–3837.
5. Brunel, D.; Nagy, J. B.; Daelen, G.; Derouane, E. G.; Geneste, P.; Vanderveken, D. J. *Appl. Catal. A* **1993**, *99*, 9–20.
6. Scaiano, J. C.; García, H. *Acc. Chem. Res.* **1999**, *32*, 783–793.
7. Corma, A.; Garcia, H. *Eur. J. Inorg. Chem.* **2004**, 1143–1164.
8. Balkus, K. J.; Gabrielov, A. G. *J. Incl. Phen. Mol. Recogn. Chem.* **1995**, *21*, 159–184.
9. Bedioui, F.; Roue, L.; Devinck, J.; Balkus, K. J. Jr. In *Zeolite Encapsulated Metal–Schiff Base Complexes. Synthesis and Electrochemical Characterization*; Weitkamp, J., Karge, H. G., Pfeifer, H., Hölderich, W., Eds.; Elsevier Science: Amsterdam, 1994; pp 917–924.
10. Schulz-Ekloff, G.; Wohrle, D.; Van Duffel, B.; Schoonheydt, R. A. *Microp. Mesop. Mater.* **2002**, *51*, 91–138.
11. Wohrle, D.; Sobbi, A. K.; Franke, O.; Schulzekloff, G. *Zeolites* **1995**, *15*, 540–550.
12. Bauld, N. L. *Tetrahedron* **1989**, *45*, 5307–5363.
13. Hassan, J.; Sevignon, M.; Gozzi, C.; Schulz, E.; Lemaire, M. *Chem. Rev.* **2002**, *102*, 1359–1469.
14. Driver, M. S.; Hartwig, J. F. *J. Am. Chem. Soc.* **1996**, *118*, 7217–7218.

15. Wolfe, J. P.; Wagaw, S.; Buchwald, S. L. *J. Am. Chem. Soc.* **1996**, *118*, 7215–7216.
16. Corma, A.; Garcia, H.; Leyva, A. *Appl. Catal. A* **2002**, *236*, 179–185.
17. Corma, A.; García, H. *J. Chem. Soc., Dalton Trans.* **2000**, 1381–1394.
18. Yamamoto, T.; Nishiyama, M.; Koie, Y. *Tetrahedron Lett.* **1998**, *39*, 2367–2370.
19. Breslin, D. T.; Fox, M. A. *J. Org. Chem.* **1994**, *59*, 7557–7561.
20. Breslin, D. T.; Fox, M. A. *J. Phys. Chem.* **1994**, *98*, 408–411.
21. Herron, N.; Stucky, G. D.; Tolman, C. A. *J. Chem. Soc., Chem. Commun.* **1986**, 1521.
22. García, H.; Martí, V.; Casades, I.; Fornés, V.; Roth, H. *Phys. Chem. Chem. Phys.* **2001**, *3*, 2955.
23. Qualye, W. H.; Lunsford, J. H. *Inorg. Chem.* **1982**, *21*, 97.
24. Herron, N.; Stucky, G. D.; Tolman, C. A. *J. Chem. Soc., Chem. Commun.* **1986**, 1521.
25. Alvaro, M.; García, H.; García, S.; Márquez, F.; Scaiano, J. C. *J. Phys. Chem.* **1997**, *101*, 3043–3051.
26. Corma, A.; Fornés, V.; García, H.; Martí, V.; Miranda, M. A. *Chem. Mater.* **1995**, *7*, 2136–2143.
27. Corma, A. *Chem. Rev.* **1995**, *95*, 559–614.
28. Mazine, V.; Heinze, J. *J. Phys. Chem. A* **2004**, *108*, 230–235.
29. Petr, A.; Kvarnstrom, C.; Dunsch, L.; Ivaska, A. *Syn. Metal.* **2000**, *108*, 245–247.
30. Jones, H. L.; Boxall, L. G.; Osteryoung, R. A. *J. Electroanal. Chem. Interfac. Electrochem.* **1972**, *38*, 476–478.
31. Domenech, A.; Alvaro, M.; Ferrer, B.; Garcia, H. *J. Phys. Chem. B* **2003**, *107*, 12781–12788.
32. Rolison, D. R. *Chem. Rev.* **1990**, *90*, 867–878.
33. Rolison, D. R.; Bessel, C. A. *Acc. Chem. Res.* **2000**, *33*, 737–744.
34. Rolison, D. B.; Bessel, C. A.; Baker, M. D.; Senaratne, C.; Zhang, J. *J. Phys. Chem.* **1996**, *100*, 8610–8611.
35. Bedioui, F.; Devynck, J.; Balkus, K. J., Jr. *J. Phys. Chem.* **1996**, *100*, 8607–8609.
36. Balkus, K. J.; Bedioui, F.; Roue, L.; Briot, E.; Devynck, J.; Bell, S. L. *J. Electroanal. Chem* **1994**, *373*, 19–29.
37. Wilkinson, F.; Kelly, G. In *Diffuse Reflectance Flash Photolysis*; Scaiano, J. C., Ed.; CRC Press: Boca Raton, 1989; p 293.
38. Kelly, G.; Willsher, C. J.; Wilkinson, F.; Netto-Ferreira, J. C.; Olea, A.; Weir, D.; Johnston, L. J.; Scaiano, J. C. *Can. J. Chem.* **1990**, *68*, 812–819.

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Tetrahedron
Tetrahedron 61 (2005) 797–802

Homogeneous catalytic aminocarbonylation of iodoalkenes and iodobenzene with amino acid esters under conventional conditions and in ionic liquids

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Received 22 September 2004; revised 2 November 2004; accepted 25 November 2004

Available online 8 December 2004

Abstract—Amino acid methyl esters were used as amine nucleophiles in palladium catalysed aminocarbonylation of iodobenzene and iodoalkenes (1-iodo-cyclohexene and 17-iodo-androst-16-ene). 2-Oxo-carboxamide type derivatives can be isolated as a result of double CO insertion by using iodobenzene as a substrate at elevated carbon monoxide pressure. On the contrary, carboxamides of expected structure were obtained exclusively in excellent yields in the whole pressure range by using iodoalkenes. The aminocarbonylation of 17-iodo-androst-16-ene in [bmim][PF_6] or [bmim][BF_4] (where bmim = 1-butyl-3-methyl-imidazolium cation) ionic liquids was also carried out and the ionic liquid–catalyst mixtures have been reused several times with only a small loss of activity.

1. Introduction

Palladium-catalysed carbonylation reactions including amino- and alkoxycarbonylation and carbonylative coupling reactions are widely used in synthetic chemistry.[1] There are a number of applications concerning the synthesis of simple building blocks and the functionalization of biologically important skeletons.[2] Aminocarbonylation plays a special role among these reactions, since those carboxamides which are hardly available via the carboxylic acid–carboxylic halide–carboxamide route (e.g., with bulky substituents at the amide nitrogen) can be synthesised from easily available starting materials. The synthesis of unsaturated carboxamides or aryl carboxamides with various structures has been published using enol-triflates/iodo-alkenes or aryl triflates/aryl halides as substrates in aminocarbonylation, respectively.[3]

The application of amino acid esters as N-nucleophiles in aminocarbonylation could be of great importance for two reasons: (i) amino acid derivatives or (oligo)peptides can be marked at the N-termini by the desired aromatic (or alkenyl) groups and (ii) skeletons of practical importance can be functionalized by amino acids protected at the carboxyl termini.

Although homogeneous carbonylation reactions may serve as an attractive tool in organic synthesis, there are serious difficulties in product separation and catalyst recovery. Among the methods (the use of various two-phase systems,[4] supported catalysts,[5] aqueous/organic media,[6] and alternative solvents like supercritical fluids,[7] fluorous solvents[8]) aiming at the reduction of these problems, ionic liquid catalysis plays an important role.[9] Homogeneous catalysis carried out in ionic liquids drew particular attention in the last few years and several examples for various palladium catalysed reactions carried out in ionic liquids have been published. However, there are only some sporadic results for the palladium catalysed aminocarbonylation of organic halides in ionic liquids.[10]

Although the N-acylation of amino acids is widely used in synthetic chemistry, to the best of our knowledge, there is only one example for the use of homogeneous

Keywords: Aminocarbonylation; Carbon monoxide; Amino acid; Palladium; Ionic liquid.
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0040–4020/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2004.11.064

aminocarbonylation of enol triflates by aminoacid derivatives as a tool for the introduction of a carboxamide moiety.[11] (This reaction was carried out using a slight excess of the *C*-protected amino acid as nucleophile, at 3 bar CO pressure (40 psi) at room temperature resulting in the products in 45–78% yields after 2 days). We found recently,[12] that homogeneous catalytic aminocarbonylation of steroidal substrates can be effectively carried out in ionic liquids as solvents even at atmospheric CO pressure using various palladium–phosphine catalytic systems.

Encouraged by the increasing importance of the selective synthesis of amino acid derivatives, we decided to investigate the possibility of extending the scope of this method to iodo-aryl/iodo-alkenyl substrates. Accordingly, the high-yielding aminocarbonylation of iodo-benzene, 1-iodo-cyclohexene and 17-iodo-androst-16-ene with amino acid methyl esters of different types is published in the present paper.

2. Results and discussion

2.1. Aminocarbonylation in conventional organic solvents

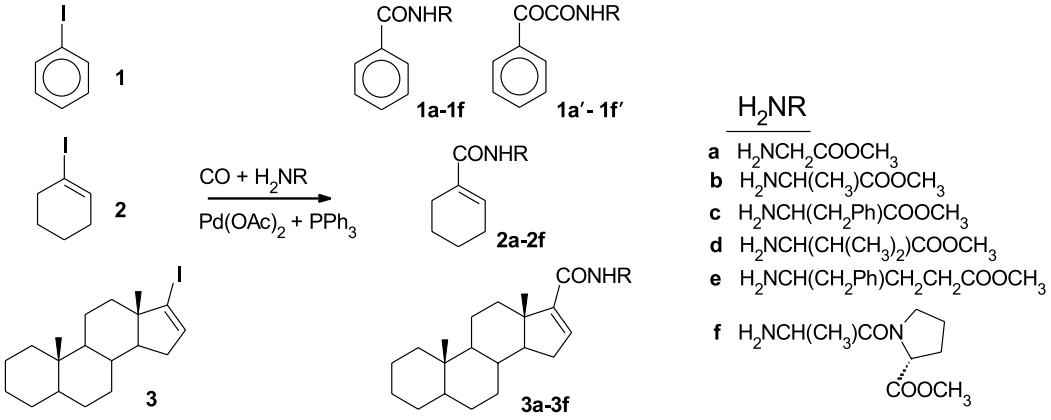
Iodo-benzene (**1**), 1-iodo-cyclohexene (**2**) and 17-iodo-androst-16-ene (**3**) were reacted with methyl glycinate (**a**), methyl alaninate (**b**), methyl phenylalaninate (**c**), methyl valinate (**d**), methyl 4-amino-4-benzyl-butanoate (**e**) and methyl *N*-alaninoyl-prolinate (**f**) under atmospheric carbon monoxide in the presence of in situ generated palladium(0)-triphenylphosphine catalysts (Scheme 1). Palladium(II) acetate was used as catalytic precursor. (The formation of Pd(0) species from the generally used $Pd(OAc)_2$–PPh_3 system has been proved by cyclic voltammetry and ^{31}P NMR.[13] The reduction of Pd(II) to Pd(0) is due to PPh_3, which is itself oxidised to triphenylphosphine oxide).

The reactivities of the two types of substrates, that of iodobenzene (**1**) and iodo-alkenes (1-iodo-cyclohexene (**2**) and 17-iodo-androst-16-ene (**3**)) are markedly different. The formation of the expected amides was observed both with **2** and **3** under atmospheric carbon monoxide at 50 °C in DMF. The resulting amides (**2a**–**2f**, **3a**–**3f**) have been obtained with practically complete conversion and isolated in 70–86% yields after column chromatography (see Section 4). However, iodobenzene (**1**) shows a rather poor reactivity towards amino acid derivatives under the same reaction conditions. (Carbonylation products have only been obtained in traces (<1%) except for **1a** which was formed in 20% yield). The isolation of the corresponding *N*-benzoyl amino acids (**1a**–**1f**) as pure compounds failed.

Under increased carbon monoxide pressure (10–40 bar) 2-oxo-carboxamides (**1a′**–**1d′**) have been obtained in excellent yields from iodobenzene (**1**). The formation of 2-oxo-carboxamides is accompanied by the formation of simple carboxamides (**1a**–**1f**) in surprisingly low amounts (less than 5%, if any detectable amount). It should be noted that 2-oxoamides present very interesting biological properties. For example, various lipophilic 2-oxoamides are potent inhibitors of digestive lipases[14–16] and phospholipases A2.[17] Most recently it has been reported that 2-oxoamides based on γ-aminoacids inhibit Group VIA phospholipase A2 and exhibit interesting in vivo anti-inflammatory and analgesic activity.[18] Carrying out the carbonylation reaction with the iodovinyl substrates (**2** and **3**) under same reaction conditions, the corresponding double-carbonylation products (**2a′**–**2d′**) were absent even at 40 bar carbon monoxide pressure.

Although the formation of 2-oxo-carboxamide can be explained in two ways, namely both the 'glyoxyl-route' and the 'acyl-aminocarbonyl-route' can be operative, detailed mechanistic studies revealed that the latter is responsible for the formation of double-carbonylation.[19,20] This way, the iodo-substrates are oxidatively added to in situ formed palladium(0) complexes. Carbon monoxide is coordinated to palladium and its insertion to Pd–C bond takes place. While benzoyl-palladium complexes might form the corresponding benzoyl-aminocarbonyl complex which leads to keto-carboxamides (**1a′**–**f′**) by reductive elimination, the alkenoyl-palladium species react directly with the amine nucleophile providing the corresponding carboxamide (**2a**–**2f** and **3a**–**3f**).

It is worth noting, that the good reactivity of the substrates with iodo-alkene functionality and the easy work-up of the reaction mixtures make these derivatives suitable for any functionalization. The use of iodo-alkenes as synthetic substitutes for the corresponding enol-triflates is supported



Scheme 1. Reaction of iodo-benzene (**1**) and iodo-alkenes (**2** and **3**) with amino acid derivatives under carbonylation conditions.

not only by green chemistry principles but also their advantageous synthetic properties as substrates in homogeneous catalytic reactions. The advantageous use of steroidal substrates with 17-iodo-16-ene functionality has been proved in a variety of palladium-catalysed homogeneous reactions.[21]

2.2. Aminocarbonylation of 3 in ionic liquids

As products obtained with homogeneous catalytic transformations of steroidal substrates cannot be separated by distillation, several repetitive chromatographic separations are needed for the complete removal of the catalyst, which is essential for compounds of pharmaceutical interest.

Recently, we have found[12] that the palladium-content of the crude steroidal product could be decreased by the use of ionic liquids as solvents during aminocarbonylation of steroidal 17-iodo-16-ene derivatives with morpholine as the nucleophile. The ionic liquid–catalyst mixture could be reused several times with only a small loss of activity.

A similar method was effectively used for the model aminocarbonylation of **3** with methyl glycinate as the nucleophile (Table 1). The steroidal substrate was reacted with methyl glycinate in the presence of a palladium catalyst and Et_3N in carbon monoxide atmosphere at 100 °C. The product could be extracted with toluene together with the unreacted starting material.

Based on our previous results, $[bmim]^+[PF_6]^-$ and $[bmim]^+[BF_4]^-$ ionic liquids were used as solvents and in situ formed palladium complexes as catalysts derived from $Pd(OAc)_2 + n PPh_3$ or $Pd(OAc)_2 + n$DPPBA (DPPBA: 4-(diphenylphosphino)benzoic acid) precursors. A relatively high phosphine/palladium ratio was essential for the efficient re-circulation of the catalyst, so the reactions were carried out at 6, 8 or 10 P/Pd ratios.

Although the reuse of the ionic liquid–catalyst mixture seems to be less effective here compared to the aminocarbonylation with morpholine, the same effects of the modification of the reaction conditions on the conversion were observed. The ionic liquid $[bmim]^+[BF_4]^-$ was found to be superior to $[bmim]^+[PF_6]^-$. The increase in the ratio of PPh_3 to palladium led to lower conversion (compare entries 1, 3, 4 in Table 1). The best results were obtained with the $Pd(OAc)_2 + 10$DPPBA catalytic system. It should be mentioned that using this phosphine no leaching of the ligand into the extract was observed.

3. Conclusions

Palladium catalysed aminocarbonylation proved to be an efficient method for the functionalization of iodobenzene and iodoalkenes using amino acid methyl esters as amine nucleophiles. The reactivity of the two types of substrates differs substantially. 2-Oxo-carboxamide type derivatives have been isolated as a result of double CO insertion by using iodobenzene, while carboxamides were obtained exclusively in excellent yields in the whole pressure range using iodoalkenes. 17-Iodo-androst-16-ene was successfully carbonylated in $[bmim][PF_6]$ or $[bmim][BF_4]$ ionic liquids and the ionic liquid–catalyst mixtures have been reused several times with only a small loss of activity.

4. Experimental

4.1. General procedures

^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Varian Inova 400 spectrometer at 400.13 and 100.62 MHz, respectively. Chemical shifts δ are reported in ppm relative to $CHCl_3$ (7.26 and 77.00 ppm for ^1H and ^{13}C, respectively). Elemental analyses were measured on a 1108 Carlo Erba apparatus.

Samples of the catalytic reactions were analysed with a Hewlett Packard 5830A gas chromatograph fitted with a capillary column coated with OV-1.

4.2. Aminocarbonylation experiments at normal pressure

In a typical experiment a solution of $Pd(OAc)_2$ (5.6 mg, 0.025 mmol), PPh_3 (13.1 mg, 0.05 mmol), 0.5 mmol iodo substrate (**1**, **2** or **3**), 0.65 mmol amino acid methyl ester (**a**, **b**, **c**, **d**, **e** or **f**) were dissolved in 10 ml DMF (10 ml) under argon. Triethylamine (0.5 ml) was added to the homogeneous yellow solution and the atmosphere was changed to carbon monoxide. The colour changed to dark red. The reaction was conducted for 6 h at 50 °C. Some metallic palladium was formed at the end of the reaction which was filtered. (A sample of this solution was immediately analysed by GC–MS.) The mixture was then concentrated

Table 1. Carbonylation of 17-iodo-5α-androst-16-ene in the presence of methyl glycinate using $Pd(OAc)_2$ + phosphine in situ catalysts in ionic liquids[a]

Entry	Solvent	Phosphine	P/Pd	Conversion (%)				
				Run 1	Run 2	Run 3	Run 4	Run 5
1	$[bmim]^+[PF_6]^-$	PPh_3	6	99	98	92	70	52
2	$[bmim]^+[BF_4]^-$	PPh_3	6	100	100	98	74	55
3	$[bmim]^+[PF_6]^-$	PPh_3	8	93	90	78	57	—
4	$[bmim]^+[PF_6]^-$	PPh_3	10	85	83	62	54	—
5	$[bmim]^+[PF_6]^-$	DPPBA	6	100	76	58	—	—
6	$[bmim]^+[BF_4]^-$	DPPBA	6	100	88	87	83	64
7	$[bmim]^+[PF_6]^-$	DPPBA	10	100	100	86	55	
8	$[bmim]^+[BF_4]^-$	DPPBA	10	100	100	98	88	70

[a] Reaction conditions: ionic liquid (600 mg), **1** (0.2 mmol), $Pd(OAc)_2$ (0.01 mmol), the phosphine (as indicated), Et_3N (0.15 ml) and methyl glycinate hydrochloride (0.4 mmol) were heated in CO atmosphere for 8 h at 100 °C.

and evaporated to dryness. The residue was dissolved in chloroform (20 ml) and washed with water (20 ml). The organic phase was thoroughly washed twice with 5% HCl (20 ml), saturated $NaHCO_3$ (20 ml), brine (20 ml), dried over Na_2SO_4 and concentrated to a yellow waxy material or a thick oil. Chromatography (silica, chloroform, than chloroform/ethanol = 1/1) yielded the desired compounds as yellow solids.

4.3. Aminocarbonylation experiments at high pressure

The DMF solution of the catalyst precursor and reactants (amounts given in Section 4.2) was transferred under argon into a 100 ml stainless steel autoclave. The reaction vessel was pressurised up to 40 bar total pressure with carbon monoxide and the magnetically stirred mixture was heated in an oil bath at 50 °C for 6 h. The work-up procedure is identical with that given above.

4.4. Aminocarbonylation in ionic liquid

In a typical procedure, 17-iodo-5α-androst-16-ene (0.2 mmol), $Pd(OAc)_2$ (0.01 mmol), PPh_3 (0.02 mmol), methyl glycinate (0.4 mmol) and $[bmim]^+[PF_6]^-$ or $[bmim]^+[BF_4]^-$ (600 mg) were placed in a Schlenk-tube equipped with a magnetic stirrer, a septum inlet and a reflux condenser with a balloon on the top. This was placed under carbon monoxide, and Et_3N (0.15 ml) was added. The reaction mixture was heated at 100 °C for 8 h. The mixture was extracted twice with 0.4 ml toluene. The extracts were analysed by GC. Any volatiles were removed from the ionic liquid in vacuo and new load of starting materials (steroid, Et_3N and methyl glycinate) for the next catalytic run were added to the ionic liquid/catalyst mixture and the atmosphere was changed to carbon monoxide. The consecutive runs were conducted for the same reaction time.

4.5. Characterization of the products

4.5.1. Methyl *N*-(benzoyl)-glycinate (1a). MS *m/z* (rel. int. %): 193 (9) (M^+), 161 (5), 134 (18), 105 (100), 77 (**35**).

4.5.2. Methyl *N*-(phenylglyoxyl)-glycinate (1a′). ^{1}H NMR ($CDCl_3$) δ: 8.31 (d, J=8.4 Hz, 2H; Ph); 7.61 (t, J=7.6 Hz, 1H, Ph); 7.46 (m, 2H, Ph); 4.16 (d, J=5.6 Hz, 2H); 3.78 (s, 3H, OCH_3). ^{13}C NMR ($CDCl_3$) δ: 186.8; 169.3; 161.8; 134.5; 133.1; 131.2; 128.5; 52.5; 41.1. IR (KBr, (cm^{-1})): 3370 (NH); 1753 (COO); 1670 (CON). MS *m/z* (rel. int. %): 221 (7), 207 (6), 116 (18), 105 (100), 77 (42). Anal. calcd for $C_{11}H_{11}NO_4$ (221.21): C, 59.73; H, 5.01; N, 6.33; Found: C, 59.92, H, 5.12; N, 6.50. Yield: 79%.

4.5.3. Methyl *N*-(benzoyl)-alaninate (1b). MS *m/z* (rel. int. %): 207 (3) (M^+), 148 (45), 105 (100), 77/25.

4.5.4. Methyl *N*-(phenylglyoxyl)-alaninate (1b′). ^{1}H NMR ($CDCl_3$) δ: 7.3–7.7 (m, 5H); 6.27 (brs, 1H, NH); 4.59 (quint., 1H, 7.2 Hz, C*H*CH_3); 3.70 (s, 3H, OCH_3); 1.31 (d, J=7.2 Hz, 3H, CHC*H*$_3$). ^{13}C NMR ($CDCl_3$) δ: 187.0; 172.4; 161.2; 134.4; 132.1; 131.1; 128.5; 52.6; 48.1; 18.0. IR (KBr, (cm^{-1})): 3380 (NH); 1725 (COO); 1645 (CON). MS *m/z* (rel. int. %): 235 (1) (M^+), 204 (1), 176 (4), 130 (44), 105 (100), 102 (25), 77 (32). Anal. calcd for $C_{12}H_{13}NO_4$ (235.24): C, 61.27; H, 5.57; N, 5.95; Found: C, 61.42, H, 5.79; N, 5.68. Yield: 74%.

4.5.5. Methyl *N*-(phenylglyoxyl)-phenylalaninate (1c′). ^{1}H NMR ($CDCl_3$) δ: 8.23 (d, J=7.6 Hz, 2H, Ph); 7.58 (t, J=8.0 Hz, 1H, Ph); 7.44 (t, J=8.0 Hz; 2H, Ph); 7.27 (m, 4H, Ph); 7.15 (d, J=6.8 Hz, 2H, Ph); 4.93 (dq; J=6.4, 2.4 Hz, 1H, NHC*H*); 3.74 (s, 3H, OCH_3); 3.23 (dd, J=11.5; 5.2 Hz, 1H, CH_aH$_b$); 3.15 (dd, J=11.5, 5.6 Hz, 1H, CH$_a$$H_b$). ^{13}C NMR ($CDCl_3$) δ: 186.9; 171.0; 161.2; 135.4; 134.5; 133.1; 131.1; 129.2; 128.7; 128.5; 127.3; 53.3; 52.5; 38.0. MS *m/z* (rel. int. %): 311 (2) (M^+), 206 (8), 178 (12), 162 (22), 146 (19), 105 (100), 81 (33), 51 (28). Anal. calcd for $C_{18}H_{17}NO_4$ (311.34): C, 69.44; H, 5.50; N, 4.50; Found: C, 69.65, H, 5.67; N, 4.31. Yield: 82%.

4.5.6. Methyl *N*-(phenylglyoxyl)-valinate (1d′). ^{1}H NMR ($CDCl_3$) δ: 8.30 (d, J=8.0 Hz, 2H, Ph); 7.60 (m, 1H, Ph); 7.4 (m, 3H, Ph +NH); 4.59 (dd, J=5.2, 9.2 Hz, 1H, CH); 2.27 (dh, J=6.4; 5.2 Hz; 1H, C*H*$(CH_3)_2$); 0.99 (d, J=6.4 Hz, 3H, CH_3); 0.96 (d, J=6.4 Hz; 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ: 187.0; 171.4; 161.5; 134.4; 133.2; 131.2; 128.5; 57.3; 52.3; 31.4; 19.0; 17.8. IR (KBr, (cm^{-1})): 3390 (NH); 1745 (COO); 1684 (amide-I), 1668 (ν (CO). MS *m/z* (rel. int. %): 263 (10) (M^+), 204 (8), 158 (18), 130 (57), 105 (100), 77 (45). Anal. calcd for $C_{14}H_{17}NO_4$ (263.29): C, 63.87; H, 6.51; N, 5.32; Found: C, 63.71, H, 6.68; N, 5.10. Yield: 74%.

4.5.7. Methyl *N*-(phenylglyoxyl)-4-amino-4-benzyl-butanoate (1e′). ^{1}H NMR ($CDCl_3$) δ: 8.2 (d, J=8 Hz, 2H, Ph(ortho)); 7.60 (t, 1H, Ph(para)); 7.4 (t, 2H, Ph(meta)); 7.15–7.30 (m, 5H, Ph); 6.9 (brs, 1H, NH); 4.30 (m, 1H, CH); 3.63 (s, 3H, OCH_3); 2.87 (d, J=6.8 Hz; CH_2); 2.40 (m, 2H, CH_2); 2.00 (m, 1H, CH_aH$_b$); 1.80 (m, 1H, CH$_a$$H_b$). ^{13}C NMR ($CDCl_3$) δ: 183.0; 173.6; 161.6; 137.1; 134.4; 133.3; 131.1; 129.4; 128.6; 128.5; 126.8; 51.8; 50.4; 41.3; 30.9; 29.1. IR (KBr, (cm^{-1})): 3380 (NH); 1720 (COO); 1688 (CON), 1660 (CO). MS *m/z* (rel. int. %): 339 (3) (M^+), 248 (22), 117 (27), 105 (100), 91 (31), 77 (43). Anal. calcd for $C_{20}H_{21}NO_4$ (339.39): C, 70.78; H, 6.24; N, 4.13; Found: C, 70.94, H, 6.50; N, 3.88. Yield: 77%.

4.5.8. Methyl *N*-(1-cyclohexenoyl)glycinate (2a). ^{1}H NMR ($CDCl_3$) δ: 6.67 (brs, 1H; =CH); 6.18 (brs, 1H, NH); 4.07 (d, J=5.2 Hz, 1H, NHCH_2); 3.74 (s, 3H, OCH_3); 2.24 (m, 2H, CH_2); 2.14 (m, 2H, CH_2); 1.68 (m, 2H, CH_2); 1.58 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$) δ: 170.7; 168.5; 134.5; 132.5; 52.3; 41.3; 25.4; 24.1; 22.1; 21.5. IR (KBr, (cm^{-1})): 3435 (NH); 1742 (COO); 1670 (CON); 1625 (C=C). MS *m/z* (rel. int. %): 197 (30) (M^+), 165 (4), 138 (11), 109 (100), 81 (61), 53 (13). Anal. calcd for $C_{10}H_{15}NO_3$ (197.23): C, 60.90; H, 7.67; N, 7.10; Found: C, 61.05, H, 7.86; N, 7.35. Yield: 75%.

4.5.9. Methyl *N*-(1-cyclohexenoyl)-alaninate (2b). ^{1}H NMR ($CDCl_3$) δ: 6.60 (brs, 1H; =CH); 6.27 (brs, 1H, NH); 4.57 (quint., 1H, J=7.2 Hz, C*H*CH_3); 3.68 (s, 3H, OCH_3); 2.18 (brs, 2H, CH_2); 2.09 (m, 2H, CH_2); 1.62 (m, 2H, CH_2); 1.58 (m, 2H, CH_2); 1.35 (d, J=7.2 Hz, 3H, CHC*H*$_3$). ^{13}C NMR ($CDCl_3$) δ: 173.7; 167.8; 134.0; 132.6; 52.2; 47.9; 25.2; 24.0; 21.9; 21.4; 18.3. IR (KBr, (cm^{-1})): 3405,3425 (NH); 1738 (COO); 1672 (CON); 1628 (C=C).

MS *m/z* (rel. int. %): 211 (12) (M^+), 152 (35), 109 (100), 81 (36), 79 (23). Anal. calcd for $C_{11}H_{17}NO_3$ (211.26): C, 62.54; H, 8.11; N, 6.63; Found: C, 62.76, H, 8.34; N, 6.47. Yield: 78%.

4.5.10. Methyl *N*-(1-cyclohexenoyl)-phenylalaninate (2c). ^{1}H NMR ($CDCl_3$) δ: 7.2 (m, 5H, Ph); 6.54 (brs, 1H, =CH); 6.17 (d, J=7.2 Hz, 1H, NH); 4.86 (dt, J=7.2 Hz, 1H, C*H*CH_2); 3.64 (s, 3H, OCH_3); 3.09 (m, 2H, CH_2Ph); 2.07 (m, 4H, CH_2CH_2); 1.58 (m, 2H, CH_2); 1.51 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$) δ: 172.0; 167.7; 135.9; 134.0; 132.5; 129.1; 128.3; 126.8; 52.9; 51.9; 37.6; 25.1; 23.8; 21.8; 21.2. IR (KBr, (cm^{-1})): 3431 (NH); 1754 (COO); 1681 (CON); 1638 (C=C). MS *m/z* (rel. int. %): 287 (4) (M^+), 228 (4), 162 (38), 109 (100), 91 (18), 81 (35), 79 (23). Anal. calcd for $C_{17}H_{21}NO_3$ (287.36): C, 71.06; H, 7.37; N, 4.87; Found: C, 71.28, H, 7.25; N, 4.59. Yield: 81%.

4.5.11. Methyl *N*-(1-cyclohexenoyl)-valinate (2d). ^{1}H NMR ($CDCl_3$) δ: 6.59 (brs, 1H, =CH); 6.13 (d, J=8.4 Hz, 1H, NH); 4.54 (dd, J=8.4, 5.2 Hz, NHC*H*); 3.66 (s, 3H, OCH_3); 2.20 (m, 1H, C*H*$(CH_3)_2$); 0.88 (d, J=6.8 Hz, 3H, CH_3); 0.85 (d, J=6.8 Hz; 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ: 172.6; 168.2; 133.8; 132.9; 56.8; 51.9; 31.3; 25.2; 24.1; 21.9; 21.4; 18.8; 17.8. IR (KBr, (cm^{-1})): 3425 (NH); 1742 (COO); 1670 (CON); 1621 (C=C). MS *m/z* (rel. int. %): 239 (3) (M^+), 180 (14), 109 (100), 81 (40), 79 (36). Anal. calcd for $C_{13}H_{21}NO_3$ (239.31): C, 65.25; H, 8.84; N, 5.85; Found: C, 65.47, H, 9.05; N, 5.70. Yield: 79%.

4.5.12. Methyl *N*-(1-cyclohexenoyl)-4-amino-4-benzyl-butanoate (2e). ^{1}H NMR ($CDCl_3$) δ: 7.27 (d, J=7.2 Hz; 2H, Ph); 7.2 (m, 3H, Ph); 6.52 (brs, 1H, =CH); 5.6 (br d, J=7.0 Hz; 1H, NH); 4.22 (m, 1H, C*H*CH_2); 3.63 (s, 3H, OCH_3); 2.9 (m, 1H, CH_aH$_b$); 2.7 (m, 1H, CH$_a$$H_b$); 2.35 (m, 4H, 2×$CH_2$); 2.13 (m, 4H, CH_2CH_2); 1.65 (m, 2H, CH_2); 1.57 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$) δ: 174.1; 168.0; 138.0, 133.2, 132.9, 129.3, 128.2, 126.3, 51.4; 49.9, 41.0; 30.8; 28.5; 25.1; 24.0; 21.9; 21.3. IR (KBr, (cm^{-1})): 3423 (NH); 1734 (COO); 1672 (CON); 1625 (C=C). MS *m/z* (rel. int. %): 315 (1) (M^+), 224 (25), 109 (100), 98 (37), 247 (29), 91 (12), 81 (26). Anal. calcd for $C_{19}H_{25}NO_3$ (315.41): C, 72.35; H, 7.99; N, 4.44; Found: C, 72.62; H, 8.20; N, 4.24. Yield: 69%.

4.5.13. Methyl *N*-(1-cyclohexenoyl)-*N'*-alaninoyl-prolinate (2f). ^{1}H NMR ($CDCl_3$) δ: 6.70 (brs, 1H, NH); 6.65 (brs, 1H, =CH); 4.80 (qi, J=6.4 Hz, 1H, C*H*CH_3); 4.52 (dd, J=8.6, 4.8 Hz, 1H, C*H*COO); 3.71 (s, 3H, OCH_3); 3.60 (m, 2H, NCH_2); 1.95–2.25 (m, 8H, 4×CH_2); 1.50–1.70 (m, 4H, 2×CH_2); 1.41 (d, J=6.4 Hz, 3H, CHC*H*$_3$). ^{13}C NMR ($CDCl_3$) δ: 172.3; 171.7; 167.4; 149.7; 134.3; 58.8; 52.3; 46.9; 46.6; 29.0; 25.4; 25.0; 24.1; 22.1; 21.5; 18.1. IR (KBr, (cm^{-1})): 3420 (NH); 1751 (COO); 1665 (CON); 1645 (CON); 1614 (C=C). MS *m/z* (rel. int. %): 308 (2) (M^+), 180 (10), 152 (21), 128 (15), 109 (100), 81 (14). Anal. calcd for $C_{16}H_{24}N_2O_4$ (308.38): C, 62.32; H, 7.84; N, 9.08; Found: C, 62.45, H, 7.59; N, 9.31. Yield: 80%.

4.5.14. Methyl *N*-(17-androst-16-enoyl)-glycinate (3a). ^{1}H NMR: 6.44 (m, 1H, 16-H); 6.13 (m, 1H, NH); 4.08 (d, J=4.9 Hz, 2H, CH_2); 3.75 (s, 3H, OCH_3); 2.20 (ddd, J=12.0, 4.2, 2.8 Hz, 1H, 15-CH_aH$_b$); 2.15–2.20 (m, 1H, 5-CH); 1.95 (dd, J=12.0, 8.0 Hz, 1H, 15-CH$_a$$H_b$); 0.69–1.67 (m, 19H, ring protons); 0.96 (s, 3H, 18-CH_3); 0.79 (m, 3H, 19-CH_3). ^{13}C NMR ($CDCl_3$) δ: 170.7; 165.8; 149.9; 137.1; 56.8; 55.1; 52.3; 47.2; 46.5; 41.0; 8.4; 36.4; 35.0; 33.8; 31.9; 31.6; 29.0; 28.9; 26.8; 22.1; 20.7; 16.5; 12.1. MS *m/z* (rel. int. %): 373 (18) (M^+), 358 (25), 285 (12), 257 (100), 161 (26), 147 (28), 121 (32), 105 (39), 91 (39), 67 (35), 55 (26). IR: 3325 (NH); 1736 (CO); 1642 (CO). Anal. calcd for $C_{23}H_{35}NO_3$ (373.54): C, 73.96; H, 9.44; N, 3.75; Found: C, 74.05, H, 9.63; N, 3.92. Yield: 88%.

4.5.15. Methyl *N*-(17-androst-16-enoyl)-alaninate (3b). ^{1}H NMR ($CDCl_3$) δ: 6.30 (brs, 2H; 16-H+NH); 4.52 (quint., 1H, J=7.1 Hz, C*H*CH_3); 3.63 (s, 3H, OCH_3); 2.05 (m, 1H, 15-CH_aH$_b$); 1.85 (m, 1H, 15-CH$_a$$H_b$); 1.35 (d, J=7.1 Hz, 3H, CHC*H*$_3$); 0.8–1.60 (m, 22H, steroidal skeleton); 0.86 (s. 3H, 18-CH_3); 0.70 (s. 3H, 19-CH_3). ^{13}C NMR ($CDCl_3$) δ: 173.6; 165.1; 149.8; 136.6; 56.6; 55.0; 52.2; 47.5; 47.0; 46.3; 38.3; 36.3; 34.8; 33.6; 31.7; 31.4; 28.8; 28.7; 26.6; 21.9; 20.5; 18.3; 16.3; 12.0. IR (KBr, (cm^{-1})): 3445 (NH); 1753 (COO); 1680 (CON); 1602 (C=C). MS *m/z* (rel. int. %): 387 (80) (M^+), 355 (92), 254 (90), 192 (100), 161 (41), 126 (92). Anal. calcd for $C_{24}H_{37}NO_3$ (387.56): C, 74.38; H, 9.62; N, 3.61; Found: C, 74.56, H, 9.40; N, 3.39. Yield: 78%.

4.5.16. Methyl *N*-(17-androst-16-enoyl)-phenylalaninate (3c). ^{1}H NMR ($CDCl_3$) δ: 7.40 (m, 1H, Ph); 7.23 (m, 1H, Ph); 7.10 (m, 1H, Ph); 6.31 (brs, 1H, 16-H); 6.05 (d, J=7.8 Hz, 1H, NH); 4.92 (q, J=7.0 Hz, 1H, NHC*H*); 3.70 (s, 3H, OCH_3); 3.1 (m, 2H, CH_2Ph); 2.2 (m, 1H, 15-CH_aH$_b$); 1.95 (m, 1H, 15-CH$_a$$H_b$); 0.8–1.65 (m, 22H, steroidal skeleton); 0.94 (s. 3H, 18-CH_3); 0.80 (s. 3H, 19-CH_3). ^{13}C NMR ($CDCl_3$) δ: 171.9; 165.0; 149.8; 136.8; 135.8; 129.1; 128.1; 126.8; 56.5; 54.9; 52.6; 52.0; 47.0; 46.2; 38.2; 37.6; 36.2; 34.7; 33.5; 31.7; 31.4; 28.8; 28.7; 26.5; 21.9; 20.4; 16.2; 11.9. IR (KBr, (cm^{-1})): 3420 (NH); 1748 (COO); 1667 (CON); 1601 (C=C). Anal. calcd for $C_{30}H_{41}NO_3$ (463.66): C, 77.71; H, 8.91; N, 3.02; Found: C, 77.93, H, 8.70; N, 3.27. Yield: 83%.

4.5.17. Methyl *N*-(17-androst-16-enoyl)-valinate (3d). ^{1}H NMR ($CDCl_3$) δ: 6.4 (brs, 1H, 16-H); 6.14 (d, J=8.4 Hz, 1H, NH); 4.55 (m, 1H, NHC*H*); 3.65 (s, 3H, OCH_3); 2.02–2.15 (m, 2H, 15-CH_aH$_b$+CH $(CH_3)_2$); 1.95 (dd, J=11.0, 8.2 Hz, 1H, 15-CH$_a$$H_b$); 0.7–1.6 (m, 22H, steroidal skeleton); 0.91 (s, 3H, 18-CH_3); 0.84 (d, J=6.4 Hz, 3H, CH_3); 0.83 (d, J=6.4 Hz; 3H, CH_3); 0.74 (s, 3H, 19-CH_3). ^{13}C NMR ($CDCl_3$) δ: 172.5; 165.5; 150.0; 136.9; 56.6; 56.4; 51.9; 47.1; 46.2; 38.3; 36.3; 34.9; 33.6; 31.7; 31.4; 31.3; 28.8; 28.7; 26.6; 21.9; 20.5; 18.8; 17.7; 16.3; 12.0. IR (KBr, (cm^{-1})): 3430 (NH); 1740 (COO); 1676 (CON); 1605 (C=C)). MS *m/z* (rel. int. %): 415 (12) (M^+), 400 (19), 356 (24), 285 (84), 269 (30), 257 (60), 161 (23), 147 (25), 121 (39), 105 (53), 95 (68), 67 (74), 55 (100). Anal. calcd for $C_{26}H_{41}NO_3$ (415.62): C, 75.14; H, 9.94; N, 3.37; Found: C, 75.29, H, 10.11; N, 3.21. Yield: 80%.

4.5.18. Methyl *N*-(17-androst-16-enoyl)-4-amino-4-benzyl-butanoate (3e). ^{1}H NMR ($CDCl_3$) δ: 7.2–7.4 (m, 5H, Ph); 6.18 (brs, 1H, 16-H); 5.50 (brs, 1H, NH); 4.22 (m, 1H, C*H*CH_2Ph); 3.62 (s, 3H, OCH_3); 2.8–2.9 (m, 2H, CH_2); 2.7 (m, 2H, CH_2); 2.16 (m, 1H, 15-CH_aH$_b$); 1.92 (m, 1H,

15-CH_aH_b); 0.8–1.65 (m, 22H, steroidal skeleton + $CHCH_2$); 0.92 (s. 3H, 18-CH_3); 0.80 (s. 3H, 19-CH_3). ^{13}C NMR ($CDCl_3$) δ: 174.1; 165.9; 150.9; 136.5; 136.1; 135.3; 132.8; 129.5; 128.5; 126.9; 56.5; 55.1; 51.7; 50.3; 47.3; 46.6; 41.1; 38.5; 36.5; 34.9; 33.8; 31.9; 31.6; 28.9; 28.8; 26.8; 22.1; 20.6; 16.5; 12.1. IR (KBr, (cm^{-1})): 3422 (NH); 1720 (COO); 1665 (CON); 1598 (C=C). Anal. calcd for $C_{32}H_{45}NO_3$ (491.71): C, 78.17; H, 9.22; N, 2.85; Found: C, 78.26, H, 9.35; N, 2.60. Yield: 67%.

4.5.19. Methyl *N*-(17-androst-16-enoyl)-*N′*-alaninoyl-prolinate (3f). ^1H NMR ($CDCl_3$) δ: 6.71 (d, $J=7.0$ Hz, NH); 6.40 (s, 1H, 16-H); 4.78 (qi, $J=6.4$ Hz, 1H, CH); 4.52 (dd, $J=8.6$, 4.8 Hz, 1H, CH); 3.68 (s, 3H, OCH_3); 3.60 (m, 2H, CH_2); 1.9–2.3 (m, 8H); 1.38 (d, $J=6.4$ Hz, CH_3); 0.8–1.65 (m, 20H, steroidal skeleton); 0.92 (s. 3H, 18-CH_3); 0.80 (s. 3H, 19-CH_3). ^{13}C NMR ($CDCl_3$) δ: 172.4; 171.7; 165.0; 150.1; 145.3; 58.7; 56.8; 55.1; 52.2; 47.2; 46.8; 46.4; 38.4; 36.4; 35.0; 33.8; 31.9; 31.5; 29.0; 28.9; 26.8; 24.9; 22.1; 20.7; 18.1; 16.5; 12.1. Anal. calcd for $C_{29}H_{44}N_2O_4$ (484.68): C, 71.87; H, 9.15; N, 5.78; Found: C, 71.67, H, 9.27; N, 5.88. Yield: 77%.

Acknowledgements

The authors thank the Hungarian Research Fund (OTKA 035047, TS044800), the Hungarian-Greek Intergovernmental Cooperation in Science and Technology (TéT GR-5/2001) and Johnson Matthey for the loan of palladium(II) acetate.

References and notes

1. Skoda-Földes, R.; Kollár, L. *Curr. Org. Chem.* **2002**, *6*, 1097–1119.
2. *Applied Homogeneous Catalysis with Organometallic Compounds*; Cornils, B., Herrmann, W. A., Eds.; Wiley-VCH: Weinheim, 1996. Beller, M., Bolm, C., Eds.; Transition Metals for Organic Synthesis; Wiley-VCH: Weinheim, 1998; Vol. I–II.
3. Colquhoun, H. M.; Thompson, D. J.; Twigg, M. V. *Carbonylation. Direct Synthesis of Carbonyl Compounds*; Plenum: New York, 1991.
4. Joó, F. Biphasic Catalysis – Homogeneous. In Horváth, I. T., Ed.; Encyclopedia of Catalysis; Wiley: London, 2002; Vol. I, pp 737–805.
5. Panster, P.; Wieland, S. In *Applied Homogeneous Catalysis with Organometallic Compounds*; Cornils, B., Herrmann, W. A., Eds.; Wiley-VCH: Weinheim, 1996; pp 605–623.
6. *Aqueous-Phase Organometallic Catalysis. Concepts and Applications*; Cornils, B., Herrmann, W. A., Eds.; Wiley-VCH: Weinheim, 1998.
7. Jessop, P. G.; Ikariya, T.; Noyori, R. *Chem. Rev.* **1999**, *99*, 475–493.
8. (a) Horváth, I. T. *Acc. Chem. Res.* **1998**, *31*, 641–650. Barthel-Rosa, L. P.; Gladysz, J. A. *Coord. Chem. Rev.* **1999**, *190–192*, 587–605.
9. (a) Welton, T. *Chem. Rev.* **1999**, *99*, 2071–2083. (b) Gordon, C. M. *Appl. Catal. A: Gen.* **2001**, *222*, 101–117. (c) Olivier-Bourbigou, H.; Magna, L. *J. Mol. Cat. A* **2002**, *182–183*, 419–437. (d) Zhao, D.; Wu, M.; Kou, Y.; Min, E. *Catal. Today* **2002**, *74*, 157–189. (e) Keim, W. *Green Chem.* **2003**, *5*, 105–111.
10. Mizushima, E.; Hayashi, T.; Tanaka, M. *Green Chem.* **2001**, *3*, 76–79.
11. Freskos, J. N.; Ripin, D. H.; Reilly, M. L. *Tetrahedron Lett.* **1993**, *34*, 255–256.
12. Skoda-Földes, R.; Takács, E.; Horváth, J.; Tuba, Z.; Kollár, L. *Green Chem.* **2003**, *5*, 643–645.
13. (a) Amatore, C.; Carre, E.; Jutand, A.; M'Barki, M. A.; Meyer, G. *Organometallics* **1995**, *14*, 5605–5614 and references cited therein. Csákai, Z.; Skoda-Földes, R.; Kollár, L. *Inorg. Chim. Acta* **1999**, *286*, 93–97.
14. Chiou, A.; Markidis, T.; Konstantinou-Kokotou, V.; Verger, R.; Kokotos, G. *Org. Lett.* **2000**, *2*, 347–350.
15. Kokotos, G.; Verger, R.; Chiou, A. *Chem. Eur. J.* **2000**, *6*, 4211–4217.
16. Kotsovolou, S.; Chiou, A.; Verger, R.; Kokotos, G. *J. Org. Chem.* **2001**, *66*, 962–967.
17. Kokotos, G.; Kotsovolou, S.; Six, D. A.; Konstantinou-Kokotou, V.; Beltzner, C. C.; Dennis, E. A. *J. Med. Chem.* **2002**, *45*, 2891–2893.
18. Kokotos, G.; Six, D. A.; Loukas, V.; Smith, T.; Konstantinou-Kokotou, V.; Hadjipavlou-Litina, D.; Kotsovolou, S.; Chiou, A.; Beltzner, C. C.; Dennis, E. A. *J. Med. Chem.* **2004**, *47*, 3615–3628.
19. Ozawa, F.; Sugimoto, T.; Yamamoto, T.; Yamamoto, A. *Organometallics* **1984**, *3*, 692–697.
20. Son, T.; Yanagihara, H.; Ozawa, F.; Yamamoto, A. *Bull. Chem. Soc. Jpn* **1988**, *61*, 1251–1258.
21. Skoda-Földes, R.; Kollár, L. *Chem. Rev.* **2003**, *103*, 4095–4129.

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Tetrahedron 61 (2005) 803–812

Regioselective synthesis of peptidic derivatives and glycolamidic esters of Methotrexate

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Received 28 July 2004; revised 17 November 2004; accepted 18 November 2004

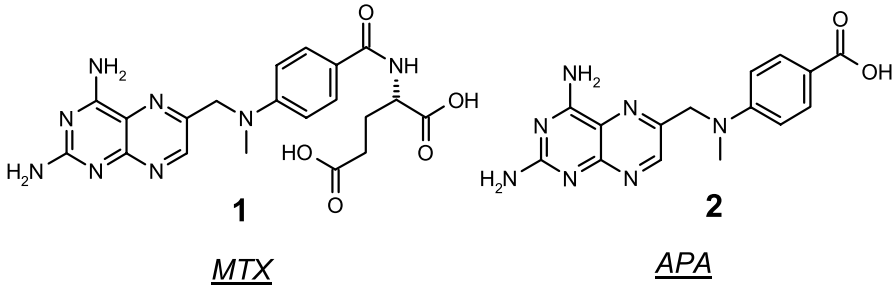
Available online 8 December 2004

Abstract—Convenient methods for regioselective syntheses of Methotrexate peptidic conjugates are described. Solid phase synthesis for derivatives of Methotrexate containing an amide bond has been applied and showed higher efficiency than liquid phase synthesis. Synthetic pathways for regioselective preparation of Glycolamidic ester derivatives of Methotrexate were also developed using 4-amino-4-deoxy-N^{10}-methylpteroic acid as starting material. These ester bonds were obtained either in solution or by using a combined liquid and solid phase synthesis.

1. Introduction

Methotrexate (MTX) (Scheme 1), a folic acid antagonist, has been extensively used in cancer chemotherapy since 1948.[1] This drug acts as an inhibitor of dihydrofolate reductase (DHFR), an essential enzyme in the biosynthesis of thymidylate, which is required for DNA replication.[2] MTX is also subsequently used in the treatment of non-neoplasmic diseases as an anti-inflammatory and/or an immunosuppressive drug.[3] The mechanism of action of MTX is well documented. It is brought into the cell by the reduced folate transporter, and it is then polyglutamylated in order to prevent efflux from the cell and to exert its cytotoxic activity by blocking the synthesis of the N5, N10-methylenetetrahydrofolate.[3]

The efficacy of MTX is hampered by its very short plasma half-life. It is administrated in relatively high doses, which often leads to drug resistance and causes non-specific toxicities in normal cells.[1] Resistance to MTX is the result of different biological phenomena in the target cell. Deficiency in the reduced folate transporter, in folyl polyglutamate synthetase (FPGS) or over-expression of gamma-glutamyl hydrolase (GGS) and efflux proteins at the surface of the cell (MRP proteins), can prevent MTX to enter into and/or stay inside the cell. In the same time, over-expression and/or mutation of DHFR can prevent MTX to exert a sufficient cytotoxic activity.[4–6] Another important side effect of MTX which has been clearly identified, is its huge chronic toxicity for the central nervous system when the drug is used concomitantly with radiations.[7]



Scheme 1. Structures of MTX and APA.

Keywords: Methotrexate; Peptidic conjugate; Regioselective synthesis; Solid phase synthesis; Vectorisation.
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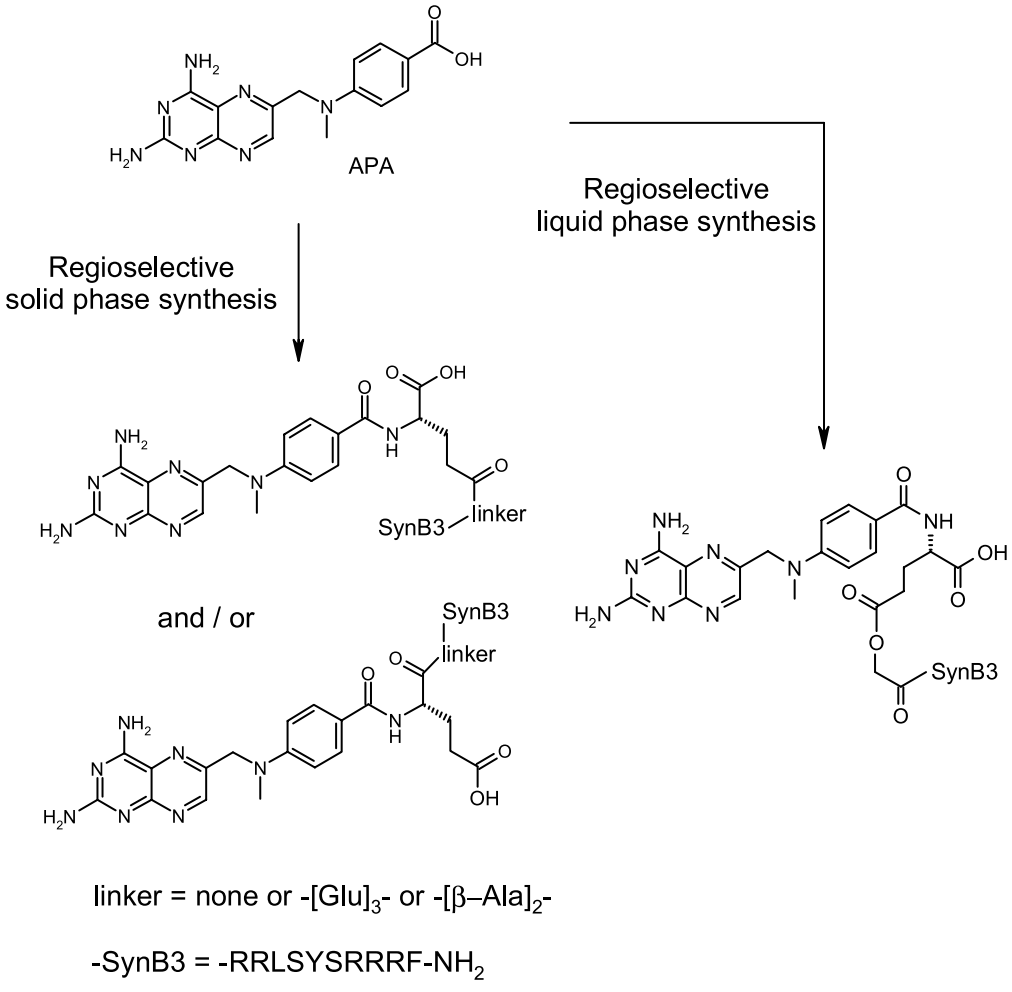
0040–4020/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2004.11.049

To overcome these problems, many experimental efforts have been made. Among them, modifications of MTX are the most employed. γ-*tert*-Butyl ester, γ-hydrazide, γ-*n*-butylamide and γ-benzylamide of MTX were synthesized in order to modify the biological behaviour of the drug.[8] Other compounds with amino acids and various esters on the two carboxylic acid functions were also prepared with different strategies, the most current one involving the use of 4-amino-4-deoxy-N^{10}-methylpteroic acid (APA) (Scheme 1) as a building block.[1,9–11] These transformations were performed in order to modify the lipophilicity of MTX and/or to study the cytotoxicity of the prodrug activated by various enzymes. In all cases, either the regioselective synthesis described involve numerous steps, or the final products are obtained after purification of the formed isomers.

Instead of modifying the core structure of MTX, vectorisation is an alternative strategy to increase the efficacy of MTX. MTX has been attached to various vectors like monoclonal antibodies,[12,13] proteins,[14] polymers[15,16] and hormonal peptides.[17–19] All the synthesis are performed in liquid phase with or without protecting groups, leading, respectively, to a single product with a relatively low yield, or to a mixture of regioisomers. More recently, Pignatello and co-workers have described the synthesis of several α- and γ-monosubstituted and α,γ-disubstituted lipoamino acid conjugates of MTX coupled with a glycolamidic ester.[20] Once again, the monosubstituted products are obtained as secondary products of the disubstituted ones, and the yields do not exceed 15% for these compounds.

Previous work in our laboratory led to the discovery of peptides derived from the Protegrin PG-1 peptide, which belongs to the family of β-stranded antimicrobial peptides.[21] These peptides have been shown to translocate efficiently through biological membranes, thus providing the basis for the development of new peptide-conjugated drugs that cross-cell membranes. In a precedent work,[22] the authors have shown that vectorized doxorubicin bypasses the P-glycoprotein pump at the luminal site of the blood-brain barrier. Moreover, using these peptidic vectors with doxorubicin is efficient in overcoming multidrug resistance. In the present paper, we report the synthesis of MTX peptidic conjugates using solid phase synthesis for the conjugate with amide bonds between the vector and the drug. A regioselective liquid phase synthesis will also be presented for conjugates containing a glycolamidic ester bond (Scheme 2).



Scheme 2. Target molecules and general strategies used.

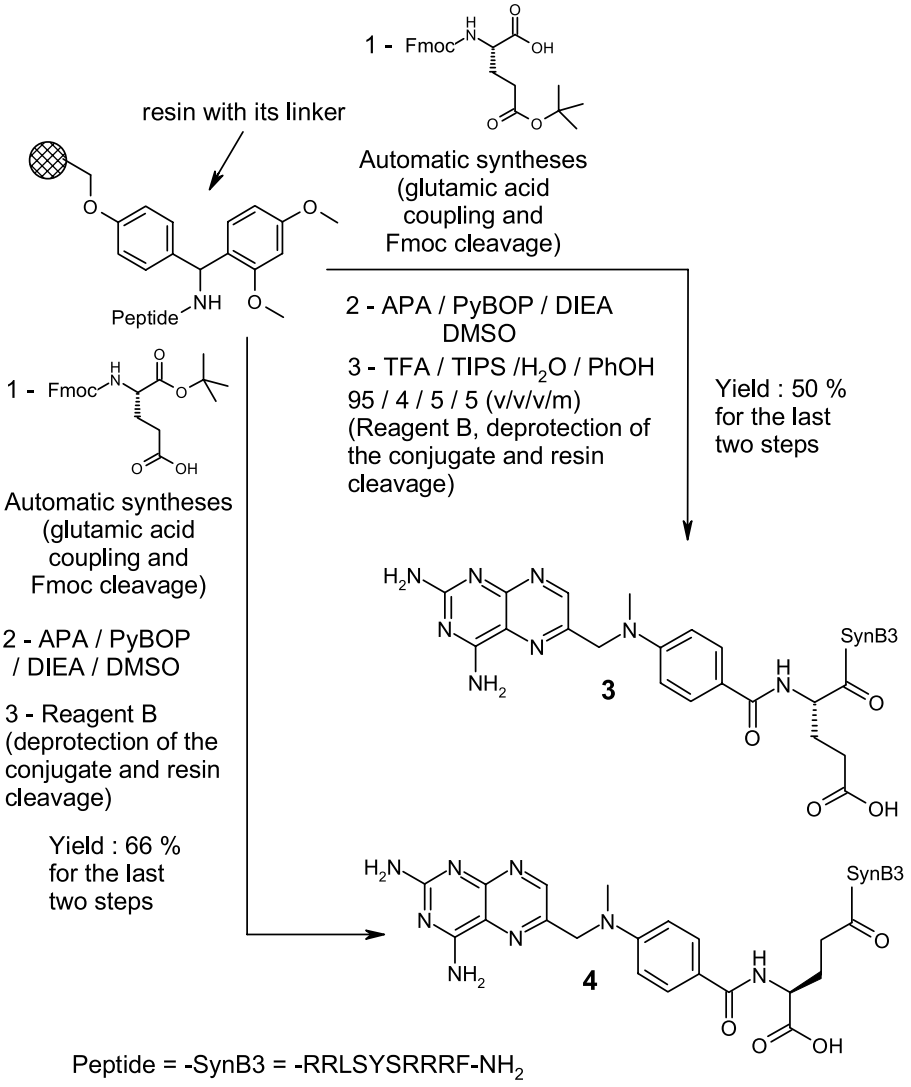
2. Results and discussion

2.1. Solid phase synthesis of MTX conjugates

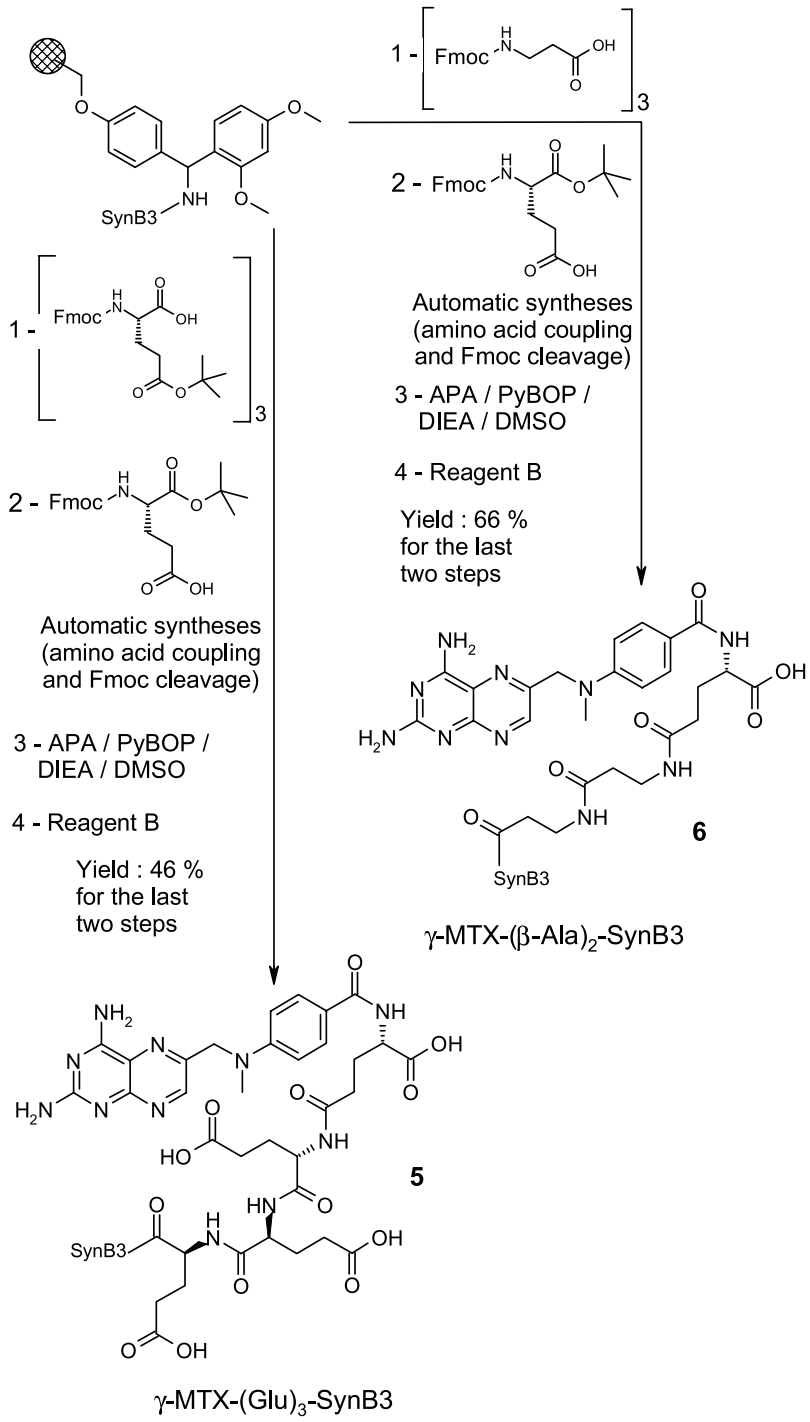
In order to selectively synthesize α- or γ-conjugates of MTX and peptide carriers, a method using solid phase synthesis has been used. Previous work had already been described for the synthesis of MTX-peptides conjugates, but the strategy used by the authors involved liquid phase synthesis of a protected MTX.[17] Use of solid phase synthesis for MTX conjugates has already been described, but in the case of MTX lipoamino acids conjugates.[20]

In the present work, two conjugates without any spacer (Scheme 3) have been synthesized. They differ in the MTX position of the vector coupling. According to the literature, the free α-carboxylic acid function of MTX seems to be important for the stability of the complex between the drug and DHFR.[24] In contrast, the γ-carboxylic acid function of MTX lies outside of the binding pocket of DHFR. As this function does not seem to be involved in the stability of the complex, it may be possible to couple the vector without affecting the biological activity of the drug. In order to verify these hypotheses, α-MTX-SynB3 **3** and γ-MTX-SynB3 **4** have been synthesized with yields of 50 and 66%, respectively. After classical peptidic synthesis using Fmoc/tBu strategy, APA was introduced using PyBOP as an activator. The two position isomers were obtained by using commercially available, correctly protected glutamic acid residues. No secondary product other than peptidic elongation ones was observed following cleavage and deprotection of the conjugate, thus showing that MTX remains stable upon drastic acidic treatment.

Using this solid phase synthesis strategy, conjugates with a peptidic spacer between MTX and the vector were also prepared (Scheme 4). A tri-glutamic acid spacer was introduced in order to enhance the biological activity of the drug. Indeed, the mechanism of action of MTX includes a step of γ-polyglutamylation by folylpolyglutamyl synthase.[3] This step could allow MTX not to be effluxed across the cell membrane by multidrug related proteins (MRP), hence increasing its intracellular half-life. Furthermore, this polyglutamylated form of MTX has been proven



Scheme 3. Solid phase synthesis of compounds α-MTX-SynB3 **3** and γ-MTX-SynB3 **4**.



Scheme 4. Solid phase synthesis of compounds γ-MTX-$(Glu)_3$-SynB3 **5** and γ-MTX-$(β\text{-Ala})_2$-SynB3 **6**.

to inhibit DHFR with the same efficacy than MTX, but has also an increased affinity for certain folate-dependent enzymes such as thymidylate synthase.

The conjugate **5** was obtained with a yield of 46%. In order to compare solid and liquid phase syntheses, an attempt was made to synthesize this compound with a final liquid phase step for the introduction of APA. The yield of the reaction was only 33%, showing the higher efficiency of solid phase synthesis. Moreover, the conversion of the reaction was only about 50%, hence decreasing the yield of the synthesis. A better conversion could not be reached because of competitive reactions of the free carboxylic acid functions.

The second derivative **6** synthesized using this strategy included a di-β-alanyl peptidic spacer. This product was designed for two reasons. Firstly, the presence of the spacer allows MTX and the peptide vector not to be too closed one to each over, which could hamper the formation of the complex between MTX and DHFR. Secondly, it has been shown in the literature that α-MTX-peptides are cleaved by many carboxypeptidases and that the rate of cleavage depends on the nature of the amino acid bearing MTX.[10,25]

It was also shown that only α-MTX-Arg is cleaved in human serum by carboxypeptidase A.[10] Arg being the N-terminal amino acid of the peptidic vector used in the present study and although the conjugates synthesized were essentially γ-isomers, this new conjugate was then synthesized to compare the biological activity of our products depending on the nature of the amino acid bearing MTX. This conjugate was obtained with a yield of 66%.

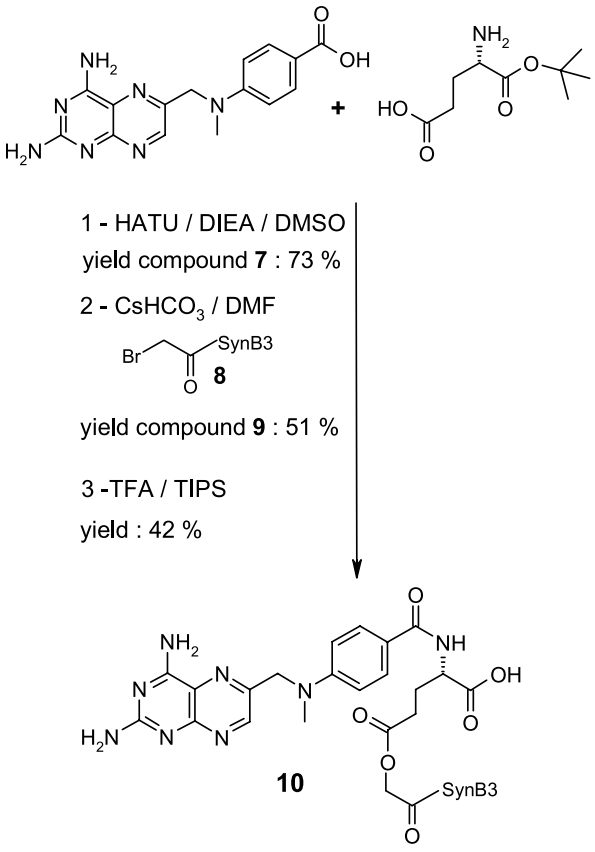
2.2. Glycolamidic ester conjugates of MTX

The difference in the biological and hydrolytic stability between amide and ester bonds prompted us to synthesize conjugates with an ester bond between the vector and MTX. Previous work in the laboratory with benzylpenicillin had already shown the usefulness of a glycolamidic ester linker for peptidic conjugation.[26] Furthermore, to our knowledge, γ-glycolamidic esters of MTX have only been obtained with low yields after separation from the diesters and the α-isomer.[20] Consequently, we elaborated a regioselective synthesis of γ-glycolamidic esters of MTX.

Two different synthetic routes have been investigated. The first one had the advantage to introduce APA during the last steps of the synthesis. There was, however, an important risk of pyroglutamate formation during deprotection of the amine function of the glutamic acid before coupling with APA. Indeed, γ-ester of glutamic acid is well known to undergo rapid lactamization under certain conditions.[27] Simplification of the synthesis scheme being, however, attractive, the synthesis was undertaken since it has been reported that the glycolamidic ester link was stable toward hydrolysis during prolonged time.[20] Cesium salt of Fmoc-Glu-OtBu was formed in solution in methanol and was reacted with 2,3,4-trichlorophenol bromoacetate[28] in solution in DMF. Without further purification, the peptide was added in the presence of DIEA to yield the desired γ-glycolamidic ester (yield: 31%, 3 steps). Deprotection of the amine function was then attempted. Unfortunately, deprotection using excess of piperidine in dry DMF only yielded the pyroglutamic compound and the hydroxy-acetic peptide. Decreasing both the amount of base and the temperature did not product any effect since the cleavage of the Fmoc protective group was immediately followed by the reaction of lactamization.

Because of the difficulties encountered, we decided to use another synthetic scheme (Scheme 5). H-Glu(OH)-OtBu was reacted with APA and HATU as an activator in DMSO to yield α-tertiobutyl-MTX **7** (yield 73%). Cesium salt of **7** formed in situ was then reacted with bromoacetyl peptide **8** obtained by reaction of bromoacetic acid with peptidyl resin, followed by deprotection and cleavage of the peptide from the resin. Compound **9** was obtained with a 51% yield as a single product as confirmed by HPLC. Cleavage of tertiobutyl protecting group was performed in a TFA/TIPS solution (95/5; v/v) and yielded the final product as a double peak in HPLC. After isolation of the two products using preparative HPLC, MALDI-MS analysis proved that the products were isomer with the same mass.

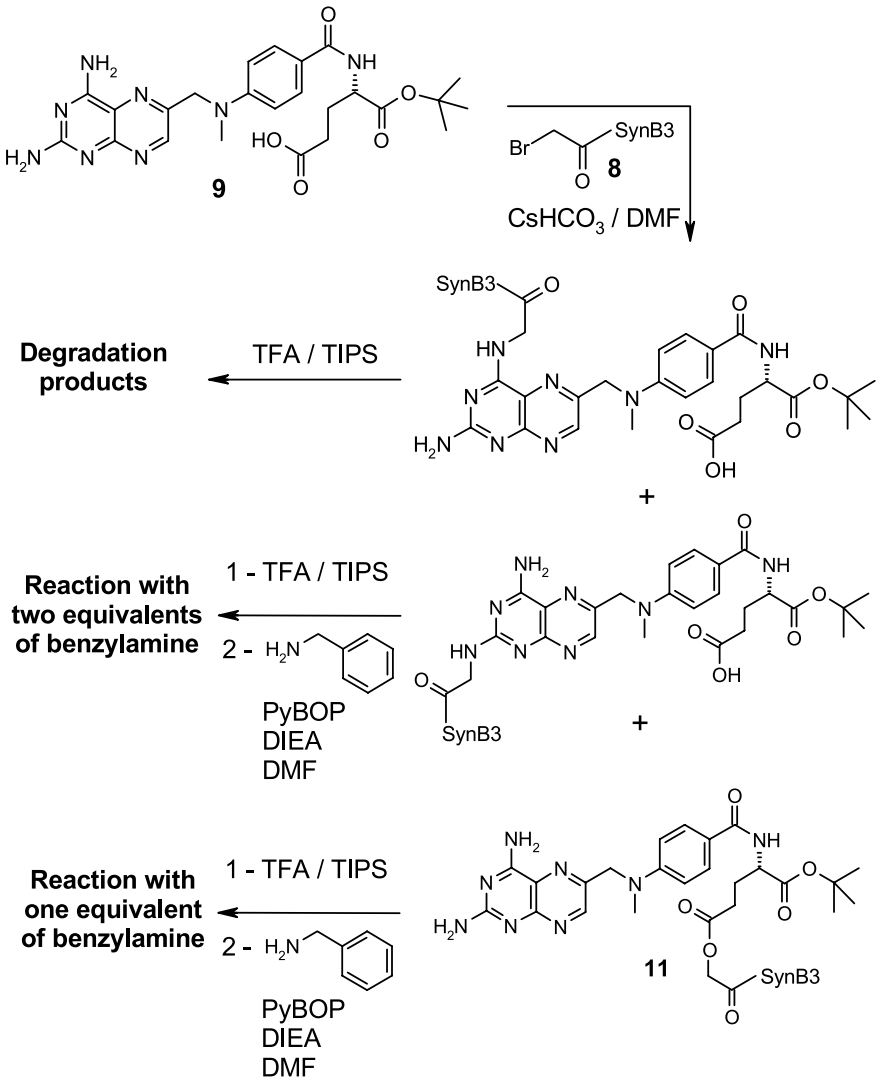
We first thought that a racemisation of the chiral glutamic acid carbon had occurred during the esterification step. To



Scheme 5. Glycolamidic ester conjugate of MTX: final synthetic route.

identify the products, we synthesized the glycolamidic conjugate with D-H-Glu(OH)-OtBu as starting material. Comparison of the final products with those obtained using L-H-Glu(OH)-OtBu proved that the two isomers were not two diastereoisomers as initially supposed. This suggests that they could actually be regioisomers. Cheung and co-workers have described the synthesis of *N*-(L-α-aminoacyl) derivatives of MTX.[29] The compounds were obtained by reaction of the di-*tert*-butyl ester of MTX with *N*-Boc-amino acid to yield mono and di substituted derivatives of MTX. Deprotection of the Boc group in acidic medium yielded only the 2-acyl products, the 4-acyl ones leading to decomposition products. Based on these observations, the two compounds obtained in our synthesis were reacted with an excess of benzylamine in the presence of PyBOP and DIEA (Scheme 6). The main product of the synthesis reacted with only 1 equiv of amine whereas the secondary product reacted with 2 equiv of benzylamine (observed in MALDI-TOF spectrometry). These findings suggest that the secondary product could possess two free carboxylic acid functions. The hypothesis of regioisomerism seems therefore to be confirmed. The secondary product was certainly the result of a nucleophilic attack of the bromoacetyl peptide by the two nitrogen of MTX in place of the carboxylate alkylated as cesium salt (Scheme 6).

Moreover, the relatively low yield of the final deprotection step (42%) could be explained by the degradation as already observed by Cheung and co-workers.[29] Separation of the products in analytical HPLC only occurred after

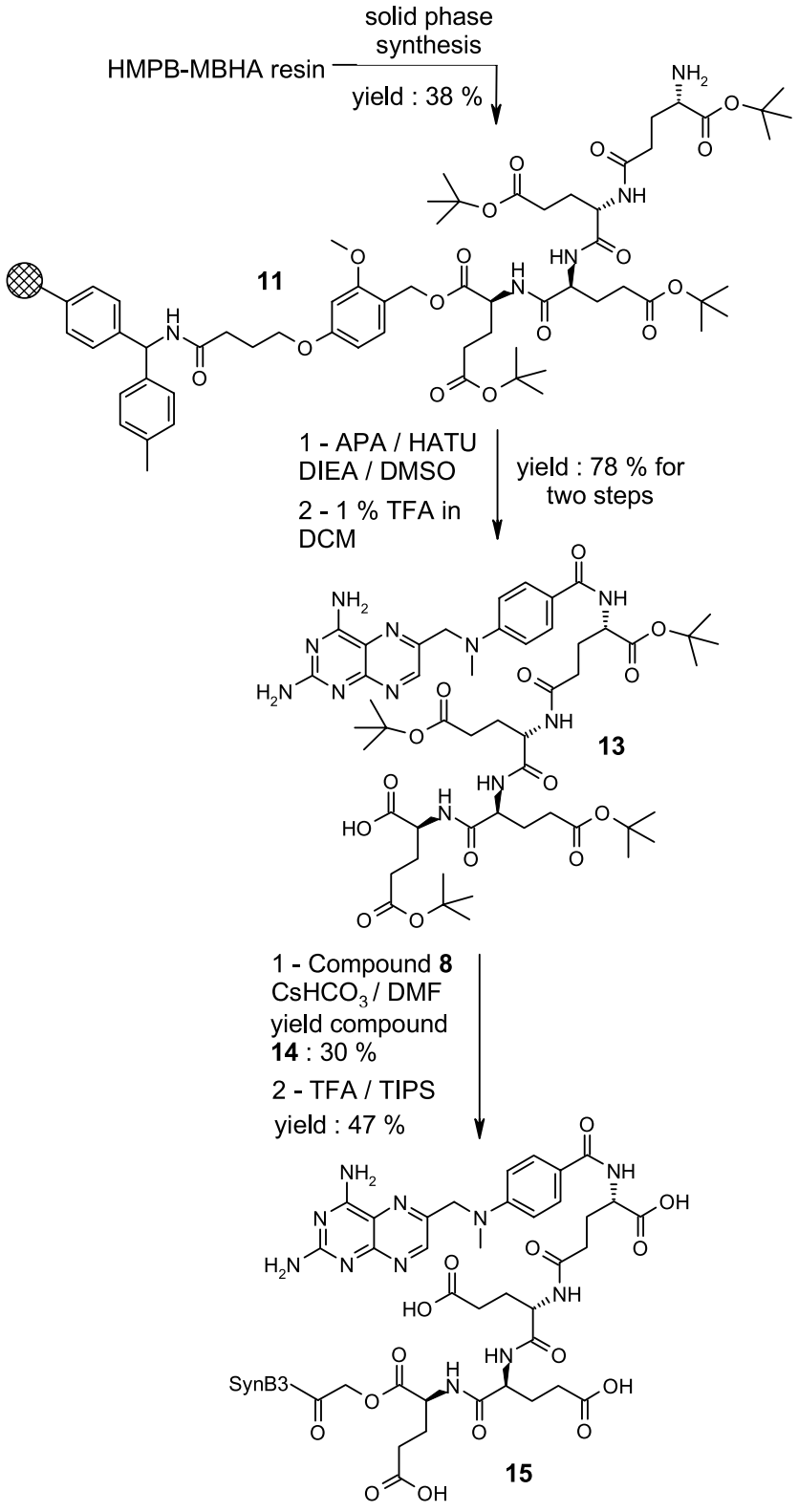


Scheme 6. Hypothesis for formation and evolution of regioisomers formed during esterification reaction.

cleavage of the tertio-butyl group. Decomposition of an alkyl derivative of MTX in acidic medium could explain the difficulties encountered during this final step, since it has been observed that 4-acyl derivatives of MTX decomposed under these conditions, whereas 2-acyl ones were stable.

Another compound containing a glycolamidic ester bond has finally been synthesized (Scheme 7, compound **15**).

This compound has been designed to be efficient against MTX resistant cell lines by taking advantage of the lability of the ester bond on the one hand, and on the other hand by delivering to the cell an active form of MTX. Indeed, the presence of the tri-glutamyl residue could bypass the resistant form of the cell where FPGS is deficient or GGS is overexpressed. The synthesis implies the use of a resin cleavable in mild acidic conditions in order to keep the protection of the carboxylic function on the glutamic acid residues. Introduction of the four correctly protected glutamic acids was made with a yield of 38%. APA was then coupled to the peptidyl resin and cleavage of the resin was made using a solution containing 1% TFA in DCM. The yield of the two steps was 78%. Once again, comparison between liquid and solid phase synthesis proved that solid phase synthesis was more efficient for the introduction of APA. The same reaction was made using a peptide already cleaved from the resin and the yield for the APA coupling step was 38%. This low yield obtained was not due to the presence of secondary products in the crude conjugate but to an important quantity of APA which did not react under the conditions used. The rest of the synthesis was the same as described for the previous glycolamidic ester conjugate. Formation of the ester bond was made using cesium hydrogencarbonate in DMF and compound **14** was obtained with a yield of 30%. Deprotection of the tertiobutyl protecting groups was then achieved to give compound **15** (yield: 47%). As expected, two final products with the same mass were identified, suggesting that regioisomers were once again present. It is also interesting to notice that the yields of the reactions with the tri-glutamic acids were below those with other derivatives in the same reaction conditions. Several hypotheses could be made as for example the steric hindrance of the numerous tertiobutyl group.



Scheme 7. Synthetic route for glycolamidic ester conjugate of tri-glutamylated MTX.

3. Conclusion

The present study details a convenient method using solid phase synthesis for the regioselective preparation of α- and γ-MTX peptide conjugates with APA as starting material. Comparison with liquid phase synthesis clearly demonstrates that yields obtained with solid phase synthesis are higher than those obtained in solution. To our knowledge, no other solid phase synthesis for MTX peptide conjugates was previously reported. The selective method used for MTX lipoamino conjugates[20] has been successfully applied to peptidic derivatives. A combined

solid and liquid phase synthesis has been developed to obtain glycolamidic conjugates of MTX in a regioselective manner. They were synthesized with good yields unlike previously reported cases.[20] These conjugates were designed in order to be efficient against MTX resistant cell lines and to increase the delivery of MTX through the blood brain barrier (BBB). We have used a member of the SynB peptide family which have been proved to transport effectively several drug-like molecules through the BBB.[26,30] Toward this goal, several studies are on going to explore the cytotoxic potency of these conjugates and the results will be published later.

4. Experimental

4.1. Materials

N,*N*-Diisopropylethylamine (DIEA), bromoacetic acid, 2,3,4-trichlorophenol, anhydrous methanol and piperidine were bought from Fluka. Dichloromethane (DCM), dimethylformamide (DMF), acetonitrile, diethyl ether, *N*,*N*-diisopropylcarbodiimide (DIPCDI), 4-amino-4-deoxy-N^{10}-methylpteroic acid and acetic acid were purchased from Sigma-Aldrich. 4-Dimethylaminopyridine (DMAP) and benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP) were obtained from Novabiochem. Cesium hydrogencarbonate was purchased from Acros Organics. The appropriate protected amino acids for solid phase synthesis and 1-hydroxybenzotriazole (HOBT) were bought from Senn Chemicals, except for Fmoc-Glu(OH)-O*t*Bu which was from Novabiochem. The resins used for solid phase synthesis were a Rink Amide Novagel HL substituted at 0.86 mmol/g and a HMPB-MBHA resin substituted at 0.82 mmol/g from Novabiochem. Prior to use, DMF was dried 24 h on molecular sieves 3 Å (extruders 1.6 mm; Prolabo).

Analytical HPLC was performed on a Beckman Gold 32 Karat System, with a Waters Symmetry Shield C18 column (250×4.6 mm; 5 μ) using the following solvent system A = H_2O/0.1% TFA and B = acetonitrile/0.1% TFA. Waters preparative system equipped with Prep LC Novapack C18 cartridges (40×10 mm; 6 μ; 60 Å) was used for preparative HPLC with the same solvent system. MALDI-TOF analysis was performed on a Voyager Elite XL System spectrometer (Perseptive Biosystems) and LC/MS/MS mass analysis was achieved on a quadripolar MicroMass Quattro Ultima spectrometer.

4.2. Synthesis

Yields calculated for the following reactions take into account TFA salts. One TFA salt was used for MTX and one per Arg residue on the peptide.

4.2.1. Solid phase synthesis. Peptides were assembled by conventional solid-phase chemistry on a Pioneer automatic synthesizer (Perseptive Biosystems) using a 9-fluorenyl-methoxycarbonyl/tertiobutyl (Fmoc/tBu) protection scheme.[23] The crude peptides and conjugates were purified on C_{18} reversed-phase preparative HPLC after trifluoro-acetic acid (TFA) cleavage/deprotection. The purity of compounds was assessed by C_{18} reversed-phase analytical HPLC and MALDI-MS. The peptide sequence was SynB3 (H-RRLSYSRRRF-NH_2, M_w 1395 g mol^{-1}).

4.2.2. General method for the coupling of APA with peptidyl resin. The last residue (APA) for solid phase synthesis of the conjugates with MTX was introduced using a manual apparatus. Briefly, starting from appropriate peptidyl resin, APA (4 equiv) was introduced using PyBOP (4 equiv) and DIEA (4 equiv) in a DMSO solution. After 4 h of reaction, the resin was washed with DMSO, DMF, DCM and dried under reduced pressure for 1 h. Cleavage and deprotection of the conjugate were performed during 3 h using a solution of TFA/H_2O/triisopropylsilane (TIPS)/phenol (88/5/4/5; v/v/v/m). 10 mL of deprotection solution were used for 60 μmol of peptide (theoretical). After precipitation of the compound with diethyl ether, the crude conjugate was purified using a C_{18} reversed-phase preparative HPLC (from 5 to 60% of buffer B in 60 min). The fractions containing the desired product were pooled and lyophilized to give the conjugate as a yellow powder. Yields and analytical characteristics of each compound are given below.

4.2.3. N'[4-[*N*-[(2,4-Diamino-6-pteridinyl)-methyl]-*N*-methylamino]benzoyl]-L-glutamic acid α-(Arg-Arg-Leu-Ser-Tyr-Ser-Arg-Arg-Arg-Phe-NH_2)-amide [3] (α-MTX-SynB3). Yield: 50%; HPLC: t_r = 6.24 min (using a separative gradient from 5 to 95% of buffer B in 15 min); MALDI-MS: *m*/*z* [DHB] 1832.1 ($M+H^+$), 1854.6 ($M+Na^+$), 1870.8 ($M+K^+$). Calcd exact mass: 1831.45 g mol^{-1}.

4.2.4. N'[4-[*N*-[(2,4-Diamino-6-pteridinyl)-methyl]-*N*-methylamino]benzoyl]-L-glutamic acid γ-(Arg-Arg-Leu-Ser-Tyr-Ser-Arg-Arg-Arg-Phe-NH_2)-amide [4] (γ-MTX-SynB3). Yield: 66%; HPLC: t_r = 6.26 min (using a separative gradient from 5 to 95% of buffer B in 15 min); MALDI-MS: *m*/*z* [DHB] 1832.6 ($M+H^+$), 1855.9 ($M+Na^+$), 1871.2 ($M+K^+$). Calcd exact mass: 1831.45 g mol^{-1}.

4.2.5. N'[4-[*N*-[(2,4-Diamino-6-pteridinyl)-methyl]-*N*-methylamino]benzoyl]-L-glutamic acid γ-(Glu-Glu-Glu-Arg-Arg-Leu-Ser-Tyr-Ser-Arg-Arg-Arg-Phe-NH_2)-amide [5] (γ-MTX-[Glu]$_3$-SynB3). Yield: 47%; HPLC: t_r = 6.33 min (using a separative gradient from 5 to 95% of buffer B in 15 min); MALDI-MS: *m*/*z* [DHB] 2218.1 ($M+H^+$). Calcd exact mass: 2217.45 g mol^{-1}.

4.2.6. N'[4-[*N*-[(2,4-Diamino-6-pteridinyl)-methyl]-*N*-methylamino]benzoyl]-L-glutamic acid γ-(β-Ala-β-Ala-Arg-Arg-Leu-Ser-Tyr-Ser-Arg-Arg-Arg-Phe-NH_2)-amide [6] (γ-MTX-[β-Ala]$_2$-SynB3). Yield: 65%; HPLC: t_r = 6.16 min (using a separative gradient from 5 to 95% of buffer B in 15 min); MALDI-MS: *m*/*z* [DHB] 1975.5 ($M+H^+$). Calcd exact mass: 1973.75 g mol^{-1}.

4.2.7. N'[4-[*N*-[(2,4-Diamino-6-pteridinyl)-methyl]-*N*-methylamino]benzoyl]-L-glutamic acid α-*tert*-butyl-ester [7] (α-MTX-OtBu). DIEA (42 μL; 0.264 mmol) and O-(7-Azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU, 60 mg; 0.158 mmol) were added to a solution of APA (50 mg; 0.132 mmol) in 1 mL of DMSO. After 25 min of stirring, 26.8 mg (0.132 mmol) of

H-Glu(OH)-OtBu solubilised in 300 μL DMSO were added. The solution was stirred at room temperature for 3 h and diethyl ether was added. The yellow precipitate was re-suspended in dry DMF and precipitated again by addition of diethyl ether. The crude MTX ester was purified on a C_{18} reverse-phase preparative HPLC (from 5 to 60% of buffer B in 60 min). The fractions containing the desired product were pooled and lyophilized to give 60 mg (yield 73%) of a 98% pure yellow powder; HPLC: $t_r=8.18$ min (using a separative gradient from 5 to 95% of buffer B in 15 min); MALDI-MS: *m/z* [DHB] 511.2 ($M+H^+$), 533.2 ($M+Na^+$), 549.1 ($M+K^+$). Calcd exact mass: 510.56 g mol^{-1}; ^1H NMR (DMSO-d_6): $\delta=12$, 14 ppm (s, 1H, –COO*H*), $\delta=8.72$ ppm (s, 1H, –C*H*=*N*– pteridine), $\delta=8.20$ ppm (d, 1H, $J=7.5$ Hz, –N*H*–), $\delta=7.72$ ppm (d, 2H, $J=8.9$ Hz, C*H* benzyl), $\delta=6.80$ ppm (d, 2H, $J=8.9$ Hz, C*H* benzyl), $\delta=4.87$ ppm (s, 2H, –CH_2–NMe), $\delta=4.27$ ppm (m, 1H, –C*H*–COOtBu), $\delta=3.45$ ppm (s, 4H, –NH_2 pteridine), $\delta=3.25$ ppm (s, 3H, CH_3–N–), $\delta=2.32$ ppm (t, 2H, $J=7.5$ Hz, –CH_2–COOH), $\delta=1.98$ ppm (m, 2H, –CH_2–CH–COOtBu), $\delta=1.39$ ppm (s, 9H, –C(CH_3)$_3$).

4.2.8. Bromoacetyl-Arg-Arg-Leu-Ser-Tyr-Ser-Arg-Arg-Arg-Phe-NH_2 [8]. The compound was synthesized using solid phase synthesis. Briefly, starting from SynB3 peptidyl resin, bromoacetyl was introduced using bromoacetic acid (4 equiv), PyBOP (4 equiv) and DIEA (4 equiv) in solution in DMF. After 4 h of reaction, the resin was washed with DMF, dichloromethane (DCM) and dried under reduced pressure for 1 h. Cleavage, deprotection and purification of the modified peptide were performed as previously described. Yield: 40% (including peptide synthesis); HPLC: $t_r=6.17$ min (using a separative gradient from 5 to 95% of buffer B in 15 min); MALDI-MS: *m/z* [DHB] 1518.1 ($M+H^+$), 1540.1 ($M+Na^+$), 1557.9 ($M+K^+$). Calcd exact mass: 1516.7 g mol^{-1}.

4.2.9. *N′*[4-[*N*-[(2,4-Diamino-6-pteridinyl)-methyl]-*N*-methylamino]benzoyl]-L-glutamic acid α-*tert*-butyl,γ-(glycolamido-Arg-Arg-Leu-Ser-Tyr-Ser-Arg-Arg-Arg-Phe-NH_2)-ester [9] (γ-MTX-[GEL-SynB3]-OtBu). 25 mg (0.049 mmol) of α-MTX-OtBu were dissolved in 125 μL of dry DMF. Solutions containing, respectively, 47.5 mg (0.245 mmol) of Cesium hydrogencarbonate in 50 μL of dry DMF and 123 mg (0.058 mmol) of bromoacetyl-SynB3 in 200 μL of dry DMF were successively added. The solution was stirred for 48 h at room temperature and after addition of 3 mL of dry DMF, the crude solution was applied on a C_{18} reversed-phase preparative HPLC (from 5 to 60% of buffer B in 60 min). The fractions containing the desired product were pooled and lyophilized to yield 65.2 mg of conjugate (yield: 51%) of a 85% pure yellow powder; HPLC: $t_r=7.11$ min (using a separative gradient from 5 to 95% of buffer B in 15 min); MALDI-MS: *m/z* [HABA] 1946.3 ($M+H^+$), 1986.3 ($M+K^+$). Calcd exact mass: 1944.56 g mol^{-1}.

4.2.10. *N′*[4-[*N*-[(2,4-Diamino-6-pteridinyl)-methyl]-*N*-methylamino]benzoyl]-L-glutamic acid γ-(glycolamido-Arg-Arg-Leu-Ser-Tyr-Ser-Arg-Arg-Arg-Phe-NH_2)-ester [10] (γ-MTX-GEL-SynB3). 65.2 mg (0.0315 mmol) of γ-MTX-[GEL-SynB3]-OtBu were suspended in 7 mL of a solution composed of TFA 95%/Triisopropylsilane (TIPS) 5% (v/v). After 30 min of stirring, TFA was evaporated to dryness under reduced pressure, and the yellow film obtained was dissolved in 5 mL of a solution containing H_2O (TFA 0.1%; v/v)/acetonitrile (TFA 0.08%; v/v) (90/10; v/v). The crude product was then applied on a C_{18} reversed-phase preparative HPLC (from 5 to 60% of buffer B in 60 min). The fractions containing the conjugate were pooled and lyophilized to yield 25 mg of a 98% pure yellow powder (yield 42%); HPLC: $t_r=6.91$ min (using a separative gradient from 5 to 95% of buffer B in 15 min); MALDI-MS: *m/z* [HABA] 1890.5 ($M+H^+$), 1930.7 ($M+K^+$). Calcd exact mass: 1888.56 g mol^{-1}.

4.2.11. H-Glu[Glu(OtBu)-Glu(OtBu)-Glu(OtBu)-HMPB-MBHA resin]-OtBu [11]. First amino acid was introduced on HMPB-MBHA resin using a manual apparatus. Briefly, 519 μL (3.435 mmol) of DIPCDI in solution in 2 mL of dry DCM were added drop wise to 2.9 g (6.87 mmol) of Fmoc-Glu(OtBu)-OH solubilised in 29 mL of dry DCM. After 30 min of stirring, DCM was evaporated to dryness and the white residue was resuspended in 30 mL of DMF. DMAP (0.168 g, 1.374 mmol) and the resin (1.676 g, 1.374 mmol) were added and the solution was stirred slowly for 3 h. The resin was filtrated, washed successively with DMF (three times), DCM (three times) diethyl ether and dried under vacuum. 2.22 g (yield 95%) of functionalized resin were obtained. The other amino acids were introduced using automatic solid phase synthesizer as previously described. Yield of the synthesis was evaluated at 38% using automatic UV titration of fluorene nucleus released.

4.2.12. *N′*[4-[*N*-[(2,4-Diamino-6-pteridinyl)-methyl]-*N*-methylamino]benzoyl]-L-glutamic acid α-*tert*-butyl-ester, γ-(Glu(OtBu)-Glu(OtBu)-Glu(OtBu)-HMPB-MBHA-resin)-amide [12]. APA (64 mg, 168 μmol) was introduced on 500 mg (130 μmol) of peptidyl resin **11** as previously described using HATU (63.8 mg, 168 μmol) and DIEA (38 μL, 260 μmol) in solution in DMSO. After stirring slowly during 4 h, the resin was washed with DMSO, DMF, DCM and dried under reduced pressure for 1 h.

4.2.13. *N′*[4-[*N*-[(2,4-Diamino-6-pteridinyl)-methyl]-*N*-methylamino]benzoyl]-L-glutamic acid α-*tert*-butyl-ester, γ-(Glu(OtBu)-Glu(OtBu)-Glu(OtBu))-amide [13]. Peptidyl resin **12** was suspended in 178 mL of DCM containing 1.8 mL of TFA. Slow stirring was maintained for 30 min and the resin was filtrated. The operation was repeated twice and the filtrates were pooled, neutralised by slow addition of ammonium hydroxyde and evaporated to dryness. The yellow residue was purified on a C_{18} reversed-phase preparative HPLC (from 5 to 60% of buffer B in 60 min). The fractions containing the desired product were pooled and lyophilized to give 120 mg (yield 78% for two steps) of a 98% pure yellow powder.

HPLC: $t_r=10.25$ min (using a separative gradient from 5 to 95% of buffer B in 15 min); MALDI-MS: *m/z* [DHB] 1066.4 ($M+H^+$), 1089.4 ($M+Na^+$), 1104.3 ($M+K^+$). Calcd exact mass: 1066.23 g mol^{-1}.

4.2.14. *N′*[4-[*N*-[(2,4-Diamino-6-pteridinyl)-methyl]-*N*-methylamino]benzoyl]-L-glutamic acid α-tert-butyl-ester,γ-(Glu(OtBu)-Glu(OtBu)-Glu(OtBu)-glycolamido-

Arg-Arg-Leu-Ser-Tyr-Ser-Arg-Arg-Arg-Phe-NH_2)-amide [14] (γ-MTX-[Glu(OtBu)$_3$-GEL-SynB3]-OtBu). Glycolamidic ester bond was formed as described for compound **7**. 119 mg (0.112 mmol) of compound **13** were dissolved in 300 μL of dry DMF. Solutions containing, respectively, 119 mg (0.56 mmol) of Cesium hydrogencarbonate in 100 μL of dry DMF and 280 mg (0.134 mmol) of bromoacetyl-SynB3 in 200 μL of dry DMF were successively added. The solution was stirred for 48 h at room temperature and after addition of 3 mL of DMF, the crude solution was applied on a C_{18} reversed-phase preparative HPLC (from 5 to 60% of buffer B in 60 min). The fractions containing the desired product were pooled and lyophilized to yield 108 mg of conjugate (yield: 30%) of a 90% pure yellow powder; HPLC: $t_r=8.15$ min (using a separative gradient from 5 to 95% of buffer B in 15 min); MALDI-MS: *m/z* [HABA] 2502.9 ($M+H^+$). Calcd exact mass: 2501.93 g mol^{-1}.

4.2.15. *N′*[4-[*N*-[(2,4-Diamino-6-pteridinyl)-methyl]-*N*-methylamino]benzoyl]-L-glutamic acid γ-(Glu-Glu-Glu-glycolamido-Arg-Arg-Leu-Ser-Tyr-Ser-Arg-Arg-Arg-Phe-NH_2)-amide [15] (γ-MTX-Glu$_3$-GEL-SynB3). 108 mg (0.035 mmol) of (γ-MTX-[Glu(OtBu)$_3$-GEL-SynB3]-OtBu) **14** were suspended in 9.6 mL of a solution composed of TFA 95%/Triisopropylsilane (TIPS) 5% (v/v). After stirring 30 min, TFA was evaporated to dryness under reduced pressure, and the yellow film obtained was dissolved in 5 mL of a solution containing H_2O (TFA 0.1%; v/v)/acetonitrile (TFA 0.08%; v/v) (90/10; v/v). The crude product was then purified on a C_{18} reversed-phase preparative HPLC (from 5 to 60% of buffer B in 60 min). The fractions containing the conjugate were pooled and lyophilized to give 48.5 mg (yield: 47%) of a 98% pure yellow powder; HPLC: $t_r=5.36$ min (using a separative gradient from 5 to 95% of buffer B in 15 min); MALDI-MS: *m/z* [HABA] 2277.8 ($M+H^+$), 2300.8 ($M+Na^+$), 2316.7 ($M+K^+$). Calcd exact mass: 2277.47 g mol^{-1}.

Acknowledgements

The authors wish to thank Dr. P. Clair and Dr. J. Temsamani for permanent interest and helpful advice.

References and notes

1. Huennekens, F. M. *Adv. Enzyme Regul.* **1997**, *37*, 77–92.
2. Hitchings, G. H.; Smith, S. L. *Adv. Enzyme Regul.* **1980**, *18*, 349–371.
3. Genestier, L.; Paillot, R.; Quemeneur, L.; Izeradjene, K.; Revillard, J. P. *Immunopharmacology* **2000**, *47*, 247–257.
4. Gorlick, R.; Cole, P.; Banerjee, D.; Longo, G.; Wei Li, W.; Hochhauser, D.; Bertino, J. R. Mechanisms of Methotrexate. Resistance in Acute Leukemia. Decreased Transport and Polyglutamylation. In *Drug Resistance in Leukemia and Lymphoma III*; Kaspers, G. J. et al, Ed.; Plenum: New York, 1999; pp 543–550.
5. Bleyer, W. A. *Cancer* **1978**, *41*, 36–51.
6. Bertino, J. R.; Göker, E.; Gorlick, R.; Li, W. W.; Banerjee, D. *Stem Cells* **1996**, *14*, 5–9.
7. Shapiro, W. R.; Allen, J. C.; Horten, B. C. *Clin. Bull.* **1980**, *10*, 49–52.
8. Rosowsky, A.; Forsch, R. A.; Uren, J.; Wick, M. *J. Med. Chem.* **1981**, *24*, 1450–1455.
9. Rosowsky, A.; Forsch, R. A.; Yu, C. S.; Lazarus, H.; Beardsley, G. P. *J. Med. Chem.* **1984**, *27*, 605–609.
10. Kuefner, U.; Lohrmann, U.; Montejano, Y.; Vitols, K. S.; Huennekens, F. M. *Adv. Enzyme Regul.* **1988**, *27*, 3–13.
11. Piper, J. R.; Montgomery, J. A. *J. Med. Chem.* **1982**, *25*, 182–187.
12. Kralovec, J.; Spencer, G.; Blair, A. H.; Mammen, M.; Singh, M.; Ghose, T. *J. Med. Chem.* **1989**, *32*, 2426–2431.
13. Hellström, I.; Hellström, K. E.; Siegall, C. B.; Trail, P. A. *Adv. Pharmacol.* **1995**, *33*, 349–388.
14. Boratynski, J.; Opolski, A.; Wietrzyk, J.; Gorski, A.; Radzikowski, C. *Cancer Lett.* **2000**, *148*, 189–195.
15. Subr, V.; Strohalm, J.; Hirano, T.; Ito, Y.; Ulbrich, K. *J. Controlled Release* **1997**, *49*, 123–132.
16. Riebeseel, K.; Biedermann, E.; Löser, R.; Breiter, N.; Hanselmann, R.; Mülhaupt, R.; Unger, C.; Kratz, F. *Bioconjugate Chem.* **2002**, *13*, 773–785.
17. Nagy, A.; Szoke, B.; Schally, A. V. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 6373–6376.
18. Janaky, T.; Juhasz, A.; Rekasi, Z.; Serfösö, P.; Pinski, J.; Bokser, L.; Srkalovic, G.; Milovanovic, S.; Redding, T. W.; Halmos, G.; Nagy, A.; Schally, A. V. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 10203–10207.
19. Szepeshazi, K.; Schally, A. V.; Juhasz, A.; Nagy, A.; Janaky, T. *Anti-Cancer Drugs* **1992**, *3*, 109–116.
20. Pignatello, R.; Spampinato, G.; Sorrenti, V.; Di Giacomo, C.; Vicari, L.; McGuire, J. J.; Russell, C. A.; Puglisi, G.; Toth, I. *Eur. J. Pharm. Sci.* **2000**, *10*, 237–245.
21. Drin, G.; Temsamani, J. *Biochim. Biophys. Acta* **2002**, *1559*, 160–170.
22. Mazel, M.; Clair, P.; Rousselle, C.; Vidal, P.; Scherrmann, J.-M.; Mathieu, D.; Temsamani, J. *Anti-Cancer Drugs* **2001**, *12*, 107–116.
23. Atherton, E.; Sheppard, R. C. In *Solid Phase Peptide Synthesis: a Practical Approach*; IRL: Oxford, 1989.
24. Bolin, J. T.; Filman, D. J.; Matthews, D. A.; Hamlin, R. C.; Kraut, J. *J. Biol. Chem.* **1982**, *257*, 13650–13662.
25. Kuefner, U.; Lorhmann, U.; Montejano, Y.; Vitols, K. S.; Huennekens, F. M. *Biochemistry* **1989**, *28*, 2288–2297.
26. Rousselle, C.; Clair, P.; Temsamani, J.; Scherrmann, J.-M. *J. Drug Target.* **2002**, *10*, 309–315.
27. Wakamiya, T.; Yoshida, D.; Mizuki, Y.; Hanatani, K.; Yamaguchi, Y.; Shimamoto, T. Study on Solid-Phase Synthesis of Compounds, Containing Hydroxyamino Acids or Saccharides, by Taking Advantage of the Characteristics of Glutamic Acid. *Peptide Science*; Aoyagi, H., Ed.; 2001, 391–394.
28. Chavanieu, A.; Ceccato, M.; Chenu, J.; Mendre, C.; Calas, B. *Protein Peptide Lett.* **1994**, *1*, 50–53.
29. Cheung, H. T. A.; Boadle, D. K.; Tran, T. Q. *Heterocycles* **1989**, *28*, 751–758.
30. Rousselle, C.; Clair, P.; Lefauconnier, J.-M.; Kaczorek, M.; Scherrmann, J.-M.; Temsamani, J. *Mol. Pharmacol.* **2000**, *57*, 679–686.

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Tetrahedron 61 (2005) 813–817

Easy route to labeled and unlabeled *R*,*R*,*R*-γ-tocopherol by aryl demethylation of α-homologues

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Received 18 August 2004; revised 27 October 2004; accepted 18 November 2004

Available online 30 November 2004

Abstract—The interest in vitamin E research is increasingly focusing on the peculiar properties of the less investigated tocopherols and their metabolites, such as γ-tocopherol, which have been revealed as very important for human health. Metabolic studies of γ-tocopherol have been constricted by its high cost and the poor availability of stable isotope-labeled forms. An efficient, inexpensive and simple route is described for the preparation of labeled and unlabeled *R*,*R*,*R*-γ-tocopherol, starting from *R*,*R*,*R*-α-tocopherol, through simple thermal decarboxylation of γ-tocopherol-5-carboxylic acid.

1. Introduction

Vitamin E is the most important fat-soluble chain-breaking antioxidant. The term vitamin E covers all tocols and tocotrienols derivatives exhibiting qualitatively the biological activity of α-tocopherol.[1] The most important members of vitamin E family for human nutrition are represented by α- and γ-tocopherol, the former having much higher vitaminic activity and bioavailability,[2–4] and thus constituting the primary and almost exclusive form in supplements. Despite, the much lower plasma concentration and bioactivity compared to α-tocopherol, as assessed in animal bioassays, recent and growing evidence suggests that γ-tocopherol has unique properties that may be very important to human health, not shared by α-tocopherol.[5] Those features do not appear to be related to its chemical antioxidant behavior, but rather reflect anti-inflammatory, antineoplastic, and natriuretic functions possibly mediated through specific binding interactions. Moreover, epidemiological data suggest that γ-tocopherol is a better negative risk factor for certain types of cancer and myocardial infarction than is α-tocopherol.[6] All these findings have given a great boost to the research in the field of vitamin E, which is currently represented more and more by in vivo studies on tocopherols metabolites and γ-tocopherol. The utilization of stable isotope-labeled analogues greatly facilitates carrying out such studies.[7] In fact, they represent a powerful tool in terms of both specificity and sensitivity, acting as probes in the body and as internal standards for accurate quantitative determinations by specific techniques like mass spectrometry,[8–10] more and more used for the characterization of such complex matrices.

Isotope-labeled forms of γ-tocopherol are not readily available, and very few papers have been reported regarding their synthesis. Woggon et al. described a preparation of [7-methyl-^{3}H,^{14}C]-γ-tocopherol that is rather long and complicated,[11] and an enzymatic route to monodeuterated γ-tocopherol on very small scale (5 mg).[12] In another work, Ingold and co-workers depicted the synthesis of *R*,*R*,*R*-d_2-γ-tocopherol in four steps from γ-tocopherol itself.[13] Though this last route proceeds in good yields, the high cost and the very low commercial availability of the starting material, the natural γ-tocopherol, make it expensive and hardly scalable. In this paper, we report a very efficient route for the preparation of trideuterated *R*,*R*,*R*-γ-tocopherol **11** from the cheap natural δ-tocopherol. The same protocol is also very convenient for large scale synthesis of unlabeled *R*,*R*,*R*-γ-tocopherol, starting from inexpensive and widely available *R*,*R*,*R*-α-tocopherol.

2. Results and discussion

In designing a route to γ-tocopherol labeled with deuterium atoms, different positions can be chosen on the basis of synthetic considerations, the reason of labeling and the analytical techniques to be used for its detection. In the last years, several ESI and APCI LC-MS/MS analytical methods

Keywords: Vitamin E; γ-Tocopherol; Labeled tocopherols.
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doi:10.1016/j.tet.2004.11.047

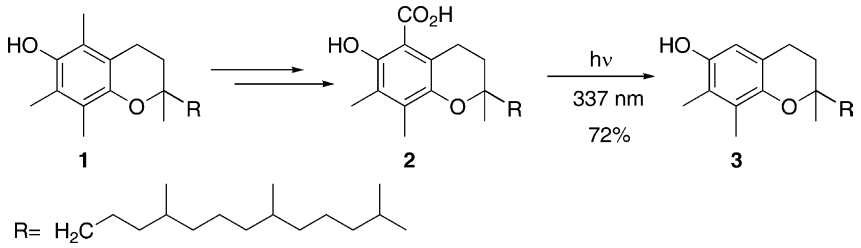


Figure 1. Transformation of α- into γ-tocopherol through photodecarboxylation of **2**.[15]

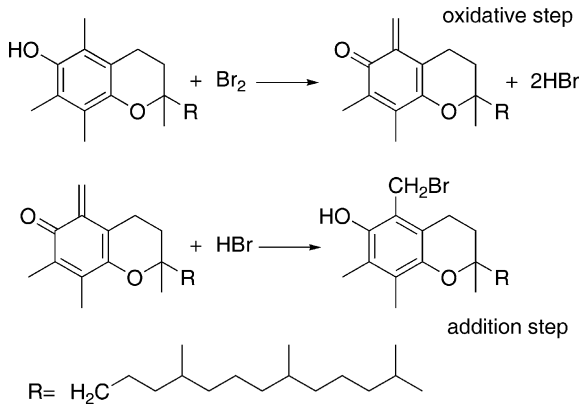
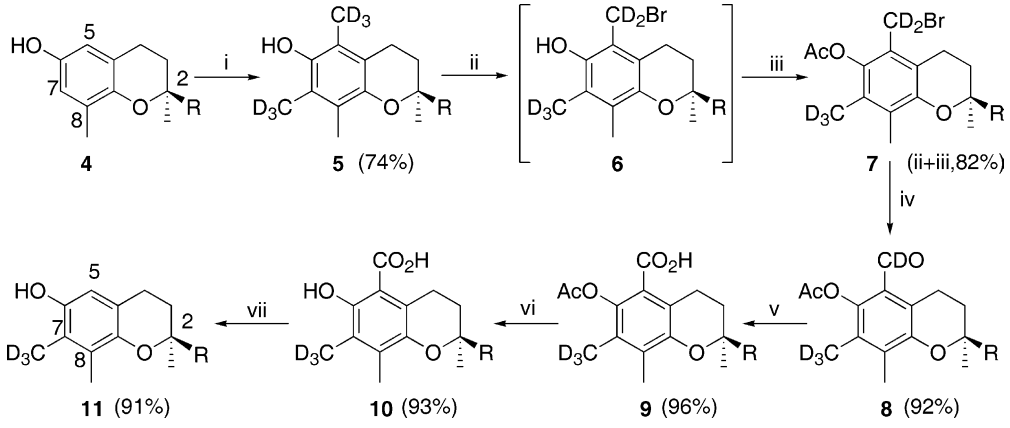


Figure 2. Proposed reaction mechanism of the bromination of α-tocopherol.[17]

have been developed successfully for tocopherols determination in various matrices, employing deuterated tocopherols as internal standards.[8–10] These results made LC-MS/MS the technique of choice for this kind of study. We planned to introduce labeling as CD_3 on one of the methyl groups of the aromatic ring, considering the degradation metabolic pathway of γ-tocopherol that results in γ-CEHC formation, that is, without modification of the chromanol ring.[14] In this way, interference of natural isotopes of the analyte on the *m/z* value of the labeled compound is avoided and d_3-γ-CEHC metabolite coming from supplementation can be traced.

Basically, two routes can be conceived for the synthesis of enantiopure labeled γ-tocopherol. One approach involves designing a suitable way for the preparation of labeled 2,3-dimethylhydroquinone, followed by the subsequent building of the chroman ring and aliphatic side chain in a stereoselective manner. In the other approach, convenient transformations without loss of enantiopurity have to be devised to introduce labeling directly on readily accessible chiral tocopherols, such as the natural ones, or their derivatives. According to Rosenau and Habicher,[15] it is possible to prepare γ-tocopherol starting from α-tocopherol through a multi-step procedure, the key point being photodecarboxylation of γ-tocopherol-5-carboxylic acid **2** (Fig. 1). Therefore, following this approach, we first prepared *R,R,R*-(5,7-$(CD_3)_2$)-α-tocopherol **5** as the precursor of the desired d_3-γ-tocopherol, introducing deuterium by $SnCl_2$-catalyzed deuteromethylation,[16] using $(CD_2O)_n$ on commercially available natural δ-tocopherol **4**. Bromination of **5** gave d_5-5-bromomethyl-γ-tocopherol **6** in almost quantitative yields.[17] According to the proposed mechanism, α-tocopherol oxidation leads to the *ortho*-quinone methide species, which adds hydrogen bromide formed in the first step, affording the benzylic brominated product (Fig. 2).

Before oxidizing the benzylic function, the phenolic hydroxyl group had to be protected in order to avoid its oxidation and relative by-products formation. Therefore, after evaporation of the solvent, acetylation was performed in the same flask under acid-catalyzed mild conditions. This prevents HBr elimination from **6**, which is highly susceptible to oxidation, bases and temperatures above 50 °C. Elimination of HBr would lead to an *ortho*-quinone methide intermediate and formation of α-tocopherol spirodimer. The acetylated product **7** was then oxidized



Scheme 1. Synthesis of *R,R,R*-(7-$^2H_3$)-γ-tocopherol **11**. (i) *i*-Pr_2O, $SnCl_2$, $(CD_2O)_n$, DCl in D_2O, 65 °C, 4 h; (ii) Br_2 in hexane, rt, 3 h; (iii) Ac_2O, AcOH, CH_2Cl_2, H_2SO_4 cat., rt, overnight; (iv) NMMO, 4 equiv, acetonitrile, rt, overnight; (v) NH_2SO_3H, $NaClO_2$ in 1,4-dioxane/H_2O, rt, 50 min; (vi) KOH 2 M in MeOH, 50 °C, 2 h; (vii) heating at 170 °C, 3 h.

following a different procedure from the one reported in literature.[15] In our hands, $KMnO_4$ oxidation under phase-transfer catalysis proved to be rather tedious and low yielding because of the difficulties encountered in the purification step. Therefore, we prepared the desired acid derivative **9** through a two steps oxidation. First, benzylic bromide **7** was reacted with anhydrous *N*-methylmorpholine-*N*-oxide (NMMO) to give aldehyde **8** in 92%, and then **8** was treated with $NH_2SO_3H/NaClO_2$ mixture, affording the acid **10**, after the saponification, in 90% overall yield from **7** (Scheme 1).

It has been reported that γ-tocopherol-5-carboxylic acid undergoes photodecarboxylation through a radical mechanism in an average yield of 72% upon irradiation at 337 nm, in the presence of iron complexes or activated titanium dioxide (Fig. 1).[15] Experiments carried out with radical starters, such as AIBN, gave only radicals coupling products, showing that only the photochemically excited form of γ-tocopherol-5-carboxylic acid is able to stabilize itself by decarboxylation. Photoirradiation requires special equipment not available in many synthetic laboratories. Therefore, we looked for an alternative procedure to remove the carboxylic group. The decarboxylation of aromatic acids is most often carried out by heating with quinoline and copper or copper salts,[18] but heating the salt of the acid[19] or the acid in an acidic medium has also been used with certain substrates.[20,21]Before trying one of those methods, we first performed a set of experiments simply heating the unlabeled compound **13** (prepared from *R*,*R*,*R*-α-tocopherol as described for its labeled counterpart, Scheme 2) under inert atmosphere. While keeping **13** at 150 °C for 3 h gave a small conversion of the acid into the desired product **14**, carrying out the thermal decarboxylation at 200 °C resulted in almost complete conversion of the acid **13**, allowing us to recover γ-tocopherol **14** in 65% yield, comparable to the yield reported using photodecarboxylation.[15] Therefore, considering these results, we performed a tuning of the reaction temperature, which showed that 170 °C was the best compromise between conversion and by-product formation. Under these conditions, both **11** and **14** were obtained with an excellent 91% yield, respectively, from **10** and **13**. Analyses carried out by chiral HPLC on a Chiracel OD-H Daicel column showed that no epimerization at the C-2 chiral center occurred throughout the synthesis, thus assessing that the *R*,*R*,*R* configuration of the starting δ- and α-tocopherol were retained. From all our experience, it can be assumed that no erosion of stereochemistry of the aliphatic side chain took place under the reaction conditions. Moreover, isotope purity of **11** was highly satisfying, resulting 97.6% by GC-MS.

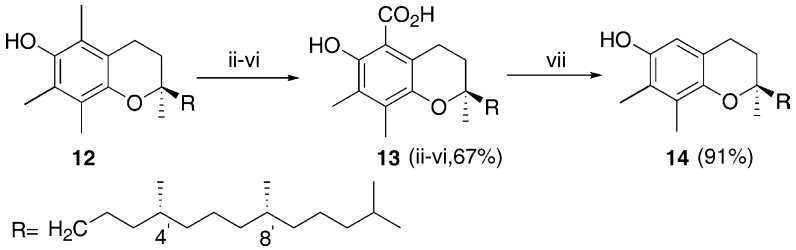
3. Conclusions

Over the last few years focus of vitamin E research has shifted significantly from the well-known bioactivity of α-tocopherol to the less investigated properties of other tocopherols and tocopherol derivatives, which have been proving very important. The high costs of commercially available γ-tocopherol and the difficulty of preparing its labeled analogue have somewhat limited the studies on this very interesting compound. For this reason, we set up an efficient, simple and scalable route that allows the preparation of d_3-*R*,*R*,*R*-γ-tocopherol and *R*,*R*,*R*-γ-tocopherol starting, respectively, from inexpensive and widely available natural δ-tocopherol and *R*,*R*,*R*-α-tocopherol. The simple thermal decarboxylation of the final step afforded the desired product in excellent yields and without loss of isomeric purity, greatly improving the previous reported procedure.

4. Experimental

4.1. General

All reactions were performed under inert atmosphere (Ar or N_2). ^1H and ^{13}C NMR spectra were recorded on a Varian Gemini 200 spectrometer (200 and 50.3 MHz, respectively). ^2H NMR spectra were recorded on a Varian VXR 300 spectrometer (46 MHz for ^2H) in $CHCl_3$ with $CDCl_3$ as internal standard. Chemical shifts are expressed on the δ scale (ppm). Deuteration level was determined by GC-MS by microSIS mode. GC-MS analyses were recorded on a Saturn 2000 GC-MS/MS Varian Chromatography System mass spectrometer connected to a 3800 Varian gas chromatograph equipped with a DB-5 capillary column (30 m×0.25 mm, 0.25 μm film thickness). Operating conditions: injector temperature 280 °C; oven program temperature 250 °C, increased at 30° min^{-1} to 300 °C and held for 7 min at 300 °C; transfer line temperature 295 °C; ion trap temperature 250 °C; emission current 10 μA; isolation window 3 amu. The APCI mass spectra were acquired on an Applied Biosystem API 4000 mass spectrometer. HPLC analyses for determination of the isomeric purity were performed on a 250×4.6 mm Chiracel OD-H column from Daicel Chem. Ind., solvent 0.5% EtOH in *n*-hexane, flow 1.0 mL/min, detection at 220 nm, using *R*,*R*,*R*- and (all-*rac*)-γ-tocopherol as standards. Commercial reagents and solvents were purchased from Aldrich, Fluka, or Merck, and purified by standard methods when necessary. Hexane was distilled over CaH_2 before use, CH_2Cl_2 over P_2O_5, *i*-Pr_2O over Na. Deuterated paraformaldehyde $(CD_2O)_n$ (≥99.5 at.% D) and DCl in D_2O (35% in DCl, 99.9 at.%



Scheme 2. Synthesis of *R*,*R*,*R*-γ-tocopherol **14**. Reactions details are the same as Scheme 1.

D) were purchased from C/D/N Isotopes (Canada), whilst $NaBD_4$ (98 at.% D) and D_2O (99.8 at.% D) from Aldrich. 2*R*,4′*R*,8′*R*-α-Tocopherol (Covitol F1490) was purchased from Henkel. All other commercial reagents were used without further purification. Column chromatography was performed on silica gel 60 (70–230 mesh). TLC was performed on silica gel Macherey–Nagel Alugram Sil G/UV_{254} (0.20 mm). All yields given refer to isolated yields.

4.1.1. (5-$^2H_3$,7-$^2H_3$)-(2*R*,4′*R*,8′*R*)-α-tocopherol (5).[16] To a solution of natural δ-tocopherol (1.95 g, 4.72 mmol) in anhydrous *i*-Pr_2O (50 mL) were added anhydrous $SnCl_2$ (13.9 g, 73.3 mmol, 15.5 equiv), DCl in D_2O (50 g, 35%, 99.9% D) and $(CD_2O)_n$ (1.1 g, 34.3 mmol, 7.28 equiv). The mixture was heated at 65 °C for 4 h, and then water was added. The aqueous phase was extracted with Et_2O (3× 100 mL). The combined organic extracts were subsequently washed to neutrality with water, dried over Na_2SO_4 and concentrated to dryness. Purification by column chromatography (Hex/EtOAc 12:1) afforded **5** (1.52 g, 74% yield) as pale yellow oil.

^{1}H NMR, ($CDCl_3$/TMS): δ 0.7–1.6 (m, 36H, C(2a)H_3 and $C_{16}H_{33}$ chain), 1.8 (m, 2H, $ArCH_2CH_2$), 2.1 (s, 3H, $ArCH_3$), 2.6 (t, *J*=6.2 Hz, 2H, $ArCH_2CH_2$). ^{2}H NMR ($CHCl_3$): δ 2.09 (s, 3D, $ArCD_3$), 2.13 (s, 3D, $ArCD_3$). ^{13}C NMR ($CDCl_3$): δ 10–11.1 (m), 11.7, 19.6, 19.7, 20.7, 21.03, 22.6, 22.7, 23.8, 24.4, 24.8, 27.9, 30.8, 31.6, 32.7, 32.8, 37.3, 37.4, 37.5, 39.4, 39.8, 74.5, 117.4, 118.4, 120.9, 122.6, 144.6, 145.6. APCI-MS (in MeOH), *m*/*z* (amu): positive ion mode, 437.6 $[M]^+$.

4.1.2. 6-*O*-Acetyl-5-(5-$^2H_2$)-bromomethyl-(7-$^2H_3$)-(2*R*,4′*R*,8′*R*)-γ-tocopherol (7). To a solution of **5** (1.38 g, 3.16 mmol) in dry hexane (25 mL) was added dropwise a solution of Br_2 (0.17 mL, 3.32 mmol, 1.05 equiv) in dry hexane (10 mL). The solution was stirred for 3 h. The solvent and the remaining Br_2 were removed in vacuo at rt, affording **6** without further purification. ^{1}H NMR of **6** was consistent with reported data.[17] In the same flask was then carried out the acetylation reaction to prepare **7**. To **6**, obtained as described above, were added CH_2Cl_2 (12 mL), AcOH (12 mL), Ac_2O (2.2 mL) and H_2SO_4 (0.2 mL). The dark mixture was stirred overnight at rt. Then water was added and CH_2Cl_2 evaporated. The aqueous phase was extracted with hexane (3×100 mL). The combined organic extracts were subsequently washed to neutrality with water, dried over Na_2SO_4 and concentrated to dryness. Purification by column chromatography (Hex/EtOAc 10:1) afforded **7** (1.45 g, 82% yield from **5**) as yellow dense oil.

^{1}H NMR, ($CDCl_3$/TMS): δ 0.95–1.8 (m, 36H, C(2a)H_3 and $C_{16}H_{33}$ chain), 1.8 (m, 2H, $ArCH_2CH_2$), 2.1 (s, 3H, $ArCH_3$), 2.37 (s, 3H, CH_3CO_2), 2.75 (t, *J*=6.6 Hz, 2H, $ArCH_2CH_2$). ^{2}H NMR ($CHCl_3$): δ 1.94 (s, 3D, $ArCD_3$), 4.36 (s, 2D, $ArCD_2Br$). $C_{31}H_{46}D_5BrO_3$ (490.7): calcd C 66.89, H 10.14, Br 14.35, found C 66.65, H 10.35, Br 14.48.

4.1.3. 6-*O*-Acetyl-5-(5-$^2H_1$)-formyl-(7-$^2H_3$)-(2*R*,4′*R*,8′*R*)-γ-tocopherol (8). To a solution of **7** (1.34 g, 2.41 mmol) in dry acetonitrile (20 mL), NMMO (1.14 g, 9.7 mmol, 4 equiv) was added. After stirring for 5 h at rt, the solvent was evaporated and the crude residue purified by column chromatography (Hex/EtOAc 15:1), affording **8** (1.17 g, 92% yield) as yellow dense oil.

^{1}H NMR, ($CDCl_3$/TMS): δ 0.95–1.6 (m, 36H, C(2a)H_3 and $C_{16}H_{33}$ chain), 1.7 (m, 2H, $ArCH_2CH_2$), 2.1 (s, 3H, $ArCH_3$), 2.3 (s, 3H, CH_3CO), 3.05 (t, *J*=6.2 Hz, 2H, $ArCH_2CH_2$). ^{2}H NMR ($CHCl_3$): δ 2.01 (s, 3D, $ArCD_3$), 10.25 (s, 1D, ArCDO). ^{13}C NMR ($CDCl_3$): δ 12.9, 13.01 (m), 19.6, 19.7, 20.4, 20.9, 22.5, 22.6, 23.8, 24.7, 27.9, 30.5, 30.7, 32.6, 32.7, 37.2, 37.3, 37.4, 39.3, 39.9, 75.8, 120.5, 122.8, 128.2, 136.6, 145.0, 149.8, 169.6, 189.9 (t, *J*=105 Hz). APCI-MS (in MeOH), *m*/*z* (amu): positive ion mode, 491.4 $[M+H]^+$, 508.6 $[M+NH_4]^+$. $C_{31}H_{46}D_4O_4$ (490.7): calcd C 75.87, H 11.09, found C 75.41, H 11.27.

4.1.4. 6-*O*-Acetyl-(7-$^2H_3$)-(2*R*,4′*R*,8′*R*)-γ-tocopherol-5-carboxylic acid (9). To a solution of **8** (490 mg, 1 mmol) in 1,4-dioxane (20 mL), NH_2SO_3H (160 mg, 1.6 mmol, 1.6 equiv) and water (7 mL) were added. After stirring for 20 min, $NaClO_2$ (180 mg, 1.4 mmol, 1.4 equiv) and water (5 mL) were added. After stirring for further 30 min, Na_2SO_3 (150 mg) was added to destroy excess of $NaClO_2$ and HOCl formed during the reaction. Water was then added and the aqueous phase was extracted with Et_2O (3× 50 mL). The combined organic extracts were subsequently washed to neutrality with water, dried over Na_2SO_4 and concentrated to dryness, giving pure **9** (485 mg, 96% yield) without further purification, as yellow oil.

^{1}H NMR, ($CDCl_3$/TMS): δ 0.95–1.6 (m, 36H, C(2a)H_3 and $C_{16}H_{33}$ chain), 1.7 (m, 2H, $ArCH_2CH_2$), 2.1 (s, 3H, $ArCH_3$), 2.28 (s, 3H, CH_3CO), 2.9 (t, *J*=6.2 Hz, 2H, $ArCH_2CH_2$), 9.2 (bs, 1H, CO_2H). ^{2}H NMR ($CHCl_3$): δ 2.01 (s, $ArCD_3$). ^{13}C NMR ($CDCl_3$): δ 12.8, 12.9 (m), 19.6, 19.7, 20.6, 21.1, 22.5, 22.7, 24, 24.4, 24.7, 27.9, 30.6, 32.7, 37.2, 37.3, 39.3, 40.1, 76, 118.3, 121.5, 128.9, 130.3, 140.5, 150, 170.1, 171.9. APCI-MS (in MeOH), *m*/*z* (amu): negative ion mode, 504.5 $[M-H]^-$. $C_{31}H_{47}D_3O_5$ (505.7): calcd C 73.62, H 10.56, found C 73.25, H 10.85.

4.1.5. (7-$^2H_3$)-(2*R*,4′*R*,8′*R*)-γ-tocopherol-5-carboxylic acid (10). To **9** (490 mg, 0.97 mmol) was added a solution of KOH in MeOH (10 mL, 2 M). The solution was heated at 50 °C for 2 h, then MeOH was evaporated and water added. The aqueous phase was extracted with Et_2O (3×50 mL). The combined organic extracts were subsequently washed to neutrality with water, dried over Na_2SO_4 and concentrated to dryness, giving pure **10** (420 mg, 93% yield) without further purification, as yellow semi-solid.

^{1}H NMR, ($CDCl_3$/TMS): δ 0.95–1.6 (m, 36H, C(2a)H_3 and $C_{16}H_{33}$ chain), 1.7 (m, 2H, $ArCH_2CH_2$), 2.1 (s, 3H, $ArCH_3$), 3.05 (t, *J*=6.2 Hz, 2H, $ArCH_2CH_2$), 9.8 (bs, 1H, ArOH), 11.1 (bs, 1H, CO_2H). ^{2}H NMR ($CHCl_3$): δ 2.01 (s, $ArCD_3$). ^{13}C NMR ($CDCl_3$): δ 11.8, 13.0 (m), 19.6, 19.7, 20.9, 22.6, 22.7, 23.6, 24, 24.4, 24.8, 27.9, 30.9, 31.4, 32.6, 32.8, 37.2, 37.3, 37.4, 39.3, 39.6, 74.6, 106.7, 119, 124, 136.1, 144.8, 155.9, 176.8. APCI-MS (in MeOH), *m*/*z* (amu): negative ion mode, 462.6 $[M-H]^-$. $C_{29}H_{45}D_3O_4$ (463.7): calcd C 75.11, H 11.08, found C 75.01, H 11.21.

4.1.6. (7-$^2H_3$)-(2*R*,4′*R*,8′*R*)-γ-tocopherol (11). Compound **10** (300 mg, 0.65 mmol) was heated at 170 °C for 3 h. After

purification of the crude residue by column chromatography (Hex/EtOAc 10:1), **11** (248 mg, 91% yield) was obtained as brown dense oil.

^{1}H NMR, ($CDCl_3$/TMS): δ 0.8–1.6 (m, 36H, C(2a)H_3 and $C_{16}H_{33}$ chain), 1.7 (m, 2H, ArCH$_2$CH_2), 2.1 (s, 3H, ArCH_3), 2.7 (t, J=6.2 Hz, 2H, ArCH_2CH$_2$), 4.7 (bs, 1H, ArOH), 6.4 (s, 1H, ArH). ^{2}H NMR ($CHCl_3$): δ 2.15 (s, ArCD_3). ^{13}C NMR ($CDCl_3$): δ 11.9 (m), 19.7, 19.8, 21.1, 22.3, 22.6, 22.7, 24, 24.4, 24.8, 27.9, 31.4, 32.7, 32.8, 37.3, 37.4, 37.45, 37.6, 39.4, 40, 40.1, 75.5, 112.2, 118.3, 121.7, 125.8, 145.7, 146.2. APCI-MS (in MeOH), *m/z* (amu): positive ion mode, 419.3 $[M]^+$. $C_{28}H_{45}D_3O_2$ (419.4): calcd C 80.13, H 12.25, found C 80.32, H 12.40. 97.6% deuteration by GC-MS (d_3 species; the remaining 2.4% accounts for d_2, d_1 and d_0 species). HPLC: 100% 2*R*-isomers (t(*R*) 16.1 min), no trace of 2*S*-isomers (t(*R*) 17.1 min) could be detected. It can be assumed that no erosion of stereochemistry of the aliphatic side chain took place under the reaction conditions.

4.1.7. (2*R*,4′*R*,8′*R*)-γ-tocopherol (14). Acid **13** was prepared as described for its labeled counterpart **10** starting from commercially available *R*,*R*,*R*-α-tocopherol. Thermal decarboxylation of **13** was accomplished (91% yield) as described for **11**. Physical and spectroscopic data were consistent with reported data for (2*R*,4′*R*,8′*R*)-γ-tocopherol.[22]

Acknowledgements

We thank Professor Th. Rosenau (University of Natural Resources and Applied Life Sciences, Wien) for providing us with an unpublished procedure from his laboratory, and Dr. G. Schiefer (DSM Nutritional Products) for HPLC analyses. The University of Pisa and Centro di Eccellenza AMBISEN are also acknowledged for financial support.

References and notes

1. IUPAC-IUB *Eur. J. Biochem.* **1982**, *123*, 473–475.
2. Bunyan, J.; McHale, D.; Green, J.; Marcinkiewicz, S. *Br. J. Nutr.* **1961**, *15*, 253–257.
3. Weiser, H.; Vecchi, M.; Schlachter, M. *Int. J. Vit. Nutr. Res.* **1986**, *56*, 45–56.
4. Weiser, H.; Riss, G.; Kormann, A. W. *J. Nutr.* **1996**, *126*, 2539–2549.
5. Jiang, Q.; Christen, S.; Shigenaga, M. K.; Ames, B. N. *Am. J. Clin. Nutr.* **2001**, *74*, 714–722.
6. Hensley, K.; Benaksas, E. J.; Bolli, R.; Comp, P.; Grammas, P.; Hamdheydari, L.; Mou, S.; Pye, Q. N.; Stoddard, M. F.; Wallis, G.; Williamson, K. S.; West, M.; Wechter, W. J.; Floyd, R. A. *Free Radic. Biol. Med.* **2004**, *36*, 1–15.
7. Kayden, H. J.; Traber, M. G. *J. Lipid Res.* **1993**, *34*, 343–358.
8. Lauridsen, C.; Leonard, S. W.; Griffin, D. A.; Liebler, D. C.; McClure, T. D.; Traber, M. G. *Anal. Biochem.* **2001**, *289*, 89–95.
9. Mottier, P.; Gremaud, E.; Guy, P. A.; Turesky, R. J. *Anal. Biochem.* **2002**, *301*, 128–135.
10. Andreoli, R.; Manini, P.; Poli, D.; Bergamaschi, E.; Mutti, A.; Niessen, W. M. A. *Anal. Biochem.* **2004**, *378*, 987–994.
11. Stocker, A.; Rüttimann, A.; Woggon, W.-D. *Helv. Chim. Acta* **1993**, *76*, 1729–1738.
12. Stocker, A.; Netscher, Th.; Rüttimann, A.; Müller, R. K.; Schneider, H.; Todaro, L. J.; Derungs, G.; Woggon, W.-D. *Helv. Chim. Acta* **1994**, *77*, 1721–1737.
13. Hughes, L.; Slaby, M.; Burton, G. W.; Ingold, K. U. *J. Labelled Compd Radiopharm.* **1990**, *28*, 1049–1057.
14. Wechter, W. J.; Kantoci, D.; Murray, E. D. J.; D'Amico, D. C.; Jung, M. E.; Wang, W. H. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 6002–6007.
15. Rosenau, Th.; Habicher, W. D. *Synlett* **1997**, *2*, 208–209.
16. Ingold, K. U.; Hughes, L.; Slaby, M.; Burton, G. W. *J. Labelled Compd. Radiopharm.* **1986**, *24*, 817–831.
17. Rosenau, Th.; Habicher, W. D. *Tetrahedron* **1995**, *51*, 7919–7926.
18. Cohen, T.; Schambach, R. A. *J. Am. Chem. Soc.* **1970**, *92*, 3189–3190.
19. Segura, P.; Bunnett, J. F.; Villanova, L. *J. Org. Chem.* **1985**, *50*, 1041–1045.
20. Huang, H. H.; Long, F. A. *J. Am. Chem. Soc.* **1969**, *91*, 2872–2875.
21. Kaeding, W. W. *J. Org. Chem.* **1964**, *29*, 2556–2559.
22. See references citation in: Baldenius, K. U.; von dem Bussche-Hünnefeld, L.; Hilgemann, E.; Hoppe, P.; Stürmer, R. In *Ullmann's Encyclopedia of Industrial Chemistry*, VCH Verlagsgesellschaft: Weinheim, 1996; Vol. A27, pp 478–488.

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Tetrahedron 61 (2005) 819–829

Charge density analysis of some processes involving intramolecular hydrogen transfer

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Received 10 September 2004; revised 10 November 2004; accepted 17 November 2004

Available online 8 December 2004

Abstract—The variations in topology of the electron density along the reaction paths of the keto–enol tautomerism of acetaldehyde, the pinacol rearrangement of protonated 1,2-ethanediol, and the unimolecular decomposition of methanediol, were studied as examples of hydrogen transfer between, respectively, C···O, C···C, and O···O atoms. The evolution of atomic charges, determined using two different atomic partitionings (AIM and Hirshfeld), indicates that the main electronic charge transfer in keto–enol tautomerism takes place between the migrating hydrogen and the carbon of the carbonyl group. The topology of the electron density demonstrates that a hydrogen-bridge structure is never formed along the reaction path of the pinacol rearrangement. The methanediol decomposition follows a concerted mechanism with very small variations in the atomic charges.

1. Introduction

Many important organic chemical reactions take place through the migration of one H atom. In this paper we study three different processes of H transfer in oxygenated compounds: between C and O atoms in the keto–enol tautomerism of acetaldehyde, between two C atoms in the pinacol rearrangement of protonated 1,2-ethanediol, and between two O atoms in the unimolecular decomposition of methanediol.

Although many theoretical studies were performed on these reactions, to our knowledge none of them included an electron density topological analysis or the computation of atomic charges to confirm the intermediate structures proposed or to describe the evolution of the charge distribution during the process. Therefore, we have analysed the formation and breaking of bonds during these processes by studying the topology of the charge density along the corresponding intrinsic reaction coordinates, IRC. Charge transfers along the IRC were studied using atomic charges calculated with two different methods: Atoms in Molecules Theory[1,2] (AIM) and the Hirshfeld scheme.[3]

Keto–enol tautomerism plays a fundamental role in several organic reactions.[4] Therefore, the mechanism of this process was extensively studied with diverse theoretical and experimental methods (see Refs. 4–6 for a review). Recently, Andrés et al. concluded, using a set of 13 theoretical levels, that the transition state, TS, for the keto–enol tautomerism of acetaldehyde could be always described (on the basis of its geometry) as a four-membered ring.[6] Using Pauling bond orders, they also concluded that the intramolecular migration of H is more advanced than the hybridization changes on heavy atoms. Nevertheless, the proposed cyclic structure was not confirmed by any criterion based on the electron charge density.

The pinacol rearrangement is another of the most studied reactions in organic chemistry. Several mechanisms have been proposed for it along the XXth century. Thus, Whitmore proposed this process takes place through several elementary steps that include the formation of an intermediate β-hydroxy carbocation by elimination of one water molecule in protonated 1,2-ethanediol.[7] W. B. Smith et al.[8,9] proposed a bridged structure for this carbocation based upon isotopic effects of the pinacol rearrangement of 1,2-dimethylene glycol and 1,1,2-trimethylene glycol. However, the mechanism proposed for the pincacol rearrangement of 1,1,2-triphenylethylene glycol included a non-cyclic carbocation.[10] Theoretical studies in the 1990s by Nakamura and Osamura[11–13] concluded that the pinacol rearrangement is more favourable through a concerted mechanism, with no intermediate cation. In the late 1990s

Keywords: Keto–enol tautomerism; Pinacol rearrangement; Methanediol decomposition; AIM theory; Hirshfeld partitioning.
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0040–4020/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2004.11.044

B. Smith[14] also rejected this intermediate form, strengthening the hypothesis of concerted mechanism.

Kent et al.[15] studied the unimolecular decomposition of methanediol to obtain water and formaldehyde in gas phase. They calculated the stability of the reagent and the products, the structure of the TS and the energy barrier for the process, making use of 12 computational levels. The study assumes a concerted mechanism and proposes a cyclic structure for the TS, with the migrating H bonded to both oxygens.

2. Computational technique

Minimum energy paths were determined for the three processes using the Gaussian 03 program.[16] The topology of the charge density was studied for several geometries (including reagents, products, and TS) along the corresponding IRC path: nine geometries for the keto–enol tautomerism of acetaldehyde, 14 for the pinacol rearrangement of protonated 1,2-ethanediol, and 15 for the unimolecular decomposition of methanediol. Atomic

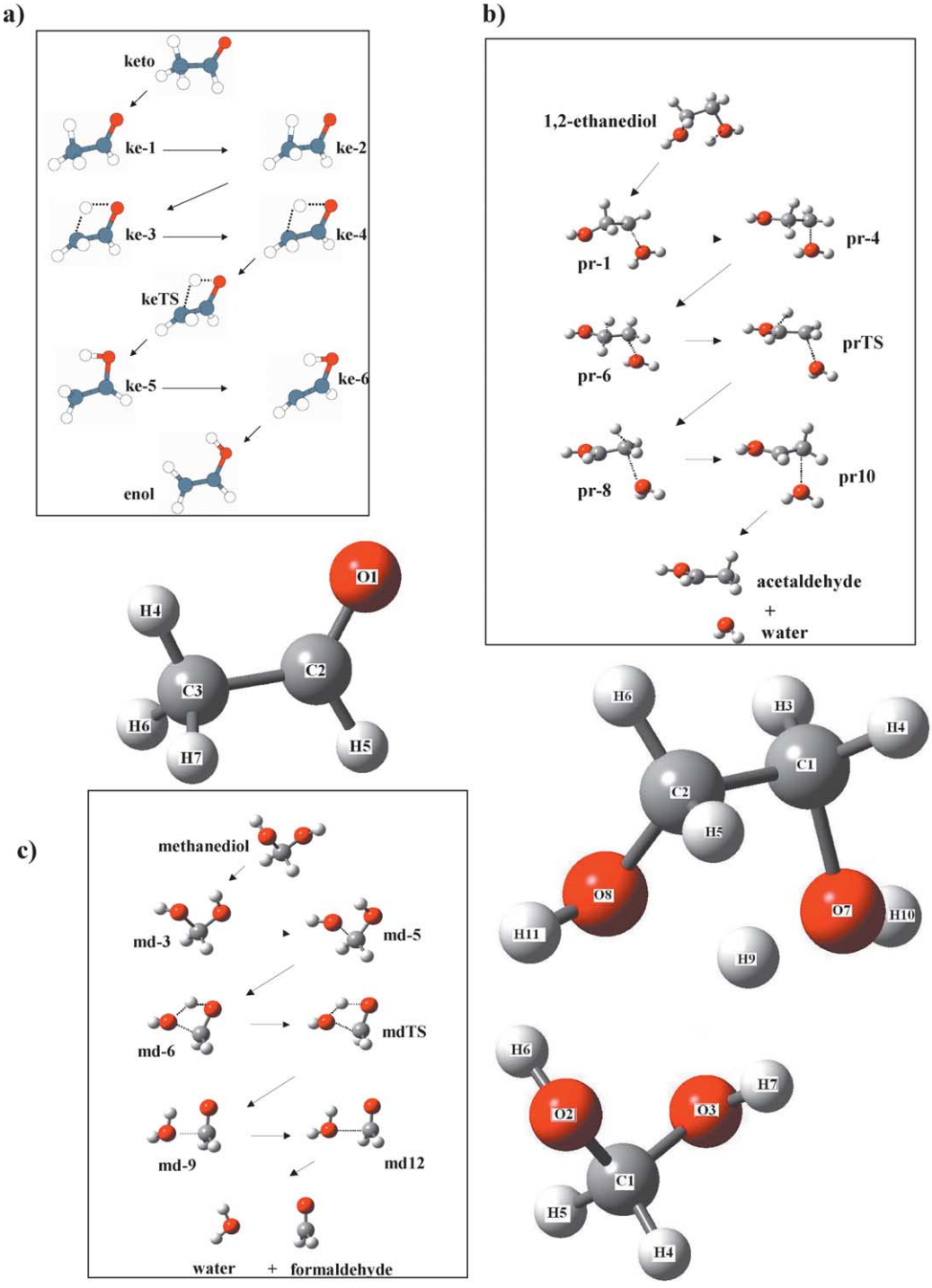

Figure 1. Plot of diverse geometries along the minimal energy path for the processes studied and atomic numbering employed: (a) keto–enol tautomerism; (b) pinacol rearrangement; (c) unimolecular decomposition of methanediol. TS are shown detailing atom numbering.

charges were obtained for every geometry according to the AIM[1,2] and Hirshfeld schemes[3] from B3LYP/6-311++G** electron densities. Figure 1 shows atom numbering and nomenclature of the intermediate geometries. For the keto–enol tautomerism the whole set of intermediate geometries studied along the IRC is shown, whereas only selected geometries are shown for pinacol rearrangement and methanediol decomposition.

According to the AIM theory an atom consists of a nucleus, which acts as an attractor for the trajectories of the gradient of the charge density vector field, $\nabla\rho(r)$, and its associated atomic basin, Ω, throughout which these trajectories spread. An atom, A, is delimited by zero flux surfaces for $\nabla\rho(r)$ and an iso-contour where the electron density vanishes. The atomic charge, q_A, is obtained by Eq. 1, where Z_A is the atomic number, through the integration of the electronic density within the atomic basin using the AIMPAC program.[17] Plots of charge density (indicating bond and ring critical points, BCP and RCP, respectively) at the TS

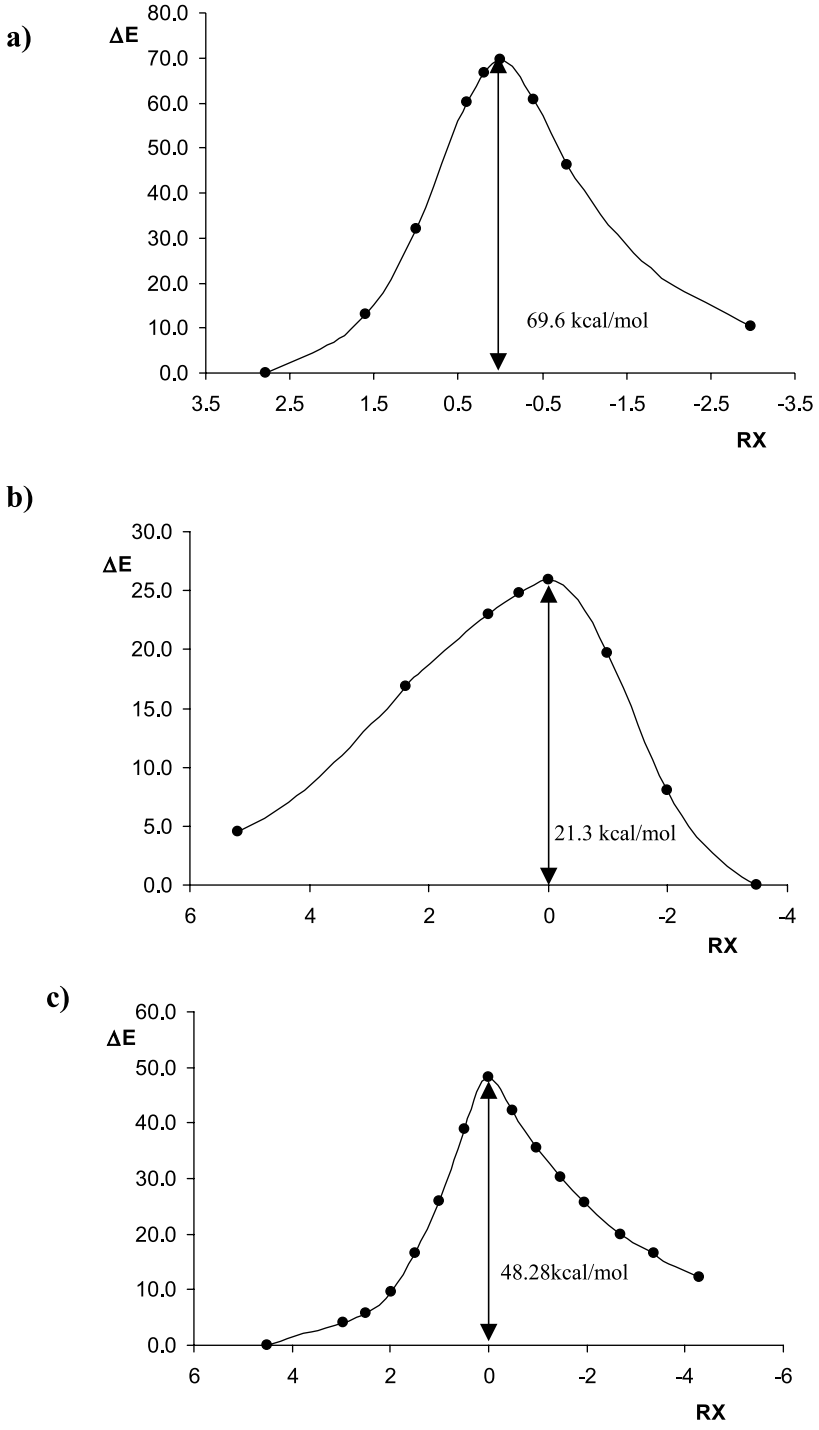

Figure 2. Variation of the molecular energy along the IRC path of: (a) the keto–enol tautomerism of acetaldehyde, (b) the pinacol rearrangement of protonated 1,2-ethanediol, and (c) the unimolecular decomposition of methanediol. Filled points correspond to geometries given in Figure 1.

were obtained with program MORPHY.[18,19]

$$q_A = Z_A - \int_{\Omega} \rho(\mathbf{r}) d\mathbf{r} \quad (1)$$

The Hirshfeld scheme[3,20–22] calculates the atomic charge making use (Eq. 2) of the atomic deformation density, $\delta\rho_A(\mathbf{r})$, which is related to the molecular deformation density, $\Delta\rho(\mathbf{r})$, by Eq. 3. $\Delta\rho(\mathbf{r})$ is defined by Eq. 4, where ρ^{mol} is the molecular electron density and ρ^{pro} is the promolecule electron density given by Eq. 5, where ρ^{at} represents the charge density of the isolated atom A placed at the same position occupied by its nucleus in the molecule. Finally, the function $w_A(\mathbf{r})$ in Eq. 3 gives the relative contribution of the atom A to the promolecule in the point $\mathbf{r}$, and is expressed by Eq. 6

$$q_A = -\int \delta\rho_A(\mathbf{r}) d\mathbf{r} \quad (2)$$

$$\delta\rho_A(\mathbf{r}) = w_A(\mathbf{r})\Delta\rho(\mathbf{r}) \quad (3)$$

$$\Delta\rho(\mathbf{r}) = \rho^{mol}(\mathbf{r}) - \rho^{pro}(\mathbf{r}) \quad (4)$$

$$\rho^{pro}(\mathbf{r}) = \Sigma\rho_A^{at}(\mathbf{r}) \quad (5)$$

$$w_A(\mathbf{r}) = \rho_A^{at}(\mathbf{r})/\rho^{pro}(\mathbf{r}) \quad (6)$$

The absolute values of the charges are very different when obtained with both methods. AIM charges are usually higher than those obtained with the Hirshfeld scheme, particularly if they correspond to electronegative atoms or atoms bonded to them. However, similar trends in calculated values have previously been found and analysed[22,23] and they do not impede common conclusions about the evolution of charges along a certain process.

3. Results and discussion

3.1. Keto–enol tautomerism

Figure 2a displays the evolution of the molecular energy during the keto–enol tautomerism. As is shown, the energy difference between the keton and the enol form, ΔE_{e-k}, is only 10.4 kcal mol^{-1} (11.1 kcal mol^{-1} after ZPVE corrections) but the intermediate steps show very high energies arising 69.6 kcal mol^{-1} above the reagent for the TS (73.0 kcal mol^{-1} after ZPVE corrections). Values previously obtained are in the range between 8.5 kcal mol^{-1} (HF/3-21G) and 14.3 kcal mol^{-1} (MP2/6-31G**) for ΔE_{e-k}, and between 71.0 kcal mol^{-1} (MP2/6-311++G(3df,2p)) and 93.9 kcal mol^{-1} (HF/6-31G) for the energy barrier, $\Delta E_{e-k}^{\neq}$.[6] The results reported here for ΔE_{e-k} are in good agreement with those obtained at the highest computational levels hitherto employed: 10.6 kcal mol^{-1} at the MP2/6-311++G(3df,2p) level,[6] 11.2 kcal mol^{-1} (G1),[24] and 11.0 kcal mol^{-1} (G2),[25] and with the experimental ΔE_{e-k} value of (10±2) kcal mol^{-1}.[26] The B3LYP/6-311++G** value for $\Delta E_{e-k}^{\neq}$ also compares satisfactorily with the experimental estimation (75 kcal mol^{-1}),[27] and with most of the values obtained at high computational levels: 71.0 at the MP2/6-311++G(3df,2p) level,[6] 79.1 at G1,[24] 76.1 (MP2(full)/6-31G*),[28] or 71.4 at the MP4(FC,SDTQ)/6-311++G**//MP2(Full)/6-31G*.[28] One $\Delta E_{e-k}^{\neq}$ value computed at the G2 level was also published (56.4 kcal mol^{-1}),[25] but the whole set of $\Delta E_{e-k}^{\neq}$ values reported in that work are too low compared to any other computational values, even with regard to those obtained at the same level by other authors (i.e., 56.0 for G1, and 55.8 for MP2(full)/6-31G*), so we think it is affected by some kind of mistake.

The variations in the main geometrical parameters are given in Table 1. The geometry of the TS exhibits a 'bridge' structure. The ring critical point obtained in the AIM study (Fig. 3) confirms the presence of a four-membered ring involving C_2, C_3, H_4, and O_1. Bond lengths for C_3–H_4 and H_4–O_1 indicate that H_4 is placed in the TS structure in an intermediate position between those corresponding to the keto and enol forms. In fact, C_3–H_4 distance is closer to that

Table 1. Variations experienced by the main geometrical features along the keto–enol tautomerisation[a]

	Keto[b]	KeTS	enol
O1–C2	1.206	7.3	15.7
C2–C3	1.504	−9.3	−17.3
H4–C3	1.090	41.6	140.3
H4–O1	2.583	−128.5	−161.8
C3–C2–O1	124.8	−14.2	2.2
H4–C3–C2	111.0	−44.2	−61.5
H4–O1–C2	55.8	21.4	53.9
O1–H4–C3	70.3	33.7	3.6
H5–C2–C3–H4	180.0	11.7	0.0
H4–O1–C2–H5	180.0	−12.3	0.0
O1–C2–C3–H4	0.0	9.1	0.0

[a] Bond distances in Å and angles in degrees. Bond distances variations are mutiplied by 10^2. Values are relative to those of the keto form.
[b] Absolute values.

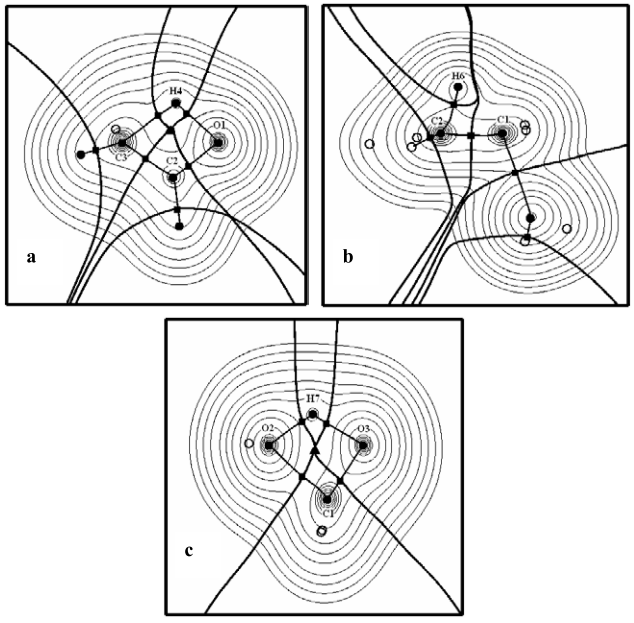

Figure 3. Plots of the B3LYP/6-311++G** electron charge density for the TS of (a) the keto–enol tautomerismn of ethanal, (b) the pinacol rearrangement of protonated 1,2-ethanediol, (c) the unimolecular decomposition of methanediol. Squares denote BCPs, triangles RCPs, open face circles are projections of nuclei and bold face ones correspond to in-plane nuclei.

in the enol, whereas H_4–O_1 distance is closer to that in the keto form. A similar behaviour is found for O_1–C_2 and C_2–C_3 bonds, which exhibit intermediate values between standard O=C and O–C distances for the first bond and between standard C–C and C=C distances for the second one. The C_3–C_2–O_1 angle decreases initially, bringing H_4 and O_1 closer together. Subsequently, its value remains constant (typical of C–C(sp^3)–O angles) until the formation of the H_4–O_1 bond in the last part of the concerted process (by then it recovers a value typical of a C–C(sp^2)–O). In spite of the significant variations in C_3–C_2–O_1, small changes are observed in the dihedral angles involving H_5, C_2, C_3, H_4, and O_1, which are practically kept in one plane during the tautomerism. H_6 and H_7 only move into that plane at the last points of the reaction (after ke-6 point, Fig. 1).

In spite of the movement of the hydrogen atoms H_6 and H_7, both the AIM and stockholder charges for these atoms remain practically constant during the reaction (maximum variations do not reach 0.04 a.u.). On the contrary, the evolution of AIM and stockholder charges for O_1, H_4, C_2, and C_3 atoms is significant (Fig. 4). According to the AIM results there is a large increase in positive charge of H_4 (0.52 a.u.), that is almost the same as the decrease in the positive charge of C_2. Both variations display a nearly linear behaviour along the IRC path and represent the most important charge transfer during the reaction. The variations of charge are negligible for O_1 (less than 0.05 a.u. along the process and less than 0.01 a.u. between the keto and the enol forms). The atomic charge of C_3 is almost the same at the beginning as at the end of the reaction but it experiences noticeable changes along the process and achieves a

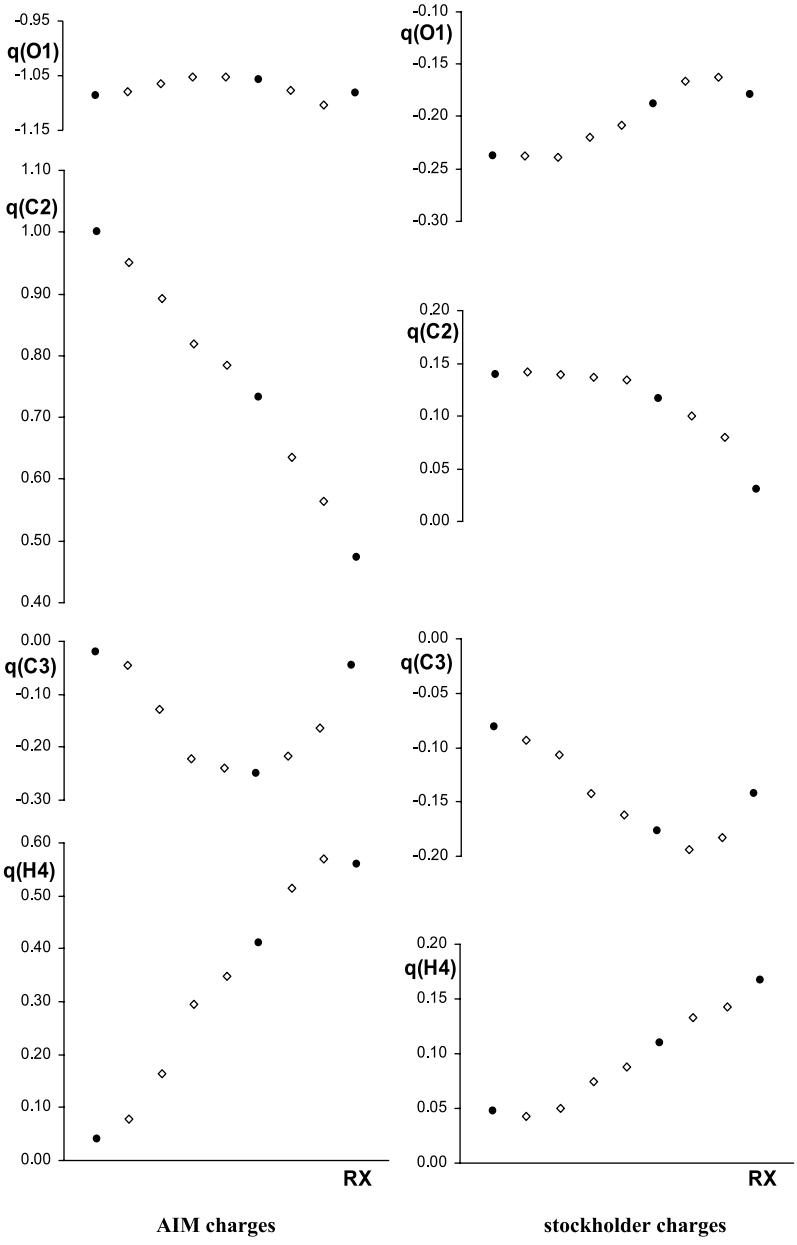

Figure 4. Variation of AIM and stockholder atomic charges along the IRC path for the keto–enol tautomerism of ethanal. All charges are in a.u. Notice that the scale is doubled for stockholder charges.

minimum value at the TS. This variation is due to the small difference between the electron population lost by H_4 and that gained by C_2 (always less than 0.11 a.u.) during the central part of the reaction. C_3 absorbs most of this electron population, but it is also spread over the remaining hydrogens. Thus, we can infer that the migration of H_4 from C_3 to O_1 is accompanied by a progressive transfer of electron charge from H_4 to C_2 which represents in the whole process more than half of the electron population of H_4 in acetaldehyde. This transfer takes place through O_1 that receives from H_4 approximately the same charge it donates to C_2.

The stockholder charges of H_4 and C_2 exhibit again similar absolute variations with opposite signs (Fig. 4). However, they are smaller than those found with the AIM method and the variations experienced by O_1 and C_3 charges between the initial and final geometries are not negligible with regard to them. Therefore, according to the Hirshfeld partitioning both O_1 and H_4 atoms transfer charge to the carbon atoms. It has to be mentioned that both AIM and stockholder descriptions differ from the NBO interpretation of the process as a delocalization of one of the O_1 lone pairs into the C_3–H_4 antibonding orbital.[28]

3.2. Pinacol rearrangement in protonated 1,2-ethanediol

Figure 2b shows the variation of the molecular energy along the IRC of the pinacol rearrangement of protonated 1,2-ethanediol to yield protonated ethanal and water. The energy difference between reagent and products, ΔE, is small (4.5 kcal mol^{-1}, 3.4 kcal mol^{-1} after ZPVE corrections) and the energy barrier, $\Delta E^{\neq}$, amounts 21.3 kcal mol^{-1} (16.5 kcal mol^{-1} after ZPVE corrections). Both values are somewhat smaller than those previously obtained at other computational levels: B3LYP/6-31G* ($\Delta E=9.4$ kcal mol^{-1}, $\Delta E^{\neq}=26.0$ kcal mol^{-1}),[14] MP2/6-31+G**//B3LYP/6-31G* ($\Delta E^{\neq}=25.7$ kcal mol^{-1}),[14] and MP2/6-31G*//HF/6-31G ($\Delta E^{\neq}=26.3$ kcal mol^{-1}).[13] Nevertheless, none of them can be considered more reliable.

The variations of the geometry during the reaction (Table 2) allow to infer the progressive formation of a water molecule since the beginning of the concerted mechanism till the end of the process. Thus, the C_1–O_7 distance increases continuously along the process. We also observe that the summation of the atomic charges for the water molecule is only zero at the end of the reaction (Fig. 5), both with AIM and stockholder charges. Both facts confirm that the process follows a concerted mechanism where the water molecule cannot be considered independent until the final point of the reaction. The wide gap observed for the stockholder charge of the water molecule at the end of the rearrangement is due to the abrupt elimination of the effects of the rest of the molecule when the water molecule is computed isolated. This effect is much smoother for AIM charges.

The geometry of the TS corresponds to a bridged structure involving the C_2, H_6, and C_1 atoms. Although H_6 adopts an intermediate position, no RCP is detected in the TS. In fact there is one bond path connecting C_2 and H_6 in the TS, but there is no bond path connecting C_1 and H_6 (Fig. 3). We have analysed the charge densities for several points of the minimum energy path that are very close to the formation of the C_1–H_6 bond and we can conclude that a ring is never formed. So, when the bond path connecting C_1 and H_6 appears, the one between C_2 and H_6 disappears. Carbon atoms exhibit sp^2 character in geometries around the TS (and the TS geometry itself), as can be inferred from C–C bond (around 1.40 Å) and H_3–C_1–H_4, H_5–C_2–O_8, and C_1–C_2–O_8 angles (all near 120°).

The variations experienced by the AIM and stockholder atomic charges along this reaction path display very similar trends if we exclude C_1 (Fig. 5). The variations for q(C1) from the reagents to the TS formation computed with AIM are opposite to those obtained with the Hirshfeld partitioning. Thus, AIM charge decreases whereas the stockholder one increases by a similar amount (Fig. 5), like it was found for the protonation of alkanols in a recent study.[23] Both methods indicate a continuous decrease of q(C1) from the TS to the products. The evolution observed for q(O8) (data not shown) from the TS to the products is also different depending on the partitioning (increases only 0.02 a.u. according to the AIM results but 0.145 a.u. according to the Hirshfeld ones).

The main charge variation in the process corresponds to that due to the formation of the water molecule from the $[OH_2]^+$ unit formed by O_7, H_9, and H_{10}. 0.25 or 0.45 a.u. have to be transferred from the rest of the molecule to this unit according to AIM and Hirshfeld partitionings, respectively, in order to form a neutral water molecule. Looking at the global process the main source for this electron transfer is always C_2. Nevertheless, the variations experienced by the atomic charges along the reaction indicate that two different stages can be considered for the electron charge reorganization: (a) most of the charge is transferred to the water molecule during the formation of the TS. This charge comes from the rest of the molecule if we look at stockholder charges, whereas C_1 is excluded as a source for electron charge and is another important acceptor according to the AIM charges; (b) less electron charge is transferred to the water molecule and a reorganization of the charge of the protonated acetaldehyde happens during the formation of the products from the TS. C_2 looses a lot of electron charge in this stage (Fig. 5) whereas H_3 and H_4 recover the charge lost during the first stage. The other atoms experience slight charge variations, if we exclude O_8 that is an important electron charge donor according to the Hirshfeld partitioning but not to AIM.

Another important trend of the process (common to both charge analyses) is the nearly constant charge shown by the atom transferred between both carbons (Fig. 5).

3.3. Unimolecular decomposition of methanediol

The energy profile of the unimolecular decomposition of methanediol (Fig. 2c) indicates the production of water and formaldehyde from this molecule in a concerted mechanism, as was assumed in a previous study of this process.[15] The difference of energy between the reagent and the products, ΔE_d, is 12.2 kcal mol^{-1} (8.8 kcal mol^{-1} after ZPVE corrections) and the barrier for the process, $\Delta E_d^{\neq}$, rises above 48 kcal mol^{-1} (43.6 kcal mol^{-1} after ZPVE

Table 2. Variations experienced by the main geometrical features along the pinacol rearrangement of protonated 1,2 ethanediol[a]

	R[b]	pr-1	pr-2	pr-3	pr-4	pr-5	prTS	pr-6	pr-7	Pr-8	pr-9	pr10	pr11	Products
C1–C2	1.525	−4.3	−5.3	−6.7	−8.4	−10.3	−12.1	−13.2	−13.3	−12.6	−11.2	−9.7	−8.7	−8.2
C1–H6	2.150	−17.3	−20.3	−25.6	−33.4	−45.2	−61.2	−77.2	−88.7	−96.4	−100.6	−102.7	−103.6	−104.0
C1–O7	1.510	28.8	36.0	44.4	52.4	59.7	66.5	72.7	78.1	83.6	89.6	96.5	104.2	
C2–H6	1.090	2.6	3.1	4.1	5.6	8.5	14.2	23.9	37.0	52.4	67.9	80.9	89.1	
C2–O8	1.430	−3.4	−3.8	−4.5	−5.4	−6.7	−8.5	−10.4	−12.2	−13.9	−15.1	−15.7	−15.9	−16.0
H9–O7	1.010	−3.7	−3.8	−4.0	−4.1	−4.2	−4.3	−4.4	−4.4	−4.5	−4.5	−4.5	−4.5	−4.5
H10–O7	0.970	0.3	0.2	0.0	−0.1	−0.2	−0.3	−0.4	−0.5	−0.5	−0.4	−0.4	−0.4	−0.5
C2–C1–H6	31.1	2.9	3.9	5.5	8.1	12.0	18.5	26.3	35.5	45.7	55.8	64.4	70.1	72.8
C1–C2–H6	109.6	−11.4	−13.2	−16.4	−21.2	−28.5	−38.6	−48.8	−57.1	−63.9	−69.3	−73.5	−76.1	
H3–C1–H4	112.5	3.0	3.8	4.6	5.4	6.2	7.2	7.9	8.1	7.3	5.5	3.0	1.2	0.3
C1–C2–O8	102.9	9.4	10.2	11.2	12.5	14.0	15.4	16.5	17.1	17.4	17.4	17.3	17.3	17.4
H5–C2–O8	110.9	3.5	3.9	4.6	5.5	6.7	7.9	8.6	8.9	8.7	8.2	7.6	7.3	7.2
H3–C1–C2–H6	314.7	−34.3	−35.5	−36.6	−37.3	−37.8	−39.7	−43.4	−48.6	−54.7	−61.4	−67.2	−70.8	
H4–C1–C2–H6	85.2	−26.5	−24.4	−21.4	−18.1	−14.1	−8.9	−3.0	3.4	10.4	17.6	23.7	27.3	
H6–C1–C2–H5	238.1	9.8	11.8	13.7	16.4	20.4	26.0	31.9	37.2	41.4	43.5	42.9	40.4	38.5
H6–C1–C2–O8	119.3	−0.4	−1.7	−3.1	−5.6	−8.8	−13.0	−17.0	−20.2	−23.1	−25.4	−26.6	−27.0	−27.0
O7–C1–C2–H6	200.2	−32.5	−31.8	−30.4	−28.6	−26.5	−24.7	−23.4	−22.9	−22.6	−22.5	−22.4	−22.7	
O7–C1–C2–O8	319.5	−33.7	−33.6	−33.6	−34.1	−35.4	−37.7	−40.4	−43.1	−45.7	−47.8	−49.1	−49.6	
H6–C2–O8–H11	287.8	0.2	−1.4	−4.4	−8.9	−15.5	−24.1	−32.4	−38.9	−44.4	−49.6	−55.6	−61.8	

[a] Bond distances in Å and angles in degrees. Bond distances variations are mutiplied by 10^2. Values are relative to those of protonated 1,2 ethanediol.
[b] Absolute values for protonated 1,2-ethanediol.

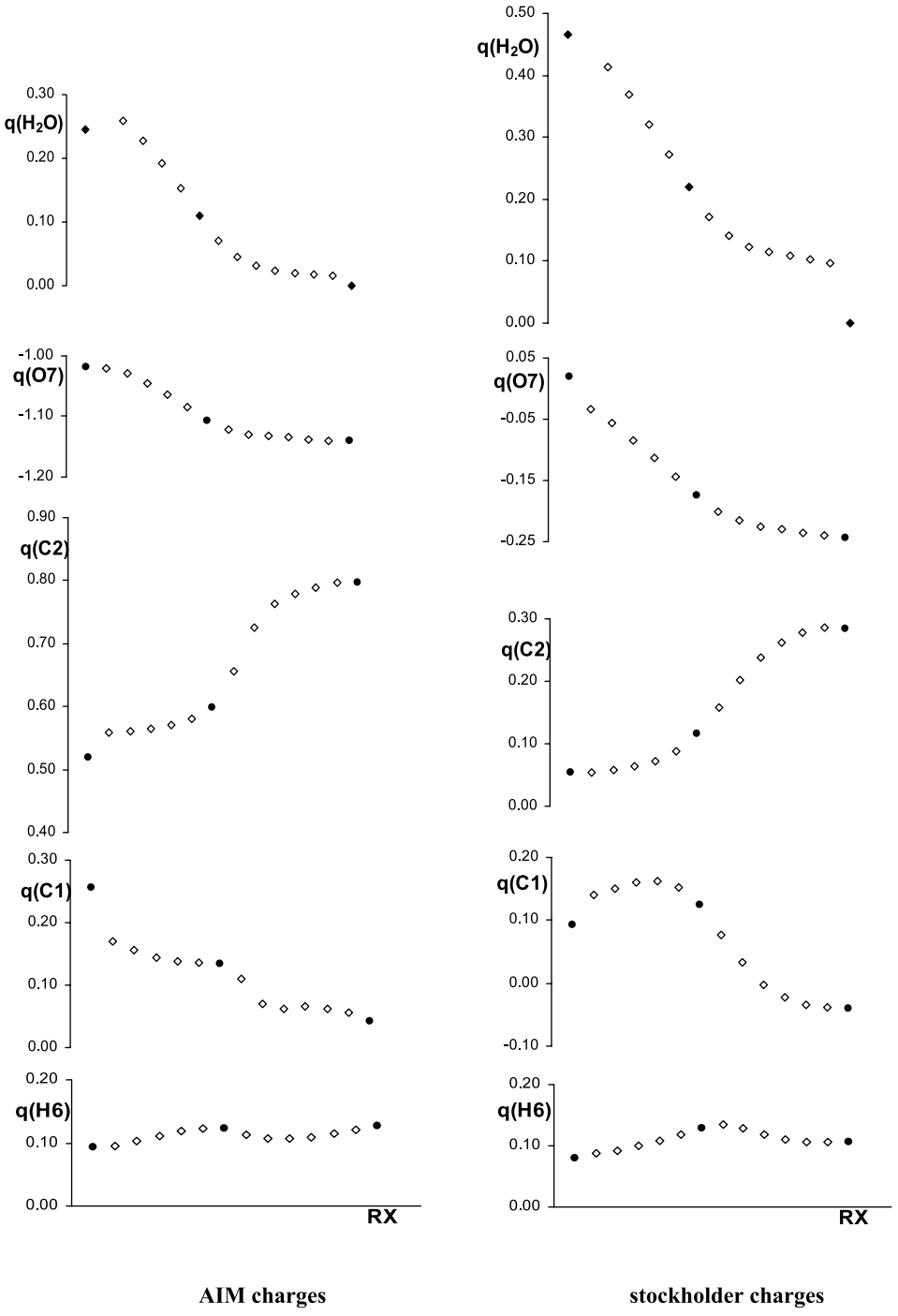

Figure 5. Variation of AIM and stockholder atomic charges along the IRC path for the pinacol rearrangement of protonated 1,2 ethanediol. All charges are in a.u.

corrections). These values are within or near the ranges of those reported by Kent et al. using 12 different computational levels:[15] 12.1–14.7 kcal mol^{-1} for ΔE_d (5.9–8.0 kcal mol^{-1} after ZPVE corrections) and 43.5–57.8 kcal mol^{-1} for $\Delta E_d^{\neq}$ after ZPVE corrections.

The reaction of decomposition implies the transfer of H_7 from O_3 to O_2 and it happens when these three atoms and C_1 are in the same plane. During the first moments of the reaction these atoms are moved towards a common plane, as can be inferred from the values of O_2–C_1–O_3–H_7, that are close to 0° from the first geometry studied (Table 3). In these first points other variations as those corresponding to O_3–H_7 or C_1–O_2 bonds are small and C_1 keeps sp^3 character as can be deduced from all the bond angles and the O_3–C_1–H_4–H_5 dihedral angle. Moreover, the analysis of the electron density does not show any bond path connecting O_2 and H_7 until the point denoted as md-6 in Table 3 and Figure 1, which is close to the TS.

Once the atoms are in the same plane, the hydrogen transfer begins and a ring structure involving C_1, O_3, O_2, and H_7

Table 3. Variations experienced by the main geometrical features along the unimolecular decomposition process of methanediol[a]

	CH_4O_2[b]	md-1	md-2	md-3	md-4	md-5	md-6	mdTS	md-7	md-8	md-9	md10	md11	md12	Products
C1–O2	1.409	0.4	2.5	8.8	16.1	23.0	27.3	29.7	33.7	40.1	47.5	55.1	67.3	78.0	
C1–O3	1.409	−0.1	−1.0	−2.4	−4.1	−6.1	−8.5	−10.4	−13.2	−16.1	−17.6	−18.7	−19.5	−19.9	−20.8
O2–H6	0.964	0.1	−0.3	−0.2	0.0	0.1	0.3	0.5	0.4	0.2	−0.3	0.6	0.1	0.3	−0.2
O2–H7	2.556	−38.9	−51.2	−63.2	−75.9	−91.2	−113.2	−136.7	−153.1	−157.2	−157.6	−158.8	−158.8	−158.4	−159.4
O3–H7	0.964	−0.1	0.0	0.1	0.3	2.0	13.0	34.7	58.0	75.3	87.3	98.0	111.1	120.9	
O2–C1–O3	112.8	−4.2	−8.1	−12.4	−16.0	−18.9	−20.2	−20.4	−20.1	−19.7	−19.6	−19.5	−19.7	−19.9	
O2–C1–H4	111.9	0.0	0.2	0.0	−1.3	−3.6	−6.1	−7.9	−10.3	−13.0	−15.1	−16.7	−18.4	−19.3	
O2–C1–H5	105.1	2.6	3.3	2.8	1.4	−0.6	−2.6	−4.1	−6.1	−8.6	−10.6	−12.3	−14.4	−15.8	
O3–C1–H4	105.1	3.7	5.4	7.3	9.4	11.6	13.4	14.4	15.6	16.5	16.9	17.1	17.2	17.2	16.9
O3–C1–H5	111.9	−2.0	−1.1	0.7	2.7	4.8	6.6	7.7	9.0	10.0	10.4	10.6	10.6	10.6	10.1
H6–O2–H7	100.0	16.5	18.5	18.2	17.3	16.5	15.4	13.3	9.8	7.5	6.9	6.4	6.2	6.1	5.1
O2–C1–O3–H7	62.6	−52.7	−58.4	−60.5	−61.7	−62.6	296.8	296.4	296.3	296.6	297.1	297.4	−62.2	−62.1	
O3–C1–H4–H5	120.7	−0.3	2.1	6.5	12.3	19.5	25.5	29.4	34.0	39.0	43.1	46.8	51.0	53.9	59.3

[a] Bond distances in Å and angles in degrees. Bond distances variations are multiplied by 10^2. Values are relative to those of methanediol.
[b] Absolute values for methanediol.

appears (confirmed by a ring critical point, Fig. 3). From this point the O_2–H_7 distance decreases achieving values close to that in the water molecule; whereas the O_3–H_7 distance increases, the bond path connecting these atoms disappears. At the same time, C_1 gets sp^2 character after the TS formation, as can be deduced from O_3–C_1–H_4 or O_3–C_1–H_5 angles (around 120°). In the last part of the reaction the modifications of the geometry are small, except for the distance between H_2O and CH_2O, which show a bond path connecting C_1 and O_2 until the reaction ends.

Figure 6 displays the variation in the charges during this process for C_1, O_2, O_3, and H_7. For the oxygen atoms both methods indicate similar variations, but differences are found for C_1 and H_7 which lead to different conclusions about the charge transfer. The charge of O_3 hardly changes if reagent and products are compared, but if the whole path is analysed a minimum charge near the TS is found. For the AIM method this variation is opposite to that found for H_7, the atom bonded to O_3, and it suggests a charge transfer between both atoms during the reaction. For O_2 and C_1 AIM differences for charges also show the same magnitude with the opposite sign, suggesting a charge transfer between them. Two stages can be defined for the charge evolution. First electron charge is taken from the hydrogen that is being transferred, H_7, and from the H-acceptor region (O_2 and H_6), to be gained by O_3 and C_1. Then, the opposite electronic rearrangement takes place after the formation of the TS and the atoms recover their initial electron population.

The profiles of the stockholder charges (Fig. 6) suggest an electron charge transfer between both oxygens through C_1 and H_7. This transfer goes first from O_2 to O_3 and then in the opposite way. In both analyses, no charge transfers exceeds 0.13 a.u.

4. Conclusions

The reported study allows us to conclude that the three processes studied follow concerted mechanisms. The keto–enol tautomerism of ethanal takes place through a four members ring TS. During the tautomerism H_4 migrates from C_3 to O_1 and transfers half of its electron charge to C_2 through O_1 according to the AIM results. According to stockholder charges both O_1 and H_4 transfer much less electron charge to the carbons. The pinacol rearrangement of protonated 1,2-ethanediol takes place through a concerted mechanism with no cyclic TS. Both AIM and Hirshfeld analyses indicate the charge of the hydrogen transferred remains practically unchanged during the process, C_2 acts as the main electron source, and two stages can be considered for the electron charge reorganization. The unimolecular decomposition of methanediol follows a concerted mechanism with a four member ring TS. Both analyses agree that the whole process takes place with very small variations of atomic charges, and the electron charge is transferred in opposite directions when the TS is built and when the products are obtained.

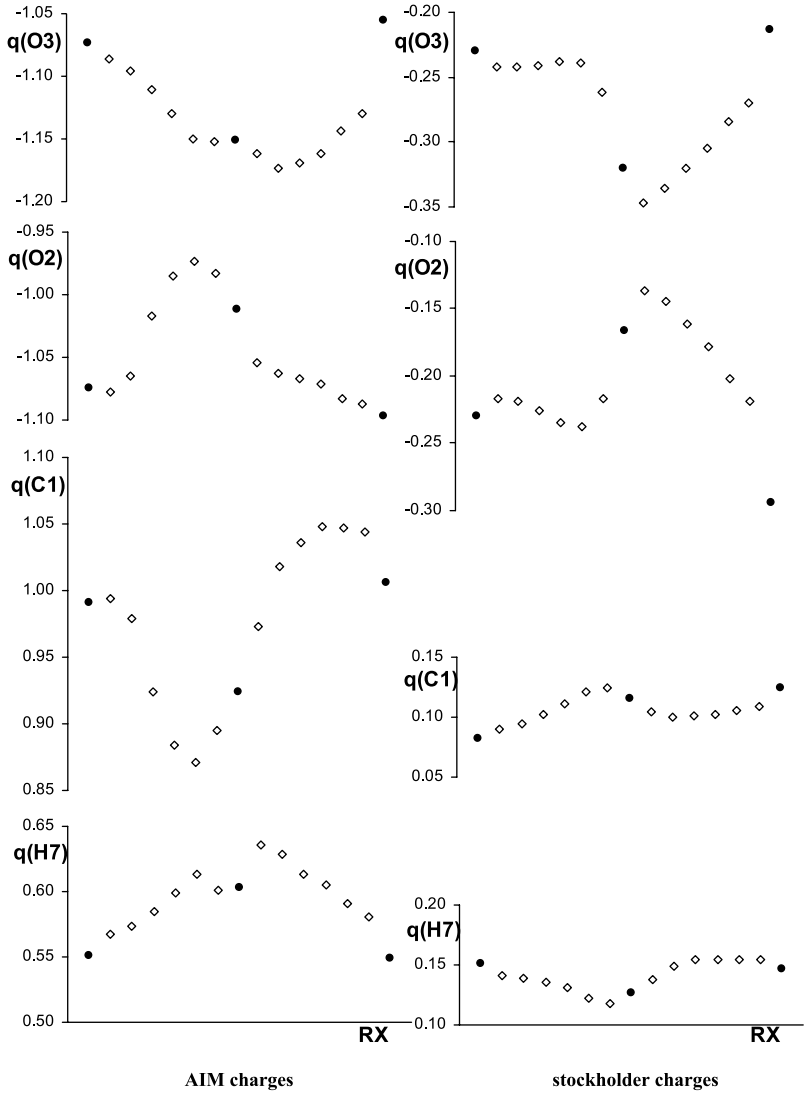

Figure 6. Variation of AIM and stockholder atomic charges along the IRC path for the unimolecular decomposition of methanediol. All charges are in a.u.

References and notes

1. Bader, R. F. W. In *Atoms in Molecules—A Quantum Theory; International Series of Monographs on Chemistry*; Oxford University Press: Oxford, 1990; Vol. 22.
2. Bader, R. F. W. *Chem. Rev.* **1991**, *91*, 893.
3. Hirshfeld, F. L. *Theor. Chim. Acta* **1977**, *44*, 129.
4. Rappoport, Z. *The Chemistry of Enols*; Wiley: New York, 1990.
5. Capon, B.; Guo, B.; Kwok, F. C.; Siddhanta, A. K.; Zucco, C. *Acc. Chem. Res.* **1988**, *21*, 135.
6. Andrés, J.; Domingo, L. R.; Picher, M. T.; Safont, V. S. *Int. J. Quant. Chem.* **1998**, *66*, 9.
7. Withmore, F. C. *J. Am. Chem. Soc.* **1932**, *54*, 3274.
8. Smith, W. B.; Bowman, R. E.; Kmet, T. J. *J. Am. Chem. Soc.* **1959**, *81*, 997.
9. Smith, W. B.; Kmet, T. J.; Rao, P. S. *J. Am. Chem. Soc.* **1961**, *83*, 2190.
10. Collins, C. J.; Rainey, W. T.; Smith, W. B.; Kay, I. A. *J. Am. Chem. Soc.* **1959**, *81*, 460.
11. Nakamura, K.; Osamura, Y. *Tetrahedron Lett.* **1990**, *31*, 251.
12. Nakamura, K.; Osamura, Y. *J. Phys. Org. Chem.* **1990**, *3*, 737.
13. Nakamura, K.; Osamura, Y. *J. Am. Chem. Soc.* **1993**, *115*, 9112.
14. Smith, W. B. *J. Phys. Org. Chem.* **1999**, *12*, 741.
15. Kent, D. R. IV; Widicus, S. L.; Blake, G. A.; Goddard, W. A. III *J. Chem. Phys.* **2003**, *119*, 5117.
16. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A. Jr.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Baboul, A. G.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98*, *Revision A.9*; Gaussian, Inc., Pittsburgh PA, 1998.
17. Bader, R. F. W. and coworkers. *AIMPAC*: *A suite of programs*

for the Theory of Atoms in Molecules; McMaster University: Hamilton, Ontario, Canada L8S 4M1. Contact bader@mcmail.cis.mcmaster.ca.
18. *MORPHY98*, *a topological analysis program* written by P. L. A. Popelier with a contribution from R. G. A. Bone (UMIST, England, EU).
19. Popelier, P. L. A. *Chem. Phys. Lett.* **1994**, *288*, 160.
20. Rousseau, B.; Peeters, A.; Van Alsenoy, C. *Chem. Phys. Lett.* **2000**, *324*, 189.
21. de Proft, F.; Vivas-Reyes, R.; Peeters, A.; Van Alsenoy, C.; Geerlings, P. *J. Comput. Chem.* **2003**, *24*, 463.
22. Maslen, E. N.; Spackman, M. A. *Aust. J. Phys.* **1985**, *38*, 273.
23. Mandado, M.; Van Alsenoy, C.; Mosquera, R. A. *J. Phys. Chem. A* **2004**, *108*, 7050.
24. Smith, B. J.; Nguyen, M. T.; Bouma, W. J.; Radom, L. *J. Am. Chem. Soc.* **1991**, *113*, 6452.
25. Suenobu, K.; Nagaoka, M.; Yamabe, T. *J. Mol. Struct. (THEOCHEM)* **1999**, *461*, 581.
26. Holmes, J. H.; Lossing, F. P. *J. Am. Chem. Soc.* **1982**, *104*, 2648.
27. Wesdemiotis, C.; McLafferty, F. W. *J. Am. Chem. Soc.* **1987**, *109*, 4760.
28. Wu, C.-C.; Lien, M.-H. *J. Phys. Chem.* **1996**, *100*, 594.

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Tetrahedron 61 (2005) 831–836

Relationship between the electrophilicity of substituting agents and substrate selectivity in Friedel–Crafts reactions

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Received 6 October 2004; revised 12 November 2004; accepted 15 November 2004

Available online 8 December 2004

Abstract—The global electrophilicity index evaluated at the ground state of benzylating and acylating agents shows a quantitative linear relationship with the experimental substrate selectivity index evaluated for a series of Friedel–Crafts reactions. The theoretical scale correctly accounts for the electrophilic activation/deactivation effects promoted by electron withdrawing and electron releasing substituents in these molecules. The predicted substrate selectivity values estimated from the knowledge of the electrophilicity index may become accurate to within 10% and less.

1. Introduction

Electrophilic aromatic substitution (EAS) reaction is one of the archetypal polar processes in organic chemistry.[1–3] The accepted two step mechanism involves the formation of an ionic intermediate, the benzenium ion,[4–6] where the attacking electrophile forms a σ bond with the substrate (also named the σ complex). Extensive studies reported by Brown showed a linear relationship between the relative stability of the σ complex and relative rates for a significant number of electrophilic substitution reactions,[7] yet there was not evidence showing that the transition states were closely related to this intermediate complex.[8] An alternative two-step mechanism involves Dewar's π-complex, where the interaction of an electrophile with the aromatic substrate forms a first weak reagent–substrate complex (outer complex) which is in equilibrium with a second structure two-electron three-center complex (π-complex). The formed complex is indeed a bridged tetracoordinated carbonium ion (benzonium ion).[9] However, studies by Olah point out to a mechanism characterized by an early formation of the π-complex, followed by the formation of the σ complex, that accounts for the low substrate but high positional selectivities observed in EAS reactions.[10,11] Furthermore, they proved that transition states of these reactions were not rigidly fixed, always resembling the Wheland intermediates, but they could frequently represent a much earlier stage of the reaction that could even corresponds in structure to the starting aromatics.[12,13] The mechanism of EAS reactions has been recently revisited by Esteves et al.[14] Based on experimental and computational results, these authors have introduced a unified mechanism involving three separate intermediates on the potential energy surface of the reaction.

One of the factors determining the reactivity in EAS processes is the electrophilicity of the attacking group. For instance, it has been shown that substituents may affect the substrate selectivity as measured by the $k_{\text{Toluene}}/k_{\text{Benzene}}$ (k_T/k_B, hereafter) ratio. Thus, while electron-donating substituent located at position *ortho* and *para* with respect to the benzylic centre, increase the k_T/k_B ratio, electron-withdrawing substituents decrease it.[12,13] On the other hand, the acylation of toluene and benzene clearly proved the importance of substituents on the electrophilicity of the substituting agent, which was reflected by high k_T/k_B ratios. The effect of the nucleophilicity of the aromatic substrate was observed to cause similar effects;[15] so that with increasingly more basic aromatics, even relatively weak electrophiles resulted in early transition states resembling more starting materials than intermediates.[12,13,15] This result opens an interesting alternative to look at the substrate selectivity in EAS reactions using static reactivity models developed around the ground states of reactants.

The second relevant aspect in the EAS reactions is the activating effect promoted by Lewis acid (LA) catalysts. This is still an active area of research, and several works

Keywords: Electrophilicity index; Electrophilicity and substrate selectivity; Electrophilic activation/deactivation in Friedel–Crafts reactions.
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0040–4020/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2004.11.038

describing a wide variety of catalysts have been recently reported.[16–19]A recent study by Sefkow and Buchs reports on the uncatalyzed Friedel–Craft alkylation of aromatic compounds through reactive benzyl cations, that are generated by thermal decomposition of aryl-benzyl-sulfamoylcarbamates.[20]

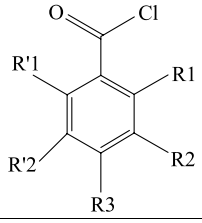
In this article we present a theoretical model to quantitatively describe the substrate selectivity in terms of the global electrophilicity of the benzylating and acylating reagents involved in the Friedel–Crafts EAS reactions, using a global electrophilicity index.[21,22] We rank, within a unique absolute scale, the global electrophilicity of a series of (10) benzylating and (7) acylating reagents. The usefulness of the theoretical scale is illustrated for the rationalization of substituent effects on the electrophilic activation/deactivation reagents and to predict the experimental substrate selectivity described by the k_T/k_B ratios of related systems.

2. Model and computational details

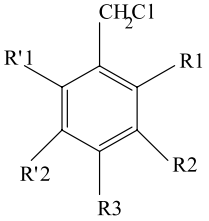
The concept of electrophilicity viewed as a reactivity index was introduced by Maynard et al. to study the reaction of the human inmunodeficiency virus type 1 (HIV-1) nucleocapsid protein p7 (NCp7) with a variety of electrophilic agents.[21] It was reformulated by Parr et al.[22] using a second order expansion of the electronic energy with respect to the charge transfer ΔN at fixed geometry. Since electrophiles are species that stabilize upon receiving an additional amount of electronic charge from the environment, there exist a minimum of energy for a particular ΔN^* value. Using this simple idea Parr et al. performed a variational calculation that led to the definition of the global electrophilicity index as $\omega = -\Delta E(\Delta N^*)$, which may be recast into the more familiar form:[21]

$$\omega = \frac{\mu^2}{2\eta} \tag{1}$$

in terms of the electronic chemical potential μ and the chemical hardness η. The ω index establishes an absolute scale of electrophilicity in the sense that the hierarchy of electrophilicity is built up from the electronic structure of molecules, independent of the nucleophilic partner, which is replaced by an unspecified environment viewed as a sea of electrons.[21] It has been successfully used to describe reactivity in different organic systems. For instance, the global electrophilicity values obtained from ω have been used to rank the electrophilicity of reagents participating in Diels–Alder and 1,3-dipolar cycloadditions reactions.[23,24] It was also found that the difference in electrophilicity for the diene/dienophile pair determined the nature of the reaction mechanism (non-polar or polar character of the process), thereby reinforcing the reliability of the ω index as a kinetic descriptor of reactivity.[23] This index is almost insensitive to solvent effects in neutral electrophiles, thus gas phase calculations suffice to establish the electrophilic power of molecules.[25] More recently, we have shown that the intrinsic electronic contribution to the substituent σ_p Hammett constants, $\sigma_e(\omega)$, can be estimated from the ω index calculated for a series of substituted ethylenes.[26] We found that electron-withdrawing substitution increased the electrophilicity power of ethylene, and that the corresponding $\sigma_e(\omega)$ values were consistently predicted as positive numbers. Our aim in this work is to illustrate how the electrophilicity index performs to quantitatively account for the observed substrate selectivity in Friedel–Craft benzylation and acylation.

General structure Acylating agents		R1	R'1	R2	R'2	R3
	1	NO_2	H	H	H	NO_2
	2	H	H	NO_2	NO_2	H
	3	F	H	H	F	H
	4	H	H	H	H	H
	5	H	H	H	H	CH_3
	6	H	H	H	H	F
	7	H	H	H	H	OCH_3

A

General structure Benzilating agents		R1	R'1	R2	R'2	R3
	1	Cl	H	H	H	H
	2	H	H	F	H	H
	3	F	H	H	H	H
	4	H	H	H	H	H
	5	H	H	H	H	F
	6	CH_3	H	H	H	H
	7	H	H	H	H	CH_3
	8	CH_3	CH_3	H	H	CH_3
	9	OCH_3	H	H	H	H
	10	H	H	H	H	OCH_3

B

Chart 1. General structure of acylating (**A**) and benzylating (**B**) agents involved in Friedel–Crafts reactions considered in this work.

All the structures included in this study are shown in Chart 1. They were optimized at the B3LYP level of theory using the Gaussian98 package of programs.[27] Several basis set, including 6-31G*, 6-311G** and 6-311++G** were used in order to test the stability of the reactivity index with respect to the basis set. The values of the electronic chemical potential and the chemical hardness were obtained from the expressions $\mu \approx (\varepsilon_H + \varepsilon_L)/2$ and $\eta \approx \varepsilon_L + \varepsilon_H$, in terms of the one electron energies of the HOMO and LUMO frontier molecular orbitals, ε_H and ε_L, respectively.[28] With these quantities at hand, the global electrophilicity at the ground state of molecules was obtained using Eq. (1).

3. Results and discussion

The global electrophilicity patterns of the substituting acylating (**A**) and benzylating (**B**) agents, commonly used in Friedel–Crafts reactions are ranked in Chart 2. Acylating agents display global electrophilicity values located at the top of the scale, within the range [2.0–4.0] eV. Benzylating agents on the other hand display lower electrophilicity values within the range [1.0–2.0] eV. In both cases it is possible to rationalize the electrophilic activating/deactivating effects promoted by substituent group in these molecules. For instance, if we start from the unsubstituted reference compound **A4** ($\omega = 2.50$ eV), substitution at R3 by

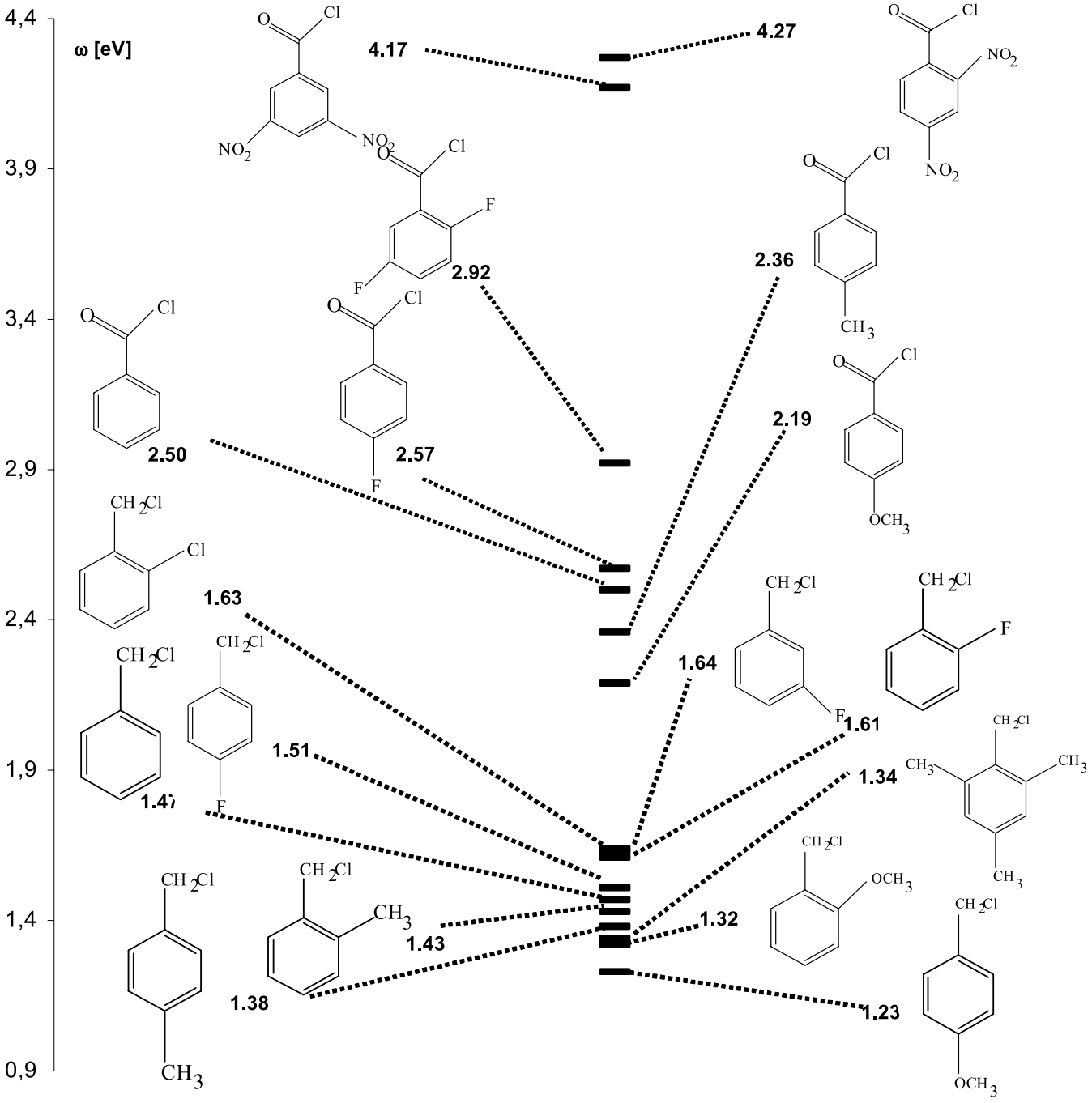


Chart 2. Theoretical scale of global electrophilicity for the acylating (**A**) and benzylating (**B**) agent series involved in Friedel–Crafts reactions considered in this work.

Table 1. Global electrophilicity values (in eV units) obtained from different basis set at the B3LYP level of theory for the ground state (ω) of acylating (**A**) and benzylating (**B**) agents[a]

Compound	(ω), Basis set			$\ln(k_T/k_B)$
	6-31G*	6-311G**	6-311++G**	
A1	3.77	3.97	4.27	3.37
A2	3.64	3.86	4.17	3.66
A3	2.45	2.75	2.92	4.57
A4	2.14	2.37	2.50	5.03
A5	2.03	2.25	2.36	5.10
A6	2.18	2.43	2.57	5.14
A7	1.84	2.05	2.19	5.45
B1	1.35	1.57	1.63	1.53
B2	1.31	1.54	1.64	1.53
B3	1.29	1.52	1.61	1.57
B4	1.20	1.40	1.47	1.84
B5	1.21	1.42	1.51	2.16
B6	1.16	1.36	1.43	2.95
B7	1.12	1.31	1.38	3.66
B8	1.10	1.28	1.34	3.67
B9	1.04	1.24	1.32	4.10
B10	0.97	1.15	1.23	4.57

[a] Experimental substrate selectivity index $\ln(k_T/k_B)$ from Ref. 10.

the weak electron releasing $-CH_3$ group results in an electrophilic deactivation in compound **A5** ($\omega=2.36$ eV). Substitution at the same position with the stronger electron releasing $-OCH_3$ group results in an even higher electrophilic deactivation in compound **A7** ($\omega=2.19$ eV). Substitutions with electron withdrawing groups show, as expected, electrophilic activation. For instance, with reference to compound **A4**, substitution at R3 with fluorine causes a slight activation of about 0.07 eV in compound **A6**, whereas substitution with two fluorine atoms at R1 and R2′ in compound **A3** results in an even higher electrophilic activation of about 0.42 eV within the ω scale. Note, however, that the most efficient activation with reference to compound **A4** is achieved by $-NO_2$ substitution at (R1, R3) and (R2, R2′) in compounds **A1** ($\omega=4.27$ eV) and **A2** ($\omega=4.17$ eV), respectively.

For the series of benzylating agents, a similar picture is obtained, yet the effect of the substituting groups is largely lower than that obtained for the acylating agent series. For instance, starting from the reference compound **B4** ($\omega=1.47$ eV), substitution at R1 with chlorine and fluorine atoms results in a moderate electrophilic activation in compounds **B1** ($\omega=1.63$ eV) and **B3** ($\omega=1.61$ eV). Note that substitution with fluorine at R2 in compound **B2** ($\omega=1.64$ eV) has a similar activating effect. Electrophilic deactivation promoted by electron releasing groups is also moderate. Substitution at R1 or R3 by a $-CH_3$ group causes a marginal deactivation in compounds **B6** ($\omega=1.43$ eV) and **B7** ($\omega=1.38$ eV), respectively. Multiple substitutions with $-CH_3$ groups at positions R1, R1′ and R3 in compound **B8** ($\omega=1.34$ eV) do not show any additional deactivating effects. Note however that, as expected, a single substitution by the stronger electron releasing $-OCH_3$ group at position R1 or R3 in compounds **B9** ($\omega=1.32$ eV) and **B10** ($\omega=1.23$ eV), respectively, results in a slightly higher electrophilic deactivation. In summary, while chemical substitution by EW and ER groups dramatically affects the electrophilic pattern of the acylating agents, this effect is markedly smaller in the series of benzylating agents. This result may be traced to an additional resonance effect promoted by the conjugated carbonyl group in the acyl series, making substituent effects more efficient than that observed for the series B, where this resonance effects is not present. Table 1 shows the global electrophilicity values for the whole series of acylating and benzylating agents at several levels of theory for the GS of the substituting agents. Note that the global electrophilicity index is computationally very stable with respect to the basis set change.

The usefulness of a reactivity scale has been clearly described and illustrated by Mayr et al.[29–31] A reactivity scale should be able of answering fundamental questions about reaction feasibility; intramolecular selectivity and other important aspects of reactivity.[29–31] Within the present approach, and for the particular cases where the activated species are very close in structure to reactants, we intend to illustrate the usefulness of the validated scale of electrophilicity to quantitatively account for the substrate selectivity observed in the acylation and benzylation

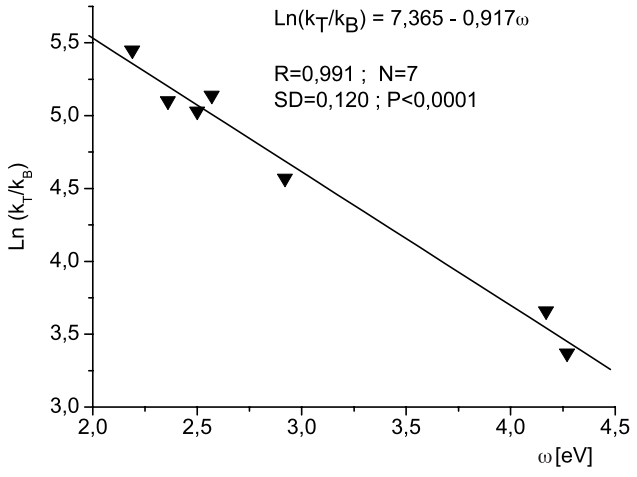


Figure 1. Comparison between the experimental substrate selectivity index $\ln(k_T/k_B)$ and the global electrophilicity evaluated at the ground state structure for the acylating agent series. Electrophilicicty values from B3LYP/6-311++G** calculations. R is the regression coefficient; N is the number of points in the regression; SD is the standard deviation and P is the probability that the observed relationship was randomly obtained.

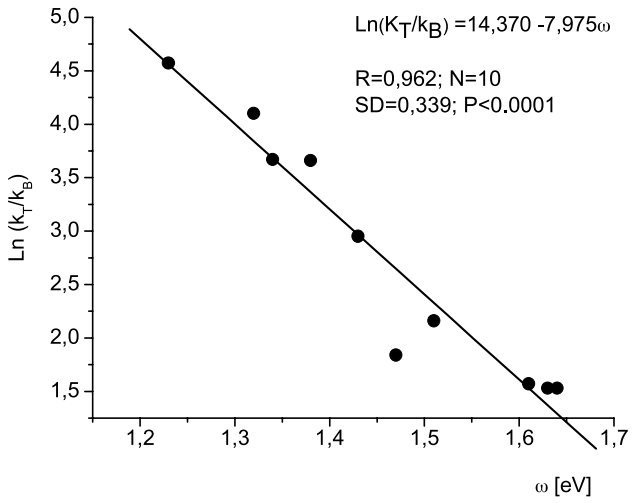


Figure 2. Comparison between the experimental substrate selectivity index $\ln(k_T/k_B)$ and the global electrophilicity evaluated at the ground state structure for the benzylating agent series. Electrophilicicty values from B3LYP/6-311++G** calculations. R is the regression coefficient; N is the number of points in the regression; SD is the standard deviation and P is the probability that the observed relationship was randomly obtained.

reaction of aromatic compounds. First of all we observe in Figures 1 and 2, that the relationship between k_T/k_B ratio and global electrophilicity index shows a negative slope. This is because strongly electrophilic reagents leads to low substrate selectivity in the form of low k_T/k_B rate coefficient ratios.[10] We compare in Figure 1 the experimental substrate selectivity described by the k_T/k_B ratio and the global electrophilicity index for the series of acylating agents evaluated at the B3LYP/6-311++G** level. The experimental data are available from literature.[10] The resulting regression equation is:

$$\mathrm{Ln}(k_T/k_B) = 7.365 - 0.917\omega \quad (R = 0.991) \tag{2}$$

In order to test the quality of the linear relationship obtained for this series, two new compounds **A8** (R3=NO_2) and **A9** (R1=CH_3, R3=CH_3, R1′=CH_3) not included in the regression shown in Figure 1 were selected from Olah's data base to test the predictive power of the model.[10] This empirical equation predicts for compound **A8**, for which ω=3.96 eV, a value $\ln(k_T/k_B)$=3.73, which is in excellent agreement with the experimental value $\ln(k_T/k_B)$=3.95. Compound **A9** on the other hand, which has an electrophilicity value ω=1.86 eV yields a predicted $\ln(k_T/k_B)$=5.66, which is again in excellent agreement with the experimental value $\ln(k_T/k_B)$=5.28.[10]

The comparison between the $\ln(k_T/k_B)$ index and the global electrophilicity at the ground state of benzylating agents evaluated at the B3LYP/6-311++G** level is displayed in Figure 2. The comparison yields the following regression equation:

$$\mathrm{Ln}(k_T/k_B) = 14.379 - 7.975\omega \quad (R = 0.959) \tag{3}$$

This empirical equation was tested for compounds **B11** (R3=Cl) and **B12** (R1=OCH_3, R2=CH_3, R3=OCH_3, R2′=CH_3 and R1′=OCH_3) not included in the regression shown in Figure 2. Compound **B11** for which ω=1.62 eV, yields a value $\ln(k_T/k_B)$=1.45, which is to be compared to the experimental one $\ln(k_T/k_B)$=1.82.[10] Compound **B12** on the other hand, for which we evaluated a global electrophilicity ω=1.26 eV yields a $\ln(k_T/k_B)$ value of 4.32, which is to be compared to the experimental one $\ln(k_T/k_B)$=4.91.[10] The predictions for the benzylating agent series are not as accurate as those for the acylating agent series, a result probably traced to the low global electrophilicity value evaluated for the activated reference compound **B4**, which show a significant deviation from linearity. This result may be probably improved going beyond the ground state of reactants, by using an activated structure closer to the transition state

These comparisons may be somehow questionable in the sense that the choice of molecules to perform the comparison is completely arbitrary. In order to make a

Table 2. Statistical data for different correlations between global electrophilicity index evaluated at the ground state (ω) and the experimental substrate selectivity index $\ln(k_T/k_B)$[a]

Line	Compounds included in the correlation	Constant	Slope	*R*	*N*	Predicted, $\ln(k_T/k_B)$
1	**A2**,**A3**,**A4**,**A5**,**A6**,**A8**,**A9**	6.8084	−0.7340	0.980	7	**A1**: 3.67 (3.37), **A7**: 5.20 (5.45)
2	**A1**,**A3**,**A4**,**A5**,**A7**,**A8**,**A9**	7.1932	−0.8168	0.977	7	**A2**: 3.79 (3.66), **A6**: 5.09 (5.14)
3	**A1**,**A2**,**A4**,**A6**,**A7**,**A8**,**A9**	7.0966	−0.8270	0.972	7	**A3**: 4.68 (4.57), **A5**: 5.14 (5.10)
4	**A2**,**A3**,**A4**,**A5**,**A6**,**A7**,**A8**	7.1548	−0.8313	0.986	7	**A1**: 3.60 (3.37), **A9**: 5.61 (5.28)
5	**A1**,**A2**,**A3**,**A4**,**A5**,**A6**,**A9**	7.0178	−0.8220	0.982	7	**A7**: 5.22 (5.41), **A8**: 3.76 (3.95)
6	**B2**,**B3**,**B4**,**B5**,**B6**,**B7**,**B8**,**B9**,**B11**,**B12**	15.1760	−8.4740	0.950	10	**B1**: 1.36 (1.53), **B10**: 4.75 (4.57)
7	**B1**,**B3**,**B4**,**B5**,**B6**,**B7**,**B8**,**B10**,**B11**,**B12**	14.8679	−8.2872	0.956	10	**B2**: 1.28 (1,53), **B9**: 3.93 (4.10)
8	**B1**,**B2**,**B4**,**B5**,**B6**,**B7**,**B9**,**B10**,**B11**,**B12**	14.8528	−8.2442	0.957	10	**B3**: 1.58 (1.57), **B8**: 3.80 (3.67)
9	**B1**,**B2**,**B3**,**B4**,**B6**,**B8**,**B9**,**B10**,**B11**,**B12**	14.5906	−8.0698	0.963	10	**B5**: 2.40 (2.16), **B7**: 3.45 (3.66)
10	**B1**,**B2**,**B3**,**B5**,**B7**,**B8**,**B9**,**B10**,**B11**,**B12**	14.7875	−8.1399	0.987	10	**B4**: 2.82 (1.84), **B6**: 3.15 (2.95)

[a] In the last column the experimental substrate selectivity index $\ln(k_T/k_B)$ is given in parentheses for comparisons.

meaningful conclusion about the relationship between electrophilicity of the substituting agents and the experimental substrate selectivity index, it is necessary to perform additional comparisons with several sets of molecules randomly selected. The result is summarized in Table 2. It may be seen that in general there exist a true linear relationship between both variables. Hence the predictive power of the generated linear relationships may become accurate to within 10% and less, thereby showing the usefulness of the present reactivity scale. There remains however some improvements that can be made by explicitly introducing the catalyst, and evaluating the global electrophilicity of molecules at a more realistic stage of the reaction, namely the Wheland complex or even at the transition state.

4. Concluding remarks

The global electrophilicity of benzylating and acylating agents participating in Friedel–Crafts electrophilic aromatic substitution reactions has been ranked within a unique absolute scale using the global electrophilicity index. The theoretical scale correctly accounts for the electrophilic activation/deactivation effects promoted by electron withdrawing and electron releasing substituents in these molecules. The comparison between global electrophilicity and the experimental relative rate coefficients ($k_{Toluene}/k_{Benzene}$) shows a quantitative linear relationship. The values of relative rate coefficients predicted from the knowledge of the global electrophilicity index may be accurate to within 10% and less.

Acknowledgements

Work supported by Fondecyt, grants 1030548 and 1010649, and Millennium Nucleus for Applied Quantum Mechanics and Computational Chemistry, project P02-004-F (Chile). L. M. thanks Departamento de Postgrado y Postítulo (Universidad de Chile) under Grants PG/101/02 and AGCI-DAAD for PhD fellowship.

References and notes

1. Olah, G. A. In *Friedel–Crafts and Related Reactions* Wiley: New York, 1964; Vol. I–IV.
2. Olah, G. A.; Kobayashi, S. *J. Am. Chem. Soc.* **1970**, *92*, 6369.
3. Carey, F. A.; Sundberg, R. J. *Advanced Organic Chemistry. Part A: Structure and Mechanisms*; Book News, 2002; p 570.
4. Wheland, G. W. *J. Am. Chem. Soc.* **1942**, *64*, 900.
5. Doering, W. v. E.; Saunders, M.; Boynton, H. G.; Earhart, H. W.; Wadley, E. F.; Edwards, W. R.; Laber, G. *Tetrahedron* **1958**, *4*, 178.
6. Stock, L. M.; Brown, H. C. *Adv. Phys. Org. Chem.* **1963**, *1*, 35.
7. Brown, H. C.; Pearsall, H. W. *J. Am. Chem. Soc.* **1952**, *74*, 191.
8. Brouwer, D. M.; Mackor, E. L.; MacLean, C. L. In Olah, G. A., Schleyer, P. v. R., Eds.; Carbonium Ions; Wiley: New York, 1970; Vol. II; Chapter 20.
9. Dewar, M. J. S. *J. Chem. Soc.* **1946**, *406*, 777.
10. Olah, G. A. *Acc. Chem. Res.* **1971**, *4*, 240.
11. Olah, G. A.; Kobayashi, S.; Tashiro, M. *J. Am. Chem. Soc.* **1972**, *94*, 7448.
12. Olah, G. A.; Tashiro, M.; Kobayashi, S. *J. Am. Chem. Soc.* **1970**, *92*, 6369.
13. Olah, G. A.; Kobayashi, S. *J. Am. Chem. Soc.* **1971**, *93*, 6964.
14. Esteves, P. M.; Carneiro, J.W. de. M.; Cardoso, S. P.; Barbosa, A. G. H.; Laali, K. K.; Rasult, G.; Prakash, G. K. S.; Olah, G. A. *J. Am. Chem. Soc.* **2003**, *125*, 4836.
15. Taylor, R. *Electrophilic Aromatic Substitution*; Wiley: Chichester, 1990.
16. Kawamura, M.; Cui, D. M.; Hayashi, T.; Shimada, S. *Tetrahedron Lett.* **2003**, *44*, 7715.
17. Firouzabadi, H.; Iranpoor, N.; Nowrouzi, F. *Tetrahedron Lett.* **2003**, *44*, 5343.
18. Singh, R. P.; Kamble, R. M.; Chandra, L.; Saravanan, P.; Singh, V. K. *Tetrahedron* **2001**, *57*, 41.
19. Cardoso, L. A. M.; Alves, W. Jr.; Gonzaga, A. R. E.; Aguiar, L. M. G.; Andrade, H. M. C. *J. Mol. Catal. A* **2004**, *209*, 189.
20. Sefkov, M.; Buchs, J. *Org. Lett.* **2003**, *5*, 193.
21. Maynard, T. A.; Huang, M.; Rice, W. G.; Covell, D. G. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *25*, 11578.
22. Parr, R. G.; Szentpály, L. v.; Liu, S. *J. Am. Chem. Soc.* **1999**, *121*, 9500.
23. Domingo, L. R.; Aurell, M. J.; Pérez, P.; Contreras, R. *Tetrahedron* **2002**, *58*, 4417.
24. Pérez, P.; Domingo, L. R.; Aurell, M. J.; Contreras, R. *Tetrahedron* **2003**, *59*, 3117.
25. Pérez, P.; Toro-Labbé, A.; Contreras, R. *J. Am. Chem. Soc.* **2001**, *123*, 5527.
26. Domingo, L. R.; Pérez, P.; Contreras, R. *J. Org. Chem.* **2003**, *68*, 6060.
27. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. J. A.; Stratmann, R. E.; Burrant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M. W.; Gill, P. M.; Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A.; Gaussian 98, Revision A.6 Gaussian, Inc.: Pittsburg, PA, 1998.
28. Parr, R. G.; Yang, W. *Density Functional Theory of Atoms and Molecules*; Oxford Science: Oxford, 1994.
29. Mayr, H.; Kempf, B.; Ofial, A. R. *Acc. Chem. Res.* **2003**, *36*, 66.
30. Mayr, H.; Müller, K.-H.; Ofial, A. R.; Buhl, M. *J. Am. Chem. Soc.* **1999**, *121*, 2418.
31. Mayr, H.; Bug, T.; Gotta, M. F.; Hering, N.; Irrgang, B.; Janker, B.; Kempf, B.; Loos, R.; Ofial, A. R.; Remennikov, G.; Schimmel, H. *J. Am. Chem. Soc.* **2001**, *123*, 9500.

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Tetrahedron 61 (2005) 837–844

Synthetic approach to pentacyclic quassinoids from communic acids, via ambracetal derivatives

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Received 26 June 2004; revised 12 November 2004; accepted 15 November 2004

Available online 30 November 2004

Abstract—Methyl (8*R*,13*S*)-8α,13:13,17-diepoxy-14,15-dinorlabdane-19-oate, easily prepared from communic acids, is a suitable intermediate for synthesizing pentacyclic quassinoids, because it enables the elaboration of the A ring and the further construction of the B–C–D ring system of these terpenoids. The cetal group is stable under the reaction conditions utilized during the elimination of the ester group and the introduction of the hydroxyl group on C-3. At the same time, it enables the regeneration of the methylketone and exocyclic double bond presented by methyl 13-oxo-14,15-dinorlabd-8(17)en-19-oate. The latter compound was previously used to construct the B–C–D-ring of these quassinoids.

1. Introduction

In a previous paper we reported the efficient preparation of the tetracyclic derivative **3**, a possible intermediate in the synthesis of pentacyclic quassinoids from communic acids (**1a–c**) via methylketone **2** (Scheme 1).[1] **3** presents the B, C and D rings suitably functionalized to construct the B–C–D–E framework of such quassinoids, but the elaboration of the A ring of quassinoids from this intermediate is likely to be difficult. Thus we have investigated how to functionalize the A ring during the early steps of the synthetic sequence, before creating the B–C–D–E system. We believed that cetal **4**, the preparation of which, from **1a–c** in a 3-step sequence, we have reported elsewhere, [1,2] could well be suitable for this purpose. Cetal **4**, bearing the methylketone and exocyclic double-bond groups as masked functions, would allow us to functionalize the A ring according to the method we have described previously.[3] Thus, the functionalized cetal **A** would be prepared and then the regeneration of methylketone and the exocyclic double bond would lead to **B**, which, following the previously developed synthetic sequence utilized to convert **2** into **3**,[1] would provide the tetracyclic intermediate **C**. The whole ring of this compound is suitably functionalized, and thus pentacyclic quassinoids, as bruceantin, may be obtained (Scheme 1).

In this paper we report a procedure to create the methylketone and exocyclic double bond groups from the cetal function in **4**, in addition to two methods to functionalize the A ring of **4**.

2. Results and discussion

We began by investigating methods to create methylketone and exocyclic double-bond groups from cetal **4**, the preparation of which from **1a–c** via the reduction derivatives **5a–b**, has now been considerably improved. **5a–b** was converted into **4** in one-pot reaction, without isolating the intermediate methylketone **2**. Our previous studies revealed that the $TiCl_4$-catalysed nucleophilic cleavage of cetal could be used to this end.[4] We assayed the reaction of **4** with $TiCl_4$ in the presence of Et_3SiH as the nucleophile under various conditions.[5] The most significant results are shown in Table 1 (Scheme 2). As can be seen, good yields of oxane derivatives **6a–b** were always obtained. Formate **7** showed similar reactivity, affording a 1:1 mixture of oxanes **8a–b** when treated at −78 °C.

The epimeric mixture **6a–b** was used as the starting material to prepare methylketone **2**. The key step in the sequence was the C–O cleavage of the α-alkoxyaldehydes **9a–b** under

Keywords: Quassinoids; Labdane diterpenes; Acetal cleavage; Wolff–Kishner elimination.
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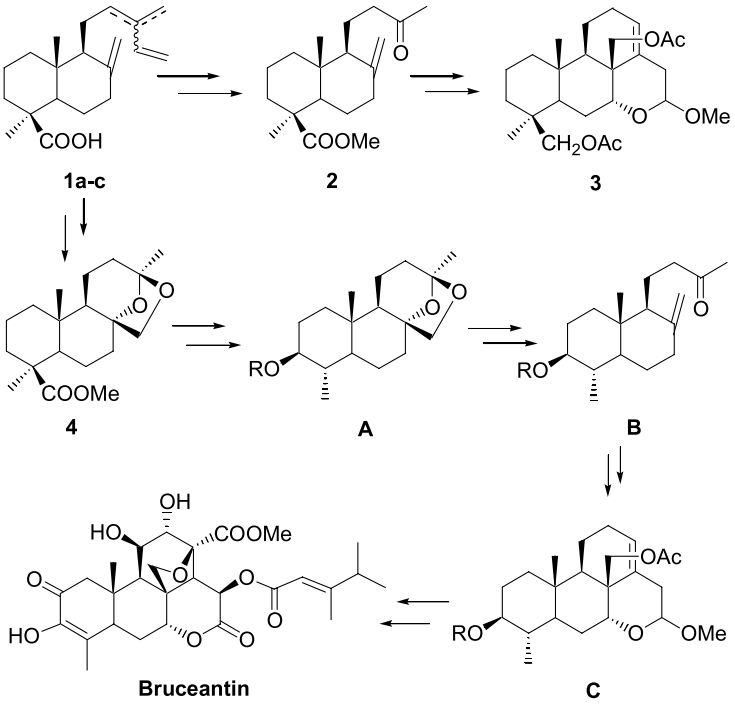
0040–4020/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2004.11.034



Scheme 1.

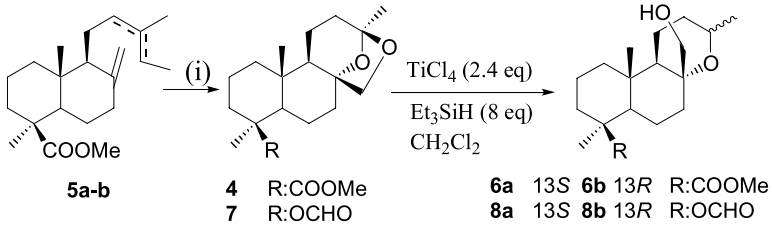
Table 1. Treatment of **4** with $TiCl_4$–Et_3SiH in CH_2Cl_2

Entry	Reaction time (h)	Temperature	**6a–6b** (% conversion)
1	1	−78 °C	1:1 (95%)
2	2	rt	1:6 (96%)
3	14	rt	0:1 (70%)

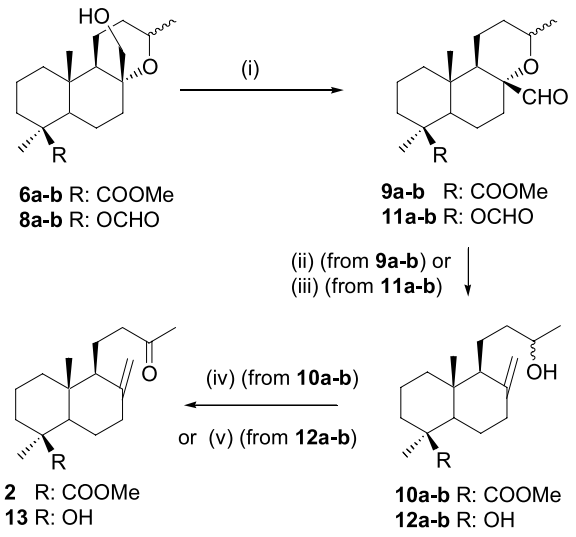
Wolff–Kishner reduction conditions (Scheme 3).[6] The treatment of **6a–b** with PCC in CH_2Cl_2 at room temperature afforded aldehydes **9a–b**, which, by treating with KOH and H_2NNH_2 in triethylene glycol at 160 °C for 2 h, yielded a mixture of alcohols **10a–b**, after esterification with diazomethane. These were transformed into methylketone **2** by Jones' reagent. In a similar way **8a–b** was transformed into methylketone **13**.

After the procedure to transform cetals **4** and **7** into the corresponding methylketones **2** and **13** had been established, we undertook the functionalization of the A rings of **4** and **7**. Two synthetic sequences were developed to achieve our objective, the key step of both being the Baeyer–Villiger rearrangement of aldehyde **15** to give the formate **7**.[3] The refluxing of a solution of **15** in CH_2Cl_2 in the presence of MCPBA afforded formate **7**, which underwent the regioselective elimination of formic acid by heating with collidine, thus yielding **16**. The treatment of **16** with SeO_2–*t*BuOOH in CH_2Cl_2 gave alcohol **17** which was converted into ketone **18** after oxidation with PCC, reduction with Raney nickel[7] and epimerization with MeONa in MeOH (Scheme 4).

We also assayed an alternative route to **18** from **7**, involving saponification, dehydration and oxidation. By treating **7** with KOH in methanol we obtained *nor*-alcohol **19**. The dehydration of **19** with MsCl and pyridine at room temperature yielded a 1:4 mixture of regiosomers **16** and **20**. By treating this under reflux with Na_2CrO_4 in the presence of NaAcO, Ac_2O and AcOH we obtained β-enone **21**, the exocyclic alkene **16** being recovered unaltered. The β-enone **21** was transformed under Birch reduction conditions into ketone **18**, which, after reduction with sodium borohydride, was transformed into **22**, which bears the characteristic A-ring functionalization pattern of the postulated intermediates in the synthesis of pentacyclic quassinoids,[8] with the appropriate configuration on C-3 and C-4 (Scheme 5).



Scheme 2. Reagents and conditions: (i) OsO_4 0.2%, *t*BuOH–H_2O, rt, 15 min; NaIO4, 90 °C, 16 h; Jones, acetone, rt, 3 h (80%).



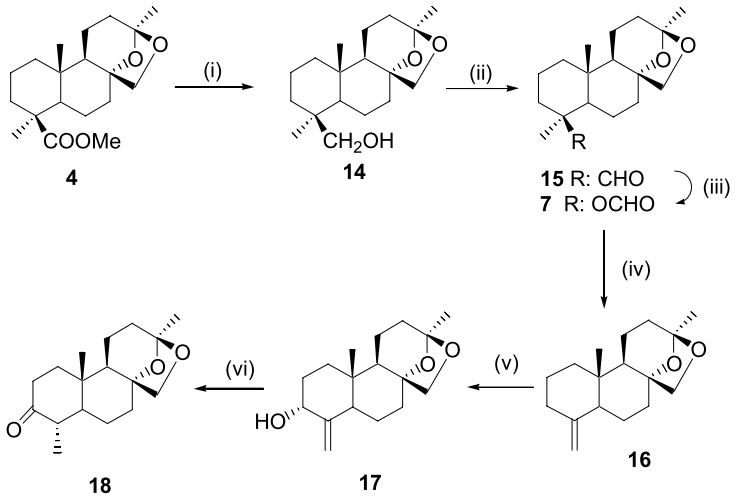
Scheme 3. Reagents and conditions: (i) PCC, CH_2Cl_2, rt, 1 h. (ii) H_2NNH_2, KOH, triethylene glycol, 160 °C, 2 h; CH_2N_2, OEt_2 (95%). (iii) H_2NNH_2, KOH, triethylene glycol, 160 °C, 2 h (95%). (iv) Jones reagent, acetone, 0 °C, 1 h (89%). (v) PCC, CH_2Cl_2, rt, 45 min (90%).

In summary, cetal **4** is a key intermediate for synthesizing pentacyclic quassinoids from communic acids (**1a–c**). This cetal is efficiently converted into the functionalized A-ring compound **22** via a 7-step sequence at a 10% overall yield. **4** is also transformed into methylketone **2**, a precursor of the B–C–D–E ring system of quassinoids, via a 4-step sequence at a 65% overall yield. The successive development of both synthetic sequences will enable to convert **4** into the intermediate **B**, which will then be transformed, following our described procedure,[1] into **C**, a very advanced precursor of quassinoids.

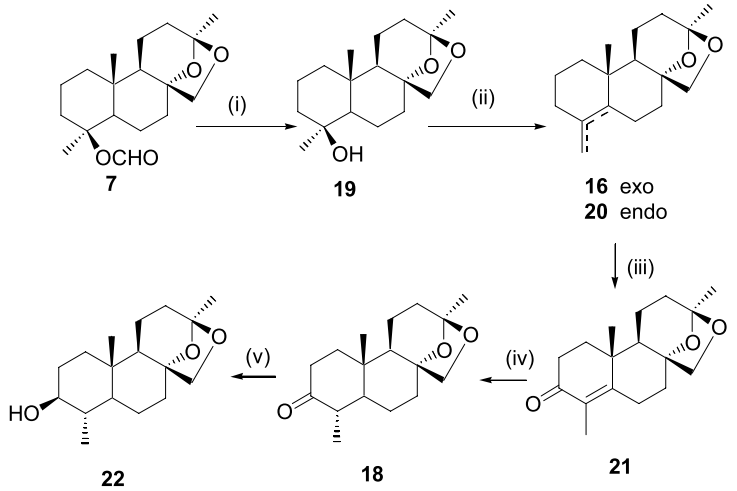
3. Experimental

3.1. General

Melting points were determined with a Kofler hot stage melting point apparatus and are uncorrected. IR spectra were obtained on Perkin–Elmer Models 782 and 983G spectrometers with samples between sodium chloride plates or as potassium bromide pellets. Proton nuclear magnetic



Scheme 4. Reagents and conditions: (i) Ref. 2, 4. (ii) PCC, CH_2Cl_2 rt, 30 min (85%). (iii) MCPBA, CH_2Cl_2, reflux, 16 h (70%). (iv) Collidine, reflux, 12 h (90%). (v) SeO_2, *t*BuOOH, CH_2Cl_2, rt, 12 h (55%). (vi) PCC, CH_2Cl_2; Raney-Ni, THF; MeONa, MeOH, reflux (58%) (yield of 3 steps).



Scheme 5. Reagents and conditions: (i) KOH, MeOH, reflux, 1 h (95%). (ii) MsCl, pyridine, rt, 16 h (90%). (iii) Na_2CrO_4, NaAcO, AcOH, Ac_2O, reflux, 2.5 h (70%). (iv) THF, *t*BuOH, Li, NH_3, −78 °C (85%). (v) $NaBH_4$, EtOH, rt, 1 h (92%).

resonance spectra were taken on a Bruker AMX 300 (300 MHz), Bruker ARX 400 (400 MHz) and Bruker AMX 500 (500 MHz) spectrometers using $CDCl_3$, and CD_3-$COCD_3$ as solvent and TMS or residual protic solvent $CHCl_3$ (δ_H=7.25 ppm) as internal reference, and the multiplicity of a signal is a singlet unless otherwise stated, when the following abbreviations are used: s, singlet; bs, broad singlet; d, doublet; bd, broad doublet; dd, double doublet; t, triplet; m, multiplet. ^{13}C NMR spectra were run at 75 MHz on Bruker AMX 300, ARX 400 and AMX 500 instruments. Chemical shifts are in ppm (δ scale) and the coupling constants are in Hertz. Carbon substitution degrees were established by DEPT pulse sequence. MS were recorded on a Hewlett–Packard 5988A spectrometer using an ionizing voltage of 70 eV. HRMS were obtained on a trisector WG AutoSpecQ spectrometer. For analytical TLC Merck silica gel 60G in 0.25 mm thick layers was used. Chromatographic separations were carried out by conventional column on Merck silica gel 60 (70–230 mesh) and by flash column on Merck silica gel 60 (230–400 mesh) using hexane–MeO^{t}Bu (H–E) mixtures of increasing polarity. Routinely, dry organic solvents were stored under argon, over freshly activated molecular sieves. Ether, benzene, and THF, were dried over sodium-benzophenone ketyl, HMPA from Na, dichloromethane over calcium hydride, and methanol from magnesium methoxide. Where necessary reactions were carried out under a nitrogen or argon atmosphere.

3.1.1. Methyl (8*R*,13*S*)-8α,13:13,17-diepoxy-14,15-dinorlabdane-19-oate (4). A 0.2% aq OsO_4 solution (47 mL) was added to a solution of **5a–b** (17 g, 53.5 mmol (17.0 g, 53.6 mmol) in *t*-BuOH (195 mL) and H_2O (82 mL), and the mixture was stirred for 15 min. Then $NaIO_4$ (60 g, 283 mmol) was added and the mixture was further stirred at 90 °C for 16 h. After filtration, the solvent was evaporated and the residue was fractionated into *t*-BuOMe (150 mL)–H_2O (40 mL). The organic phase was successively washed with 10% aq K_2CO_3 (2×50 mL) and brine (2×50 mL), dried over anhydrous Na_2SO_4 and evaporated to give a crude (16.26 g).

A 2 M solution of Jones' reagent (8 mL) was added slowly to a stirred solution of the above crude (16.26 g) in acetone (75 mL) until orange colour permanence, and the mixture was further stirred at room temperature for 3 h. Then it was poured into H_2O-ice (150 mL) and extracted with Et_2O (4× 50 mL). The organic phase was successively washed with satd aq Na_2CO_3 solution (3×50 mL) and brine (2×50 mL), dried over anhydrous Na_2SO_4 and evaporated to give **4** as a colourless oil (13.8 g, 80%). Compound **4** had identical spectroscopic properties to those reported in the literature.

3.1.2. Methyl (8*R*,13*S*)-8α,13-epoxy-17-hydroxy-14,15-dinorlabdan-19-oate (6a) and methyl (8*R*,13*R*)-8α,13-epoxy-17-hydroxy-14,15-dinorlabdan-19-oate (6b). *Procedure A*: Et_3SiH (0.76 mL, 4.8 mmol) was added to a solution of **4** (0.23 g, 0.75 mmol) in CH_2Cl_2 (10 mL) under an argon atmosphere at −78 °C and the mixture was stirred at this temperature for 10 min. Then a 1.0 M $TiCl_4$ solution (1.45 mL, 1.45 mmol) was added and the reaction was stirred for 1 h. A satd $NaHCO_3$ solution (2 mL) was slowly added and the mixture was stirred for an additional 2 h. The mixture was extracted with ether (3×10 mL) and washed with water (3×10 mL) and brine (3×10 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered and evaporated to give a crude residue which after chromatography (hexane–ether, 3:2) yielded 96 mg (42%) of **6a**, 95 mg (41%) of **6b** and 30 mg (13%) of starting material. **6a**: $[\alpha]_D$=+21.73° (*c* 5.45, CH_2Cl_2). IR (film, cm^{-1}) ν_{max}: 3451, 2948, 2873, 2852, 1725, 1466, 1449, 1378, 1315, 1233, 1207, 1190, 1154, 1044, 1001, 984, 958, 851, 834, 774, 735, 719. ^1H NMR ($CDCl_3$, 400 MHz) δ: 0.51 (3H, s, Me-20), 0.85–1.10 (3H, m), 1.12 (3H, d, *J*=6.1 Hz, Me-16), 1.16 (3H, s, Me-18), 1.2–2.00 (11H, m), 2.00–2.20 (3H, m), 3.59 (1H, dd, *J*=11.1, 1.68 Hz, H-17), 3.61 (3H, s, COO*Me*) 3.67 (1H, dc, *J*=6.1, 2.6 Hz, H-13), 3.91 (1H, d, *J*=11.0 Hz, H-17). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 177,6 (*C*OOMe), 76.7 (C-8), 66.1 (C-13), 58.2 (C-17), 56.7* (C-9), 56.3* (C-5), 51.1 (COO*Me*), 43.8 (C-4), 39.4 (C-1), 38.1 (C-3), 37.2 (C-10), 35.9 (C-12), 34.8 (C-6), 28.5 (C-18), 22.4 (C-16), 21.2 (C-7), 19.2 (C-2), 18.3 (C-11), 13.5 (C-20). FAB HRMS *m/z* calcd for $C_{17}H_{28}O_3Na$ (M^+ + Na) 347.219830, found 347.219691. **6b**: $[\alpha]_D$=+48.84° (*c* 5.1, CH_2Cl_2). IR (film, cm^{-1}) ν_{max}: 3439, 2948, 2874, 1725, 1467, 1447, 1381, 1326, 1234, 1210, 1151, 1101, 1063, 1037, 956, 822. ^1H NMR ($CDCl_3$, 400 MHz) δ: 0.61 (3H, s, Me-20), 0.90–1.11 (3H, m), 1.17 (3H, d, *J*=6.1 Hz, Me-16), 1.20 (3H, s, Me-18), 1.4–2.00 (12H, m), 2.15–2.25 (2H, m), 3.45 (1H, dd, *J*=10.5, 1.2 Hz, H-17), 3.62 (1H, d, *J*= 10.7 Hz, H-17), 3.64 (3H, s, COO*Me*), 4.02 (1H, dc, *J*=6.1, 2.8 Hz, H-13). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 177,7 (*C*OOMe), 76.1 (C-8), 66.3 (C-13), 63.8 (C-17), 56.9* (C-9), 50.2* (C-5), 51.3 (COO*Me*), 43.8 (C-4), 39.3 (C-1), 38.1 (C-3), 37.8 (C-10), 37.2 (C-12), 29.6 (C-6), 28.7 (C-18), 22.8 (C-16), 21.4 (C-7), 19.2 (C-2), 16.0 (C-11), 12.9 (C-20). FAB HRMS *m/z* calcd for $C_{17}H_{28}O_3Na$ (M^+ + Na) 347.219830, found 347.219704.

Procedure B: Et_3SiH (3.93 mL, 24.8 mmol) was added to a solution of **4** (1 g, 3.1 mmol) in CH_2Cl_2 (60 mL) under an argon atmosphere at room temperature, and the mixture was stirred at this temperature for 10 min. Then a 1.0 M $TiCl_4$ solution (7.44 mL, 7.44 mmol) was added, and the reaction was stirred for 2 h. A satd $NaHCO_3$ solution (5 mL) was slowly added and the mixture was stirred for an additional 2 h. The mixture was extracted with ether (3×50 mL) and washed with water (3×30 mL) and brine (3×30 mL). The ethereal phase was dried over anhydrous Na_2SO_4, filtered and evaporated to give a crude residue which after chromatography (hexane–ether, 3:2) yielded 100 mg of **6a**, 545 mg of **6b** and 330 mg of starting material.

3.1.3. (8*R*,13*S*)-8α,13-Epoxy-17-hydroxy-14,15.19-trinorlabdan-4β-yl formate (8a) and (8*R*,13*R*)-8α,13-Epoxy-17-hydroxy-14,15.19-trinorlabdan-4β-yl formate (8b). Et_3SiH (1.23 mL, 8 equiv) was added to a solution of **7** (0.3 g, 0.97 mmol) in CH_2Cl_2 (15 mL) under an argon atmosphere at −78 °C and the mixture was stirred at this temperature for 10 min. Then a 1.0 M $TiCl_4$ solution (2.33 mL, 2.4 mmol) was added, and the reaction was stirred for 1 h. A satd $NaHCO_3$ solution (2 mL) was slowly added and the mixture was stirred for an additional 2 h. Following the same work-up used for **4** and after chromatography (hexane–ether, 1:1) 143 mg of **8a** and 140 mg of **8b** were obtained (95%). **8a**: $[\alpha]_D$=+52.10° (*c*

0.6, CH_2Cl_2). IR (film, cm^{-1}) ν_{max}: 3494, 2931, 2870, 1721, 1650, 1466, 1448, 1203, 1164, 1062, 1043, 957. ^1H NMR ($CDCl_3$, 400 MHz) δ: 0.87 (3H, s, Me-20), 0.96–1.24 (4H, m), 1.16 (3H, d, $J=6.1$ Hz, Me-16), 1.30 (1H, dt, $J=14.4$, 4.7 Hz), 1.54 (3H, s, Me-18), 2.24 (1H, dt, $J=12.9$, 3.3 Hz), 2.69 (1H, dt, $J=12.5$, 1.6 Hz), 2.85 (1H, bs), 3.48 (1H, dd, $J=10.4$, 2.7 Hz, H-17), 3.60 (1H, d, $J=10.4$ Hz, H-17), 3.99 (1H, m, H-13), 8.05 (1H, s, OC*H*O). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 160.4 (O*C*HO), 83.9 (C-4), 75.9 (C-7), 66.4 (C-13), 64.1 (C-17), 56.5* (C-9), 50.2* (C-5), 38.2 (C-12), 36.9 (C-10), 36.9 (C-3), 35.8 (C-1), 29.40 (C-7), 26.2 (C-18), 22.7 (C-16), 19.2 (C-2), 17.6 (C-11), 15.8 (C-6), 14.7 (C-20). FAB HRMS *m/z* calcd for $C_{18}H_{30}O_4Na$ (M^+ + Na) 333.204179, found 333.2046033. **8b**: $[\alpha]_D=+25.48°$ (*c* 2.2, CH_2Cl_2). IR (film, cm^{-1}) ν_{max}: 3458, 2932, 2871, 1720, 1449, 13.77, 1203, 1174, 1055, 1038, 958, 875, 735. ^1H NMR ($CDCl_3$, 400 MHz) δ: 0.82 (3H, s, Me-20), 0.96–1.90 (15H, m), 1.17 (3H, d, $J=5.8$ Hz, Me-16), 1.54 (3H, s, Me-18), 2.22 (1H, dt, $J=12.7$, 3.3 Hz), 2.70 (1H, bd, $J=14.7$ Hz), 3.65 (1H, d, $J=11.1$ Hz, H-17), 3.70 (1H, dc, $J=5.8$, 2.6 Hz, H-13), 3.99 (1H, dd, $J=11.1$, 2.1 Hz, H-17), 8.05 (1H, s, OC*H*O). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 160.4 (O*C*HO), 84.1 (C-4), 76.7 (C-8), 66.4 (C-13), 58.7 (C-17), 56.6* (C-9), 56.6* (C-5), 38.4 (C-12), 37.0 (C-10), 35.9 (C-3), 35.7 (C-1), 34.7 (C-7), 26.2 (C-18), 22.5 (C-16), 19.2 (C-2), 18.2 (C-11), 17.8 (C-6), 15.7 (C-20). FAB HRMS *m/z* calcd for $C_{18}H_{30}O_4Na$ (M^+ + Na) 333.204179, found 333.2046033.

3.1.4. Methyl (8*R*,13*R*)-8α,13-epoxy-17-oxo-14,15-dinorlabdan-19-oate (9b). PCC (167 mg, 1.2 equiv) was added to a solution of **6b** (0.2 g, 0.64 mmol) in CH_2Cl_2 (7 mL) under argon atmosphere, and the mixture was stirred at room temperature for 1 h. Then it was filtered through a short silica gel column (eluted with ether) and the solvent was evaporated under reduced pressure, affording 179 mg (90%) of the aldehyde **9b**. $[\alpha]_D=+46.66°$ (*c* 1.2, CH_2Cl_2). IR (film, cm^{-1}) ν_{max}: 3428, 2948, 2874, 2851, 1725, 1675, 1465, 1449, 1377, 1329, 1232, 1153, 1093, 1032, 984, 942, 820, 774. ^1H NMR ($CDCl_3$, 400 MHz) δ: 0.63 (3H, s, Me-20), 0.80–1.10 (2H, m), 1.14 (3H, d, $J=6.2$ Hz, Me-16), 1.18 (3H, s, Me-18), 1.2–2.00 (11H, m), 2.01–2.33 (3H, m), 3.60 (3H,s, COO*Me*), 4.06 (1H, dc, $J=6.3$, 3.0 Hz, H-13), 9.70 (1H, s, H-17). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 199.8 (C-17), 177,5 (*C*OOMe), 78.6 (C-8), 66.8 (C-13), 56.4* (C-9), 51.3 (COO *C*H_3), 50.1* (C-5), 43.8 (C-4), 39.2 (C-1), 38.3 (C-10), 38.1 (C-3), 35.2 (C-12), 30.4 (C-6), 28.6 (C-18), 22.7 (C-16), 21.1 (C-7), 19.1 (C-2), 15.7 (C-11), 13.1 (Me-10). FAB HRMS *m/z* calcd for $C_{19}H_{30}O_4Na$ (M^+ + Na) 345.204179, found 345.204352.

3.1.5. Methyl (13*R*)-13-Hydroxy-14,15-dinor-8(17)-labden-19-oate (10b). Hydrazine hydrate (0.1 mL) and KOH (104 mg, 6 equiv) was added to a solution of **9b** (100 mg, 0.31 mmol) in triethyleneglycol (5 mL) and the mixture was heated at 160 °C for 2 h. Then the reaction mixture was diluted with water (5 mL) and extracted with *t*BuOMe (3× 10 mL). The combined organic phases were washed with HCl 1 N (2×10 mL) and brine (3×10 mL), and dried over anhydrous Na_2SO_4, filtered, and evaporated. The residue was treated with a solution of diazomethane in ether to give 91 mg (95%) of **10b**. $[\alpha]_D=+66.90°$ (*c* 3.5, CH_2Cl_2). IR (film, cm^{-1}) ν_{max}: 3441, 3080, 2931, 2851, 1725, 1644, 1465, 1451, 1383, 1332, 1316, 1228, 1187, 1154, 1137, 1091, 1031, 988, 889, 808, 775, 666. ^1H NMR ($CDCl_3$, 300 MHz) δ: 0.53 (3H, s, Me-20), 1.00–1.17 (2H, m), 1.19 (3H, d, $J=6.2$ Hz, Me-16), 1.20 (3H, s, Me-18), 1.21–2.1 (11H, m), 2.19 (1H,dc, $J=12.0$, 1.5 Hz), 2.37–2.46 (1H, m), 3.63 (3H, s, COO*Me*), 3.79 (1H, m, H-13), 4.56 (1H, s, H-17), 4.87 (1H, s, H-17). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 177.8 (C-19), 148.1 (C-8), 106.7 (C-17), 68.5 (C-13), 56.5 (C-9), 56.2 (C-5), 51.2 (COO*Me*), 44.4 (C-4), 40.4 (C-10), 39.4 (C-1), 38.9 (C-3), 38.5 (C-12), 38.4 (C-7), 28.9 (C-18), 26.4 (C-6), 23.8 (C-16), 20.1 (C-2), 19.8 (C11), 12.6 (C-20). FAB HRMS *m/z* calcd for $C_{19}H_{32}O_3Na$ (M^+ + Na) 331.224915, found 331.224512.

3.1.6. Methyl 13-oxo-14,15-dinor-8(17)-labden-19-oate (2). A 2 M solution of Jones' reagent (8 mL) was added slowly to a stirred solution of **10b** (0.2 g, 0.75 mmol) in acetone (10 mL) at 0 °C, until orange colour permanence, and the mixture was further stirred at room temperature for 1 h. Then it was poured into H_2O-ice (5 mL) and extracted with Et_2O (3×10 mL). The organic phase was successively washed with satd $NaHCO_3$ (2×10 mL) and brine (2× 10 mL), dried over anhydrous Na_2SO_4 and evaporated to give a crude which after treating with a diazomethane solution in *t*BuOMe afforded **2** as a colourless oil (186 mg, 89%).

3.1.7. (13*R*)-8α,13-Epoxy-17-oxo-14,15.19-trinorlabdan-4β-yl formate (11b). PCC (250 mg) was added to a solution of **8b** (0.3 g, 0.96 mmol) in CH_2Cl_2 (10 mL) under argon atmosphere and the mixture was stirred at room temperature for 1 h. Following the same work-up used for **15**, 268 mg of formate **11b** was obtained (90%). $[\alpha]_D=-3.36°$ (*c* 0.95, CH_2Cl_2). IR (film, cm^{-1}) ν_{max}: 2931, 2871, 1722, 1644, 1448, 1378, 1254, 1203, 1175, 1114, 1062, 1020, 865, 731. ^1H NMR ($CDCl_3$, 400 MHz) δ: 0.89 (3H, s, Me-20), 0.98–1.10 (3H, m), 1.16 (3H, d, $J=6.1$ Hz, Me-16), 1.31 (1H, bt, $J=13.7$ Hz), 1.55 (3H, s, Me-18), 1.45–1.94 (8H, m), 2.37 (1H, dt, $J=12.9$, 3.2 Hz), 2.73 (1H, bd, $J=15.0$ Hz), 4.08 (1H, m, H-13), 8.00 (1H, s, OC*H*O), 9.75 (1H, s, H-17). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 198.5 (C-17), 160.3 (O*C*HO), 83.8 (C-4), 78.4 (C-8), 66.4 (C-13), 55.9* (C-9), 49.6* (C-5), 37.9 (C-12), 37.7 (C-10), 35.6 (C-3), 34.2 (C-1), 30.40 (C-7), 26.2 (C-18), 22.7 (C-16), 19.2 (C-2), 17.5 (C-11), 15.4 (C-6), 14.8 (Me-10). FAB HRMS *m/z* calcd for $C_{18}H_{28}O_4Na$ (M^+ + Na) 331.188529, found 331.188773.

3.1.8. (13*R*)-14,15,19-Trinor-8(17)-labden-4β,13-diol (12b). Hydrazine hydrate (0.1 mL) and KOH (104 mg, 6 equiv) was added to a solution of **11b** (100 mg, 0.31 mmol) in triethyleneglycol (5 mL) and the mixture was heated at 160 °C for 2 h. Following the same work-up used for **9a–b**, and after chromatography (hexane–ether 3:7) 82 mg of **12b** was obtained (95%). $[\alpha]_D=+36.28°$ (*c* 0.35, CH_2Cl_2). IR (film, cm^{-1}) ν_{max}: 3408, 2931, 2847, 1643, 1454, 1374, 1263, 1186, 1125, 1089, 1021, 938, 888, 803, 739. ^1H NMR ($CDCl_3$, 400 MHz) δ: 0.86 (3H, s, Me-20), 1.09 (1H, dt, $J=14.7$, 5.5 Hz), 1.20 (3H, s, Me-18), 1.21 (3H, d, $J=6.2$ Hz, Me-16), 1.25–1.90 (15H, m), 2.03 (1H, dc, $J=12.9$, 5.1 Hz), 2.47 (1H, dc, $J=12.9$, 2.3 Hz), 3.80 (1H, m, H-13), 4.55 (1H, s, H-17), 4.90 (1H, d, $J=1.3$ Hz, H-17). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 148.3 (C-8), 107.1 (C-17), 72.4 (C-4), 69.0 (C-13), 56.2 (C-9), 53.8 (C-5), 41.0

(C-12), 39.7 (C-10), 38.7 (C-1), 38.7 (C-3), 37.9 (C-7), 31.1 (C-18), 23.4 (C-11), 23.6 (C-16), 19.9 (C-2), 18.6 (C-6), 14.1 (C-20). FAB HRMS *m/z* calcd for $C_{17}H_{30}O_2Na$ (M^+ + Na) 289.214350, found 289.213850.

3.1.9. 4β-Hydroxy-14,15,19-trinor-8(17)-labden-13-one (13). PCC (48 mg, 1.2 equiv) was added to a solution of **12b** (50 mg, 0.19 mmol) in CH_2Cl_2 (5 mL) under argon atmosphere, and the mixture was stirred at room temperature for 45 min. Following the same work-up used for **6a–b**, 45 mg of **13** was obtained (90%). $[\alpha]_D = +38.53°$ (*c* 0.25, CH_2Cl_2). IR (film, cm^{-1}) ν_{max}: 3499, 3078, 2930, 2849, 1713, 1643, 1453, 1374, 1261, 1185, 1162, 1096, 1022, 940, 884, 804. ^1H NMR ($CDCl_3$, 400 MHz) δ: 0.87 (3H, s, Me-20), 1.05–1.30 (3H, m), 1.19 (3H, s, Me-18), 1.37–1.95 (10H, m), 2.00 (1H, dt, J=13.1, 5.0 Hz), 2.13 (3H, s, H-16), 2.3–2.4 (1H, m), 2.45 (1H, bd, J=11.5 Hz), 2.60 (1H, ddd, J=9.7, 5.8, 3.9 Hz), 4.49 (1H, s, H-17), 4.89 (1H, s, H-17). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 209.5 (C-13), 147.9 (C-8), 107.1 (C-17), 72.3 (C-4), 55.4 (C-9), 53.7 (C-5), 43.0 (C-12), 39.7 (C-10), 41.0 (C-3), 38.5 (C-1), 37.8 (C-7), 31.1 (C-18), 30.1 (C-16), 23.3 (C-11), 18.6 (C-2), 17.5 (C-6), 14.0 (C-20). FAB HRMS *m/z* calcd for $C_{17}H_{28}O_2Na$ (M^+ + Na) 287.198700, found 287.199107.

3.1.10. (8*R*,13*S*)-8α,13:13,17-Diepoxy-14,15-dinorlabdan-19-al (15). PCC (1.76 g, 1.2 equiv) was added to a solution of **14** (2.0 g, 6.79 mmol) in CH_2Cl_2 (35 mL) under argon atmosphere, and the mixture was stirred at room temperature for 30 min. Then it was filtered through a short silica gel column (eluted with ether) and solvent was evaporated under reduced pressure affording 1.68 g of the aldehyde **15** (85%). $[\alpha]_D = +27.16°$ (*c* 0.4, CH_2Cl_2). IR (film, cm^{-1}) ν_{max}: 2943, 2870, 1717, 1447, 1393, 1269, 1215, 1194, 1159, 1107, 1067, 1029, 979, 921, 865, 818, 739. ^1H NMR ($CDCl_3$, 400 MHz) δ: 0.76 (3H, s, Me-20), 1.05 (3H, s, CH_3-18), 0.83–1.40 (7H, m), 1.43 (3H, s, Me-16), 1.44–1.85 (8H, m), 1.96–2.07 (2H, m), 2.17 (1H, bd, J=14.2 Hz), 3.42 (1H, d, J=7.1 Hz, H-17), 4.29 (1H, d, J=7.1, H-17), 9.72 (1H, s, H-19). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 205.3 (C-19), 106.3 (C-13), 82.2 (C-8), 73.4 (C-17), 56.0 (C-9), 52.3 (C-5), 48.3 (C-4), 37.7 (C-10), 38.3 (C-12), 36.31 (C-3), 36.0 (C-1), 34.3 (C-7), 24.4 (C-18), 24.2 (C-16), 19.8 (C-2), 18.2 (C-6), 17.9 (C-11), 13.8 (C-20). FAB HRMS *m/z* calcd for $C_{17}H_{28}O_3Na$ (M^+ + Na) 293.211670, found 293.211242.

3.1.11. (8*R*,13*S*)-8α,13:13,17-Diepoxy-14,15,19-trinorlabdan-4β-yl formate (7). A solution of MCPBA (2.5 g, 2.5 equiv) in CH_2Cl_2 (20 mL) was added to a solution of the aldehyde **15** (1.1 g, 3.76 mmol) in CH_2Cl_2 (20 mL) and the mixture was stirred under reflux for 16 h. The solvent was evaporated under reduced pressure to give a crude which was dissolved in *t*BuOMe (100 mL) and washed with satd $NaHCO_3$ (5×30 mL), and brine (3×30 mL). The organic phase was dried (Na_2SO_4) and concentrated to give a crude, which after chromatography on silica gel (25% ether–hexane) afforded 0.81 g of **7** (70%). $[\alpha]_D = +23.82°$ (*c* 0.75, CH_2Cl_2). IR (film, cm^{-1}) ν_{max}: 2985, 2935, 2872, 1721, 1449, 1386, 1266, 1204, 1161, 1111, 1027, 866, 816. ^1H NMR ($CDCl_3$, 400 MHz) δ: 0.86–1.07 (3H, m), 0.99 (3H, s, Me-20), 1.33 (1H, dt, J=14.3, 4.4 Hz), 1.42 (3H, s, Me-16), 1.44–1.52 (3H, m), 1.56 (3H, s, Me-18), 1.59–1.96 (8H, m), 2.68 (1H, bd, J=14.7 Hz), 3.40 (1H, d, J=7.1 Hz, H-17), 4.37 (1H, d, J=7.1 Hz, H-17), 8.02 (1H, s, OC*H*O). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 160.5 (O*C*HO), 106.1 (C-13), 83.9 (C-4), 82.3 (C-8), 73.5 (C-17), 55.7 (C-9), 52.9 (C-5), 37.3 (C-10), 38.0 (C-12), 35.9 (C-1), 35.9 (C-3), 35.7 (C-7), 27.2 (C-18), 24.2 (C-16), 19.4 (C-2), 17.4 (C-6), 17.3 (C-11), 14.5 (C-20). FAB HRMS *m/z* calcd for $C_{18}H_{28}O_4Na$ (M^+ + Na) 331.188529, found 331.187695.

3.1.12. (8*R*,13*S*)-8α,13:13,17-Diepoxy-14,15,19-trinor-4(18)-labdene (16). A solution of formate **7** (0.5 g, 1.66 mmol) in collidine (6 mL) was stirred under reflux for 12 h. The mixture was cooled to room temperature, diluted with *t*BuOMe (20 mL) and washed with 2 N HCl (5×10 mL) and brine (3×20 mL). The organic layer was dried and concentrated under reduced pressure to afford 373 mg (90%) of the *nor*-olefin **16**. $[\alpha]_D = +49°$ (*c* 3.0, CH_2Cl_2). IR (film, cm^{-1}) ν_{max}: 3079, 2931, 2869, 1789, 1738, 1646, 1451, 1385, 1238, 1206, 1157, 1111, 1069, 1030, 979, 916, 890, 867, 817. ^1H NMR ($CDCl_3$, 400 MHz) δ: 0.70 (3H, s, Me-20), 0.80–1.00 (1H, m), 1.16 (1H, dt, J=12.8, 4.9 Hz), 1.42 (3H, s, Me-16), 1.50–1.90 (12H, m), 1.95 (1H, dt, J=11.1, 3.2 Hz), 2.29 (1H, bd, J=10.7 Hz), 3.41 (1H, d, J=7.0 Hz, H-17), 4.32 (1H, d, J=7.1 Hz, H-17), 4.42 (1H, s, H-18), 4.72 (1H, s, H-18). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 149.9 (C-4), 106.4 (C-13), 106.3 (C-18), 82.6 (C-8), 73.6 (C-17), 51.5 (C-9), 51.5 (C-5), 38.6 (C-3), 36.4 (C-12), 36.3 (C-1), 35.1 (C-7), 29.8 (C-10), 24.3 (C-16), 22.7 (C-6), 22.5 (C-2), 18.4 (C-11), 12.6 (C-20). FAB HRMS *m/z* calcd for $C_{17}H_{28}O_2Na$ (M^+ + Na) 263.201105, found 263.200116.

3.1.13. (8*R*,13*S*)-8α,13:13,17-Diepoxy-14,15,19-trinor-4(18)-labden-3α-ol (17). A mixture of **16** (250 mg, 0.89 mmol) and SeO_2 (74 mg, 0.75 equiv) in EtOH (10 mL) was added at 0 °C to a 5.5 M solution of *t*-BuOOH in undecane (0.162 mL, 1 equiv). The mixture was stirred at room temperature for 12 h, the solvent evaporated and the residue was fractionated into *t*-BuOMe (30 mL) and water (10 mL). The organic extract was washed with water (3×30 mL) and brine (3×30 mL) and dried (Na_2SO_4), filtered and evaporated to give a crude which after chromatography (hexane–ether, 3:7) gave 136 mg (55%) of **17** as a colourless oil. $[\alpha]_D = +41.13°$ (*c* 2.0, CH_2Cl_2). IR (film, cm^{-1}) ν_{max}: 3442, 3076, 2982, 2937, 2871, 1788, 1712, 1650, 1448, 1386, 1268, 1236, 1199, 1166, 1148, 1113, 1099, 1068, 1028, 984, 967, 940, 894, 865, 817, 736, 701, 680, 600. ^1H NMR ($CDCl_3$, 400 MHz) δ: 0.67 (3H, s, Me-20), 1.20–1.33 (2H, m), 1.42 (3H, s, Me-16), 1.50–1.80 (10H, m), 1.85–1.92 (2H, m), 2.42 (1H, bdd, J=12.0, 1.6 Hz), 3.41 (1H, d, J=7.0 Hz, H-17), 4.27 (1H, t, J=2.8 Hz, H-3), 4.31 (1H, d, J=7.1 Hz, H-17), 4.57 (1H, bs, H-18), 4.96 (1H, bs, H-18). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 151.1 (C-4), 109.4 (C-18), 106.4 (C-13), 82.5 (C-8), 73.6 (C-17), 72.9 (C-3), 51.2 (C-9), 45.0 (C-5), 39.2 (C-10), 36.2 (C-12), 34.9 (C-1), 32.6 (C-7), 29.1 (C-2), 24.3 (C-16), 22.0 (C-6), 18.4 (C-11), 11.8 (C-20). FAB HRMS *m/z* calcd for $C_{16}H_{26}O_3Na$ (M^+ + Na) 279.196020, found 279.196468.

3.1.14. (4*S*,8*R*,13*S*)-8α,13:13,17-Diepoxy-14,15,19-trinorlabdan-3-one (18). PCC (126 mg, 1.2 equiv) was added to a stirred solution of **17** (136 mg, 0.49 mmol) in

CH_2Cl_2 (35 mL) under argon atmosphere, and the mixture was stirred at room temperature for 45 min. Then it was filtered through a short silica gel column (eluted with ether) and the solvent was evaporated under reduced pressure affording a crude which was dissolved in THF (30 mL) and then Raney nickel (1.5 g) (Fluka, cat no. 83440) was added.[†] The mixture was stirred at room temperature for 30 min and filtered through a silica gel bed, affording a crude which was dissolved in a solution of MeONa (40 mg, 1.5 equiv) in MeOH (10 mL). The mixture was stirred at reflux for 1 h and the solvent was evaporated at vacuum, the crude was dissolved in ether (30 mL) and washed with water (3× 30 mL) and brine (3×30 mL) and the ethereal phase was dried over anhydrous Na_2SO_4, filtered and evaporated to give a crude which after chromatography (hexane–ether, 7:3) yielded 79 mg (58%) of **18**. $[\alpha]_D=+25.2°$ (*c* 0.6, CH_2Cl_2). IR (film, cm^{-1}) ν_{max}: 2980, 2949, 2939, 2867, 1708, 1448, 1392, 1367, 1352, 1332, 1305, 1269, 1234, 1199, 1179, 1163, 1134, 1112, 1089, 1066, 1021, 977, 941, 922, 894, 866, 817, 742. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1.00 (3H, d, $J=6.6$ Hz, Me-18), 1.07 (3H, s, Me-20), 1.10–1.40 (2H, m), 1.41 (3H, s, Me-16), 1.42–1.52 (2H, m), 1.55–1.93 (7H, m), 1.99 (1H, ddd, $J=13.3$, 6.7, 2.4 Hz), 2.26 (1H, dda, $J=12.7$, 5.9 Hz), 2.36 (1H, dd, $J=5.6$, 2.5 Hz), 2.42 (1H, ddd, $J=13.3$, 6.7, 0.8 Hz), 3.43 (1H, d, $J=$ 7.0 Hz, H-17), 4.35 (1H, d, $J=7.0$ Hz, H-17). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 212.4 (C-3), 106.5 (C-13), 82.1 (C-8), 73.5 (C-17), 52.8 (C-9), 51.2 (C-5), 44.7 (C-4), 38.4 (C-12), 37.1 (C-2), 36.9 (C-10), 36.1 (C-1), 35.1 (C-7), 24.2 (C-16), 23.6 (C-6), 18.3 (C-11), 12.5 (C-20), 11.8 (C-18). FAB HRMS *m*/*z* calcd for $C_{17}H_{26}O_3Na$ (M^+ +Na) 279.196020, found 279.196479.

3.1.15. (8*R*,13*S*)-8α,13:13,17-Diepoxy-14,15,19-trinor-labdan-4β-ol (19). To a solution of formate **7** (0.7 g, 2.27 mmol) in MeOH (15 mL) a 2 N solution of KOH in MeOH (7 mL) was added and the mixture was refluxed for 1 h. The solvent was evaporated and the crude was diluted with ether (50 mL) and washed with water (3×30 mL) and brine (3×30 mL). The organic layer was dried and concentrated under reduced pressure to afford 0.64 g (95%) of the *nor*-alcohol **19**. $[\alpha]_D=+24.46°$ (*c* 0.5, CH_2Cl_2). IR (film, cm^{-1}) ν_{max}: 3485, 2935, 2872, 1713, 1453, 1385, 1304, 1267, 1189, 1164, 1098, 1066, 1027, 976, 937, 922, 865, 815, 786, 744, 683. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.94 (2H, dt, $J=13.2$, 3.8 Hz), 1.03 (3H, s, Me-20), 1.07 (1H, dd, $J=12.5$, 2.1 Hz), 1.21 (3H, s, Me-18), 1.25–1.40 (2H, m), 1.42 (3H, s, Me-16), 1.43–1.50 (2H, m), 1.55–1.98 (10H, m), 3.39 (1H, d, $J=7.1$ Hz, H-17), 4.37 (1H, d, $J=7.1$ Hz, H-17). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 106.1 (C-13), 82.5 (C-8), 73.7 (C-17), 71.9 (C-4), 53.9 (C-9), 52.8 (C-5), 37.2 (C-10), 41.0 (C-3), 38.4 (C-12), 36.1 (C-1), 35.8 (C-7), 31.07 (C-18), 24.3 (C-16), 19.21 (C-6), 17.6 (C-2), 17.5 (C-11), 14.4 (C-20). FAB HRMS *m*/*z* calcd for $C_{17}H_{28}O_3Na$ (M^+ +Na) 303.193615, found 303.194263.

3.1.16. (8*R*,13*S*)-8α,13:13,17-Diepoxy-14,15,19-trinor-4-labdene (20). MsCl (0.25 mL, 1.5 equiv) was added to a solution of alcohol **19** (0.6 g, 2.14 mmol) in pyridine (10 mL) at 0 °C and the mixture was stirred at room temperature for 16 h. Then the mixture was poured into ice and diluted with ether (50 mL). The organic layer was washed with 2 N HCl (3×20 mL), water (3×15 mL), saturated $NaHCO_3$ (3×15 mL) and brine (3×10 mL). Then it was dried (anh. Na_2SO_4) and concentrated under reduced pressure to give a crude which was after chromatography on silica gel (hexane–ether 85:15) afforded 0.51 g (90%) of a 4:1 mixture of *nor*-olefins **20** and **16**. **20**: $[\alpha]_D=+73.9°$ (*c* 2.5, CH_2Cl_2). IR (film, cm^{-1}) ν_{max}: 2934, 2869, 1799, 1728, 1575, 1449, 1386, 1268, 1239, 1206, 1166, 1130, 1097, 1029, 891, 865, 819, 751. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0.99 (3H, s, Me-20), 1.10–1.35 (2H, m), 1.41 (3H, s, Me-16), 1.61 (3H, s, Me-18), 1.45–2.1 (14H, m), 2.59 (1H, bdd, $J=10.5$, 3.3 Hz), 3.48 (1H, dd, $J=7.0$, 1.2 Hz, H-17), 4.40 (1H, d, $J=7.0$ Hz, H-17). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 134.4 (C-5), 125.6 (C-4), 106.6 (C-13), 82.7 (C-8), 73.3 (C-17), 52.0 (C-9), 38.1 (C-12), 37.7 (C-10), 36.2 (C-3), 35.7 (C-1), 32.7 (C-7), 24.3 (C-16), 23.3 (C-6), 20.1 (C-20), 19.6 (C-18), 18.7 (C-2), 18.6 (C-11). FAB HRMS *m*/*z* calcd for $C_{17}H_{24}O_3Na$ (M^+ +Na) 263.201105, found 263.201103.

3.1.17. (8*R*,13*S*)-8α,13:13,17-Diepoxy-14,15,19-trinor-4-labden-3-one (21). To a solution of the mixture of **20** and **16** (250 mg, 1 mmol) in benzene (15 mL), sodium chromate (216 mg, 1.4 equiv), sodium acetate (390 mg, 5 equiv), acetic anhydride (1.5 mL), and glacial acetic acid (1.2 mL) were added, and the mixture was stirred under reflux for 2.5 h. The reaction mixture was poured into ice and extracted with ether (3×50 mL). The combined organic phases were washed with satd $NaHCO_3$ (3×30 mL) and brine (2×30 mL). The ethereal phase was dried over anhydrous Na_2SO_4, filtered and evaporated to give a crude residue which after chromatography (hexane–ether, 45:55) yielded 185 mg (70%) of **21**. $[\alpha]_D=+63.82°$ (*c* 3.25, CH_2Cl_2). IR (film, cm^{-1}) ν_{max}: 2946, 2874, 1709, 1667, 1614, 1449, 1386, 1359, 1316, 1267, 1239, 1202, 1166, 1139, 1090, 1029, 990, 972, 920, 898, 863, 821, 751, 699, 599. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0.70–1.00 (1H, m), 1.18 (3H, s, Me-20), 1.44 (3H, s, Me-16), 1.50–1.90 (8H, m), 1.80 (3H, s, Me-18), 1.98 (1H, dt, $J=13.2$, 3.0 Hz), 2.41 (1H, dd, $J=5.4$, 3.3 Hz), 2.46 (1H, d, $J=5.1$ Hz), 2.81 (1H, m), 3.57 (1H, d, $J=6.6$ Hz, H-17), 4.43 (1H, d, $J=6.2$ Hz, H-17). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 198.5 (C-3), 160.5 (C-5), 129.3 (C-4), 106.9 (C-13), 81.6 (C-8), 72.9 (C-17), 51.4 (C-9), 39.1 (C-10), 36.0 (C-12), 35.5 (C-2), 33.1 (C-1), 34.0 (C-7), 25.6 (C-6), 24.2 (C-16), 18.1 (C-20), 18.1 (C-11), 11.2 (C-18). FAB HRMS *m*/*z* calcd for $C_{17}H_{24}O_3Na$ (M^+ +Na) 277.180370, found 277.180137.

3.1.18. (4*S*,8*R*,13*S*)-8α,13:13,17-Diepoxy-14,15,19-trinorlabdan-3-one (18). To a solution of ketone **21** (250 mg, 0.9 mmol) in 20 mL of liquid NH_3 was added under an argon atmosphere 10 mL of THF, 1 mL of *t*BuOH and Li (63 mg, 10 equiv) at −78 °C. The mixture was stirred at −45 °C for 3 h. Then the cooling bath was removed to allow the solution to warm to room temperature. The mixture was diluted with ether (50 mL) and washed with water (3×25 mL) and brine (3×25 mL) and the ethereal phase was dried over anhydrous Na_2SO_4, filtered and evaporated to give a crude residue which after

[†] The Raney nickel was weighed as an aqueous slurry after removing four fifty of water.

chromatography (hexane–ether, 7:3) yielded 200 mg (85%) of **18**.

3.1.19. (4*S*,8*R*,13*S*)-8α,13:13,17-Diepoxy-14,15,19-trinorlabdan-3β-ol (22). Sodium borohydride (16 mg, 4 equiv) was added to a solution of ketone **18** (120 mg, 0.43 mmol) in EtOH (5 mL) and the mixture was stirred at room temperature for 1 h. Then 20 mL of ether was added and the organic phase was washed with water (5×10 mL) and brine (3×10 mL), and the ethereal phase was dried over anhydrous Na_2SO_4, filtered and evaporated to give 111 mg (92%) of **22**. $[\alpha]_D = +25.73°$ (*c* 0.25, CH_2Cl_2). IR (film, cm^{-1}) ν_{max}: 3446, 2933, 2870, 1727, 1453, 1386, 1267, 1237, 1206, 1164, 1139, 1106, 1081, 1027, 973, 915, 894, 864, 819, 741. ^1H NMR ($CDCl_3$, 300 MHz) δ: 0.87 (3H, s, Me-20), 1.00 (3H, d, $J=6.4$ Hz, Me-18), 1.10–1.13 (1H, m), 1.25–1.40 (2H, m), 1.43 (3H, s, Me-16), 1.45–1.93 (14H, m), 3.14 (1H, ddd, $J=11.1$, 9.8, 5.1 Hz, H-3), 3.41 (1H, d, $J=7.0$, 2.1 Hz, H-17), 4.33 (1H, d, $J=7.0$ Hz, H-17). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 106.3 (C-13), 82.6 (C-8), 76.4 (C-3), 73.7 (C-17), 51.6 (C-9), 50.8 (C-5), 38.5 (C-4), 36.7 (C-10), 36.3 (C-12), 36.2 (C-1), 30.3 (C-2), 35.5 (C-7), 22.3 (C-16), 22.3 (C-6), 17.9 (C-11), 15.3 (C-18), 12.9 (C-20). FAB HRMS *m/z* calcd for $C_{17}H_{28}O_3Na$ ($M^+ + Na$) 281.211670, found 281.211175.

Acknowledgements

The authors thank the Spanish Ministry of Science and Technology for its financial support (Project PPQ2002-03308).

References and notes

1. Barrero, A. F.; Alvarez-Manzaneda, E. J.; Alvarez-Manzaneda, R.; Chahboun, R.; Meneses, R.; Cuerva, J. M.; Aparicio, M.; Romera, J. L. *Org. Lett.* **2001**, *3*, 647–650.
2. Barrero, A. F.; Altarejos, J.; Alvarez-Manzaneda, E. J.; Ramos, J. M.; Salido, S. *Tetrahedron* **1993**, *49*, 9525–9534.
3. Barrero, A. F.; Alvarez-Manzaneda, E. J.; Alvarez-Manzaneda, R.; Chahboun, R.; Meneses, R.; Aparicio, M. *Synlett* **1999**, 713–716.
4. Barrero, A. F.; Alvarez-Manzaneda, E. J.; Romera Santiago, J. L.; Chahboun, R. *Synlett* **2003**, 2313–2316.
5. (a) Kotsuki, H.; Ushio, Y.; Kadota, I.; Ochi, M. *Chem. Lett.* **1988**, 927–930. (b) Kotsuki, H.; Ushio, Y.; Kadota, I.; Ochi, M. *J. Org. Chem.* **1989**, *54*, 5153–5161. (c) Kotsuki, H. *Synlett* **1992**, 97–106. (d) Ishihara, K.; Mori, A.; Yamamoto, H. *Tetrahedron Lett.* **1987**, *28*, 6613–6616. (e) Ishihara, K.; Mori, A.; Yamamoto, H. *Tetrahedron* **1990**, *46*, 4595–4612. (f) Kim, Y.; Mundy, B. P. *J. Org. Chem.* **1982**, *47*, 3356–3357. (g) Masaki, Y.; Serizawa, Y.; Nagata, K.; Kaji, K. *Chem. Lett.* **1983**, 1601–1602. (h) Lewis, M. D.; Cha, J. K.; Kishi, Y. *J. Am. Chem. Soc.* **1982**, *104*, 4976–4978. (i) Fotsch, C. H.; Chamberlin, A. R. *J. Org. Chem.* **1991**, *56*, 4141–4147.
6. Szmant, H. H. *Angew. Chem. Int. Ed.* **1968**, *7*, 120–128.
7. Barrero, A. F.; Alvarez-Manzaneda, E. J.; Chahboun, R.; Meneses, R. *Synlett* **1999**, 1663–1666.
8. Chiu, C. K.-F.; Govindan, S. V.; Fuchs, P. L. *J. Org. Chem.* **1994**, *59*, 311–323.

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Tetrahedron
Tetrahedron 61 (2005) 845–851

New biologically active linear triterpenes from the bark of three new-caledonian *Cupaniopsis* species

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Received 8 September 2004; revised 10 November 2004; accepted 15 November 2004

Available online 30 November 2004

Abstract—In the course of automated screening for small-molecule agonists to peroxisome proliferator-activated receptor-γ (PPAR-γ), 10 new linear triterpenes **1**–**10** have been isolated from the bark of three neocaledonian *Cupaniopsis* species, SAPINDACEAE. The structures were elucidated by extensive mono- and bi-dimensional spectroscopy and mass spectrometry.

1. Introduction

The peroxisome proliferator-activated receptor (PPAR) is a member of the nuclear hormone receptor superfamily of ligand-activated transcription factors that are related to retinoid, steroid and thyroid hormone receptors. PPAR-γ is probably the isoform focusing most of the attention, since it has become clear that agonists to this isoform could play a therapeutic role in diabetes, obesity, inflammation and cancer.[1] The most described endogenous ligand of PPAR-γ is a prostaglandin, and most of known ligands of the PPAR family are lipophilic coumpounds.[2] In the recent years, a series of PPAR-γ pharmacophores have been developed by chemists, but the high competition in this field encourages the discovery of novel chemical entities.

In an effort to find new naturally occurring PPAR-γ ligands, we screened a series of 1200 plant extracts prepared from species belonging to the New-Caledonian biodiversity. In this report, we describe the isolation, structure elucidation and biological activities of ten new linear triterpenes (**1**–**10**) named cupaniopsins A–E and analogues from the bark of *Cupaniopsis trigonocarpa* Radlk. and Guillaumin, *C. azantha* Radlk. and *C. phallacrocarpa* F. Adema, SAPINDACEAE family. Biological activity was evaluated on fractions obtained from a preliminary standard HPLC fractionation. Subsequent bioactivity-directed fractionation resulted in the isolation of a family of compounds after repeated preparative HPLC.

The genus *Cupaniopsis* consists of some 60 species distributed from South-East Asia to the West pacific region including Australia, Papua-New-Guinea and New-Caledonia. Except for two papers dealing with lipid content,[3,4] the chemical constituents of the genus *Cupaniopsis* are not known.

2. Results and discussion

2.1. Isolation

Ground bark of *C. trigonocarpa*, *C. azantha* and *C. phallacrocarpa* were extracted by ethyl acetate to give crude extracts. A small amount of each extract was submitted to a rapid filtration on a polyamide cartridge in order to remove polyphenols and tannins. The filtered extracts were then submitted to a C-18 HPLC fractionation using a gradient mobile phase of acetonitrile/water 50:50–100:0 in order to obtain nine fractions. Filtered crude extracts and fractions were distributed into 96 deep well mother plates. Bioassay performed on daughter plates allowed to detect the active fractions. These fractions were accumulated by repetitive preparative HPLC and 10 compounds (**1**–**10**) were obtained after further purifications. Based on NMR data obtained from ^{1}H, ^{13}C, COSY, HMQC and HMBC experiments (see Tables 1 and 2 for ^{1}H and ^{13}C NMR data) the compounds could be identified as linear triterpenoids.

Keywords: Triterpenoids; Sapindaceae; Cupaniopsins; *Cupaniopsis trigonocarpa*; *C. azantha*; *C. phallacrocarpa*; PPAR-γ.
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doi:10.1016/j.tet.2004.11.036

Table 1. [1]H NMR spectroscopic data for the compounds **1–10**

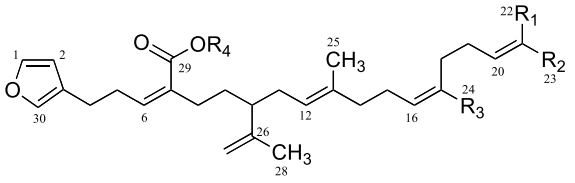
	δ_c and multiplicity									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1-H	7.32, t (1.6)	7.33, s	7.33, t (1.6)	4.20, d (6.8)	7.34, s	7.33, s	7.33, s	4.18, m	7.33, s	
2-H	6.26, s	6.26, s	6.26, s	5.61, t (6.8)	6.27, s	6.26, s	6.26, s	5.59, t (6.7)	6.26, s	5.90, m
4-H	2.53, t (7.4)	2.52, m	2.53, t (7.4)	2.18, m	2.54, t (7.3)	2.54, t (7.4)	2.54, t (7.4)	2.20, t (7.8)	2.57, m	2.54, m
5-H	2.72, dt (7.2, 7.4)	2.73, m	2.66, dt (7.0, 7.4)	2.20, m	2.75, dt (7.1, 7.3)	2.75, dt (7.0, 7.4)	2.75, dt (7.0, 7.4)	2.58, dt (7.6, 7.8)	2.66, dt (7.1, 7.2)	2.72, m
6-H	5.97, t (7.2)	5.98, t (7.3)	5.85, t (7.0)	5.37, m	6.01, t (7.1)	5.99, t (7.0)	5.99, t (7.0)	5.95, t (7.6)	5.85, t (7.1)	5.79, t (7.3)
8-H	2.08/2.20, m	2.09/2.20, mm	2.05/2.20, m	1.93/2.01, m	1.96/2.20, m	1.96/2.20, m	1.96/2.20, m	2.04/2.21, m	2.03/2.18, m	2.08/2.20, m
9-H	1.40/1.50, m	1.38/1.55, m	1.40/2.01, m	1.37/1.43, m	1.46/1.51, m	1.38/1.46, m	1.38/1.46, m	1.46/1.51, m	1.44/1.49. m	1.38/1.44, m
10-H	2.01, m	2.01, m	2.01, m	2.02, m	2.02, m	2.01, m	2.01, m	2.02, m	2.02, m	2.03, m
11-H	2.01, m	2.02, m	2.01, m	2.02, m	2.02, m	2.02, m	2.02, m	1.99, m	2.04, m	2.04, m
12-H	5.09, m	5.08, m	5.07, m	5.06, m	5.08, m	5.02, m	5.02, m	5.06, m	5.06, m	5.07, m
14-H	2.08, m	2.05, m	2.08, m	1.97, m	1.98, m	2.02, m	2.02, m	1.95, m	2.08, m	2.07, m
15-H	2.30, m	2.53, m	2.28, m	2.05, m	1.98, m	2.03, m	2.03, m	1.98, m	2.53, m	2.57, m
16-H	6.85, t (7.3)	5.97, t (7.3)	6.84, t (7.3)	5.08, m	5.10, m	5.08, m	5.08, m	5.10, m	5.99, t (7.1)	5.97, t (7.1)
18-H	2.08, m	2.25, m	2.28, m	1.96, m	1.94, m	2.02, m	2.02, m	1.95, m	2.24, m	2.24, m
19-H	2.30, m	2.10, m	2.08, m	2.05, m	2.06, m	2.03, m	2.03, m	1.98, m	2.11, m	2.08, m
20-H	5.11, m	5.09, m	5.14, m	5.07, m	5.09, m	5.27, m	5.35, m	5.08, m	5.08, m	5.08, m
22-H	1.59, s	1.58, s	1.58, s	1.59, s	1.59, m	4.08, m	1.65, s	1.59, s	1.57, s	1.57, s
23-H	1.66, s	1.67, s	1.66, s	1.67, s	1.66, s	1.78, s	3.99, m	1.67, s	1.66, s	1.66, s
24-H				1.58, s	1.58, s	1.57, s	1.57, s	1.58, s		
25-H	1.58, s	1.58, s	1.58, s	1.58, s	1.57, s	1.56, s	1.56, s	1.58, s	1.58, s	1.57, s
27-H	4.63/4.73, br.s	4.63/4.73, br.s	4.64/4.74, br.s	4.68/4.76, br.s	4.64/4.74, br.s	4.64/4.74, br.s	4.64/4.74, br.s	4.64/4.74, br.s	4.65/4.75, br.s	4.67/4.77, br.s
28-H	1.60, s	1.60, s	1.58, s	1.62, s	1.60, s	1.60, s	1.60, s	1.60, s	1.60, s	1.59, s
29-H				4.01, s						
30-H	7.21, s	7.21, s	3.72, s	4.15, s	7.22, s	7.22, s	7.22, s	4.16, m	3.72, s	3.73, s
31-H			7.21, s						7.21, s	4.75, s

[1]H NMR in $CDCl_3$, 600 MHz for compounds **1–4** and **6–8**, 300 MHz for compounds **5**, **9** and **10**; chemical shifts in ppm, (*J* in Hertz).

Table 2. ^{13}C NMR spectroscopic data for the compounds **1–10**

	δ_c and multiplicity									
C	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1	142.8, d	142.8, d	142.7, d	58.6, t	142.8, t	142.7, t	142.7, t	58.2, t	142.7, d	174.0, s
2	110.9, d	110.9, d	110.9, d	127.1, d	110.9, d	110.9, d	110.9, d	127.3, d	110.9, d	116.1, d
3	124.2, s	124.2, s	124.3, s	143.1, s	122.8, s	124.5, s	124.5, s	142.0, s	124.3, s	169.6, s
4	24.6, t	24.6, t	24.6, t	35.6, t	24.6, t	24.5, t	24.5, t	35.2, t	24.8, t	28.3, t
5	29.8, t	29.9, t	29.7, t	25.9, t	29.9, t	29.7, t	29.7, t	28.4, t	29.9, t	27.1, t
6	143.9, d	143.9, d	140.7, d	125.7, d	144.3, d	144.4, d	144.4, d	142.0, d	140.1, d	138.5, d
7	132.0, s	131.9, s	132.7, s	140.0, s	135.5, s	131.7, s	131.7, s	132.6, s	132.8, s	134.6, s
8	32.4, t	32.6, t	32.5, t	26.2, t	32.3, t	31.9, t	31.9, t	32.4, t	32.3, t	32.7, t
9	31.4, t	31.5, t	32.2, t	31.6, t	32.1, t	31.9, t	31.9, t	32.3, t	32.6, t	32.3, t
10	46.9, d	47.1, d	47.2, d	47.9, d	47.3, d	47.1, d	47.1, d	47.4, d	47.4, d	47.5, d
11	32.1, t	32.2, t	32.1, t	32.2, t	32.3, t	32.1, t	32.1, t	32.1, t	32.3, t	32.3, t
12	124.0, d	123.5, d	124.2, d	122.8, d	124.4, d	125.0, d	125.0, d	122.8, d	123.7, d	123.6, d
13	134.3, s	135.0, s	134.1, s	135.6, s	131.9, s	135.0, s	135.0, s	134.9, s	134.7, s	134.9, s
14	38.4, t	39.3, t	38.5, t	39.8, t	39.7, t	39.1, t	39.1, t	39.7, t	39.3, t	39.6, t
15	26.7, t	28.8, t	26.7, t	26.8, t	26.8, t	26.4, t	26.4, t	26.7, t	28.3, t	28.3, t
16	145.4, d	144.8, d	145.2, d	124.4, d	124.2, d	124.0, d	124.0, d	124.0, d	145.6, d	145.3, d
17	131.2, s	130.9, s	131.0, s	135.0, s	134.8, s	136.0, s	136.0, s	135.0, s	130.5, s	130.6, s
18	27.3, t	34.4, t	27.6, t	39.8, t	39.8, t	39.1, t	39.1, t	39.7, t	34.7, t	34.4, t
19	27.6, t	27.8, t	27.4, t	26.7, t	26.7, t	26.0, t	26.0, t	26.7, t	28.0, t	27.7, t
20	123.6, d	123.4, d	123.9, d	124.2, d	124.2, d	128.4, d	126.3, d	124.0, d	123.4, d	123.5, d
21	132.2, s	132.3, s	132.3, s	131.3, s	131.2, s	134.0, s	134.0, s	131.2, s	132.4, s	132.4, s
22	17.6, q	17.2, q	17.6, q	17.2, q	17.7, q	61.4, t	13.7, q	17.7, q	17.8, q	17.9, q
23	25.7, q	25.7, q	25.5, q	25.7, q	25.6, q	21.5, q	69.0, t	25.7, q	25.8, q	25.8, q
24	173.4, s	173.4, s	171.8, s	16.0, q	16.2, q	16.2, q	16.2, q	15.9, q	171.9, s	171.9, s
25	16.1, q	16.2, q	16.1, q	16.2, q	16.0, q	16.1, q	16.1, q	16.2, q	16.2, q	16.5, q
26	147.2, s	147.3, s	146.9, s	147.4, s	147.2, s	147.1, s	147.1, s	147.2, s	147.1, s	147.2, s
27	111.5, t	111.5, t	111.8, t	111.6, t	111.7, t	111.7, t	111.7, t	111.7, t	111.7, t	111.9, t
28	18.5, q	18.5, q	18.2, q	18.5, t	18.3, q	18.1, q	18.1, q	18.3, q	18.4, q	18.5, q
29	173.4, s	172.9, s	168.5, s	67.1, t	172.0, s	173.6, s	173.6, s	172.0, s	168.5, s	168.2, s
30	139.0, d	139.0, d	51.2, q	60.9, t	138.9, d	138.9, d	138.9, d	60.2, t	51.4, t	51.9, t
31			138.9, d						138.9, d	73.2, t

^{13}C NMR in $CDCl_3$, 150 MHz for compounds **1–4**, **6–8**; 75 MHz for compounds **5**, **9** and **10**; chemical shifts in ppm.



Compound	Δ^{16}	R^1	R^2	R^3	R^4
1	E	CH_3	CH_3	COOH	H
2	Z	CH_3	CH_3	COOH	H
3	E	CH_3	CH_3	COOH	CH_3
5	Z	CH_3	CH_3	CH_3	H
6	Z	CH_2OH	CH_3	CH_3	H
7	Z	CH_3	CH_2OH	CH_3	H
9	Z	CH_3	CH_3	COOH	CH_3

Figure 1. Structure of compounds **1–3**, **5–7** and **9**.

2.2. Structure elucidation of compounds 1–10

The extract of *C. trigonocarpa* afforded four triterpenes (compounds **1–4**), (Figs. 1 and 3). Compound **1** possessed a molecular formula $C_{30}H_{42}O_5$ supported by HRESIMS of the $[M+Na]^+$ ion peak at *m/z* 505.2918 (calcd 505.2930). The IR spectrum of **1** showed absorption bands for carbonyl groups at 1684 cm^{-1}. The UV spectrum showed an absorption band at 218 nm. These data are both consistent with a α,β-unsaturated carboxyl group. The ^1H NMR and ^{13}C NMR spectra of compound **1** suggested a furano-triterpene. The presence of a β-substituted furan ring is deduced from the presence of a fragment ion at *m/z* 81 (C_5H_5O, pyrylium ion) in its EIMS and from the characteristic pattern of the downfield signals in its ^1H NMR spectrum (δ 6.26, 7.21 and 7.32) (Table 1). The ^{13}C NMR spectrum, with carbon multiplicities determined by a DEPT135 experiment, confirmed the presence of two carboxyl groups (both δ 173.4), 10 olefinic carbons (one methylene, four methines, five quaternary) and four aromatic carbons (three CH and one quaternary C) which accounted for all degrees of unsaturation involved by the molecular formula. HMBC and ^1H–^1H COSY correlations (Fig. 2) observed between the six olefinic protons and the α,β-insaturated carbonyl groups or α,β-unsaturated methyl groups allowed to propose the molecular structure **1**. The geometry of the Δ^6 and Δ^{16} double bonds were assigned as (*Z*) and (*E*), respectively, by the high field chemical shift value of 6-H and low-field chemical shift value of 16-H when compared with those of the model compounds (*E*)- and (*Z*)-methyl-2-pentenoic acids,[5] confirmed by observation of cross peaks between 6-H and 8-H_2 in the NOESY spectrum. The geometry of the Δ^{12} double bond could be deduced to be (*E*) from the absence of cross peak between 12-H and 25-H_3 whereas correlations between proton 20-H at δ H 5.11 and 23-H_3 at δ 1.66 are well observed. It was not possible to determine the absolute configuration of the asymmetric C-10. It is interesting to note that the two protons of the methylenes 8 and 9 are not equivalent suggesting that free rotation is hindered by steric repulsion between the carbonyl 29 and the methyl 28. The compound **1** was named *Z,E,E*-cupaniopsin A.

The HRESIMS indicated a molecular formula of $C_{30}H_{42}O_5$ for **2**, deduced from the ion peak at *m/z* 505.2910 $[M+Na]^+$ (calcd 505.2930). Most NMR signals of compounds **2** were nearly identical to those of **1**. The ^1H NMR spectrum of **2** displayed a signal at δ 5.97 (t, $J=7.3$ Hz) for the chemical shift of proton 16 suggesting that the Δ^{16} double bond was (*Z*) which was confirmed by the NOESY spectrum where cross peak was observed between signal at δ 5.97 (16-H) and 2.25 (18-H_2). Consequently, compound **2** is the Δ^{16} isomer of cupaniopsin A, named *Z,E,Z*-cupaniopsin A.

The HRESIMS of **3** revealed an ion peak at *m/z* 519.3103 $[M+Na]^+$ (calcd: 519.3086) giving the molecular formula $C_{31}H_{44}O_5$ and suggesting the presence of an additional CH_2 when compared with **1** and **2**. The ^1H NMR spectrum revealed the presence of a methoxy group at δ 3.72. HMBC correlations observed between the carbonyl at δ 168.5 and the vinylic proton at δ 5.85 (6-H) and the methoxy group at δ 3.72 (30-H_3) allowed to propose structure **3**. Other HMBC and NOESY correlations were comparable with those of *Z,E,E*-cupaniopsin A and thus were fully in agreement with the proposed structure. Compound **3** was identified as the 29-methylester of **1**.

The HRESIMS of compound **4** revealed an ion peak at *m/z* 481.3661 $[M+Na]^+$ (calcd: 481.3658) giving the molecular formula $C_{30}H_{50}O_3$. Compound **4** (Fig. 3) differs from other compounds in the series by the absence of the furan ring and the carboxylic acid groups deduced from the ^1H NMR and ^{13}C NMR spectra. The IR spectrum of **4** showed absorption bands for hydroxy groups at 3420 cm^{-1}. The ^1H NMR revealed one extra olefinic proton at δ 5.61 (t, $J=$ 6.8 Hz) and the presence of three hydroxymethyl groups at δ 4.2, 4.15 and 4.01 and an additional methyl was easily observed in the ^{13}C NMR at δ 16.0 (C-24). In the HMBC

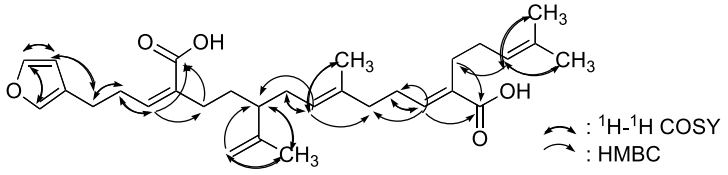
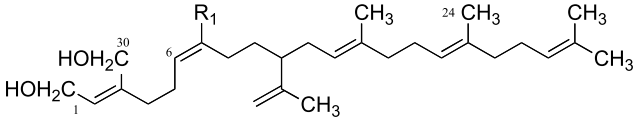


Figure 2. Selected HMBC and COSY correlations for **1**.



Compound	Δ^6	R^1
4	E	CH_2OH
8	Z	COOH

Figure 3. Structure of compounds **4** and **8**.

spectrum the proton at δ 5.61 (2-H) showed correlations with the carbons at δ 60.9 (C-30), 58.6 (C-1) and 35.6 (C-4) suggesting a $(HOCH_2)CH{=}C(CH_2OH)CH_2-$ terminal moiety. Furthermore the HMBC spectrum displayed correlations between 6-H/C-4, C-5, C-8, C-29 and between proton 16-H/C-24 indicating that the previous two carboxylic acid groups were replaced by one primary alcohol and one methyl group. In the NOESY spectrum cross peaks were observed between 2-H and 4-H_2, 6-H and 29-H_2, 16-H and 18-H_2 suggesting the *Z*, *E*, *E* geometry of the Δ^2, Δ^6 and Δ^{16} double bonds, respectively. The geometry of the Δ^{12} (*E*) could be deduced from the absence of any cross peak between 12-H and 25-H_3. Compound **4** was named *Z*,*E*,*E*,*E*-cupaniopsin B.

From the extract of *C. azantha* compounds **5**–**8** were isolated (Figs. 1 and 3). The spectral data for compound **5** were almost identical to those of cupaniopsin A. The ESIMS (positive ion mode) of compound **5** showed an ion peak at *m/z* 475.3 $[M+Na]^+$. In the ^{13}C NMR spectrum **5** differs from **1** by the presence of only one carbonyl carbon at δ 172.0 and an additional methyl at δ 16.2. Examination of the ^1H–^1H COSY and HMBC spectra allowed us to propose the structure of 17-descarboxy-cupaniopsin A for compound **5**. The geometry of Δ^6 and Δ^{12} double bonds could be deduced from identical chemical shifts between compounds **1** and **5** and for the Δ^{16} double bond between **4** and **5** sharing same substructures. Compound **5** was named *Z*,*E*,*Z*-cupaniopsin C.

Compounds **6** and **7** were analyzed by NMR spectrometry as a 1:1 non-separable mixture. The ESIMS (positive ion mode) of the mixture **6** and **7** showed one major peak at *m/z* 491.37 $[M+Na]^+$ suggesting a probable additional hydroxy group when compared with **5**. A careful examination of the spectral data and their comparison with other compounds of the series allowed us to confirm the presence of the hydroxy group in the terminal part of the molecule and to propose structures **6** and **7** for these compounds (Fig. 1). In the HMBC spectrum, the methylene groups at δ 4.08 (**6**) (s, δ_C 61.4) correlated with the vinylic carbons at δ 128.4 (C-20) and 134.0 (C-21) and the methyl group at δ 21.5 (C-23) whereas the methylene groups at δ 3.99 (**7**) (s, δ_C 69.0) correlated with the vinylic carbons at δc 126.3 (C-20) and 134.0 (C-21) and the methyl group at δ 13.7 (C-22). In the ^1H–^1H COSY spectrum, protons at δ 5.27 (**6**) and 5.35 (**7**) (m, 20-H) displayed correlations with signals at δ 1.78 for compound **6** and 1.65 for compound **7** (23-H_3 and 22-H_3, respectively). The geometry of Δ^{20} for compounds **6** and **7**, (*Z*) and (*E*), respectively, could be deduced from the chemical shifts of the olefinic methyls 21.4 and 13.7, respectively.[6] Consequently compounds **6** and **7** were identified as the 22-hydroxy-and 23-hydroxy derivatives of *Z*,*E*,*Z*-cupaniopsin C **5**.

Most NMR signals of compounds **8** were nearly identical to those of **4** characterized by the absence of a furan ring at the terminal part of the molecule. The main difference was given by the IR spectrum of **8** which showed absorption band for a carbonyl group at 1701 cm^{-1}. This was confirmed in the ^{13}C NMR spectrum by the presence of a peak at δ 172.0 and by the fact that only two oxygen bearing-carbons are displayed instead of three for compound **4**. The ESIMS (positive ion mode) of compound **8** showed a molecular ion peak at *m/z* 472.3. In the HMBC spectrum correlations between signals at δ 5.95 (6-H), 2.04 and 2.21 (8-H_2) and the carbonyl at δ 172.0 (C-29) indicated the position of the carboxylic acid at C-7. Other HMBC correlations, identical with those of compound **4** allowed us to propose structure **8**. The geometry of the double bonds was confirmed by the observation of cross peak between signals at δ 5.59 (2-H) and 2.20 (4-H_2) for Δ^2 (*Z*), and deduced from the absence of any cross peak between the two olefinic protons in positions 12 and 16 and the methyl groups in positions 13 and 17 in the NOESY spectrum suggesting that Δ^{12} and Δ^{16} were both *E*. Finally the chemical shift at δ 5.95 for proton 6-H was in favor of a (*Z*) geometry for Δ^6 double bond.[5] Compound **8** was named *Z*,*Z*,*E*,*E*-cupaniopsin D.

The extract of *C. phallacrocarpa* afforded compounds **9** and **10** (Figs. 1 and 4). Most NMR signals of compounds **9** were nearly identical to those of **3** characterized by the presence of a methyl carboxylate in position 7. The ESIMS of **9** revealed the main peak at *m/z* 519.2 $[M+Na]^+$. The ^1H NMR spectrum of **9** displayed a chemical shift of 5.99 for proton 16 suggesting that Δ^{16} double bond was (*Z*), which was confirmed in the NOESY spectrum where a cross peak was observed between 16-H and 18-H_2. HMBC and ^1H-^1H COSY correlations were identical with those observed for compound **3**. Consequently compound **9** is the Δ^{16} isomer of compound **3** named *Z*,*E*,*Z*-cupaniopsin A methyl ester.

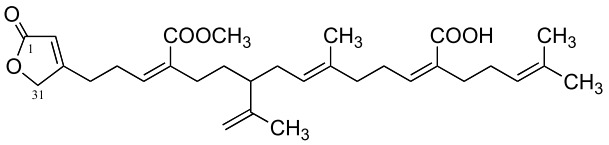


Figure 4. Structure of compound **10**.

The HRESIMS indicated a molecular formula of $C_{31}H_{44}O_6$ for **10**, deduced from the ion peak at *m/z* 535.3035 $[M+Na]^+$ (calcd 535.3036) with consequently a same degree of unsaturation as compound **1**. Examination of the ^1H and ^{13}C NMR spectra revealed the absence of a furan ring but the presence of three carbonyls. The ^1H–^1H COSY spectrum showed weak correlations between the olefinic proton at δ 5.90 (s, 2-H) and methylene 31-H_2 and between the latter and methylene 4-H_2. In the HMBC spectrum, correlations between methylene 31-H_2 and carbons C-1, C-2 and C-3, and correlations between methylene 4-H_2 and carbons C-2, C-3, C-5, C-6 and C-31 allowed us to propose a substituted α,β-unsaturated lactone for the west terminal part of compound **10**. The methylene groups C-31 at δ 73.2 was shifted downfield when compared with compounds **4** and **8**, which is fully in agreement with the chemical shift observed in a lactone moiety.[7] Other HMBC correlations for **10** were almost identical with those of compound **3** for the central part, and compound **2** for the east terminal moiety allowing us to propose structure **10**. The geometry of the Δ^6 and Δ^{16} double bonds were both assigned as (*Z*) by comparison between the high-field chemical shifts of H-6 and H-16 olefinic protons and those of the model compounds (*E*)- and (*Z*)-methyl-2-pentenoic acids.[5] It was confirmed in the

NOESY spectrum by the presence of cross peaks between the vinylic proton 6-H and the methylene groups 8-H_2, and between the vinylic protons 16-H and the methylene group 18-H_2. The geometry of the Δ^{12} double bond could be deduced to be (*E*) by absence of cross peak between H-12 and 25-H_3. Compound **10** was named *Z*,*E*,*Z*-cupaniopsin E.

3. Biological activity

The binding affinities of compounds **1**–**10** for PPAR-γ were evaluated by competition against an isotopically labeled reference compound (rosiglitazone), as described earlier.[8] Results are given by the K_i value which is the inhibition constant of a compound determined at equilibrium in competition with a reference ligand.

The K_i obtained for the different compounds are summarized in Table 3, indicating that five of the 10 compounds exhibited potent binding activity on PPAR-γ, the most potent being *Z*,*E*,*E*-cupaniopsin A **1**. The overall structural pattern of these compounds is consistent with the general properties of PPAR-γ ligands, being most often lipophilic, long polyunsaturated hydrocarbon chains. Moreover, examination of the results allowed us to determine some structure/activity relationships. A better activity is observed when the furan ring is present in the structure. Cupaniopsins B, D and E are less active (**4**, **8** and **10**, respectively) than compounds **1**–**3**, **5** and **9**. The mixture of compounds **6**+**7** is significantly less active, consequently it appears that the terminal hydroxyl contributes to greatly decrease the biological activity. The presence of two carboxyls in positions **7** and **17** are necessary for a strong binding activity; compounds which possess either a methyl carboxylate in position 7 (compounds **3** and **9**) or a methyl group in position 17 (compound **5**) are significantly less active. Finally, compounds **1** and **3** are more potent that compounds **2** and **9**, respectively, which indicate that Δ^{16} geometry (*E*) is more favorable than (*Z*) for the biological activity. Details on biological activity studies will be described later.

Table 3. Biological activity data obtained for **1**–**10**

Cpd	K_i (nM)
1	15
2	44
3	190
4	>10,000
5	240
6+**7**	>10,000
8	>10,000
9	480
10	2900

4. Conclusions

In New-Caledonia the genus *Cupaniopsis* includes 28 species. The bark crude extracts of three of them exhibit a significant activity on PPAR-γ receptor, a nuclear receptor involved in diabetes, obesity, inflammation and cancer. Chemical studies have led to the identification of ten linear triterpenes, named cupaniopsins. This is the first report of linear furanotriterpenes isolated from plant kingdom. Linear furanotriterpenes have been previously isolated from several species of sponges.[9–11] Their isolation is a consequence of a random screening of New-Caledonian plant extracts on a given receptor associated with the use of a new strategy for the search of new active natural products.

5. Experimental

5.1. General

NMR spectra were recorded with a Bruker spectrometer (300 MHz for ^{1}H and 75 MHz for ^{13}C (compounds **5**, **9** and **10**) and 600 MHz for ^{1}H and 150 MHz for ^{13}C (compounds **1**–**4** and **6**–**8**) using $CDCl_3$ as solvent. ESIMS was obtained on a Navigator mass Thermoquest. HRESIMS were obtained on a MALDI-TOF spectrometer (Voyager-De STR; Perseptive Biosystems). The HPLC separations were performed using a Waters autopurification equipped with a UV–Vis diode array detector (190–600 nm) and a Pl-ELS 1000 ELSD detector Polymer laboratory. IR spectra were obtained on a Nicolet FTIR 205 spectrophotometer. The UV spectra were recorded on a Perkin–Elmer Lamba 5 spectrophotometer.

5.2. Plant material

Bark of *C. trigonocarpa* was collected in December 1998 in the sclerophyllous forest of Tiea, North Province, *C. phallacrocarpa* in October 1995 in the dense rain forest of Aoupinié, North Province, East coast, and *C. azantha* in July 1998 in 'Forêt Nord', South Province, West coast, New-Caledonia by one of us (M.L). The corresponding voucher specimens LIT 0672, LIT 0051 and LIT 0565 are kept at the Herbarium of the Botanical and Tropical Ecology Department of the IRD Center, Noumea, New-Caledonia.

5.3. Extraction and isolation

A fraction of the ethyl acetate extract prepared from air-dried and ground bark of each species was dissolved by a small amount of methanol and loaded onto a polyamide cartridge. The cartridge was washed with methanol and the organic solvent was removed under vacuum. The organic extract was then solved in DMSO (15 mg in 300 μL), filtered through a 0.45 μm nylon and injected on a semi-preparative Kromasil® C-18 column (250×10 mm I.D, 5 μm). A gradient mobile phase consisting of acetonitrile/water 50:50–100:0 acetonitrile at 3 mL/min in 40 min followed by 100% acetonitrile in 30 min was used in order to obtain nine fractions (24 mL each). The detection was performed using a diode array detector and an evaporative light scattering detector after that 1/1000 of the eluant was split. The filtered crude extract and the nine fractions were evaporated under vacuum and dissolved with an appropriate amount of DMSO at a concentration of 10 mg/mL and distributed into a 96 deep wells mother plate. Daughter plates at a concentration of 1 mg/mL were prepared and used for the bioassay. Further separations were performed on the active fractions (fr. 5 and 6, 40–52 min for *C. trigonocarpa* and *C. phallacrocarpa*, fr. 5 for *C. azantha*).

From 360 mg of the filtered extract of barks of *C. trigonocarpa*, 86 mg of the active fractions were obtained after repeated injections on a preparative Kromasil® C-18 column (250×21.2 mm I.D, 5 μ) at 21.2 ml/min. Twenty compounds ranging from 0.1 to 19 mg were separated using a preparative column with a gradient consisting of acetonitrile/water 70:30–100:0 acetonitrile in 40 min. The bioassay revealed that compounds **1**–**4** at retention time of 19, 20.5, 29.5 and 24 min, respectively, were the active molecules. Finally isolation of the other compounds of the series have been performed from the crude ethyl acetate extracts of the two others species after having established appropriate analytical conditions and compared their UV spectra with those of compounds **1**–**4**. Compounds **5**–**8** were isolated from *C. azantha* using a gradient acetonitrile/water 80:20–100:0 in 65 min, 0.1% TFA, at 4.7 mL/min for **5** (retention time of 40.20 min) and isocratic mobile phase acetonitrile/water 80:20, 0.1% TFA at 4.7 mL/min for compounds **6**+**7** (retention time of 18.15 min) and **8** (retention time 20.62 min). Compounds **9** (retention time of 37.5 min) and **10** (retention time at 14.0 min) were isolated from *C. phallacrocarpa* using the same isocratic mobile phase.

5.3.1. Compound 1. Gummy solid. $[\alpha]_D^{25}=+4.2$ ($c=1$, CH_3OH); UV ($CHCl_3$) λ_{max} (ε) 200 (11800), 218 (9330); IR (KBr) υ_{max}: 2926, 1684 cm^{-1}. ^1H NMR ($CDCl_3$, 600 MHz and ^{13}C NMR ($CDCl_3$, 150 MHz) see Tables 1 and 2. HRESIMS, *m/z* $[M+Na]^+$ 505.2918 ($C_{30}H_{42}O_5Na$ calcd for 505.2930).

5.3.2. Compound 2. Gummy solid. $[\alpha]_D^{25}=+4.8$ ($c=0.8$, CH_3OH); UV ($CHCl_3$) λ_{max} (ε) 200 (10900), 218 (7000); IR (KBr) υ_{max}: 2963, 1694 cm^{-1}. ^1H NMR ($CDCl_3$, 600 MHz and ^{13}C NMR ($CDCl_3$, 150 MHz) see Tables 1 and 2. HRESIMS, *m/z* $[M+Na]^+$ 505.2910 ($C_{30}H_{42}O_5Na$ calcd for 505.2930).

5.3.3. Compound 3. Gummy solid. $[\alpha]_D^{25}=+4.5$ ($c=1.0$, CH_3OH); UV ($CHCl_3$) λ_{max} (ε) 200 (8600), 221 (5700); IR (KBr) υ_{max}: 3419, 2926, 1715 cm^{-1}. ^1H NMR ($CDCl_3$, 600 MHz and ^{13}C NMR ($CDCl_3$, 150 MHz) see Tables 1 and 2. HRESIMS, *m/z* $[M+Na]^+$ 519.3103 ($C_{31}H_{44}O_5Na$ calcd for 519.3086).

5.3.4. Compound 4. Gummy solid. $[\alpha]_D^{25}=+3.3$ ($c=1$, CH_3OH); UV (MeOH) λ_{max} (ε) 203 (37500); IR (KBr) υ_{max}: 3366, 2926 cm^{-1}. ^1H NMR ($CDCl_3$, 600 MHz and ^{13}C NMR ($CDCl_3$, 150 MHz) see Tables 1 and 2. HRESIMS, *m/z* $[M+Na]^+$ 481.3661 ($C_{30}H_{50}O_3Na$ calcd for 481.3658).

5.3.5. Compound 5. Gummy solid. $[\alpha]_D^{25}=+3.1$ ($c=0.7$, CH_3OH); UV ($CHCl_3$) λ_{max} (ε) 200 (8600); IR (KBr) υ_{max}: 2929, 1702 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz and ^{13}C NMR ($CDCl_3$, 75 MHz) see Tables 1 and 2. EIMS, *m/z* $[M+Na]^+$ 475.39 ($C_{30}H_{44}O_3Na$).

5.3.6. Compounds 6+7. Gummy solid. UV ($CHCl_3$) λ_{max} (ε) 200 (10700), IR (KBr) υ_{max}: 3420, 2924, 1716 cm^{-1}, ^1H NMR ($CDCl_3$, 600 MHz and ^{13}C NMR ($CDCl_3$, 150 MHz) see Tables 1 and 2. EIMS, *m/z* $[M+Na]^+$ 491.37 ($C_{30}H_{44}O_4Na$).

5.3.7. Compound 8. Gummy solid. $[\alpha]_D^{25}=+3.2$ ($c=0.65$, CH_3OH); UV (MeOH) λ_{max} (ε) 260 (45400); IR (KBr) υ_{max}: 3419, 2926, 1701 cm^{-1}. ^1H NMR ($CDCl_3$, 600 MHz and ^{13}C NMR ($CDCl_3$, 150 MHz) see Tables 1 and 2. EIMS, *m/z* $[M+Na]^+$ 472.36 ($C_{30}H_{48}O_4Na$).

5.3.8. Compound 9. Gummy solid. $[\alpha]_D^{25}=+4.4$ ($c=0.56$, CH_3OH); UV (MeOH) λ_{max} (ε) 210 (46900), IR (KBr) υ_{max}: 3419, 2926, 1716 cm^{-1}, ^1H NMR ($CDCl_3$, 300 MHz and ^{13}C NMR ($CDCl_3$, 75 MHz) see Tables 1 and 2. EIMS, *m/z* 519.2 $[M+Na]^+$ ($C_{31}H_{44}O_5Na$).

5.3.9. Compound 10. Gummy solid. $[\alpha]_D^{25}=+25.7$ ($c=0.21$, CH_3OH), UV (MeOH) λ_{max} (ε) 213 (48400), IR (KBr) υ_{max}: 3420, 2933, 1734 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz and ^{13}C NMR ($CDCl_3$, 75 MHz) see Tables 1 and 2. HRESIMS, *m/z* $[M+Na]^+$ 535.3035 ($C_{31}H_{44}O_6Na$ calcd for 535.3036).

5.4. Biological activity

The binding affinity of the compounds was evaluated on PPAR-γ by competition against an isotopically labeled reference compound (rosiglitazone), as described earlier.[8] Details on biological activity studies will be described later.

Acknowledgements

The authors are very grateful to Dr. Tanguy Jaffré and Dr. Jean-Marie Veillon of the Botany and Plant Ecology Department, Research Institute for the Development, Nouméa, for their help in the identification of the plants, Vincent Dumontet, Bruno Fogliani and Pelenato Maituku, for their technical assistance in the collection of the various species.

References and notes

1. Murphy, G. J.; Holder, J. C. *Trends Pharmacol. Sci.* **2000**, *21*, 469–474.
2. Nosjean, O.; Boutin, J. A. *Cell. Signal* **2002**, *14*, 573–583.
3. Finkelstein, S.; Grubb, P. J. *Seed Sci. Res.* **2002**, *12*, 173–180.
4. Mustafa, J.; Gupta, A.; Agarwal, R.; Osman, S. M. *J. Am. Oil Chem. Soc.* **1986**, *63*, 671–672.
5. Bohlmann, F.; Zdero, C. *Chem. Ber.* **1974**, *107*, 2912–2922.
6. Kwak, J. H.; Kyung, B. L.; Schmitz, F. J. *J. Nat. Prod.* **2001**, *64*, 1081–1083.
7. Shiina, J.; Nihiyama, S. *Tetrahedron* **2003**, *59*, 6039–6044.
8. Ferry, G.; Bruneau, V.; Beauverger, P.; Goussard, M.; Rodriguez, M.; Lamamy, V.; Dromaint, S.; Canet, E.; Galizzi, J. P.; Boutin, J. A. *Eur. J. Pharmacol.* **2001**, *417*, 77–89.
9. Erdogan, I.; Sener, B. *Acta Pharma. Turcica.* **2001**, *43*, 17–19.
10. Bifulco, G.; Bruno, I.; Minale, L.; Riccio, R.; Debitus, C.; Bourdy, G.; Vassas, A.; Lavayre, J. *J. Nat. Prod.* **1995**, *58*, 1444–1449.
11. Cimino, G.; de Stefano, S.; Minale, L. *Tetrahedron* **1972**, *28*, 1315–1324.

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Tetrahedron 61 (2005) 853–861
Tetrahedron

Reaction of some 4,6-dimethoxyindoles with nitric acid: nitration and oxidative dimerisation

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Received 30 August 2004; revised 25 October 2004; accepted 12 November 2004

Available online 10 December 2004

Abstract—A range of 3-substituted-4,6-dimethoxyindoles bearing electron-withdrawing groups in either the 2- or 7-position, can be nitrated using nitric acid in acetonitrile, to give 7-nitro and 2-nitro-indoles, respectively. Those without electron-withdrawing groups undergo oxidative dimerisation at C7, if 2,3-disubstituted, and at C2, if *N*-methylated and unsubstituted at C2.

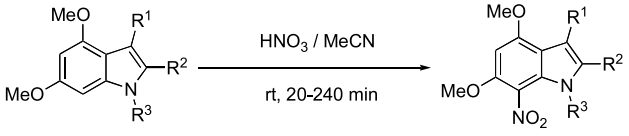
1. Introduction

The nitration of indoles has been the subject of investigation for many years, and is difficult to achieve in a clean and selective manner.[1–3] We have recently reported that selective nitration can be carried out in the case of 3-substituted-4,6-dimethoxyindoles, provided that an electron-withdrawing substituent is also present.[4] Thus, if the electron-withdrawing substituent is at C2, nitration takes place at C7, whereas if the electron-withdrawing substituent is at C7, nitration occurs at C2. The very effective reagent used in these experiments was concentrated nitric acid on silica. Many years ago, we found that concentrated nitric acid in acetonitrile was a highly successful reagent for the oxidation of active methylene compounds,[5] possibly via intermediate nitro-aliphatic structures. We therefore decided to investigate the use of this reagent on activated 4,6-dimethoxyindoles.

2. Results and discussion

2.1. Reaction of 4,6-dimethoxyindoles with C2 electron-withdrawing substituents

The 3-aryl-4,6-dimethoxyindoles **1–6**, each containing carbonyl substituents at C2, underwent smooth reaction with concentrated nitric acid in acetonitrile at room temperature over 20–240 min to give the related 7-nitro compounds **7–12** in yields of 63–85%. None of the indoles **1–6** have been reported previously, but are minor variants of published compounds.[6] All the nitro compounds **7–12** are new, but the methyl ester analog of compound **9** has already been reported.[4] It is significant that 7-nitration still occurs in high yield for the *N*-methylindole **6**. The facility of this reagent system is comparable to the use of nitric acid on silica, being extremely simple: the only potential problem lies in monitoring the progress of the reaction, so as not to allow it to proceed for too long. In most cases, especially for the faster reactions, there is a useful colour change from yellow to green, as the conversion is completed.



	R^1	R^2	R^3		Yield %
1	4-BrC_6H_4	COMe	H	**7**	75
2	4-BrC_6H_4	CO_2Et	H	**8**	66
3	4-BrC_6H_4	$COCO_2Et$	H	**9**	69
4	4-BrC_6H_4	COCONHBu	H	**10**	72
5	4-BrC_6H_4	$COCONEt_2$	H	**11**	63
6	4-BrC_6H_4	$COCO_2Et$	Me	**12**	85

2.2. Reaction of 4,6-dimethoxyindoles with C7 electron-withdrawing substituents

The 3-aryl-4,6-dimethoxyindoles **13–18**, each containing carbonyl substituents at C7, underwent smooth reaction

Keywords: Indoles; Nitric acid; Nitration; Oxidative dimerisation.
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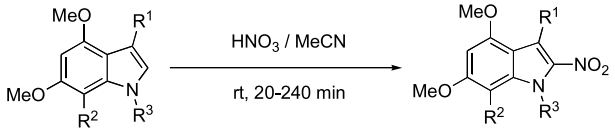
doi:10.1016/j.tet.2004.11.026

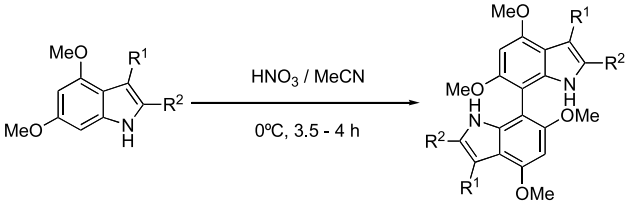
with concentrated nitric acid in acetonitrile at room temperature over 20–240 min to give the related 2-nitro compounds **19**–**24** in yields of 35–88%. Indoles **13** and **14** have been reported previously,[7,8] while indoles **15**–**18** have not, but are minor variants of published compounds.[6] The nitro compound **19** has been reported,[4] but compounds **20**–**24** are new, and expand the range of rare 2-nitro-indoles. The unusually low yield of 35% occurs in the case of the most highly activated 3-(4-methoxyphenyl)-substituted indole **14**. Again, it is significant that the *N*-methylindole **18** undergoes 2-nitration in high yield.



	R^1	R^2	R^3		Yield %
13	4-BrC_6H_4	CHO	H	**19**	65
14	4-$MeOC_6H_4$	CHO	H	**20**	35
15	4-BrC_6H_4	$COCO_2Et$	H	**21**	85
16	4-BrC_6H_4	COCONHBu	H	**22**	68
17	4-BrC_6H_4	$COCONEt_2$	H	**23**	74
18	4-BrC_6H_4	$COCO_2Et$	Me	**24**	88

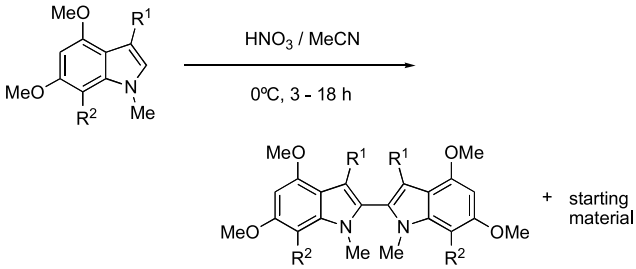
2.3. Reaction of 4,6-dimethoxyindoles without electron-withdrawing substituents

Nitration of activated indoles without the stabilizing influence of electron-withdrawing substituents has been found to be very difficult to control.[4] Nevertheless, the simple conditions of concentrated nitric acid in acetonitrile warranted their investigation, in case clean and selective reactions could be achieved. Thus the 2,3-disubstituted indoles **25**–**27** were found to undergo smooth reaction over 3.5–4 h at 0 °C, to afford the 7,7′-biindolyls **28**–**30** in high yield. While the dimer **28** is a very well known compound,[9] accessible by oxidative dimerisation from indole **25** using a wide range of quinone and other oxidizing agents, dimers **29** and **30** are new. Indeed, attempts to generate them by quinone oxidation of indoles **26** and **27** respectively, have failed, presumably because of the susceptibility of the 2-methyl group to indiscriminate oxidation.[10]



	R^1	R^2		Yield %
25	Ph	Ph	**28**	86
26	Ph	Me	**29**	82
27	4-BrC_6H_4	Me	**30**	82

Reactions of 3-substituted-4,6-dimethoxyindoles with nitric acid in acetonitrile were found to be indiscriminate and led to complex product mixtures, unless the indole nitrogen was substituted by a methyl group. The new *N*-methylindoles **31**–**34** underwent reaction at 0 °C during 3–18 h to afford the 2,2′-biindolyls **35**–**38** in 25–72% yields. The structures of the 2,2′-biindolyls **35**–**38** were evident from their ^1H and ^{13}C NMR and mass spectra, and the compounds were fully characterized. Although the yields of the 2,2′-biindolyls are only moderate good recovery of starting material can also be achieved. Also the reactions are clean and the methodology is very direct and compares favourably with the copper coupling of 2-iodo-1-methylindole.[11]



	R^1	R^2		Yield %	Yield %
31	Me	H	**35**	35	39
32	Ph	H	**36**	25	59
33	4-BrC_6H_4	H	**37**	63	24
34	4-BrC_6H_4	Me	**38**	72	22

Attempts to extend this methodology to non-activated indoles were not successful. Treatment of 1-methylindole and 1,3-dimethylindole with concentrated nitric acid in acetonitrile rapidly switched from no reaction to the formation of complex product mixtures. Thus it seems that the methoxy groups play an important role in activating both the 2- and 7-positions in the nitration and oxidative coupling reactions. In the coupling reactions, the nitric acid could be acting as an oxidizing agent to generate a radical cation, which leads to dimerisation of radicals at C2 and C7. It is also significant that dimerisation of *N*-methylindoles **31**–**33**, which are potentially capable of dimerising at C2 or C7, actually occurs selectively at C2. Presumably the reaction is sterically controlled, as the *N*-methyl analog of indole **25** cannot be oxidatively dimerised by reaction with quinones, even though the *N*-methyl analog of the 7,7′-dimer **28** can be prepared as a stable compound by direct methylation of compound **28**.[9]

3. Conclusions

Concentrated nitric acid in acetonitrile offers simple and effective conditions for the conversion of a range of relatively activated indoles into related nitro compounds. Some more highly activated indoles, provided that they are substituted at nitrogen, undergo very useful oxidative dimerisation processes at C7 or at C2. The nitration reaction provides an effective route to relatively rare nitro-indoles, while the oxidative coupling process has particular potential for future development.

4. Experimental

Melting points were determined by an Electrothermal melting point apparatus and were uncorrected. The ^{1}H and ^{13}C NMR, and DEPT spectra were recorded on Bruker DPX 300 MHz spectrometer in $CDCl_3$ using tetramethylsilane as an internal standard. Infrared spectra were recorded on a Spectrum GX FT-IR system 2000 (Perkin–Elmer) spectrometer. Elemental analyses were performed on a Perkin Elmer Elemental Analyser 2400 CHN. Mass spectra were recorded on a Finnigan MAT INCOS 50 mass spectrometer. The ESITOf mass spectra were obtained from a Micromass LCT mass spectrometer. Merck silica gel 60 PF_{254} was used for preparative thin layer chromatography (PLC). Acetonitrile and 65% nitric acid were commercial materials.

4.1. Preparation of indole substrates

4.1.1. 2-Acetyl-3-(4-bromophenyl)-4,6-dimethoxyindole 1. To a solution of 3-(4-bromophenyl)-4,6-dimethoxyindole (105.3 mg, 0.317 mmol) in dry dichloromethane (10 mL) at 0 °C was added $SnCl_4$ (107.3 mg, 0.412 mmol) and the mixture stirred at 0 °C for 45 min. To this solution was added acetyl chloride (36.2 mg, 0.432 mmol) and the mixture stirred at 0 °C for 2 h. After pouring into water, the mixture was extracted with dichloromethane. The organic layer was collected, washed with water, dried over $MgSO_4$ and evaporated under reduced pressure. The crude product was purified by chromatography eluting with 7/3 v/v dichloromethane/hexane and recrystallization. Pale yellow solid, mp 237–238 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.05 (br s, 1H, NH), 7.44 and 7.22 (2d, 4H, 4-BrArH), 6.33 (d, 1H, H7, $J=1.59$ Hz), 6.03 (d, 1H, H5, $J=1.65$ Hz), 3.79 and 3.53 (2s, 6H, 2×OMe), 1.92 (s, 3H, COMe). IR (KBr): ν_{max} 3329, 2924, 1623, 1577, 1523, 1278, 1205, 1131, 817 cm^{-1}. EIMS: *m/z* (relative intensity) 375 ($[M+2]^+$, 70), 373 (M^+, 69), 360 (11), 358 (10), 294 (18), 279 (100). Anal. calcd for $C_{18}H_{16}BrNO_3$: C, 57.77; H, 4.31; N, 3.74. Found: C, 57.68; H, 4.55; N, 3.82.

4.2. General procedure for the reaction of 3-(4-bromophenyl)-4,6-dimethoxyindole with oxalyl chloride followed by alcohol or amine

3-(4-Bromophenyl)-4,6-dimethoxyindole (1.0 equiv) was dissolved in anhydrous dichloromethane and the solution was cooled at 0 °C in ice. Oxalyl chloride (1.2 equiv) was added rapidly and the solution was allowed to stir at this temperature. The alcohol or amine (10.0 equiv) was added and the mixture warmed to room temperature or reflux. Water was added when the reaction was completed and the solution was extracted with CH_2Cl_2. The organic extract was washed with water and saturated NaCl, dried over $MgSO_4$, and concentrated under reduced pressure. The crude product was purified by column chromatography (ethyl acetate/hexane) and recrystallization (CH_2Cl_2/light petroleum).

4.3. Compounds 2, 3, and 15

According to the general procedure, 3-(4-bromophenyl)-4,6-dimethoxyindole (0.205 g, 0.618 mmol) in dichloromethane (20 mL) was treated with oxalyl chloride (94.1 mg, 0.742 mmol) at 0 °C for 20 min, followed by addition of absolute ethanol (0.21 mL, 6.18 mmol) and heating to reflux for 2 h. Chromatography and elution with 3:7 v/v ethyl acetate/hexane and recrystallization afforded three products.

4.3.1. Ethyl 3-(4-bromophenyl)-4,6-dimethoxyindole-2-carboxylate 2 (0.017 g, 7%). Pale yellow solid, mp 219–221 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.86 (br s, 1H, NH), 7.39 and 7.25 (2d, 4H, 4-BrArH), 6.36 (d, 1H, H7, $J=1.73$ Hz), 6.07 (d, 1H, H5, $J=1.71$ Hz), 4.12 (q, 2H, OCH_2CH_3, $J=7.12$ Hz), 3.77 and 3.57 (2s, 6H, 2×OMe), 1.09 (t, 3H, OCH_2CH_3, $J=7.12$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 160.7 (*C*OOEt), 160.3 (*C*OMe), 156.2 (*C*OMe), 137.6 (Cq), 133.7 (Cq), 132.7 (2×CH), 129.6 (2×CH), 123.5 (Cq), 121.1 (Cq), 120.8 (Cq), 112.6 (Cq), 93.0 (C7), 85.8 (C5), 60.6 (OCH_2CH_3), 55.5 (OMe), 55.1 (OMe), 14.0 (OCH_2CH_3). IR (KBr): ν_{max} 3337, 2925, 1662, 1587, 1344, 1262, 1218, 1151, 1095 cm^{-1}. EIMS: *m/z* (relative intensity) 405 ($[M+2]^+$, 69), 403 (M^+, 80), 359 (100), 357 (92), 278 (52), 250 (61). HRMS calcd for $[C_{19}H_{18}BrNO_4+H]^+$: 404.0497. Found: 404.0490.

4.3.2. Ethyl 3-(4-bromophenyl)-4,6-dimethoxyindole-2-glyoxylate 3 (0.102 g, 35%). Pale yellow solid, mp 140–141 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.48 (br s, 1H, NH), 7.41 and 7.22 (2d, 4H, 4-BrArH), 6.33 (d, 1H, H7, $J=1.81$ Hz), 6.03 (d, 1H, H5, $J=1.81$ Hz), 3.70 (q, 2H, OCH_2CH_3, $J=7.16$ Hz), 3.78 and 3.56 (2s, 6H, 2×OMe), 1.09 (t, 3H, OCH_2CH_3, $J=7.16$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 164.0 (Ar*C*OCOOEt), 162.4 (ArCO*C*OOEt and *C*OMe), 156.9 (*C*OMe), 139.9 (Cq), 132.6 (2×CH), 132.4 (Cq), 130.1 (2×CH), 128.7 (Cq), 127.1 (Cq), 121.9 (Cq), 113.3 (Cq), 93.8 (C7), 85.6 (C5), 62.1 (OCH_2CH_3), 55.6 (OMe), 55.1 (OMe), 13.6 (OCH_2CH_3). IR (KBr): ν_{max} 3326, 2929, 1742, 1603, 1571, 1523, 1207, 1155, 1133 cm^{-1}. EIMS: *m/z* (relative intensity) 433 ($[M+2]^+$, 25), 431 (M^+, 28), 360 (55), 358 (58), 279 (100). Anal. calcd for $C_{20}H_{18}BrNO_5$: C, 55.57; H, 4.20; N, 3.24. Found: C, 55.78; H, 4.27; N, 3.41.

4.3.3. Ethyl 3-(4-bromophenyl)-4,6-dimethoxyindole-7-glyoxylate 15 (0.092 g, 31%). Pale yellow solid, mp 181–182 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.59 (br s, 1H, NH), 7.51 and 7.43 (2d, 4H, 4-BrArH), 7.11 (d, 1H, H2, $J=2.13$ Hz), 6.20 (s, 1H, H5), 4.33 (q, 2H, OCH_2CH_3, $J=7.14$ Hz), 3.96 and 3.95 (2s, 6H, 2×OMe), 1.44 (t, 3H, OCH_2CH_3, $J=7.14$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 185.1 (Ar*C*OCOOEt), 166.0 (ArCO*C*OOEt), 162.2 (*C*OMe), 162.1 (*C*OMe), 138.5 (Cq), 134.2 (Cq), 131.0 (2×CH), 130.7 (2×CH), 122.0 (C2), 120.1 (Cq), 118.1 (Cq), 110.6 (Cq), 100.8 (Cq), 87.4 (C5), 61.5 (OCH_2CH_3), 56.9 (OCH_3), 55.5 (OMe), 14.2 (OCH_2CH_3). IR (KBr): ν_{max} 3428, 2977, 1729, 1616, 1583, 1536, 1309, 1212, 1180, 1083 cm^{-1}. EIMS: *m/z* (relative intensity) 433 ($[M+2]^+$, 37), 431 (M^+, 34), 360 (97), 358 (100). Anal. calcd for $C_{20}H_{18}BrNO_5$: C, 55.57; H, 4.20; N, 3.24. Found: C, 55.97; H, 4.49; N, 3.28.

4.4. Compounds 4 and 16

According to the general procedure, 3-(4-bromophenyl)-4,6-dimethoxyindole (0.450 g, 1.35 mmol) in dichloromethane (20 mL) was treated with oxalyl chloride

(206.4 mg, 1.63 mmol) at 0 °C for 30 min, followed by addition of *n*-butylamine (1.35 mL, 13.5 mmol) and warming to room temperature for 2 h. Chromatography and elution with 3:7 v/v ethyl acetate/hexane and recrystallization afforded two products.

4.4.1. *N*-*n*-Butyl 3-(4-bromophenyl)-4,6-dimethoxyindole-2-glyoxylamide 4 (0.143 g, 23%). Yellow solid, mp 139–140 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 11.58 (br s, 1H, NH), 7.51 and 7.34 (2d, 4H, 4-BrArH), 6.40 (d, 1H, H7, J=1.66 Hz), 6.10 (d, 1H, H5, J=1.66 Hz), 3.88 and 3.65 (2s, 6H, 2×OMe), 3.35 (q, 2H, NHCH_2CH$_2$CH$_2$CH$_3$, J=6.64 Hz), 1.53 and 1.38 (2m, 4H, NHCH$_2$CH_2CH_2CH$_3$), 0.94 (t, 3H, NHCH$_2$CH$_2$CH$_2$CH_3, J=7.26 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.8 (Ar*C*OCONHBu^n), 163.5 (*C*OMe), 162.2 (*C*OMe), 156.7 (ArCO*C*ONHBu^n), 139.7 (Cq), 133.7 (Cq), 132.2 (2×CH), 130.0 (2×CH), 129.7 (Cq), 126.9 (Cq), 121.4 (Cq), 113.2 (Cq), 93.8 (C7), 85.7 (C5), 55.6 (OMe), 55.1 (OMe), 39.0 (NH*C*H$_2$CH$_2$CH$_2$CH$_3$), 31.1 (NHCH$_2$*C*H$_2$CH$_2$CH$_3$), 19.9 (NHCH$_2$CH$_2$*C*H$_2$CH$_3$), 13.6 (NHCH$_2$CH$_2$CH$_2$*C*H$_3$). IR (KBr): ν_{max} 3328, 2958, 2930, 1619, 1513, 1480, 1317, 1212, 1158 cm^{-1}. EIMS: *m*/*z* (relative intensity) 460 ([M+2]$^+$, 45), 458 (M$^+$, 48), 360 (61), 358 (61), 279 (100). Anal. calcd for $C_{22}H_{23}BrN_2O_4$: C, 57.53; H, 5.05; N, 6.10. Found: C, 57.90; H, 5.27; N, 6.51.

4.4.2. *N*-*n*-Butyl 3-(4-bromophenyl)-4,6-dimethoxyindole-7-glyoxylamide 16 (0.291 g, 40%). Yellow solid, mp 219–220 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.44 (br s, 1H, NH), 7.50 and 7.42 (2d, 4H, 4-BrArH), 7.07 (d, 1H, H2), 6.19 (s, 1H, H5), 5.95 (br t, 1H, NHBu^n), 3.95 and 3.92 (2s, 6H, 2×OMe), 3.45 (q, 2H, NHCH_2CH$_2$CH$_2$CH$_3$, J=6.72 Hz), 1.65 and 1.47 (2m, 4H, NHCH$_2$CH_2CH_2CH$_3$), 1.00 (t, 3H, NHCH$_2$CH$_2$CH$_2$CH_3, J=7.28 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 190.0 (Ar*C*OCONHBu^n), 167.8 (ArCO*C*ONHBu^n), 162.2 (*C*OMe), 161.6 (*C*OMe), 138.7 (Cq), 134.2 (Cq), 131.0 (2×CH), 130.7 (2×CH), 121.7 (C2), 120.0 (Cq), 118.0 (Cq), 110.5 (Cq), 101.4 (Cq), 87.9 (C5), 57.1 (OMe), 55.4 (OMe), 39.0 (NH*C*H$_2$CH$_2$CH$_2$CH$_3$), 31.5 (NHCH$_2$*C*H$_2$CH$_2$CH$_3$), 20.0 (NH CH$_2$CH$_2$*C*H$_2$CH$_3$), 13.8 (NHCH$_2$CH$_2$CH$_2$*C*H$_3$). IR (KBr): ν_{max} 3405, 3284, 2958, 1667, 1583, 1559, 1538, 1360, 1325, 1221, 1116, 802 cm^{-1}. EIMS: *m*/*z* (relative intensity) 460 ([M+2]$^+$, 42), 458 (M$^+$, 45), 360 (99), 358 (100). Anal. calcd for $C_{22}H_{23}BrN_2O_4$: C, 57.53; H, 5.05; N, 6.10. Found: C, 57.70; H, 5.14; N, 6.16.

4.5. Compounds 5 and 17

According to the general procedure, 3-(4-bromophenyl)-4,6-dimethoxyindole (0.200 g, 0.602 mmol) in dichloromethane (20 mL) was treated with oxalyl chloride (91.7 mg, 0.722 mmol) at 0 °C for 30 min followed by addition of *N*,*N*-diethylamine (0.63 mL, 60.2 mmol) and warming to room temperature for 2 h. Chromatography and elution with 6:4 v/v ethyl acetate/hexane and recrystallization afforded two products.

4.5.1. *N*,*N*-Dimethyl 3-(4-bromophenyl)-4,6-dimethoxyindole-2-glyoxylamide 5 (0.046 g, 17%). Yellow solid, mp 235–236 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.22 (br s, 1H, NH), 7.39 and 7.21 (2d, 4H, 4-BrArH), 6.34 (s, 1H, H7), 6.30 (s, 1H, H5), 3.79 and 3.54 (2s, 6H, 2×OMe), 3.00–2.93 (m, 4H, N(CH_2CH$_3$)$_2$), 1.02 and 0.78 (2t, 6H, N(CH$_2$CH_3)$_2$, J=7.10 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 182.4 (Ar*C*OCONEt$_2$), 165.9 (ArCO*C*ONEt$_2$), 162.0 (*C*OMe), 157.0 (*C*OMe), 139.4 (Cq), 132.7 (2×CH), 132.0 (Cq), 129.9 (2×CH), 127.7 (Cq), 126.8 (Cq), 121.9 (Cq), 113.6 (Cq), 93.6 (C7), 85.1 (C5), 55.6 (OMe), 55.2 (OMe), 42.5 (N(*C*H$_2$CH$_3$)$_2$), 38.9 (N(*C*H$_2$CH$_3$)$_2$), 13.9 (N(CH$_2$*C*H$_3$)$_2$), 12.4 (N(CH$_2$*C*H$_3$)$_2$). IR (KBr): ν_{max} 3314, 2982, 2937, 1644, 1609, 1575, 1529, 1381, 1255, 1207, 1152, 1133, 810 cm^{-1}. EIMS: *m*/*z* (relative intensity) 460 ([M+2]$^+$, 19), 458 (M$^+$, 24), 360 (30), 358 (30), 279 (100). Anal. calcd for $C_{22}H_{23}BrN_2O_4$: C, 57.53; H, 5.05; N, 6.10. Found: C, 57.57; H, 5.26; N, 6.23.

4.5.2. *N*,*N*-Dimethyl 3-(4-bromophenyl)-4,6-dimethoxyindole-7-glyoxylamide 17 (0.133 g, 49%). Yellow solid, mp 234–235 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.69 (br s, 1H, NH), 7.41 and 7.35 (2d, 4H, 4-BrArH), 7.03 (d, 1H, H2), 6.12 (s, 1H, H5), 3.86 and 3.85 (2s, 6H, 2×OMe), 3.45 (br s, 2H, N(CH_2CH$_3$)$_2$), 3.20 (q, 2H, N(CH_2CH$_3$)$_2$, J=6.98 Hz), 1.21 and 1.09 (2t, 6H, N(CH$_2$CH_3)$_2$, J=7.13, 7.08 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 190.0 (Ar*C*OCONEt)$_2$, 168.4 (ArCO*C*ONEt)$_2$, 161.9 (*C*OMe), 161.5 (*C*OMe), 138.7 (Cq), 134.3 (Cq), 131.0 (2×CH), 130.7 (2×CH), 121.9 (C7), 120.0 (Cq), 117.9 (Cq), 110.6 (Cq), 101.7 (Cq), 87.6 (C5), 56.7 (OMe), 55.5 (OMe), 41.7 (N(*C*H$_2$CH$_3$)$_2$), 38.2 (N(*C*H$_2$CH$_3$)$_2$), 13.4 (N(CH$_2$*C*H$_3$)$_2$), 12.7 (N(CH$_2$*C*H$_3$)$_2$). IR (KBr): ν_{max} 3370, 2974, 2929, 1639, 1607, 1577, 1537, 1359, 1220, 1090 cm^{-1}. EIMS: *m*/*z* (relative intensity) 460 ([M+2]$^+$, 33), 458 (M$^+$, 35), 360 (87), 358 (100). Anal. calcd for $C_{22}H_{23}BrN_2O_4$: C, 57.53; H, 5.05; N, 6.10. Found: C, 57.44; H, 5.08; N, 6.24.

4.6. Compounds 6 and 18

According to the general procedure, 3-(4-bromophenyl)-4,6-dimethoxy-1-methylindole (0.170 g, 0.491 mmol) in dichloromethane (10 mL) was treated with oxalyl chloride (74.8 mg, 0.590 mmol) at 0 °C for 30 min followed by addition of ethanol (10 mL) and heating to reflux for 2 h. Chromatography and elution with 1:1:3 v/v/v ethyl acetate/dichloromethane/hexane and recrystallization afforded two products.

4.6.1. Ethyl 3-(4-bromophenyl)-4,6-dimethoxy-1-methylindole-2-glyoxylate 6 (0.074 g, 34%). Yellow solid, mp 138–139 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.40 and 7.18 (2d, 4H, 4-BrArH), 6.26 (d, 1H, H7), 6.06 (d, 1H, H5), 3.95 and 3.84 (2s, 6H, 2×OMe), 3.56 (s, 3H, NMe), 3.54 (q, 2H, OCH_2CH$_3$, J=7.21 Hz), 1.05 (t, 3H, OCH$_2$CH_3, J=7.21 Hz). IR (KBr): ν_{max} 2941, 1737, 1639, 1614, 1509, 1256, 1211, 1181, 1153 cm^{-1}. EIMS: *m*/*z* (relative intensity) 447 ([M+2]$^+$, 17), 445 (M$^+$, 17), 374 (28), 372 (28), 293 (100). Anal. calcd for $C_{21}H_{20}BrNO_5$: C, 56.52; H, 4.52; N, 3.14. Found: C, 56.72; H, 4.57; N, 3.29.

4.6.2. Ethyl 3-(4-bromophenyl)-4,6-dimethoxy-1-methylindole-7-glyoxylate 18 (0.112 g, 51%). Pale yellow solid, mp 162–163 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.39 and 7.28 (2d, 4H, 4-BrArH), 6.78 (s, 1H, H2), 6.15 (s, 1H, H5), 4.33 (q, 2H, OCH_2CH$_3$, J=7.11 Hz), 3.82 and 3.78 (2s, 6H, 2×OMe), 3.58 (s, 3H, NMe), 1.34 (t, 3H, OCH$_2$CH_3,

J=7.12 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 185.0 (Ar*C*O-COOEt), 165.1 (ArCO*C*OOEt), 160.9 (*C*OMe), 159.6 (*C*OMe), 136.7 (Cq), 134.3 (Cq), 131.2 (2×CH), 130.6 (2×CH), 129.2 (C2), 120.0 (Cq), 117.0 (Cq), 112.8 (Cq), 104.2 (Cq), 87.8 (C5), 61.8 (OCH_2CH_3), 57.2 (OMe), 55.3 (OMe), 38.1 (NMe), 14.2 (OCH_2CH_3). IR (KBr): ν_{max} 2946, 1741, 1631, 1578, 1542, 1290, 1212, 1036, 815 cm^{-1}. EIMS: *m/z* (relative intensity) 447 ([M+2]$^+$, 37), 445 (M$^+$, 37), 374 (98), 372 (100), 294 (26), 293 (88). Anal. calcd for $C_{21}H_{20}BrNO_5$: C, 56.52; H, 4.52; N, 3.14. Found: C, 56.75; H, 4.59; N, 3.25.

4.7. General procedure for methylation of 4,6-dimethoxyindoles

To a suspension of sodium hydride (1.2 equiv) in dry dimethylsulfoxide under nitrogen was added a solution of the indole (1.0 equiv) in dry dimethylsulfoxide and the mixture stirred at room temperature for 1 h. Methyl iodide (1.2 equiv) was added and the mixture stirred at room temperature for 18 h. Water was added and the solution was extracted with CH_2Cl_2. The organic layer was washed with water and saturated NaCl, dried over $MgSO_4$, and concentrated under reduced pressure. The crude product was purified by column chromatography (ethyl acetate/dichloromethane/hexane) and recrystallization (CH_2Cl_2/light petroleum).

4.7.1. 4,6-Dimethoxy-1,3-dimethylindole 31. According to the general procedure, 4,6-dimethoxy-3-methylindole (0.350 g, 1.83 mmol) in dimethylsulfoxide (30 mL) was treated with sodium hydride (52.7 mg, 2.20 mmol) for 1 h followed by addition of methyl iodide (0.14 mL, 2.20 mmol). Chromatography and elution with 7:3 v/v dichloromethane/hexane and recrystallization afforded **31** (0.334 g, 89%). Colourless solid, mp 105–106 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.47 (s, 1H, H2), 6.23 (s, 1H, H7), 6.08 (s, 1H, H5), 3.79 and 3.78 (2s, 6H, 2×OMe), 3.54 (s, 3H, NMe), 2.33 (s, 3H, 3-Me). IR (KBr): ν_{max} 2965, 2931, 1619, 1589, 1459, 1315, 1265, 1214, 1146, 1105 cm^{-1}. EIMS: *m/z* (relative intensity) 206 ([M+1]$^+$, 17), 205 (M$^+$, 100), 191 (18), 190 (61), 162 (33), 147 (22). Anal. calcd for $C_{12}H_{15}NO_2$: C, 70.22; H, 7.37; N, 6.82. Found: C, 70.56; H, 7.39; N, 7.04.

4.7.2. 4,6-Dimethoxy-1-methyl-3-phenylindole 32. According to the general procedure, 4,6-dimethoxy-3-phenylindole (0.258 g, 1.01 mmol) in dimethylsulfoxide (10 mL) was treated with sodium hydride (29.1 mg, 1.21 mmol) for 1 h followed by addition of methyl iodide (0.07 mL, 1.21 mmol). Chromatography and elution with 6:3 v/v dichloromethane/hexane and recrystallization afforded **32** (0.226 g, 83%). Colourless solid, mp 119–120 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.53 (d, 2H, PhH, J=7.33 Hz), 7.28 (t, 2H, PhH, J=7.48 Hz), 7.16 (t, 1H, PhH, J=7.23 Hz), 6.82 (s, 1H, H2), 6.23 (d, 1H, H7, J=1.47 Hz), 6.19 (d, 1H, H5, J=1.36 Hz), 3.82 and 3.73 (2s, 6H, 2×OMe), 3.67 (s, 3H, NMe). IR (KBr): ν_{max} 2961, 2934, 1623, 1599, 1585, 1541, 1505, 1333, 1210, 1151, 1048, 802, 767, 703 cm^{-1}. EIMS: *m/z* (relative intensity) 269 ([M+1]$^+$, 19), 268 (M$^+$, 100), 252 (51), 237 (14), 224 (22), 221 (16). Anal. calcd for $C_{17}H_{17}NO_2$: C, 76.38; H, 6.41; N, 5.24. Found: C, 76.70; H, 6.57; N, 5.33.

4.7.3. 3-(4-Bromophenyl)-4,6-dimethoxy-1-methylindole 33. According to the general procedure, 3-(4-bromophenyl)-4,6-dimethoxyindole (0.358 g, 1.08 mmol) in dimethylsulfoxide (10 mL) was treated with sodium hydride (31.3 mg, 1.30 mmol) for 1 h followed by addition of methyl iodide (0.08 mL, 1.30 mmol). Chromatography and elution with 1/2/6 v/v/v ethyl acetate/dichloromethane/hexane and recrystallization afforded **33** (0.34 g, 81%). Pale yellow solid, mp 144.5–146 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.39 (s, 4H, 4-BrArH), 6.81 (s, 1H, H2), 6.32 (d, 1H, H7, J=1.66 Hz), 6.19 (d, 1H, H5, J=1.63 Hz), 3.82 and 3.73 (2s, 6H, 2×OMe), 3.66 (s, 3H, NMe). IR (KBr): ($_{max}$ 3001, 2934, 1617, 1582, 1541, 1214, 1153, 835 cm^{-1}. EIMS: *m/z* (relative intensity) 347 ([M+2]$^+$, 77), 345 (M$^+$, 77), 332 (17), 330 (19), 304 (12), 302 (13), 251 (100), 236 (25), 208 (16). Anal. calcd for $C_{17}H_{16}BrNO_2$: C, 58.97; H, 4.66; N, 4.05. Found: C, 59.38; H, 4.36; N, 3.71.

4.7.4. 3-(4-Bromophenyl)-4,6-dimethoxy-1,7-dimethylindole 34. To a solution of 3-(4-bromophenyl)-4,6-dimethoxy-7-carboxaldehyde **13** (123.4 mg, 0.34 mmol) in ethanol (10 mL) was added hydrazine 35% solution in water (0.32 mL, 3.43 mmol) at reflux. After 3 h, to this solution was added KOH (384.4 mg, 6.85 mmol) in ethylene glycol (10 mL) and the mixture warmed to 150 °C for 18 h. After cooling to room temperature, the reaction mixture was neutralised with dilute hydrochloric acid and extracted several times with CH_2Cl_2. The combined organic layer was washed with H_2O, saturated NaCl solution, then dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by chromatography and elution with 1/1 v/v dichloromethane/hexane to give 3-(4-bromophenyl)-4,6-dimethoxy-7-methylindole (93.6 mg, 79%).

According to the general procedure, 3-(4-bromophenyl)-4,6-dimethoxy-7-methylindole (0.053 g, 0.154 mmol) in dimethylsulfoxide (5 mL) was treated with sodium hydride (4.2 mg, 0.184 mmol) for 1 h followed by methyl iodide (0.01 mL, 0.184 mmol). Chromatography and elution with 7/3 v/v dichloromethane/hexane and recrystallization afforded **34** (0.053 g, 95%). Colourless solid, mp 194–195 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.48 and 7.43 (2d, 4H, 4-BrArH), 6.80 (s, 2H, H2), 6.37 (s, 2H, H5), 4.01 and 3.90 (2s, 6H, 2×OMe), 3.80 (s, 3H, NMe), 2.61 (s, 3H, ArMe). ^{13}C NMR (75 MHz, $CDCl_3$): δ 154.1 (*C*OMe), 152.3 (*C*OMe), 137.9 (Cq), 135.1 (Cq), 131.1 (2×CH), 130.4 (2×CH), 128.4 (C2), 119.3 (Cq), 115.4 (Cq), 112.2 (Cq), 102.5 (Cq), 90.6 (C5), 57.9 (OMe), 55.2 (OMe), 37.1 (NMe), 10.2 (ArMe). IR (KBr): ν_{max} 2996, 2935, 2840, 1613, 1587, 1545, 1512, 1467, 1333, 1210, 1121, 1088, 1040, 794 cm^{-1}. EIMS: *m/z* (relative intensity) 361 ([M+2]$^+$, 96), 359 (M$^+$, 100), 346 (42), 344 (35), 266 (54), 264 (58), 250 (27). Anal. calcd for $C_{18}H_{18}BrNO_2$: C, 60.01; H, 5.04; N, 3.89. Found: C, 59.69; H, 4.93; N, 3.87.

4.8. General procedure for nitration or oxidative dimerisation

To a solution of the indole in acetonitrile was added 65% nitric acid with stirring at 0 °C or room temperature. The reaction was closely monitored by TLC. Water was added when the reaction was completed and the solution was

extracted with CH_2Cl_2. The organic layer was washed with water and saturated NaCl solution, then dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by chromatography and recrystallization from dichloromethane/hexane.

4.8.1. 2-Acetyl-3-(4-bromophenyl)-4,6-dimethoxy-7-nitroindole 7. Following the general procedure, treatment of indole **1** (0.040 g, 0.107 mmol) in acetonitrile (10 mL) with 65% nitric acid (0.08 mL) at room temperature for 120 min afforded nitroindole **7** (0.034 g, 75%) purified by chromatography and elution with 4:1 v/v dichloromethane/hexane and recrystallization. Pale green solid, mp 278–279 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.72 (br s, 1H, NH), 7.60 and 7.29 (2d, 4H, 4-BrArH), 6.19 (s, 1H, H5), 4.11 and 3.78 (2s, 6H, 2×OMe), 2.03 (s, 3H, COMe). ^{13}C NMR (75 MHz, $CDCl_3$): δ 189.5, 162.1, 153.6, 133.4, 132.5, 132.1, 131.9, 131.0, 123.4, 122.2, 118.4, 113.4, 88.4, 57.3, 55.9, 28.4. IR (KBr): ν_{max} 3411, 2924, 1642, 1575, 1543, 1365, 1223 cm^{-1}. EIMS: *m/z* (relative intensity) 420 ($[M+2]^+$, 100), 418 (M^+, 89), 324 (84). HRMS calcd for $[C_{18}H_{15}BrN_2O_5+Na]^+$: 441.0062. Found: 441.0075.

4.8.2. Ethyl 3-(4-bromophenyl)-4,6-dimethoxy-7-nitroindole-2-carboxylate 8. Following the general procedure, treatment of indole **2** (0.042 g, 0.105 mmol) in acetonitrile (15 mL) with 65% nitric acid (0.12 mL) at room temperature for 30 min afforded nitroindole **8** (0.031 g, 66%) purified by chromatography and elution with 4:1 v/v dichloromethane/hexane and recrystallization. Pale yellow solid, mp 227–228 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.66 (br s, 1H, NH), 7.28 and 7.51 (2d, 4H, 4-BrArH), 6.21 (s, 1H, H5), 4.26 (q, 2H, OCH_2CH_3, J=7.12 Hz), 4.10 and 3.81 (2s, 6H, 2×OMe), 1.22 (t, 3H, OCH_2CH_3, J=7.11 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 161.7, 160.4, 158.9, 132.4, 132.4, 130.0, 124.1, 123.0, 121.5, 118.5, 112.7, 88.4, 61.0, 57.3, 55.9, 14.1. IR (KBr): ν_{max} 3456, 2924, 1715, 1623, 1578, 1469, 1288, 1225 cm^{-1}. EIMS: *m/z* (relative intensity) 450 ($[M+2]^+$, 100), 448 (M^+, 97), 422 (47), 420 (55), 374 (29), 372 (30), 323 (40). HRMS calcd for $[C_{19}H_{17}BrN_2O_6+Na]^+$: 471.0168. Found: 471.0152.

4.8.3. Ethyl 3-(4-bromophenyl)-4,6-dimethoxy-7-nitroindole-2-glyoxylate 9. Following the general procedure, treatment of indole **3** (0.067 g, 0.154 mmol) in acetonitrile (10 mL) with 65% nitric acid (0.08 mL) at room temperature for 240 min afforded nitroindole **9** (0.051 g, 69%) purified by chromatography and elution with 1:1:2 v/v/v ethyl acetate/dichloromethane/hexane and recrystallization. Pale yellow solid, mp 221–223 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 11.09 (br s, 1H, NH), 7.54 and 7.29 (2d, 4H, 4-BrArH), 6.23 (s, 1H, H5), 3.89 (q, 2H, OCH_2CH_3, J=7.10 Hz), 4.13 and 3.84 (2s, 6H, 2×OMe), 1.20 (t, 3H, OCH_2CH_3, J=7.18 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 176.5, 163.1, 162.3, 160.3, 134.0, 132.4, 131.2, 130.4, 128.2, 128.1, 122.5, 118.4, 112.9, 88.8, 62.5, 57.4, 56.0, 13.6. IR (KBr): ν_{max} 3395, 2924, 1742, 1614, 1575, 1317, 1276, 1206 cm^{-1}. EIMS: *m/z* (relative intensity) 478 ($[M+2]^+$, 17), 476 (M^+, 15), 405 (34), 403 (34), 324 (100). Anal. calcd for $C_{20}H_{17}BrN_2O_7$: C, 50.33; H, 3.59; N, 5.87. Found: C, 50.26; H, 3.72; N, 5.74.

4.8.4. *N*-*n*-Butyl 3-(4-bromophenyl)-4,6-dimethoxy-7-nitroindole-2-glyoxylamide 10. Following the general procedure, treatment of indole **4** (0.064 g, 0.139 mmol) in acetonitrile (10 mL) with 65% nitric acid (0.08 mL) at room temperature for 20 min afforded nitroindole **10** (0.051 g, 73%) purified by chromatography and elution with 7:3 v/v ethyl acetate/hexane and recrystallization. Pale yellow solid, mp 194–196 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 12.97 (br s, 1H, NH), 7.36 (br t, 1H, CON*H*Bu^n), 7.53 and 7.28 (2d, 4H, 4-BrArH), 6.19 (s, 1H, H5), 4.11 and 3.82 (2s, 6H, 2×OMe), 3.37 (q, 2H, $NHCH_2CH_2CH_2CH_3$, J=6.66 Hz), 1.54 and 1.37 (2m, 2H, $NHCH_2CH_2CH_2CH_3$), 0.93 (t, 3H, $NHCH_2CH_2CH_2CH_3$, J=7.18 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.3, 160.1, 162.3, 162.1, 133.6, 132.5, 131.9, 131.5, 130.4, 129.4, 128.0, 121.9, 118.6, 112.7, 88.6, 57.4, 55.9, 39.2, 31.1, 20.0, 13.6. IR (KBr): ν_{max} 3390, 3357, 2959, 2934, 1651, 1619, 1570, 1517, 1322, 1290, 1228, 1200 cm^{-1}. EIMS: *m/z* (relative intensity) 505 ($[M+2]^+$, 31), 503 (M^+, 33), 405 (54), 403 (52), 324 (100). Anal. calcd for $C_{22}H_{22}BrN_3O_6$: C, 52.39; H, 4.40; N, 8.33. Found: C, 51.91; H, 4.46; N, 8.05. HRMS calcd for $[C_{22}H_{22}BrN_3O_6+H]^+$: 504.0770. Found: 504.0762.

4.8.5. *N*,*N*-Dimethyl 3-(4-bromophenyl)-4,6-dimethoxy-7-nitroindole-2-glyoxylamide 11. Following the general procedure, treatment of indole **5** (0.062 g, 0.133 mmol) in acetonitrile (10 mL) with 65% nitric acid (0.16 mL) at room temperature for 20 min afforded nitroindole **11** (0.042 g, 63%) purified by chromatography and elution with 1:1 v/v ethyl acetate/hexane and recrystallization. Pale yellow solid, mp 214–215 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.73 (br s, 1H, NH), 7.42 and 7.18 (2d, 4H, 4-BrArH), 6.11 (s, 1H, H5), 4.03 and 3.70 (2s, 6H, 2×OMe), 3.06–2.93 (m, 4H, $N(CH_2CH_3)_2$), 1.06 and 0.78 (2t, 6H, $N(CH_2CH_3)_2$, J=7.10 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 182.2, 165.2, 162.3, 160.0, 133.7, 132.4, 130.9, 130.2, 128.9, 126.4, 122.4, 118.3, 113.3, 88.6, 57.4, 56.0, 42.5, 39.1, 13.9, 12.3. IR (KBr): ν_{max} 3414, 2924, 1648, 1619, 1569, 1542, 1366, 1302, 1274, 1224 cm^{-1}. EIMS: *m/z* (relative intensity) 505 ($[M+2]^+$, 7), 503 (M^+, 7), 405 (23), 403 (26), 324 (100). Anal. calcd for $C_{22}H_{22}BrN_3O_6$: C, 52.39; H, 4.40; N, 8.33. Found: C, 52.65; H, 4.40; N, 8.34.

4.8.6. Ethyl 3-(4-bromophenyl)-4,6-dimethoxy-1-methyl-7-nitroindole-2-glyoxylate 12. Following the general procedure, treatment of indole **6** (0.025 g, 0.057 mmol) in acetonitrile (10 mL) with 65% nitric acid (0.08 mL) at room temperature for 60 min afforded nitroindole **12** (0.024 g, 85%) purified by chromatography and elution with 4:1 v/v dichloromethane/hexane and recrystallization. Pale yellow solid, mp 208–209 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.51 and 7.23 (2d, 4H, 4-BrArH), 6.22 (s, 1H, H5), 4.02 and 3.85 (2s, 6H, 2×OMe), 3.74 (s, 3H, NMe), 3.66 (q, 2H, OCH_2CH_3, J=7.14 Hz), 1.15 (t, 3H, OCH_2CH_3, J=7.14 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 179.3 (Ar*C*O-COOEt), 163.2 (ArCO*C*OOEt), 158.6 (COMe), 154.4 (COMe), 133.0 (2×CH), 130.2 (2×CH), 132.9 (Cq), 131.4 (Cq), 130.4 (Cq), 128.7 (Cq), 122.4 (Cq), 121.5 (Cq), 112.5 (Cq), 88.1 (C5), 62.3 (OCH_2CH_3), 57.3 (OMe), 55.7 (OMe), 33.7 (NMe), 13.5 (OCH_2CH_3). IR (KBr): ν_{max} 2992, 1737, 1646, 1621, 1569, 1524, 1323, 1223, 1206, 804 cm^{-1}. EIMS: *m/z* (relative intensity) 492 ($[M+2]^+$, 25), 490 (M^+, 26), 419 (37), 417 (40), 339 (34), 338 (100).

HRMS calcd for $[C_{21}H_{19}BrN_2O_7+Na]^+$: 513.0273. Found: 513.0273.

4.8.7. 3-(4-Bromophenyl)-4,6-dimethoxy-2-nitroindole-7-carboxaldehyde 19. Following the general procedure, treatment of indole **13** (0.098 g, 0.273 mmol) in acetonitrile (10 mL) with 65% nitric acid (0.08 mL) at room temperature for 60 min afforded nitroindole **19** (0.072 g, 65%) purified by chromatography and elution with 4:1 v/v dichloromethane/hexane and recrystallization. Pale yellow solid, mp 284–286 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 11.21 (br s, 1H, NH), 10.36 (s, 1H, CHO), 7.54 and 7.30 (2d, 4H, 4-BrArH), 6.18 (s, 1H, H5), 4.04 and 3.80 (2s, 6H, 2× OMe). ^{13}C NMR (75 MHz, $CDCl_3$): δ 187.6, 166.4, 163.5, 135.7, 134.3, 132.0, 130.5, 130.2, 122.3, 118.6, 111.2, 103.6, 88.3, 56.4, 55.7. IR (KBr): ν_{max} 3409, 2869, 1649, 1602, 1495, 1299, 1247, 1219, 1151 cm^{-1}. EIMS: *m/z* (relative intensity) 406 ($[M+2]^+$, 64), 404 (M^+, 78), 376 (30), 374 (32), 295 (68), 280 (100). Anal. calcd for $C_{17}H_{13}BrN_2O_5$: C, 50.39; H, 3.23; N, 6.91. Found: C, 50.75; H, 3.42; N, 6.55.

4.8.8. 4,6-Dimethoxy-3-(4-methoxyphenyl)-2-nitroindole-7-carboxaldehyde 20. Following the general procedure, treatment of indole **14** (0.102 g, 0.327 mmol) in acetonitrile (10 mL) with 65% nitric acid (0.08 mL) at room temperature for 120 min afforded nitroindole **20** (0.041 g, 35%) purified by chromatography and elution with 1:1 v/v ethyl acetate/hexane and recrystallization. Pale yellow solid, mp 241–242 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 11.10 (br s, 1H, NH), 10.28 (s, 1H, –CHO), 7.31 and 6.88 (2d, 4H, 4-MeOArH), 6.09 (s, 1H, H5), 3.96, 3.81, and 3.73 (3s, 12H, 3×OMe). ^{13}C NMR (75 MHz, $CDCl_3$): δ 187.7, 166.5, 163.8, 159.5, 135.8, 134.5, 131.9, 123.1, 120.3, 112.8, 111.6, 103.5, 88.1, 56.4, 55.8, 55.2. IR (KBr): ν_{max} 3406, 2924, 2852, 1649, 1598, 1509, 1302, 1238, 1217, 1175 cm^{-1}. EIMS: *m/z* (relative intensity) 357 ($[M+1]^+$, 19), 356 (M^+, 100), 326 (30), 295 (21), 280 (13). HRMS calcd for $[C_{18}H_{16}N_2O_6+Na]^+$: 379.0906. Found: 379.0909.

4.8.9. Ethyl 3-(4-bromophenyl)-4,6-dimethoxy-2-nitroindole-7-glyoxylate 21. Following the general procedure, treatment of indole **15** (0.069 g, 0.158 mmol) in acetonitrile (10 mL) with 65% nitric acid (0.08 mL) at room temperature for 240 min afforded nitroindole **21** (0.064 g, 85%) purified by chromatography and elution with 1:1:2 v/v/v ethyl acetate/dichloromethane/hexane and recrystallization. Yellow solid, mp 209–210 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 11.27 (br s, 1H, NH), 7.57 and 7.33 (2d, 4H, 4-BrArH), 6.19 (s, 1H, H5), 4.44 (q, 2H, $COOCH_2CH_3$, J=7.15 Hz), 4.01 and 3.83 (2s, 6H, 2×OMe), 1.43 (t, 3H, OCH_2CH_3, J=7.10 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 184.7, 165.5, 165.2, 164.3, 135.8, 135.2, 132.0, 130.6, 130.1, 122.5, 118.8, 111.9, 100.2, 88.9, 61.8, 57.0, 56.0, 14.2. IR (KBr): ν_{max} 3407, 2984, 1744, 1636, 1579, 1309, 1232, 1207, 1161, 817 cm^{-1}. EIMS: *m/z* (relative intensity) 478 ($[M+2]^+$, 17), 476 (M^+, 18), 447 (31), 445 (31), 405 (84), 403 (83), 374 (73), 372 (72), 293 (100). HRMS calcd for $[C_{20}H_{17}BrN_2O_7+Na]^+$: 499.0117. Found: 499.0118.

4.8.10. *N*-*n*-Butyl 3-(4-bromophenyl)-4,6-dimethoxy-2-nitroindole-7-glyoxylamide 22. Following the general procedure, treatment of indole **16** (0.098 g, 0.212 mmol) in acetonitrile (10 mL) with 65% nitric acid (0.08 mL) at room temperature for 20 min afforded nitroindole **22** (0.0731 g, 68%) purified by chromatography and elution with 7:3 v/v ethyl acetate/hexane and recrystallization. Yellow solid, mp 245–246 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.93 (br s, 1H, NH), 7.49 and 7.18 (2d, 4H, 4-BrArH), 6.45 (br t, 1H, $NHBu^n$), 6.13 (s, 1H, H5), 4.00 and 3.77 (2s, 6H, 2×OMe), 3.47 (q, 2H, $NHCH_2CH_2CH_2CH_3$, J=6.63 Hz), 1.67 and 1.50 (2m, 4H, $NHCH_2CH_2CH_2CH_3$), 1.02 (t, 3H, $NHCH_2CH_2CH_2CH_3$, J=7.28 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 190.0, 166.8, 165.9, 163.7, 135.5, 135.3, 132.1, 130.5, 129.8, 122.5, 118.9, 111.5, 100.5, 89.2, 57.1, 55.9, 39.0, 31.5, 20.0, 13.8. IR (KBr): ν_{max} 3412, 3271, 2933, 1652, 1626, 1578, 1497, 1371, 1298, 1230, 1164, 986 cm^{-1}. EIMS: *m/z* (relative intensity) 505 ($[M+2]^+$, 3), 503 (M^+, 33), 460 (26), 458 (29), 405 (20), 403 (18), 360 (93), 358 (100). Anal. calcd for $C_{22}H_{22}BrN_3O_6$: C, 52.39; H, 4.40; N, 8.33. Found: C, 52.63; H, 4.29; N, 7.95. HRMS calcd for $[C_{22}H_{22}N_3O_6Br+H]^+$: 504.0770. Found: 504.0764.

4.8.11. *N*,*N*-Dimethyl 3-(4-bromophenyl)-4,6-dimethoxy-2-nitroindole-7-glyoxylamide 23. Following the general procedure, treatment of indole **17** (0.050 g, 0.109 mmol) in acetonitrile (15 mL) with 65% nitric acid (0.12 mL) at room temperature for 20 min afforded nitroindole **23** (0.041 g, 74%) purified by chromatography and elution with 7:3 v/v ethyl acetate/hexane and recrystallization. Yellow solid, mp 244–246 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 11.37 (br s, 1H, NH), 7.48 and 7.23 (2d, 4H, 4-BrArH), 6.11 (s, 1H, H5), 3.90 and 3.73 (2s, 6H, 2×OMe), 3.45 (br q, 2H, $N(CH_2CH_3)_2$), 3.12 (q, 2H, $N(CH_2CH_3)_2$, J=7.03 Hz), 1.22 and 1.13 (2t, 6H, $N(CH_2CH_3)_2$, J=7.08 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 189.4, 167.6, 165.3, 163.7, 135.6, 135.4, 132.0, 130.5, 130.1, 122.3, 118.7, 111.8, 101.0, 88.9, 56.7, 55.8, 41.7, 38.3, 13.4, 12.6. IR (KBr): ν_{max} 3404, 2927, 1646, 1631, 1596, 1578, 1466, 1299, 1241, 1165 cm^{-1}. EIMS: *m/z* (relative intensity) 505 ($[M+2]^+$, 9), 503 (M^+, 10), 405 (100), 403 (90), 324 (24). Anal. calcd for $C_{22}H_{22}BrN_3O_6$: C, 52.39; H, 4.40; N, 8.33. Found: C, 52.34; H, 4.56; N, 8.18. HRMS calcd for $[C_{22}H_{22}BrN_3O_6+Na]^+$: 526.0590. Found: 526.0597.

4.8.12. Ethyl 3-(4-bromophenyl)-4,6-dimethoxy-1-methyl-2-nitroindole-7-glyoxylate 24. Following the general procedure, treatment of indole **18** (0.050 g, 0.112 mmol) in acetonitrile (10 mL) with 65% nitric acid (0.12 mL) at room temperature for 120 min afforded nitroindole **24** (0.049 g, 88%) purified by chromatography and elution with 1:1:1 v/v/v ethyl acetate/dichloromethane/hexane and recrystallization. Yellow solid, mp 212.5–213 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.54 and 7.26 (2d, 4H, 4-BrArH), 6.23 (s, 1H, H5), 4.45 (q, 2H, OCH_2CH_3, J=7.10 Hz), 3.96 and 3.75 (2s, 6H, 2×OMe), 3.68 (s, 3H, NMe), 1.41 (t, 3H, OCH_2CH_3, J=7.10 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 184.6 (Ar*C*OCOOEt), 164.5 (ArCO-*C*OOEt), 164.5 (COMe), 162.0 (COMe), 137.8 (Cq), 131.9 (2×CH), 130.9 (Cq), 130.5 (2×CH), 122.1 (Cq), 120.1 (Cq), 111.4 (Cq), 103.5 (Cq), 88.9 (C5), 62.1 (OCH_2CH_3), 57.0 (OMe), 55.7 (OMe), 37.7 (NMe), 14.2 (OCH_2CH_3). IR (KBr): ν_{max} 2982, 1728, 1664, 1579, 1497, 1311, 1294, 1227, 1155, 1055, 800 cm^{-1}. EIMS: *m/z* (relative intensity)

492 ([M+2]$^+$, 13), 490 (M$^+$, 14), 419 (99), 417 (100), 293 (27). HRMS calcd for [$C_{21}H_{19}BrN_2O_7$+Na]$^+$: 513.0273. Found: 513.0264.

4.8.13. 7,7′-Bi(4,6-dimethoxy-2,3-diphenyl)indolyl 28. According to the general procedure, treatment of indole **25** (0.150 g, 0.456 mmol) in acetonitrile (15 mL) with 65% nitric acid (0.12 mL) at 0 °C for 4 h afforded dimer **28** (0.128 g, 86%) purified by chromatography and elution with 1:1 v/v dichloromethane/hexane and recrystallization. Colourless solid, mp 295–296 °C (lit. 290 °C)[9]. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.01 (br s, 2H, NH), 7.46–7.15 (m, 20H, PhH), 6.49 (s, 2H, H5), 3.83 and 3.79 (2s, 12H, 4×OMe). ^{13}C NMR (75 MHz, $CDCl_3$): 154.8, 154.1, 137.0, 136.0, 132.9, 132.8, 131.4, 128.2, 128.0, 127.2, 126.8, 125.8, 114.9, 113.5, 98.7, 90.7, 57.6, 55.4. IR (KBr): ν_{max} 3456, 2995, 2931, 2836, 1599, 1425, 1330, 1137, 698 cm^{-1}. EIMS: *m/z* (relative intensity) 657 ([M+1]$^+$, 11), 656 (M$^+$, 24), 419 (99), 417 (100), 293 (33).

4.8.14. 7,7′-Bi(4,6-dimethoxy-2-methyl-3-phenyl)indolyl 29. According to the general procedure, treatment of indole **26** (0.200 g, 0.750 mmol) in acetonitrile (20 mL) with 65% nitric acid (0.16 mL) at 0 °C for 3.5 h afforded dimer **29** (0.163 g, 82%) purified by chromatography and elution with 1:1 v/v dichloromethane/hexane and recrystallization. Pale yellow solid, mp 291–293 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.68 (br s, 2H, NH), 7.49–7.24 (m, 10H, PhH), 6.47 (s, 2H, H5), 3.79 (2s, 12H, 4×OMe), 2.28 (s, 6H, Me). ^{13}C NMR (75 MHz, $CDCl_3$): δ 153.7, 153.5, 136.3, 136.1, 130.9, 130.0, 127.0, 125.3, 114.0, 112.3, 98.7, 90.7, 57.8, 55.3, 12.1. IR (KBr): ν_{max} 3456, 2934, 1601, 1427, 1326, 1211, 1123 cm^{-1}. EIMS: *m/z* (relative intensity) 533 ([M+1]$^+$, 37), 532 (M$^+$, 100), 486 (54), 267 (68), 252 (57) Anal. calcd for $C_{34}H_{32}N_2O_4$: C, 76.67; H, 6.06; N, 5.26. Found: C, 76.31; H, 6.13; N, 5.23.

4.8.15. 7,7′-Bi(4,6-dimethoxy-2-methyl-3-(4-bromophenyl)indolyl 30. According to the general procedure, treatment of indole **27** (0.100 g, 0.289 mmol) in acetonitrile (10 mL) with 65% nitric acid (0.08 mL) at 0 °C for 4 h afforded dimer **30** (0.082 g, 82%) purified by chromatography and elution with 1:1 v/v dichloromethane/hexane and recrystallization. Pale yellow solid, mp 282–283 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.68 (br s, 2H, NH), 7.49 and 7.33 (2d, 8H, 4-BrArH), 6.46 (s, 2H, H5), 3.81 and 3.78 (2s, 12H, 4×OMe), 2.25 (s, 6H, Me). ^{13}C NMR (75 MHz, $CDCl_3$): δ 153.6, 136.3, 135.0, 132.5, 130.1, 119.3, 112.9, 111.9, 98.6, 90.5, 57.7, 55.2, 12.1. IR (KBr): ν_{max} 3458, 2936, 1591, 1428, 1327, 1212, 1135 cm^{-1}. EIMS: *m/z* (relative intensity) 692 ([M+2]$^+$, 49), 690 (M$^+$, 100), 688 (51), 644 (48), 345 (37). Anal. calcd for $C_{34}H_{30}Br_2N_2O_4$: C, 59.15; H, 4.38; N, 4.06. Found: C, 59.00; H, 4.70; N, 3.95.

4.8.16. 2,2′-Bi(4,6-dimethoxy-1,3-dimethyl)indolyl 35. According to the general procedure, treatment of indole **31** (0.100 g, 0.488 mmol) in acetonitrile (10 mL) with 65% nitric acid (0.08 mL) at 0 °C for 3 h afforded dimer **35** (0.034 g, 35%) purified by chromatography and elution with 1:1 v/v dichloromethane/hexane and recrystallization. Colourless solid, mp 167–169 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.41 (d, 2H, H7, J=1.64 Hz), 6.25 (d, 2H, H5, J=1.64 Hz), 3.94 and 3.92 (2s, 12H, 4×OMe), 3.42 (s, 6H, 2×NMe), 2.29 (s, 6H, 2×Me). ^{13}C NMR (75 MHz, $CDCl_3$): δ 157.5, 155.5, 138.9, 125.3, 113.0, 112.4, 91.0, 85.1, 55.7, 55.2, 30.4, 11.5. IR (KBr): ν_{max} 2936, 1622, 1581, 1257, 1212, 1150, 803 cm^{-1}. EIMS: *m/z* (relative intensity) 409 ([M+1]$^+$, 13), 408 (M$^+$, 49), 393 (23), 174 (15), 105 (100), 77 (55). HRMS calcd for [$C_{24}H_{28}N_2O_4$+H]$^+$: 409.2127. Found: 409.2132.

4.8.17. 2,2′-Bi(4,6-dimethoxy-1-methyl-3-phenyl)indolyl 36. According to the general procedure, treatment of indole **32** (0.081 g, 0.302 mmol) in acetonitrile (10 mL) with 65% nitric acid (0.08 mL) at 0 °C for 18 h afforded dimer **36** (0.020 g, 25%) purified by chromatography and elution with 1:1 v/v dichloromethane/hexane and recrystallization. Colourless solid, mp>320 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.32–7.13 (m, 10H, PhH), 6.31 (s, 2H, H7), 6.21 (s, 2H, H5), 3.89 and 3.79 (2s, 12H, 4×OMe), 3.14 (s, 6H, 2×NMe). ^{13}C NMR (75 MHz, $CDCl_3$): δ 157.5, 154.9, 139.0, 135.5, 130.3, 128.0, 127.1, 125.3, 118.1, 110.7, 92.0, 85.2, 55.6, 50.1, 30.4. IR (KBr): ν_{max} 3449, 2931, 1617, 1583, 1323, 1257, 1214, 1150, 696 cm^{-1}. EIMS: *m/z* (relative intensity) 533 ([M+1]$^+$, 6), 532 (M$^+$, 17), 456 (7), 165 (100), 149 (25). HRMS calcd for [$C_{34}H_{32}N_2O_4$+Na]$^+$: 555.2260. Found: 555.2252.

4.8.18. 2,2′-Bi(3-(4-bromophenyl)-4,6-dimethoxy-1-methyl)indolyl 37. According to the general procedure, treatment of indole **33** (0.102 g, 0.296 mmol) in acetonitrile (10 mL) with 65% nitric acid (0.08 mL) at 0 °C for 18 h afforded dimer **37** (0.064 g, 63%) purified by chromatography and elution with 3:2 v/v dichloromethane/hexane and recrystallization. Pale yellow solid, mp 259–261 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.25 and 7.08 (2d, 8H, 4-BrArH), 6.38 (d, 2H, H7, J=1.71 Hz), 6.31 (d, 2H, H5, J=1.71 Hz), 3.92 and 3.80 (2s, 12H, 4×OMe), 3.26 (s, 6H, 2×NCH$_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 157.9, 154.8, 139.1, 134.3, 131.7, 130.2, 125.7, 119.4, 117.6, 110.4, 92.3, 85.2, 55.6, 55.1, 30.6. IR (KBr): ν_{max} 3447, 2933, 1622, 1582, 1506, 1327, 1258, 1213, 1151, 1074 cm^{-1}. EIMS: *m/z* (relative intensity) 692 ([M+2]$^+$, 33), 690 (M$^+$, 56), 688 (30), 361 (99), 359 (100), 265 (91). Anal. calcd for $C_{34}H_{30}Br_2N_2O_4$: C, 59.15; H, 4.38; N, 4.06. Found: C, 59.29; H, 4.72; N, 4.27.

4.8.19. 2,2′-Bi(3-(4-bromophenyl)-4,6-dimethoxy-1,7-dimethyl)indolyl 38. According to the general procedure, treatment of indole **34** (0.052 g, 0.0145 mmol) in acetonitrile (10 mL) with 65% nitric acid (0.08 mL) at 0 °C for 10 h afforded dimer **38** (0.038 g, 72%) purified by chromatography and elution with 1:1 v/v dichloromethane/hexane and recrystallization. Colourless solid, mp 302–303 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.18 and 6.78 (2d, 8H, 4-BrArH), 6.35 (s, 2H, H5), 3.92 (s, 6H, OMe), 3.72 (s, 12H, ArOMe and NMe), 2.62 (s, 6H, ArMe). ^{13}C NMR (75 MHz, $CDCl_3$): δ 154.7, 152.5, 138.5, 134.4, 131.7, 129.7, 128.2, 119.2, 117.7, 112.3, 102.2, 90.3, 57.7, 55.0, 34.0, 10.7. IR (KBr): ν_{max} 3449, 2930, 1611, 1587, 1510, 1459, 1333, 1246, 1215, 1181, 1133, 1078 cm^{-1}. EIMS: *m/z* (relative intensity) 720 ([M+2]$^+$, 53), 718 (M$^+$, 100), 716 (53), 372 (53), 370 (53). Anal. calcd for $C_{36}H_{34}Br_2N_2O_4$: C, 60.18; H, 4.77; N, 3.90. Found: C, 59.79; H, 4.68; N, 3.80.

Acknowledgements

We thank the Thailand Research Fund for the award of a Royal Golden Jubilee Ph.D. scholarship to T. K., and the Australian Research Council for financial support.

References and notes

1. *Indoles-Part Two*; Houlihan, W. J., Ed.; Wiley: New York, 1972; pp 540–550.
2. Sundberg, R. J. *The Chemistry of Indoles*; Academic: New York, 1970; pp 11–14.
3. Joule, J. A. Indole and its Derivatives. In *Science of Synthesis*, Thieme: Stuttgart, 2001; Vol. 10, pp 361–652.
4. Jones, A. W.; Purwono, B.; Bowyer, P. K.; Mitchell, P. S. R.; Kumar, N.; Nugent, S. J.; Jolliffe, K. A.; Black, D. StC. *Tetrahedron* **2004**, in press.
5. Black, D. StC.; Strauch, R. J. *Aust. J. Chem.* **1988**, *41*, 1613–1619.
6. Black, D. StC.; Kumar, N.; McConnell, D. B. *Tetrahedron* **1996**, *52*, 8925–8936.
7. Black, D. StC.; Kumar, N.; Wong, L. C. H. *Synthesis* **1986**, 474.
8. Black, D. StC.; Bowyer, M. C.; Catalano, M. M.; Ivory, A. J.; Keller, P. A.; Kumar, N.; Nugent, S. J. *Tetrahedron* **1994**, *50*, 10497–10508.
9. Black, D. StC.; Choy, A.; Craig, D. C.; Ivory, A. J.; Kumar, N. *J. Chem. Soc., Chem. Commun.* **1989**, 111–112.
10. Wahyuningsih, T. D. Ph.D. Thesis, The University of New South Wales, 2004.
11. Bergman, J.; Eklund, N. *Tetrahedron* **1980**, *36*, 1439–1443.

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Cyclic peptides containing a δ-sugar amino acid—synthesis and evaluation as artificial receptors

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Received 25 August 2004; revised 21 October 2004; accepted 12 November 2004

Available online 10 December 2004

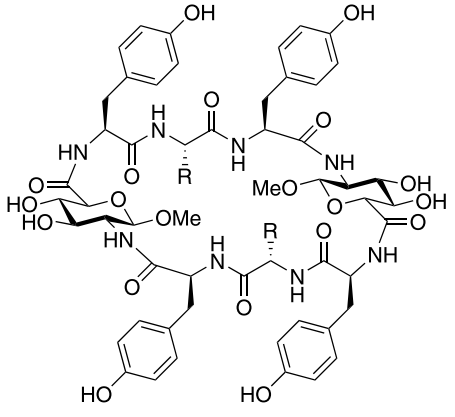
Abstract—An Fmoc-protected δ-sugar amino acid, prepared by oxidation of a glucosamine derivative, was coupled to three different tripeptide *tert*-butyl esters (H-Tyr-Tyr-Tyr-O^{t}Bu, H-Tyr-Glu(OBzl)-Tyr-O^{t}Bu and H-Tyr-Arg(Mtr)-Tyr-O^{t}Bu) and the resulting sugar amino acid/amino acid hybrids were transformed into dimers that were subsequently cyclized to give three C_2-symmetric macrocycles. The macrocycles were deprotected and their binding properties towards *p*-nitrophenyl glycosides, nucleotides, and purines were examined. Of the ligands screened, only some of the purines showed weak, but significant, binding.

1. Introduction

Interactions of small ligands, such as carbohydrates, metabolites or hormones, with binding sites in proteins are vital to life processes and the synthesis of artificial receptors that mimic such interactions has been an ongoing goal in many research groups for a long time.[1] A basic design for biomimetic artificial receptors involves amphiphilic molecules, often macrocycles, with both polar and non-polar regions, thus enabling interactions with both polar and non-polar regions of a ligand.

Sugar amino acids[2–5] are carbohydrates that contain at least one amino and one carboxylic acid functionality, which allows for the use of peptide coupling chemistry in order to combine them with amino acids or other building blocks. Sugar amino acids have been used to prepare cyclic homo-oligomers[6–8] and cyclic sugar amino acid/amino acid hybrids,[9–15] that have been used in various studies. It has been proposed that such molecules could be interesting artificial receptors, and in one case it has been shown that a cyclodextrin-like cyclic hexamer could bind to benzoic acid and *p*-nitrophenol in water, although no binding constants were given.[6] We decided to explore the use of sugar amino acids as polar structural elements combined with non-polar aromatic amino acids for the construction of amphiphilic molecules as biomimetic receptors.

Herein, we report the synthesis of polyamphiphilic water-soluble macrocyclic sugar amino acid/amino acid hybrid molecules **1a–c** (see Fig. 1) and an investigation of their binding properties against biomolecules. We chose to use the δ-sugar amino acid obtained by oxidation of a partially protected methyl β-glycoside of glucosamine together with the aromatic amino acid tyrosine as building blocks for our macrocycles. The δ-sugar amino acid was chosen because of its extended geometry, which prevents turn formation and presumably thus gives rise to more accessible cores of the macrocycles. In addition, we introduced amino acids with charged side chains to enhance solubility and potentially



1a: R = tyrosine side chain
1b: R = CH_2CH_2COOH
1c: R = $CH_2CH_2CH_2NHC(NH)NH_2$

Figure 1. Synthesized macrocycles.

Keywords: Sugar amino acids; Macrocycles; Cyclic peptides; Artificial receptors; Molecular recognition.
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doi:10.1016/j.tet.2004.11.024

also binding. Two monosaccharides and six amino acids were used in each macrocyclic ring in order to obtain macrocycles large enough to form a central pocket where ligands might bind.

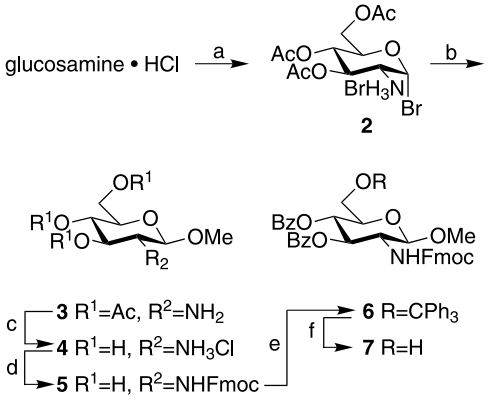
2. Results and discussion

2.1. Synthesis

The synthetic strategy towards the macrocycles involved two building blocks for each macrocycle, a C-protected tripeptide and an amino sugar precursor, which upon oxidation gives the N-protected sugar amino acid (SAA). The tripeptide and the sugar amino acid were coupled together to give a linear sugar amino acid/amino acid hybrid, which was then transformed into a dimer that was subsequently cyclized to give the desired macrocycle. To achieve this, it was necessary to use orthogonal protecting groups for N- and C-protection. The use of the base-labile Fmoc group and acid-labile *tert*-butyl esters met this requirement.

The starting material glucosamine hydrochloride was transformed into the known tri-*O*-acetylated methyl pyranoside **3** in a two-step procedure using a combination of previously described methods[16,17] (Scheme 1). Attempts to deacetylate **3** using base-catalyzed transesterfication with Me_2NEt in MeOH gave ca. 12% of an *N*-acetyl side-product, while acidic transesterfication cleanly produced known hydrochloride **4**.[18] The amine was selectively protected using *N*-(9-fluorenylmethoxycarbonyloxy)-succinimide (Fmoc-OSu) to give **5**. The primary hydroxyl group was protected as the triphenylmethyl ether and the secondary hydroxyl groups as benzoates to give **6**. Cleavage of the triphenylmethyl ether using hydrogen bromide in acetic acid completed the synthesis of sugar amino acid precursor **7**. A similar sequence leading to the α anomer of the same sugar amino acid has been disclosed.[19]

Three different tripeptide *tert*-butyl esters **10a–c** were prepared in solution in good yields (Scheme 2) to serve as the required C-protected tripeptides. Peptide couplings were made using *N*-(3-dimethylaminopropyl)-*N*′-ethyl carbodiimide hydrochloride (EDC·HCl), 1-hydroxybenzotriazole (HOBt) and *N*-methylmorpholine in THF, *tert*-butyl esters were cleaved with 33% TFA in CH_2Cl_2 using Et_3SiH as a scavenger,[20] and piperidine was used to cleave the Fmoc group. The 4-methoxy-2,3,6-trimethylbenzenesulfonyl (Mtr) group has previously been reported to be stable at 25% TFA in CH_2Cl_2,[15] and this was also the case at 33% TFA in CH_2Cl_2.



Scheme 1. Synthesis of the δ-sugar amino acid precursor **7**: (a) AcBr (neat), 3 days, 82%; (b) MeOH, pyridine, 1 h, 76%; (c) HCl/MeOH, 24 h, 95%; (d) Fmoc-OSu, $NaHCO_3$, 15 h, 76%; (e) chlorotriphenylmethane, pyridine, 85 °C, 2 h, then BzCl, pyridine, r.t., 4 h, 63%; (f) HBr/AcOH, 3 min, 81%.

Sugar amino acid precursor **7** was oxidized with Jones's reagent and the crude sugar amino acid was directly coupled to a C-protected tripeptide **10a–c** (Scheme 3) to give sugar amino acid/amino acid hybrids **14a–c**. The hybrids **14a–c** were either deprotected at the N-terminal using DBU in the presence of a solid-phase thiol as a scavenger for the liberated dibenzofulvene[21] to give **15a–c** or at the C-terminal using TFA/Et_3SiH/CH_2Cl_2[20] to give **16a–c**. The N-deprotected hybrids **15a–c** and the C-deprotected hybrids **16a–c** were coupled together using *N*,*N*′-diisopropylcarbodiimide (DIC) and HOBt to give linear dimers **17a–c**. The EDC·HCl/HOBt/*N*-methylmorpholine coupling protocol that had been used earlier in the synthetic scheme gave excessive epimerization in this coupling (ca. 20% epimer of **17a** formed according to NMR) and could not be used here. The DIC/HOBt protocol without base has been reported to give good results in difficult couplings[22] and gave good results with **17a–c** (no epimerization according to ^{1}H NMR spectrum). The linear dimers were N-deprotected as above to give **18a–c**.

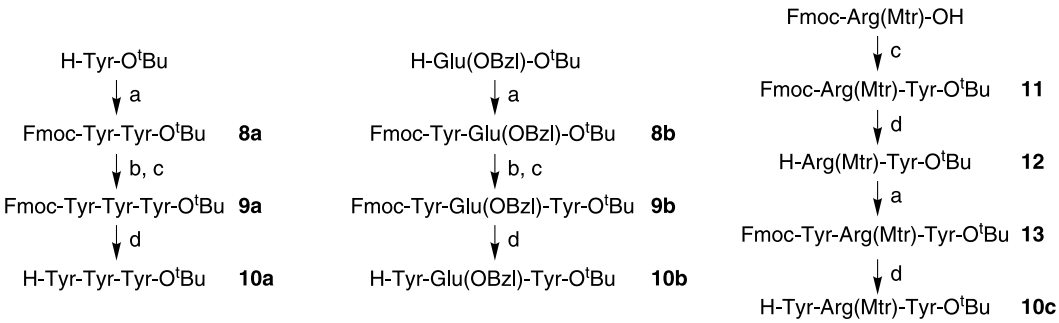
In order to evaluate cyclization conditions, a portion of **18a** was C-deprotected and initial cyclization attempts were made using the following conditions:

(a) EDC·HCl/HOBt and *N*-methylmorpholine in THF.
(b) DIC/HOBt both with and without *N*,*N*-diisopropylethylamine (DIPEA) in both THF and DMF.
(c) Diphenylphosphoryl azide (DPPA) both with $NaHCO_3$ in DMF and with DIPEA in THF.
(d) 1-[bis-(Dimethylamino)methylene]-1*H*-benzotriazolium tetrafluoroborate 3-oxide (TBTU)[†], HOBt, and DIPEA in THF.
(e) 1-[bis-(Dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium hexafluorophosphate 3-oxide (HATU) with both DIPEA and 2,4,6-collidine in THF.
(f) 1-(1-Pyrrolidinyl-1*H*-1,2,3-triazolo[4,5-*b*]pyridinylmethylene)pyrrolidinium hexafluorophosphate 3-oxide (HAPyU)[‡] with both DIPEA and 2,4,6-collidine in THF.

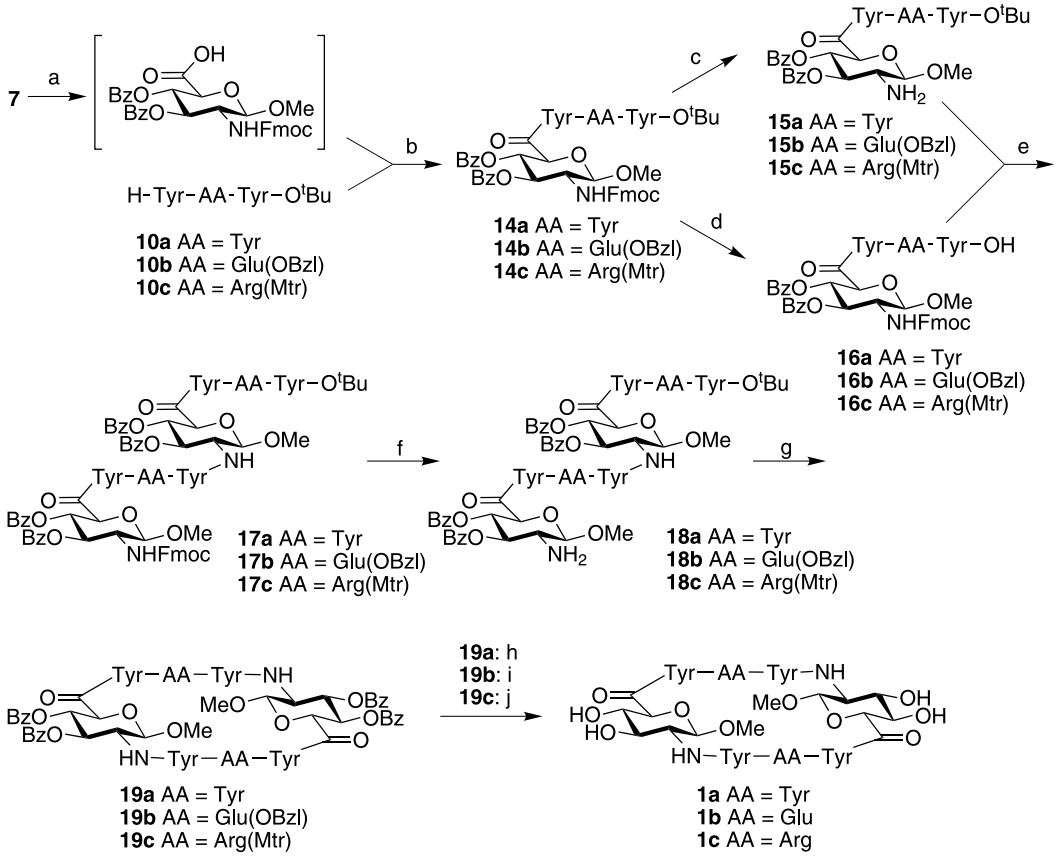
All attempts were carried out at 1 mM concentration of the linear starting material. Only TBTU, HATU, and HAPyU

[†] HATU and HBTU were long believed to be uronium salts, but have been shown to be guanidinium salts when prepared using the conventional methods.[23] This is likely to be true for TBTU and HAPyU as well. We have chosen to name all these reagents as guanidinium salts.

[‡] HAPyU was prepared from 1-hydroxy-7-azabenzotriazole potassium salt (KOAt)[23] and commercially available chloro-*N*,*N*,*N*′,*N*′-bis(tetramethylene)-formamidinium hexafluorophosphate using Knorr's method for the preparation of similar coupling reagents.[24,25]



Scheme 2. Synthesis of tripeptide *tert*-butyl esters **10a–c**: (a) Fmoc-Tyr-OH, EDC·HCl, HOBt, *N*-methylmorpholine, THF, 16 h, 83–88%; (b) TFA, Et_3SiH, CH_2Cl_2, 4 h; (c) H-Tyr-O^{t}Bu, EDC·HCl, HOBt, *N*-methylmorpholine, THF, 16 h, 83–94%; (d) piperidine, CH_2Cl_2, 30 min, 83–90%.



Scheme 3. Synthesis of macrocycles **1a–c**: (a) CrO_3, H_2SO_4(aq), acetone, 1.5 h; (b) EDC·HCl, HOBt, *N*-methylmorpholine, THF, 16 h, 45–64% (two steps); (c) DBU, *N*-(2-mercaptoethyl) aminomethyl polystyrene, 6 h, 82–99%; (d) TFA, Et_3SiH, CH_2Cl_2, 3–4 h; (e) DIC, HOBt, THF, 16 h, 48–72%; (f) DBU, *N*-(2-mercaptoethyl)aminomethyl polystyrene, 6 h, 88–99%; (g) (i) TFA, Et_3SiH, CH_2Cl_2, 3–4 h; (ii) HAPyU, DIPEA, THF, 2 h, 39–53%; (h) NaOMe/MeOH, 24 h, 61%; (i) (i) HCOOH, Pd black, MeOH, 15 min; (ii) NaOMe/MeOH, 24 h, 81%; (j) (i) TFA, PhSMe, 24 h; (ii) NaOMe/MeOH, 24 h, 59%.

gave the macrocycle **19a** as a major product according to MALDI-TOF analysis of the reaction mixtures.

The HATU and HAPyU reagents have been shown to give less epimerization than TBTU in the cyclization of pentapeptides[26] and these reagents were thus investigated further. Furthermore, it has been shown that DIPEA gives less epimerization than 2,4,6-collidine for the cyclization of pentapeptides by HAPyU,[26] while for segment condensations the opposite is true.[27] Hence, both DIPEA and 2,4,6-collidine were evaluated as bases. In the cyclization of **18a** to **19a**, DIPEA gave a higher yield and less epimerization. Compounds **18b–c** could also be cyclized to **19b–c** using this method (see Table 1). In the cyclization of **18b** to **19b**, HAPyU gave a better yield than HATU.

The ^{1}H NMR spectra of the protected macrocycles **19a–c** only gave poorly resolved spectra with broad peaks at room temperature, presumably due to slow conformational exchange. Resolved spectra of **19a** and **19b** could be obtained at 150 and 120 °C, respectively, but compound **19c** only gave poorly resolved spectra even at these temperatures.

Macrocycle **19a** was deprotected using 10 mM NaOMe in

Table 1. Cyclization conditions, yields and epimerizations

Starting material	Reagents[a]	Reaction time (h)[b]	Isolated yield (%)[c]	Epimerization
18a	HAPyU/2,4,6-collidine	3	35	5% isolated yield
18a	HAPyU/DIPEA	1	37	Not observed
18b	HAPyU/DIPEA	3	53	Traces on TLC
18b	HATU/DIPEA	2.5	32	Traces on TLC
18c	HAPyU/DIPEA	4	34	Traces on TLC

[a] At room temperature in THF with 1 mM concentration of linear starting material.
[b] Reactions were monitored with MALDI-TOF until the deprotected starting material was consumed.
[c] For both C-deprotection and cyclization.

MeOH to afford **1a** in 61% yield. The deprotection of macrocycle **19b** started with the cleavage of the benzyl esters using catalytic transfer hydrogenation with palladium black and formic acid as the hydrogen source,[28] followed by treatment of the crude product with NaOMe/MeOH to give **1b** in 81% yield. In the case of the macrocycle **19c**, the Mtr groups were first cleaved using neat TFA with thioanisole as a scavenger[29] and the crude product was then treated with NaOMe/MeOH to afford **1c** in 59% yield after HPLC purification.

2.2. Conformational analysis

The deprotected macrocycles **1a–c** all gave well resolved NMR spectra at room temperature. Macrocycle **1a** in MeOH-d_4 and macrocycles **1b–c** in D_2O all gave the expected ^{1}H NMR spectra for symmetrical compounds. In addition to the major peaks, macrocycle **1c** in also gave smaller peaks at 2.81 and 2.56 ppm, as well as some overlapping smaller peaks at 3.74 ppm and in the aromatic region. Heating of **1c** in DMSO-d_6 brought the ^{1}H NMR signals to coalescence, which shows that the multiple peaks of **1c** at ambient temperature were due to slow conformational exchange (Fig. 2).

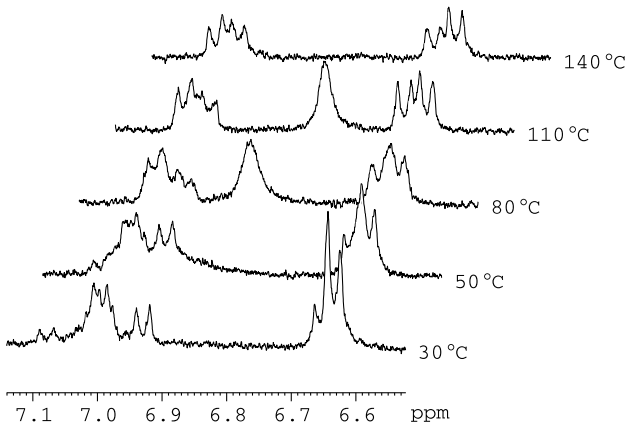


Figure 2. The aromatic region of the ^{1}H NMR spectrum of **1c** at different temperatures (400 MHz, DMSO-d_6).

Monte–Carlo conformational searches were performed on **1a–c** using MacroModel 8.5 (MMFFs force field with water as solvent, 20,000 steps, all backbone torsions were selected for random variation) to give for each macrocycle 110–240 conformers within 5 kcal/mol of the global minimum. When these conformers were studied, a coherent picture emerged. The dominant low-energy conformers for macrocycles **1a–c** were twisted, oblong structures with extended sugar amino acids and turns formed by the tripeptides (Fig. 3). In the conformers with lowest energy, the axial hydrogen atoms in the two sugar amino acids were facing each other, but conformers where one of the sugar amino acids had rotated to place the hydroxyl groups in position to form hydrogen bonds to the tripeptide, or to the other sugar amino acid, were also found. Hydrogen bonds were occasionally found within the tripeptides, but no pattern could be discerned. The $^3J_{HH}$ coupling constants for all three macrocycles indicate that the sugar amino acids are in the $^4C_1$ conformation. This was the case for the calculated conformers of **1a–b**, but many conformers of macrocycle **1c** deviated from the expected $^4C_1$ conformation of the pyranose rings. As there is no support for this in the coupling constants, we conclude that it is an artefact in the calculations possibly induced by the strong hydrogen bond formed between the arginine and tyrosine side chains.

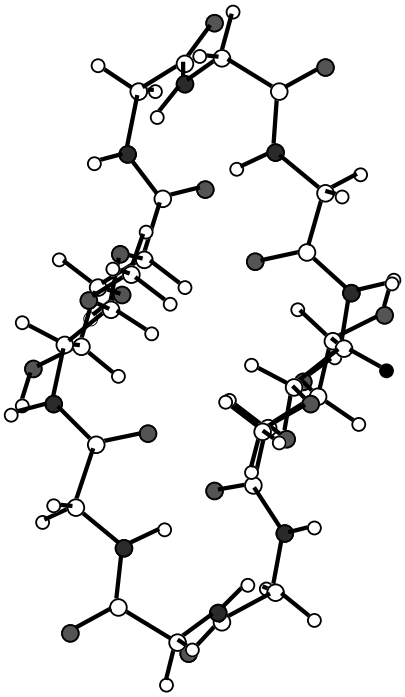


Figure 3. Calculated global energy minimum of macrocycle **1a** (side chains omitted for clarity).

2.3. Molecular recognition properties

Compound **1a** was not soluble in water and its binding properties were not examined. Macrocycles **1b–c** were screened using NMR titrations against a number of putative ligands: *p*-nitrophenyl glycosides, nucleotides, aromatic

amino acids, aromatic amines and purines. Of all the ligands screened, only **1b** and caffeine and **1c** and the purine nucleotides 2′-deoxyadenosine 5′-monophosphate (dAMP) and 2′-deoxyguanosine 5′-monophosphate (dGMP) showed weak, but significant, interactions ($K_a \approx 10\ M^{-1}$). For comparison, reference peptide Ac-Tyr-Arg-Tyr-OMe[§] was also titrated with dAMP and dGMP and was found to bind more weakly ($K_a \approx 5\ M^{-1}$). The binding is most likely due to hydrophobic interaction between the purine and tyrosine rings, and the small increase in affinity for **1c** is thus due to its dimeric cyclic structure and/or the presence of the sugar amino acid moieties. However, the weak affinities preclude conclusions regarding detailed structure–affinity relationships.

3. Conclusions

We have described the synthesis of three δ-sugar amino acid/tripeptide dimeric macrocycles and evaluated their binding properties. Macrocycles **1b–c** were found to bind some purine derivatives with weak, but significant, binding constants. Apparently, the structures have to be modified in order to present binding sites pre-organized for higher affinity binding of biomolecules. However, although the binding is weak, this shows that sugar amino acid containing peptides can act as artificial receptors and serves as a starting point for further research.

4. Experimental

4.1. General methods

THF and CH_2Cl_2 were dried over 4 Å molecular sieves before use and MeOH was dried over 3 Å molecular sieves before use. Other solvents were not dried unless specified. Matrex 35–70 μm 60 Å silica (Millipore) was used for flash chromatography. Sephadex LH-20 in CH_2Cl_2/MeOH 1:1 was used for size-exclusion chromatography. Sep-Pak Plus C_{18} cartridges (Waters) were used for solid-phase extraction. Chemical shifts are reported relative to Me_4Si and were calculated using the residual solvent peak as a reference. NMR spectra were assigned with the help of correlation spectroscopy (COSY). All compounds were estimated to be >95% pure by ^{1}H NMR spectroscopy.

4.2. Preparation of sugar amino acid precursor 7

4.2.1. Tri-*O*-acetyl-2-amino-2-deoxy-α-D-glucopyranosyl bromide hydrobromide (2).[16,17] Acetyl bromide (70 mL, 0.94 mol) was added to D-glucosamine hydrochloride (21.9 g, 101.6 mmol, dried 24 h in vacuo at 70 °C over P_2O_5) and the mixture was stirred for 3 days at room temperature. Residual acetyl bromide was removed in vacuo (water aspirator) and the crude product was dissolved in hot chloroform (distilled from P_2O_5) and filtered while still hot. Crystals began to form as the solution cooled and diethyl ether was added to the stirred solution until the product precipitated from the mixture to afford **2** (37.6 g, 82%) as small white needles. Mp 143–148 °C (dec.), lit.[16] 149–150 °C (dec.); $[\alpha]_D^{22} = +130$ (*c* 1.0, acetone), lit.[5] $[\alpha]_D = +148.4$ (*c* 5.01, acetone); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.66 (br s, 3H, NH_3), 7.10 (d, *J*=3.6 Hz, 1H, H^1), 5.50 (t, *J*=9.8 Hz, 1H, H^3), 5.25 (t, *J*=9.7 Hz, 1H, H^4), 4.33 (m, 2H, H^5+H^6), 4.17 (d, *J*=10.9 Hz, H^6), 3.93 (dd, *J*=10.3, 3.7 Hz, 1H, H^5), 2.26, 2.13, 2.09 (3s, 3H each, OAc)[¶]; HRMS (FAB) calcd for $C_{12}H_{18}BrNO_7Na$ (M−HBr+Na): 390.0164; found 390.0171.

4.2.2. Methyl tri-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranoside (3). Hydrobromide **2** (37.6 g, 84 mmol) was dissolved in MeOH (800 mL) and pyridine (8 mL, distilled from CaH_2) was added. After 1 h, toluene (150 mL) was added and the mixture was concentrated. The residue was dissolved in chloroform (750 mL), washed with Na_2CO_3-(aq) (5%, 2×100 mL) and water (100 mL), dried over Na_2SO_4, and concentrated. The crude product was recrystallized in chloroform/heptane to give **3** (20.3 g in three crops, 76%) as small white needles. Mp 146–148 °C, lit.[17] 151–152 °C; $[\alpha]_D^{22} = +14$ (*c* 1.0, MeOH), lit.[17] $[\alpha]_D^{27} = +15$ (*c* 1, MeOH); ^{1}H NMR spectrum is in agreement with published data[17]; HRMS (FAB) calcd for $C_{13}H_{21}NO_8Na$ (M+Na): 342.1165, found 342.1158.

4.2.3. Methyl 2-amino-2-deoxy-β-D-glucopyranoside hydrochloride (4). Acetyl chloride (67 mL, 0.95 mol) was slowly added to MeOH (320 mL) at 0 °C. Compound **3** (11.2 g, 35.1 mmol) was added and the solution was stirred for 24 h at room temperature. The solution was concentrated and the crude product was recrystallized in MeOH/EtOAc to give **4** (7.64 g in two crops, 95%) as small white needles. Mp 192–193 °C, lit.[18] 190 °C; $[\alpha]_D^{22} = -25$ (*c* 1.0, water), lit.[18] $[\alpha]_D^{22} = -23.4$ (*c* 1, water); ^{1}H NMR (300 MHz, D_2O) δ 4.51 (d, *J*=8.6 Hz, 1H, H^1), 3.79 (dd, *J*=12.4, 2.0 Hz, 1H, H^6), 3.61 (dd, *J*=12.4, 5.2 Hz, 1H, H^6), 3.54 (dd, *J*=10.5, 8.3 Hz, 1H, H^3), 3.45 (s, 3H, OMe), 3.34 (m, 2H, H^4+H^5), 2.88 (dd, *J*=10.6, 8.5 Hz, 1H, H^2); HRMS (FAB) calcd for $C_7H_{15}NO_5Na$ (M−HCl+Na): 216.0848; found 216.0855.

4.2.4. Methyl 2-(9-fluorenylmethoxycarbonyl)amino-2-deoxy-β-D-glucopyranoside (5). Compound **4** (2.78 g, 12.1 mmol) was dissolved in water (22 mL) and $NaHCO_3$ (1.02 g, 12.1 mmol) was added. After the evolution of gas had ceased, additional $NaHCO_3$ (1.02 g, 12.1 mmol), acetone (22 mL), and *N*-(9-fluorenylmethoxycarbonyloxy)-succinimide (4.08 g, 12.1 mmol) were added. The reaction mixture was stirred overnight and solidified during the reaction. The product was suspended in water (100 mL) and chloroform (100 mL), filtered off, and washed with water and chloroform. The crude product was recrystallized in methanol to give **5** (3.81 g in three crops, 76%) as tiny white needles. Mp 163–164 °C; $[\alpha]_D^{22} = -20$ (*c* 0.5, MeOH); ^{1}H NMR (400 MHz, MeOH-d_4) δ 7.79 (d, *J*=7.5 Hz, 2H, Fmoc), 7.68 (d, *J*=6.9 Hz, 2H, Fmoc), 7.38 (t, *J*=7.2 Hz, 2H, Fmoc), 7.30 (t, *J*=7.5 Hz, 2H, Fmoc), 4.28 (m, 4H, 3× Fmoc-H+H^1), 3.88 (dd, *J*=11.9, 2.1 Hz, 1H, H^6), 3.68 (dd, *J*=11.8, 5.8 Hz, 1H, H^6), 3.46 (s, 3 H, OMe), ~3.34

[§] Ac-Tyr-Arg(Mtr)-Tyr-OMe was prepared from Ac-Tyr-OH, H-Arg(Mtr)-OtBu and H-Tyr-OMe analogously to the synthesis of **9a–b**. The Mtr group was cleaved as in the synthesis of **1c**.

[¶] In agreement with previously published data,[30] but a COSY experiment shows that the signals had been incorrectly assigned.

($H^2+H^3+H^4+H^5$, partially obscured by solvent signal); HRMS (FAB) calcd for $C_{22}H_{25}NO_7Na$ (M+Na): 438.1529; found 438.1530.

4.2.5. Methyl 3,4-di-*O*-benzoyl-2-(9-fluorenylmethoxycarbonyl)amino-2-deoxy-6-*O*-triphenylmethyl-β-D-glucopyranoside (6). Compound **5** (4.00 g, 9.63 mmol) was dissolved in pyridine (200 mL, distilled from CaH_2). Chlorotriphenylmethane (8.05 g, 28.9 mmol) was added and the mixture was stirred at 85 °C for 2 h. Benzoyl chloride (5.6 mL, 48.2 mmol) was added at 0 °C and the mixture was stirred for 3.5 h in room temperature. The mixture was poured over ice and the product was extracted with EtOAc (3×300 mL). The extract was washed with H_2SO_4(aq) (0.5 M, 2×200 mL), $NaHCO_3$(aq) (sat., 2×200 mL) and water (100 mL), dried over Na_2SO_4 and evaporated. The product was purified with flash chromatography (toluene/EtOAc 10:1, $R_f=0.20$) and lyophilized from benzene to give **6** (5.27 g, 63%) as a fluffy white powder. $[\alpha]_D^{22}=-20$ (*c* 0.4, MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.82 (d, $J=7.5$ Hz, 2H, Ar), 7.77 (d, $J=7.5$ Hz, 2H, Ar), 7.63 (m, 4H, NH+3×Ar), 7.53 (m, 2H, Ar), 7.37 (m, 14H, Ar), 7.17 (m, 10H, Ar), 5.49 (m, 2H, H^3+H^4), 4.68 (d, $J=8.4$ Hz, 1H, H^1), 4.22 (m, 2H, Fmoc), 4.04 (t, $J=6.5$ Hz, 1H, Fmoc), 3.93 (br d, $J=7.8$ Hz, 1H, H^5), 3.82 (q, $J=9.2$ Hz, 1H, H^2), 3.50 (s, 3H, OMe), ~3.28 (H^6, obscured by HDO signal), 2.95 (dd, $J=10.0$, 3.4 Hz, 1H, H6); HRMS (FAB) calcd for $C_{55}H_{47}NO_9Na$ (M+Na): 888.3149; found 888.3161.

4.2.6. Methyl 3,4-di-*O*-benzoyl-2-(9-fluorenylmethoxycarbonyl)amino-2-deoxy-β-D-glucopyranoside (7). Compound **6** (3.66 g, 4.23 mmol) was dissolved in glacial acetic acid (150 mL) and HBr in AcOH (4.1 M, 2.1 mL, 8.5 mmol) was added. The mixture was stirred for 3 min and then poured over ice. The product was extracted with chloroform (4×100 mL) and the extract was dried over $MgSO_4$ and evaporated. The product was purified with flash chromatography (toluene/EtOAc 2:1, $R_f=0.14$) and lyophilized from benzene to give **7** (2.14 g, 81%) as a fluffy white powder. $[\alpha]_D^{22}=-41$ (*c* 0.5, MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.83 (m, 4H, Bz-*o*+2×Fmoc-H), 7.76 (d, $J=7.7$ Hz, 2H, Bz-*o*), 7.65–7.30 (m, 11H, Bz-*m*+Bz-*p*+NH+4×Fmoc-H), 7.14 (q, $J=7.6$ Hz, 2H, Fmoc), 5.50 (t, $J=9.9$ Hz, 1H, H^3), 5.25 (t, $J=9.8$ Hz, 1H, H^4), 4.89 (t, $J=5.7$ Hz, 1H, OH), 4.64 (d, $J=8.4$ Hz, 1H, H^1), 4.18 (m, 2H, Fmoc), 4.01 (t, $J=6.7$ Hz, 1H, Fmoc), 3.72 (m, 2H, H^2+H^5), 3.54 (m, 2H, 2×H^6), 3.44 (s, 3H, OMe); HRMS (FAB) calcd for $C_{36}H_{33}NO_9Na$ (M+Na): 646.2053; found 646.2059.

4.3. Preparation of tripeptide *tert*-butyl esters 11a–c

4.3.1. Fmoc-Tyr-Tyr-O^{t}Bu (8a). Fmoc-Tyr-OH (700 mg, 1.74 mmol) was dissolved in THF (17 mL) and H-Tyr-O^{t}Bu (412 mg, 1.74 mmol), HOBt (234 mg, 1.74 mmol), EDC·HCl (349 mg, 1.82 mmol) and *N*-methylmorpholine (0.380 mL, 3.47 mmol) were added. The mixture was stirred overnight and then evaporated. The residue was dissolved in MeOH and impregnated on silica. The product was purified with flash chromatography (toluene/EtOAc 2:1, $R_f=0.20$) to give **8a** (948 mg, 88%) as a white amorphous solid. $[\alpha]_D^{22}=-16$ (*c* 0.5, MeOH); ^{1}H NMR (MeOH-d_4, 300 MHz) δ 7.77 (d, $J=7.5$ Hz, 2H, Fmoc), 7.56 (d, $J=7.7$ Hz, 2H, Fmoc), 7.37 (t, $J=7.5$ Hz, 2H, Fmoc), 7.28 (m, 2H, Fmoc), 7.03 (d, $J=8.5$ Hz, 2H, Tyr-H^δ), 7.00 (d, $J=8.4$ Hz, 2H, Tyr-H^δ), 6.68 (d, $J=8.5$ Hz, 2H, Tyr-H^ε), 6.67 (d, $J=8.5$ Hz, 2H, Tyr-H^ε), 4.45 (t, $J=7.0$ Hz, 1H, Tyr-H^α), 4.30 (m, 2H, 1×Tyr-H^α+1×Fmoc-H), 4.16 (m, 2H, Fmoc), 3.05–2.85 (m, 3H, 3×Tyr-H^β), 2.71 (dd, $J=13.9$, 9.3 Hz, 1H, Tyr-H^β), 1.37 (s, 9H, O^{t}Bu). HRMS (FAB) calcd for $C_{37}H_{39}N_2O_7$ (M+H): 623.2757; found 623.2748.

4.3.2. Fmoc-Tyr-Tyr-Tyr-O^{t}Bu (9a). Compound **8a** (951 mg, 1.53 mmol) was dissolved in CH_2Cl_2 (12 mL) and Et_3SiH (0.61 mL, 3.8 mmol) and TFA (6 mL) were added. The mixture was stirred for 4 h and coevaporated with toluene. The crude free acid was dissolved in THF (14 mL) and H-Tyr-O^{t}Bu (363 mg, 1.53 mmol), HOBt (206 mg, 1.53 mmol), EDC·HCl (308 mg, 1.60 mmol) and *N*-methylmorpholine (0.340 mL, 3.06 mmol) were added. The mixture was stirred overnight and then evaporated. The residue was dissolved in MeOH and impregnated on silica. The product was purified with flash chromatography (toluene/MeOH 5:1, $R_f=0.36$) to give **9a** (1.13 g, 94%) as a white amorphous solid. $[\alpha]_D^{22}=-22$ (*c* 0.5, MeOH); ^{1}H NMR (MeOH-d_4, 300 MHz) δ 7.77 (d, $J=7.5$ Hz, 2H, Fmoc), 7.55 (d, $J=7.5$ Hz, 2H, Fmoc), 7.37 (t, $J=7.5$ Hz, 2H, Fmoc), 7.26 (m, 2H, Fmoc), 7.00 (d, $J=8.5$ Hz, 6H, Tyr-H^δ), 6.66 (m, 6H, Tyr-H^ε), 4.55 (t, $J=6.4$ Hz, 1H, Tyr-H^α), 4.42 (t, $J=6.9$ Hz, 1H, Tyr-H^α), 4.20 (m, 4H, 1×Tyr-H^α+Fmoc), 3.05–2.75 (m, 5H, 5×Tyr-H^β), 2.66 (dd, $J=14.5$, 9.5 Hz, 1H, Tyr-H^β), 1.35 (s, 9H, O^{t}Bu); HRMS (FAB) calcd for $C_{46}H_{48}N_3O_9$ (M+H): 786.3391; found 786.3398.

4.3.3. H-Tyr-Tyr-Tyr-O^{t}Bu (10a). Compound **9a** (2.27 g, 2.89 mmol) was suspended in CH_2Cl_2 (80 mL) and piperidine (14.3 mL) was added. After stirring for 30 min, toluene (50 mL) was added and the mixture was evaporated. The residue was dissolved in CH_2Cl_2 and purified with flash chromatography (toluene/MeOH 3:1, $R_f=0.13$) to give **10a** (1.42 g, 87%) as a white foam. $[\alpha]_D^{24}=-14$ (*c* 0.5, MeOH); ^{1}H NMR (MeOH-d_4, 300 MHz) δ 6.97 (m, 6H, Tyr-H^δ), 6.67 (m, 6H, Tyr-H^ε), 4.56 (dd, $J=7.9$, 6.0 Hz, 1H, Tyr-H^α), 4.44 (t, $J=7.1$ Hz, 1H, Tyr-H^α), 3.44 (dd, $J=8.0$, 5.0 Hz, 1H, Tyr-H^α), 3.00–2.70 (m, 5H, 5×Tyr-H^β), 2.52 (dd, $J=13.8$, 8.0 Hz, 1H, Tyr-H^β), 1.38 (s, 9H, O^{t}Bu); HRMS calcd for $C_{31}H_{38}N_3O_7$ (M+H): 564.2701; found 564.2710.

4.3.4. Fmoc-Tyr-Glu(OBzl)-O^{t}Bu (8b). The title compound was prepared from Fmoc-Tyr-OH (1.26 g, 3.12 mmol) and H-Glu(OBzl)-O^{t}Bu·HCl (1.03 g, 3.12 mmol) using the method described in the synthesis of **8a**, but using an additional equivalent of base to neutralize the hydrochloride salt. The product was purified with flash chromatography (toluene/EtOAc 3:1, $R_f=0.19$) to give **8b** (1.82 g, 86%) as a white foam. $[\alpha]_D^{22}=-16$ (*c* 0.5, MeOH); ^{1}H NMR (MeOH-d_4, 300 MHz) δ 7.77 (d, $J=7.5$ Hz, 2H, Fmoc), 7.55 (d, $J=6.6$ Hz, 2H, Fmoc), 7.37 (d, $J=7.4$ Hz, 2H, Fmoc), 7.27 (m, 7H, Fmoc+Bzl), 7.06 (d, $J=8.4$ Hz, 2H, Tyr-H^δ), 6.68 (d, $J=8.3$ Hz, 2H, Tyr-H^ε), 5.06 (m, 2H, Bzl), 4.30 (m, 3H, Tyr-H^α+Glu-H^α+1×Fmoc-H), 4.16 (m, 2H, Fmoc), 3.02 (dd, $J=13.9$ Hz, $J=5.2$ Hz, 1H, Tyr-H^β), 2.77 (dd, $J=14.0$, 9.4 Hz, 1H, Tyr-H^β), 2.43 (t,

J=7.4 Hz, 2H, Glu-H$^{\gamma}$), 2.15 (m, 1H, Glu-H$^{\beta}$), 1.90 (m, 1H, Glu-H$^{\beta}$), 1.43 (s, 9H, O^{t}Bu); HRMS (FAB) calcd for $C_{40}H_{43}N_2O_8$ (M+H): 679.3019; found 679.3014.

4.3.5. Fmoc-Tyr-Glu(OBzl)-Tyr-O^{t}Bu (9b). The title compound was prepared from **8b** (1.73 g, 2.55 mmol) and H-Tyr-O^{t}Bu (605 mg, 2.55 mmol) using the method described in the synthesis of **9a**. The product was purified with flash chromatography (toluene/EtOAc 3:2, R_f=0.11) to give **9b** (1.77 g, 83%) as a white foam. $[\alpha]_D^{22}=-15$ (c 0.5, MeOH); ^{1}H NMR (MeOH-d$_4$, 300 MHz) δ 7.77 (d, J= 7.5 Hz, 2H, Fmoc), 7.55 (dd, J=7.3, 3.0 Hz, 2H, Fmoc), 7.37 (t, J=7.4 Hz, 2H, Fmoc), 7.28 (m, 7H, Fmoc+Bzl), 7.04 (d, J=8.2 Hz, 2H, Tyr-H$^{\delta}$), 7.02 (d, J=8.2 Hz, 2H, Tyr-H$^{\delta}$), 6.69 (d, J=8.5 Hz, 2H, Tyr-H$^{\varepsilon}$), 6.67 (d, J= 8.4 Hz, 2H, Tyr-H$^{\varepsilon}$), 5.05 (m, 2H, Bzl), 4.41 (m, 2H, Tyr-H$^{\alpha}$+Glu-H$^{\alpha}$), 4.28 (m, 2H, 1×Tyr-H$^{\alpha}$+1×Fmoc-H), 4.25 (m, 2H, Fmoc), 2.92 (m, 3H, 3×Tyr-H$^{\beta}$), 2.75 (dd, J=13.8, 9.5 Hz, 1H, Tyr-H$^{\beta}$), 2.40 (t, J=7.7 Hz, 2H, Tyr-H$^{\gamma}$), 2.07 (m, 1H, Glu-H$^{\beta}$), 1.89 (m, 1H, Glu-H$^{\beta}$), 1.35 (s, 9H, O^{t}Bu); HRMS (FAB) calcd for $C_{49}H_{52}N_3O_{10}$ (M+H): 842.3653; found 842.3646.

4.3.6. H-Tyr-Glu(OBzl)-Tyr-O^{t}Bu (10b). The Fmoc group of **9b** (1.74 g, 2.06 mmol) was removed using the same method as in the synthesis of **10a**. The product was purified using flash chromatography (EtOAc/MeOH 40:1, R_f=0.21) to give **10b** (1.05 g, 88%) as a white foam. $[\alpha]_D^{22}=-19$ (c 0.5, MeOH); ^{1}H NMR (MeOH-d$_4$, 300 MHz) δ 7.32 (m, 5H, Bzl), 7.03 (d, J=8.5 Hz, 2H, Tyr-H$^{\delta}$), 6.99 (d, J=8.5 Hz, 2H, Tyr-H$^{\delta}$), 6.68 (d, J=8.5 Hz, 4H, Tyr-H$^{\varepsilon}$), 5.11 (s, 2H, Bzl), 4.40 (m, 2H, Tyr-H$^{\alpha}$+Glu-H$^{\alpha}$), 3.50 (dd, J=7.5, 5.3 Hz, 1H, Tyr-H$^{\alpha}$), 3.00–2.80 (m, 3H, Tyr-H$^{\beta}$), 2.68 (dd, J=13.6, 7.6 Hz, 1H, Tyr-H$^{\beta}$), 2.36 (t, J=7.7 Hz, 2H, Glu-H$^{\gamma}$), 2.05 (m, 1H, Glu-H$^{\beta}$), 1.87 (m, 1H, Glu-H$^{\beta}$), 1.36 (s, 9H, O^{t}Bu). HRMS (FAB) calcd for $C_{34}H_{42}N_3O_8$ (M+H): 620.2972; found 620.2969.

4.3.7. Fmoc-Arg(Mtr)-Tyr-O^{t}Bu (11). The title compound was prepared from Fmoc-Arg(Mtr)-OH (1.82 g, 2.99 mmol) and H-Tyr-O^{t}Bu (709 mg, 2.99 mmol) using the method described in the synthesis of **8a**. The product was purified with flash chromatography (toluene/EtOAc 1:3, R_f=0.17) to give **11** (2.06 g, 83%) as a white foam. $[\alpha]_D^{22}=-6$ (c 0.5, MeOH); ^{1}H NMR (300 MHz, MeOH-d$_4$) δ 7.78 (d, J= 7.5 Hz, 2H, Fmoc), 7.64 (t, J=5.6 Hz, 2H, Fmoc), 7.37 (t, J=7.4 Hz, 2H, Fmoc), 7.28 (t, J=7.4 Hz, 2H, Fmoc), 7.00 (d, J=8.4 Hz, 2H, Tyr-H$^{\delta}$), 6.67 (d, J=8.3 Hz, 2H, Tyr-H$^{\varepsilon}$), 6.62 (s, 1H, Mtr-ArH), 4.45 (t, J=7.0 Hz, 1H, Tyr-H$^{\alpha}$), 4.34 (m, 2H, Fmoc), 4.19 (t, J=6.5 Hz, 1H, Fmoc), 4.05 (t, J=6.9 Hz, 1H, Arg-H$^{\alpha}$), 3.78 (s, 3H, Mtr-OMe), 3.14 (br s, 2H, Arg-H$^{\delta}$), 2.01 (m, 2H, Tyr-H$^{\beta}$), 2.66 (s, 3H, Mtr-Me), 2.60 (s, 3H, Mtr-Me), 2.09 (s, 3H, Mtr-Me), 1.68 (m, 1H, Arg-H$^{\beta}$), 1.54 (m, 1H, Arg-H$^{\beta}$), 1.47 (m, 2H, Arg-H$^{\gamma}$), 1.35 (s, 9H, O^{t}Bu); HRMS (FAB) calcd for $C_{44}H_{54}N_5O_9S$ (M+H): 828.3642; found 828.3654.

4.3.8. H-Arg(Mtr)-Tyr-O^{t}Bu (12). The Fmoc group of **11** (2.01 g, 2.42 mmol) was removed using the same method as in the synthesis of **10a**. The product was purified using flash chromatography (EtOAc/MeOH 10:1, R_f=0.31) to give **12** (1.33 g, 90%) as a white foam. $[\alpha]_D^{22}=+7$ (c 0.5, MeOH); ^{1}H NMR (300 MHz, MeOH-d$_4$) δ 7.01 (d, J=8.5 Hz, 2H, Tyr-H$^{\delta}$), 6.69 (d, J=8.5 Hz, 2H, Tyr-H$^{\varepsilon}$), 6.65 (s, 1H, Mtr-ArH), 4.48 (dd, J=8.1, 6.5 Hz, 1H, Tyr-H$^{\alpha}$), 3.82 (s, 3H, Mtr-OMe), ~3.28 (Arg-H$^{\alpha}$, partially obscured by solvent signal), 3.12 (br s, 2H, Arg-H$^{\delta}$), 2.98 (dd, J=13.9, 6.4 Hz, 1H, Tyr-H$^{\beta}$), 2.86 (dd, J=13.9, 8.2 Hz, 1H, Tyr-H$^{\beta}$), 2.66 (s, 3H, Mtr-Me), 2.60 (s, 3H, Mtr-Me), 2.12 (s, 3H, Mtr-Me), 1.58 (m, 1H, Arg-H$^{\beta}$), 1.45 (m, 3H, 1×Arg-H$^{\beta}$+2×Arg-H$^{\gamma}$), 1.38 (s, 9H, O^{t}Bu); HRMS (FAB) calcd for $C_{29}H_{44}N_5O_7S$ (M+H): 606.2961; found 606.2958.

4.3.9. Fmoc-Tyr-Arg(Mtr)-Tyr-O^{t}Bu (13). The title compound was prepared from Fmoc-Tyr-OH (854 mg, 2.12 mmol) and **12** (1.28 g, 2.12 mmol) using the method described in the synthesis of **8a**. The product was purified with flash chromatography (toluene/EtOAc 1:4, R_f=0.16) to give **13** (1.74 g, 83%) as a white foam. $[\alpha]_D^{22}=-8$ (c 0.5, MeOH); ^{1}H NMR (300 MHz, MeOH-d$_4$) δ 7.76 (d, J= 7.6 Hz, 2H, Fmoc), 7.55 (d, J=7.1 Hz, 2H, Fmoc), 7.36 (t, J=7.4 Hz, 2H, Fmoc), 7.26 (t, J=7.2 Hz, 2H, Fmoc), 7.04 (d, J=7.8 Hz, 2H, Tyr-H$^{\delta}$), 7.01 (d, J=8.2 Hz, 2H, Tyr-H$^{\delta}$), 6.68 (d, J=8.0 Hz, 2H, Tyr-H$^{\varepsilon}$), 6.67 (d, J=8.1 Hz, 2H, Tyr-H$^{\varepsilon}$), 6.61 (s, 1H, Mtr-ArH), 4.42 (t, J=7.2 Hz, 1H, Tyr-H$^{\alpha}$), 4.35 (dd, J=8.3, 5.1 Hz, 1H, Arg-H$^{\alpha}$), 4.31 (m, 2H, 1×Tyr-H$^{\alpha}$+1×Fmoc), 4.16 (m, 2H, Fmoc), 3.78 (s, 3H, Mtr-OMe), 3.12 (br s, 2H, Arg-H$^{\delta}$), 2.94 (m, 3H, Tyr-H$^{\beta}$), 2.75 (dd, J=13.8, 9.9 Hz, 1H, Tyr-H$^{\beta}$), 2.65 (s, 3H, Mtr-Me), 2.59 (s, 3H, Mtr-Me), 2.08 (s, 3H, Mtr-Me), 1.74 (m, 1H, Arg-H$^{\beta}$), 1.58 (m, 1H, Arg-H$^{\beta}$), 1.48 (m, 2H, Arg-H$^{\gamma}$), 1.35 (s, 9H, O^{t}Bu); HRMS (FAB) calcd for $C_{53}H_{63}N_6O_{11}S$ (M+H): 991.4276; found 991.4250.

4.3.10. H-Tyr-Arg(Mtr)-Tyr-O^{t}Bu (10c). The Fmoc group of **13** (1.60 g, 1.61 mmol) was removed using the same method as in the synthesis of **10a**. The product was purified using flash chromatography (EtOAc/MeOH 10:1+2% Me_2NEt, R_f=0.21) to give **10c** (1.02 g, 83%) as a white foam. $[\alpha]_D^{22}=-10$ (c 0.5, MeOH); ^{1}H NMR (300 MHz, MeOH-d$_4$) δ 7.03 (d, J=8.4 Hz, 2H, Tyr-H$^{\delta}$), 7.02 (d, J= 8.4 Hz, 2H, Tyr-H$^{\delta}$), 6.71 (d, J=8.4 Hz, 2H, Tyr-H$^{\varepsilon}$), 6.68 (d, J=8.4 Hz, 2H, Tyr-H$^{\varepsilon}$), 6.65 (s, 1H, Mtr-ArH), 4.44 (t, J=7.2 Hz, 1H, Tyr-H$^{\alpha}$), 4.37 (dd, J=8.1 Hz, J=5.7 Hz, 1H, Arg-H$^{\alpha}$), 3.82 (s, 3H, Mtr-OMe), 3.67 (dd, J=8.1, 5.4 Hz, 1H, Tyr-H$^{\alpha}$), 3.12 (br t, J=4.5 Hz, 2H, Arg-H$^{\delta}$), 2.92 (m, 3H, Tyr-H$^{\beta}$), 2.72 (dd, J=14.0, 8.0 Hz, 1H, Tyr-H$^{\beta}$), 2.66 (s, 3H, Mtr-Me), 2.61 (s, 3H, Mtr-Me), 2.12 (s, 3H, Mtr-Me), 1.73 (m, 1H, Arg-H$^{\beta}$), 1.59 (m, 1H, Arg-H$^{\beta}$), 1.48 (m, 2H, Arg-H$^{\gamma}$), 1.37 (s, 9H, O^{t}Bu); HRMS (FAB) calcd for $C_{38}H_{53}N_6O_9S$ (M+H): 769.3595; found 769.3610.

4.4. Synthesis of the macrocycles 1a–c

4.4.1. Fmoc-SAA(di-*O*-Bz)-Tyr$_3$-O^{t}Bu (14a). Compound **7** (1.60 g, 2.56 mmol) was dissolved in acetone (380 mL) and the mixture was cooled to 0 °C. Jones's reagent (4 M, 25.6 mL, prepared by dissolving 12.0 g CrO_3 and 6.9 mL concd H_2SO_4 in 23.1 mL water) was added. The solution was stirred at room temperature for 1.5 h and then quenched by addition of MeOH (100 mL). The mixture was carefully evaporated (caution: bumping) and the residue was dissolved in water (200 mL) and EtOAc (200 mL). The phases were separated and the aqueous phase was extracted with EtOAc (2×200 mL). The organic phases were

combined and washed with water (2×200 mL), dried over Na_2SO_4 and evaporated. The crude oxidation product was dissolved in THF (45 mL) and H-Tyr-Tyr-Tyr-O^{t}Bu **10a** (1.44 g, 2.56 mmol), HOBt (0.346 g, 2.56 mmol), EDC·HCl (0.515 g, 2.69 mmol), and *N*-methylmorpholine (0.56 mL, 5.12 mmol) were added. After 16 h, the mixture was concentrated, dissolved in MeOH and impregnated on silica. The product was purified with flash chromatography (Toluene/EtOAc 2:3, R_f=0.10) to give **14a** (1.37 g, 45%). as a white amorphous solid $[\alpha]^{22}_D=-5$ (*c* 0.5, MeOH); ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ 9.22 (s, 1H, Tyr-OH), 9.13 (s, 1H, Tyr-OH), 9.12 (s, 1H, Tyr-OH), 8.29 (d, *J*=7.3 Hz, 1H, NH), 8.12 (d, *J*=8.2 Hz, 1H, NH), 7.97 (d, *J*=8.2 Hz, 1H, NH), 7.83 (d, *J*=6.7 Hz, 2H, Fmoc), 7.79 (d, *J*=7.9 Hz, 2H, Bz-*o*), 7.75 (d, *J*=7.2 Hz, 2H, Bz-*o*), 7.62 (m, 3H, Bz-*p*+NH), 7.51 (d, *J*=7.6 Hz, 1H, Fmoc), 7.42 (t, *J*=7.7 Hz, 4H, Bz-*m*), 7.38 (d, *J*=8.8 Hz, 1H, Fmoc), 7.34 (td, *J*=7.5, 3.4 Hz, 2H, Fmoc), 7.15 (m, 2H, Fmoc), 7.01 (d, *J*=8.4 Hz, 2H, Tyr-H$^\delta$), 6.95 (d, *J*=8.5 Hz, 2H, Tyr-H$^\delta$), 6.90 (d, *J*=8.4 Hz, 2H, Tyr-H$^\delta$), 6.67 (d, *J*=8.5 Hz, 2H, Tyr-H$^\varepsilon$), 6.59 (d, *J*=8.4 Hz, 2H, Tyr-H$^\varepsilon$), 6.55 (d, *J*=8.4 Hz, 2H, Tyr-H$^\varepsilon$), 5.50 (t, *J*=9.9 Hz, 1H, SAA-H^3), 5.33 (t, *J*=9.6 Hz, 1H, SAA-H^4), 4.68 (t, *J*=8.3 Hz, 1H, SAA-H^1), 4.43 (m, 2H, 2×Tyr-H$^\alpha$), 4.27 (m, 3H, 1×Tyr-H$^\alpha$+SAA-H^5+1×Fmoc-H), 4.15 (dd, *J*=10.6, 6.9 Hz, 1H, Fmoc), 4.02 (t, *J*=6.5 Hz, 1H, Fmoc), 3.73 (q, *J*=9.2 Hz, SAA-H^2), 3.42 (s, 3H, OMe), 2.78 (m, 5H, 5×Tyr-H$^\beta$), 2.58 (dd, *J*=14.7, 8.1 Hz, 1H, Tyr-H$^\beta$), 1.31 (s, 9H, O^{t}Bu); HRMS (FAB) calcd for $C_{67}H_{66}N_4O_{16}Na$ (M+Na): 1205.4372; found 1205.4388.

4.4.2. H-SAA(di-*O*-Bz)-Tyr$_3$-O^{t}Bu (15a). Compound **14a** (400 mg, 0.338 mmol) was dissolved in THF (10 mL) and *N*-(2-mercaptoethyl)aminomethyl polystyrene (2.0 mmol/g, 1.69 g) and DBU (76 μL, 0.507 mmol) were added. After stirring the mixture for 6 h, the solid phase was filtered off and washed with THF (2×8 mL) and MeOH (2×8 mL). The filtrate and washings were combined and evaporated. The residue was dissolved in CH_2Cl_2/MeOH 9:1 and filtered through silica. Evaporation of the filtrate gave **15a** (306 mg, 94%) as a yellowish amorphous solid. $[\alpha]^{22}_D=-13$ (*c* 0.5, MeOH); ^{1}H NMR (MeOH-d$_4$, 300 MHz) δ 7.92 (d, *J*=7.8 Hz, 2H, Bz-*o*), 7.84 (d, *J*=7.8 Hz, 2H, Bz-*o*), 7.50 (m, 2H, Bz-*p*), 7.35 (m, 4H, Bz-*m*), 6.99 (d, *J*=8.5 Hz, 4H, Tyr-H$^\delta$), 6.89 (d, *J*=8.5 Hz, 2H, Tyr-H$^\delta$), 6.69 (d, *J*=8.5 Hz, 2H, Tyr-H$^\varepsilon$), 6.68 (d, *J*=8.5 Hz, 2H, Tyr-H$^\varepsilon$), 6.61 (d, *J*=8.5 Hz, 2H, Tyr-H$^\varepsilon$), 5.50 (t, *J*=9.8 Hz, 1H, SAA-H^3), 5.38 (t, *J*=9.6 Hz, 1H, SAA-H^4), 4.42 (m, 4H, SAA-H^1+3×Tyr-H$^\alpha$), 4.20 (d, *J*=9.8 Hz, 1H, SAA-H^5), 3.56 (s, 3H, OMe), 3.02 (dd, *J*=10.1, 8.06 Hz, 1H, SAA-H^2), 2.86 (m, 5H, 5×Tyr-H$^\beta$), 2.66 (dd, *J*=13.7, 7.9 Hz, 1H, Tyr-H$^\beta$), 1.34 (s, 9H, O^{t}Bu); HRMS (FAB) calcd for $C_{52}H_{56}N_4O_{14}Na$ (M+Na): 983.3690; found 983.3687.

4.4.3. Fmoc-SAA(di-*O*-Bz)-Tyr$_3$-SAA(di-*O*-Bz)-Tyr$_3$-O^{t}Bu (17a). Compound **14a** (313 mg, 0.265 mmol) was dissolved in CH_2Cl_2 (4 mL) and Et_3SiH (105 μL, 0.66 mmol) and TFA (2 mL) were added. The mixture was stirred for 4 h and coevaporated with toluene. The crude free acid was dissolved in THF (3 mL) and **15a** (255 mg, 0.265 mmol), HOBt (35.8 mg, 0.265 mmol), and DIC (50 μL, 0.32 μmol) were added. The mixture was stirred for 16 h and then concentrated. The residue was dissolved in MeOH and impregnated on silica. The product was purified using flash chromatography (CH_2Cl_2/MeOH 10:1, R_f=0.27) followed by size-exclusion chromatography to give **17a** (347 mg, 65%) as a white amorphous solid. $[\alpha]^{22}_D=-8$ (*c* 0.5, MeOH); ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ 9.20 (s, 1H, Tyr-OH), 9.10 (s, 2H, 2×Tyr-OH), 9.07 (s, 1H, Tyr-OH), 9.06 (s, 1H, Tyr-OH), 9.06 (s, 1H, Tyr-OH), 8.33 (d, *J*=8.8 Hz, 1H, NH), 8.27 (d, *J*=7.3 Hz, 1H, NH), 8.11 (d, *J*=8.7 Hz, 1H, NH), 7.93 (m, 4H, 4×NH), 7.74 (m, 10H, Bz-*o*+2×Fmoc-H), 7.55 (m, 5H, Bz-*p*+NH), 7.39 (m, 12H, Bz-*m*+4×Fmoc-H), 7.12 (m, 2H, Fmoc), 6.99 (d, *J*=8.4 Hz, 2H, Tyr-H$^\delta$), 6.90 (m, 6H, Tyr-H$^\delta$), 6.78 (d, *J*=9.8 Hz, 2H, Tyr-H$^\delta$), 6.75 (d, *J*=9.1 Hz, 2H, Tyr-H$^\delta$), 6.65 (d, *J*=8.3 Hz, 2H, Tyr-H$^\varepsilon$), 6.51 (m, 10H, Tyr-H$^\varepsilon$), 5.51 (t, *J*=9.8 Hz, 1H, SAA-H^3), 5.46 (t, *J*=9.9 Hz, 1H, SAA-H^3), 5.33 (t, *J*=9.7 Hz, 1H, SAA-H^4), 5.28 (t, *J*=9.9 Hz, 1H, SAA-H^4), 4.71 (d, *J*=8.3 Hz, 1H, SAA-H^1), 4.64 (d, *J*=7.8 Hz, 1H, SAA-H^1), 4.31 (m, 9H, 2×SAA-H^5+1×Fmoc-H+6×Tyr-H$^\alpha$), 4.11 (m, 2H, SAA-H2+1×Fmoc), 3.99 (t, *J*=6.4 Hz, 1H, Fmoc), 3.69 (q, *J*=9.1 Hz, 1H, SAA-H^2), 3.39 (s, 3H, OMe), 3.37 (s, 3H, OMe), 2.82–2.20 (m, 12H, Tyr-H$^\beta$), 1.28 (s, 9H, O^{t}Bu); HRMS (FAB) calcd for $C_{115}H_{112}N_8O_{29}Na$ (M+Na): 2091.7433; found 2091.7444.

4.4.4. H-SAA(di-*O*-Bz)-Tyr$_3$-SAA(di-*O*-Bz)-Tyr$_3$-O^{t}Bu (18a). The title compound was prepared from compound **17a** (334 mg, 0.161 mmol) using the method described in the synthesis of **15a** to give **18a** (285 mg, 95%) as a yellowish amorphous solid. $[\alpha]^{21}_D=-11$ (*c* 0.5, MeOH); ^{1}H NMR (MeOH-d$_4$, 300 MHz) δ 7.86 (m, 8H, Bz-*o*), 7.49 (m, 4H, Bz-*p*), 7.35 (m, 8H, Bz-*m*), 6.97 (m, 6H, Tyr-H$^\delta$), 6.90 (d, *J*=8.7 Hz, 2H, Tyr-H$^\delta$), 6.81 (d, *J*=8.8 Hz, 2H, Tyr-H$^\delta$), 6.79 (d, *J*=8.9 Hz, 2H, Tyr-H$^\delta$), 6.69 (d, *J*=8.3 Hz, 6H, Tyr-H$^\varepsilon$), 6.61 (d, *J*=8.5 Hz, 4H, Tyr-H$^\varepsilon$), 6.57 (d, *J*=8.5 Hz, 2H, Tyr-H$^\varepsilon$), 5.73 (t, *J*=10.0 Hz, SAA-H^3), 5.42 (m, 3H, SAA-H^3+2×SAA-H^4), 4.77 (d, *J*=8.3 Hz, 1H, SAA-H^1), 4.50–4.30 ppm (m, 7H, SAA-H^1+6×Tyr-H$^\alpha$), 4.26 (d, *J*=10.0 Hz, 1H, SAA-H^5), 4.15 (dd, *J*=10.6, 8.4 Hz, 1H, SAA-H^2), 4.12 (d, *J*=9.9 Hz, 1H, SAA-H^5), 3.56 (s, 3H, OMe), 3.48 (s, 3H, OMe), 3.04 (dd, *J*=10.2, 8.0 Hz, 1H, SAA-H^2), 2.95-2.40 (m, 12H, Tyr-H$^\beta$), 1.34 (s, 9H, O^{t}Bu); HRMS (FAB) calcd for $C_{100}H_{102}N_8O_{27}Na$ (M+Na): 1869.6752; found 1869.6736.

4.4.5. Cyclo[SAA(di-*O*-Bz)-Tyr$_3$-SAA(di-*O*-Bz)-Tyr$_3$] (19a). Compound **18a** (37.6 mg, 20.3 μmol) was dissolved in CH_2Cl_2 (1.6 mL) and Et_3SiH (8.1 μL, 51 μmol) and TFA (0.8 mL) were added. The mixture was stirred for 4 h and coevaporated with toluene. The crude product was dissolved in THF (20 mL) and DIPEA (10 μL, 61 μmol) and HAPyU (10.6 mg, 24.4 μmol) were added. The mixture was stirred for 1 h at room temperature and then evaporated. The product was purified by flash chromatography (CH_2Cl_2/MeOH 6:1, R_f=0.29) followed by size-exclusion chromatography to give **19a** (13.3 mg, 37%) as a white amorphous solid. $[\alpha]^{23}_D=-6$ (*c* 0.5, MeOH); ^{1}H NMR (DMSO-d$_6$, 400 MHz, 150 °C) δ 8.47 (s, 2H, Tyr-OH), 8.41 (s, 2H, Tyr-OH), 8.34 (s, 2H, Tyr-OH), 7.86 (d, *J*=8.4 Hz, 2H, NH), 7.80 (t, *J*=7.2 Hz, 8H, Bz-*o*), 7.47 (m, 4H, Bz-*p*), 7.41 (d, *J*=8.0 Hz, 2H, NH), 7.34 (m, 10H, Bz-*m*+2×NH), 7.05 (d, *J*=8.3 Hz, 2H, NH), 6.89 (d, *J*=8.5 Hz, 4H, Tyr-H$^\delta$), 6.83 (d, *J*=8.4 Hz, 4H, Tyr-H$^\delta$), 6.79 (d, *J*=8.3 Hz, 4H,

Tyr-H$^{\delta}$), 6.66 (d, J=8.5 Hz, 4H, Tyr-H$^{\varepsilon}$), 6.60 (d, J=8.4 Hz, 4H, Tyr-H$^{\varepsilon}$), 6.52 (d, J=8.5 Hz, 4H, Tyr-H$^{\varepsilon}$), 5.85 (t, J=10.0 Hz, 2H, SAA-H^{3}), 5.66 (t, J=9.6 Hz, 2H, SAA-H^{4}), 5.10 (d, J=8.0 Hz, 2H, SAA-H^{1}), 4.49 (d, J=9.7 Hz, 2H, SAA-H^{5}), 4.35 (m, 4H, Tyr-H$^{\alpha}$), 4.13 (q, J=8.4 Hz, 2H, SAA-H^{2}), 4.01 (q, J=7.9 Hz, 2H, Tyr-H$^{\alpha}$), 3.48 (s, 6H, OMe), 2.9–2.6 (m, 12H, Tyr-H$^{\beta}$); HRMS (FAB) calcd for $C_{96}H_{92}N_8O_{26}Na$ (M+Na): 1795.6020; found 1795.6010.

4.4.6. Cyclo(SAA-Tyr$_3$-SAA-Tyr$_3$) (1a). Compound **19a** (89.1 mg, 50.3 μmol) was dissolved in MeOH (18 mL) and NaOMe/MeOH (1 M, 180 μL) was added. The solution was stirred for 18 h, then neutralised with AcOH and evaporated. The residue was purified using size-exclusion chromatography on a short column to afford **1a** (41.7 mg, 61%) as a white amorphous solid. $[\alpha]_D^{21}=-15$ (c 0.5, MeOH); ^{1}H NMR (MeOH-d$_4$, 300 MHz) δ 6.97 (m, 12H, Tyr-H$^{\delta}$), 6.70 (m, 12H, Tyr-H$^{\varepsilon}$), 4.75 (d, J=8.5 Hz, 2H, SAA-H^{1}), 4.52 (dd, J=9.4, 5.1 Hz, 2H, Tyr-H$^{\alpha}$), 4.41 (dd, J=8.5, 4.7 Hz, 2H, Tyr-H$^{\alpha}$), 4.29 (t, J=6.8 Hz, 2H, Tyr-H$^{\alpha}$), 3.82 (d, J=9.7 Hz, 2H, SAA-H^{5}), 3.78 (t, J=9.4 Hz, 2H, SAA-H^{3}), 3.41 (t, J=9.4 Hz, 2H, SAA-H^{4}), 3.33 (s, 6H, OMe), ~3.3 (SAA-H^{2}, obscured by solvent signal), 3.05–2.80 (m, 10H, Tyr-H$^{\beta}$), 2.54 (dd, J=14.1, 9.4 Hz, 2H, Tyr-H$^{\beta}$); HRMS (FAB) calcd for $C_{68}H_{76}N_8O_{22}Na$ (M+Na): 1379.4972; found 1379.4955.

4.4.7. Fmoc-SAA(di-OBz)-Tyr-Glu(OBzl)-Tyr-O^{t}Bu (14b). The title compound was prepared from **7** (1.09 g, 1.74 mmol) and **10b** (1.08 g, 1.74 mmol) using the method described in the synthesis of **14a**. The product was purified with flash chromatography (toluene/EtOAc 1:1, R_f=0.13) to give **14b** (1.39 g, 64%) as a white amorphous solid. $[\alpha]_D^{22}=-12$ (c 0.5, MeOH); ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ 9.19 (s, 1H, Tyr-OH), 9.11 (s, 1H, Tyr-OH), 8.19 (d, J=7.0 Hz, 1H, NH), 8.15 (d, J=7.9 Hz, 1H, NH), 8.14 (d, J=8.0 Hz, 1H, NH), 7.80 (d, J=7.8 Hz, 2H, Fmoc), 7.75 (d, J=7.3 Hz, 2H, Bz-*o*), 7.68 (d, J=7.3 Hz, 2H, Bz-*o*), 7.52 (m, 4H, Bz-*p*+1×Fmoc-H+1×NH), 7.35 (m, 12H, 1×Fmoc-H+Bz-*m*+Bzl), 7.12 (m, 2H, Fmoc), 6.98 (d, J=8.4 Hz, 2H, Tyr-H$^{\delta}$), 6.94 (d, J=8.5 Hz, 2H, Tyr-H$^{\delta}$), 6.64 (d, J=8.5 Hz, 2H, Tyr-H$^{\varepsilon}$), 6.57 (d, J=8.4 Hz, 2H, Tyr-H$^{\varepsilon}$), 5.46 (t, J=9.9 Hz, 1H, SAA-H^{3}), 5.32 (t, J=9.7 Hz, 1H, SAA-H^{4}), 5.04 (s, 2H, Bzl), 4.65 (d, J=8.3 Hz, 1H, SAA-H^{1}), 4.44 (dd, J=12.5, 7.5 Hz, 1H, Tyr-H$^{\alpha}$), 4.31 (d, J=10.0 Hz, 1H, SAA-H^{5}), 4.22 (m, 3H, Tyr-H$^{\alpha}$+Glu-H$^{\alpha}$+1×Fmoc-H), 4.13 (dd, J=10.7, 6.9 Hz, 1H, Fmoc), 3.99 (t, J=6.7 Hz, 1H, Fmoc), 3.71 (q, J=9.3 Hz, 1H, SAA-H^{2}), 3.40 (s, 3H, OMe), 2.81 (m, 3H, 3×Tyr-H$^{\beta}$), 2.71 (dd, J=14.2, 7.9 Hz, 1H, Tyr-H$^{\beta}$), 2.19 (m, 2H, Glu-H$^{\gamma}$), 1.81 (m, 1H, Glu-H$^{\beta}$), 1.64 (m, 1H, Glu-H$^{\beta}$), 1.26 (s, 9H, O^{t}Bu); HRMS (FAB) calcd for $C_{70}H_{70}N_4O_{17}Na$ (M+Na): 1261.4634; found 1261.4623.

4.4.8. H-SAA(di-OBz)-Tyr-Glu(OBzl)-Tyr-O^{t}Bu (15b). The title compound was prepared from **14b** (720 mg, 0.581 mmol) using the method described in the synthesis of **15a** to give **15b** (534 mg, 90%) as a yellowish amorphous solid. $[\alpha]_D^{22}=-24$ (c 0.5, MeOH); ^{1}H NMR (MeOH-d$_4$, 300 MHz) δ 7.91 (d, J=7.9 Hz, 2H, Bz-*o*), 7.81 (d, J=8.5 Hz, 2H, Bz-*o*), 7.50 (m, 2H, Bz-*p*), 7.32 (m, 9H, Bz-*m*+Bzl), 7.01 (d, J=8.4 Hz, 4H, Tyr-H$^{\delta}$), 6.69 (d, J=8.5 Hz, 2H, Tyr-H$^{\varepsilon}$), 6.67 (d, J=8.4 Hz, 2H, Tyr-H$^{\varepsilon}$), 5.48 (t, J=9.6 Hz, 1H, SAA-H^{3}), 5.40 (t, J=9.5 Hz, 1H, SAA-H^{4}), 5.08 (s, 2H, Bzl), 4.44 (d, J=8.0 Hz, 1H, SAA-H^{1}), 4.49 (t, J=6.6, 1H, Tyr-H$^{\alpha}$), 4.40 (t, J=6.9 Hz, 1H, Tyr-H$^{\alpha}$), 4.25 (m, 2H, SAA-H^{5}+Glu-H$^{\alpha}$), 3.55 (s, 3H, OMe), 2.94 (m, 5H, SAA-H^{2}+Tyr-H$^{\beta}$), 2.26 (m, 2H, Glu-H$^{\gamma}$), 1.95 (m, 1H, Glu-H$^{\beta}$), 1.77 (m, 1H, Glu-H$^{\beta}$), 1.39 (s, 9H, O^{t}Bu); HRMS (FAB) calcd for $C_{55}H_{60}N_4O_{15}Na$ (M+Na): 1039.3953; found 1039.3960.

4.4.9. Fmoc-SAA(di-OBz)-Tyr-Glu(OBzl)-Tyr-SAA(di-OBz)-Tyr-Glu(OBzl)-Tyr-O^{t}Bu (17b). The title compound was prepared from **14b** (604 mg, 0.487 mmol) and **15b** (496 mg, 0.487 mmol) using the method described in the synthesis of **17a**. The product was purified with flash chromatography (CH_2Cl_2/MeOH 15:1, R_f=0.18) followed by size-exclusion chromatography to give **17b** (767 mg, 72%) as a white amorphous solid. $[\alpha]_D^{22}=+4$ (c 0.5, DMSO); ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ 9.23 (s, 1H, Tyr-OH), 9.16 (s, 1H, Tyr-OH), 9.13 (s, 1H, Tyr-OH), 9.10 (s, 1H, Tyr-OH), 8.36 (d, J=8.5 Hz, 1H, NH), 8.25 (d, J=7.1 Hz, 1H, NH), 8.20 (m, 2H, 2×NH), 8.14 (d, J=7.7 Hz, 1H, NH), 8.09 (d, J=7.3 Hz, 1H, NH), 7.85 (m, 3H, 2×Fmoc-H+NH), 7.77 (m, 4H, Bz-*o*), 7.70 (m, 4H, Bz-*o*), 7.55 (m, 6H, 4×Bz-*p*+1×NH+1×Fmoc-H), 7.37 (m, 21H, Bz-*m*+3×Fmoc-H+2×Bzl), 7.15 (m, 2H, Fmoc), 7.01 (d, J=8.5 Hz, 2H, Tyr-H$^{\delta}$), 6.97 (d, J=8.5 Hz, 2H, Tyr-H$^{\delta}$), 6.97 (d, J=8.6 Hz, 2H, Tyr-H$^{\delta}$), 6.77 (d, J=8.4 Hz, 2H, Tyr-H$^{\delta}$), 6.66 (d, J=8.5 Hz, 2H, Tyr-H$^{\varepsilon}$), 6.60 (d, J=8.4 Hz, 2H, Tyr-H$^{\varepsilon}$), 6.59 (d, J=8.1 Hz, 2H, Tyr-H$^{\varepsilon}$), 6.47 (d, J=8.4 Hz, 2H, Tyr-H$^{\varepsilon}$), 5.51 (t, J=9.8 Hz, 1H, SAA-H^{3}), 5.47 (t, J=10.1 Hz, 1H, SAA-H^{3}), 5.37 (t, J=9.8 Hz, 1H, SAA-H^{4}), 5.31 (t, J=9.7 Hz, 1H, SAA-H^{4}), 5.06 (s, 2H, Bzl), 5.05 (s, 2H, Bzl), 4.71 (d, J=8.2 Hz, 1H, SAA-H^{1}), 4.66 (d, J=8.2 Hz, 1H, SAA-H^{1}), 4.44 (m, 3H, SAA-H^{5}+2×Tyr-H$^{\alpha}$), 4.29 (m, 5H, SAA-H^{5}+2×Tyr-H$^{\alpha}$+1×Glu-H$^{\alpha}$+1×Fmoc-H), 4.13 (m, 3H, SAA-H^{2}+1×Glu-H$^{\alpha}$+1×Fmoc-H), 4.02 (t, J=6.5 Hz, 1H, Fmoc), 3.71 (q, J=9.4 Hz, 1H, SAA-H^{2}), 3.41 (s, 3H, OMe), 3.38 (s, 3H, OMe), 2.75 (m, 7H, 7×Tyr-H$^{\beta}$), 2.56 (dd, J=15.3, 11.2 Hz, 1H, Tyr-H$^{\beta}$), 2.23 (t, J=8.0 Hz, 2H, Glu-H$^{\gamma}$), 2.10 (m, 2H, Glu-H$^{\gamma}$), 1.83 (m, 1H, Glu-H$^{\beta}$), 1.70 (m, 2H, Glu-H$^{\beta}$), 1.56 (m, 1H, Glu-H$^{\beta}$), 1.28 (s, 9H, O^{t}Bu); HRMS (FAB) calcd for $C_{121}H_{120}N_8O_{31}Na$ (M+Na): 2203.7957; found 2203.7947.

4.4.10. H-SAA(di-OBz)-Tyr-Glu(OBzl)-Tyr-SAA(di-OBz)-Tyr-Glu(OBzl)-Tyr-O^{t}Bu (18b). The title compound was prepared from **17b** (676 mg, 0.310 mmol) using the method described in the synthesis of **15a** to give **18b** (599 mg, 99%) as a yellowish amorphous solid. $[\alpha]_D^{22}=-23$ (c 0.5, MeOH); ^{1}H NMR (MeOH-d$_4$, 400 MHz) δ 7.72 (d, J=8.5 Hz, 2H, Bz-*o*), 7.67 (d, J=8.4 Hz, 2H, Bz-*o*), 7.63 (d, J=8.5 Hz, 2H, Bz-*o*), 7.58 (d, J=8.4 Hz, 2H, Bz-*o*), 7.28 (m, 4H, Bz-*p*), 7.12 (m, 18H, Bz-*m*+Bzl), 6.81 (m, 6H, Tyr-H$^{\delta}$), 6.63 (d, J=8.5 Hz, 2H, Tyr-H$^{\delta}$), 6.48 (m, 6H, Tyr-H$^{\varepsilon}$), 6.33 (d, J=8.5 Hz, 2H, Tyr-H$^{\varepsilon}$), 5.51 (t, J=10.0 Hz, 1H, SAA-H^{3}), 5.29 (t, J=9.8 Hz, 1H, SAA-H^{3}), 5.24 (t, J=9.7 Hz, 1H, SAA-H^{4}), 5.20 (t, J=9.6 Hz, 1H, SAA-H^{4}), 4.87 (s, 4H, Bzl), 4.55 (d, J=8.4 Hz, 1H, SAA-H^{1}), 4.30 (t, J=6.5 Hz, 1H, Tyr-H$^{\alpha}$), 4.23 (m, 3H, SAA-H^{1}+2×Tyr-H$^{\alpha}$), 4.16 (dd, J=9.1 Hz, J=5.0 Hz, Tyr-H$^{\alpha}$), 4.06 (m, 3H, 2×SAA-H^{5}+Glu-H$^{\alpha}$), 3.93 (m, 2H, SAA-H^{2}+Glu-H$^{\alpha}$), 3.34 (s, 3H, OMe), 3.25 (s, 3H, OMe),

2.83 (dd, $J=10.1$ Hz, $J=8.0$ Hz, 1H, SAA-H^2), 2.72 (m, 6H, 6×Tyr-H^β), 2.55 (dd, $J=14.2$, 5.0 Hz, 1H, Tyr-H^β), 2.26 (dd, $J=13.9$, 9.4 Hz, 1H, Tyr-H^β), 2.07 (m, 2H, 2× Glu-H^γ), 1.94 (m, 2H, 2×Glu-H^γ), 1.72 (m, 1H, Glu-H^β), 1.57 (m, 3H, 3×Glu-H^β), 1.14 (s, 9H, O^{t}Bu); HRMS (FAB) calcd for $C_{106}H_{110}N_8O_{29}Na$ (M+Na): 1981.7276; found 1981.7268.

4.4.11. Cyclo[SAA(di-OBz)-Tyr-Glu(OBzl)-Tyr-SAA-(di-OBz)-Tyr-Glu(OBzl)-Tyr] (19b). The title compound was prepared from **18b** (314 mg, 0.166 mmol) using the method described in the synthesis of compound **19a**. The product was purified with flash chromatography (CH_2Cl_2/MeOH 12:1, $R_f=0.25$) followed by size-exclusion chromatography to give **19b** (166 mg, 53%) as a white foam. $[\alpha]_D^{22}=-3$ (*c* 0.5, MeOH); ^{1}H NMR (DMSO-d_6, 400 MHz, 120 °C) δ 8.49 (br s, 2H, Tyr-OMe), 8.33 (br s, 2H, Tyr-OMe), 7.80 (m, 10H, Bz-*o*+2×NH), 7.48 (m, 6H, Bz-*p*+ 2×NH), 7.32 (m, 18H, Bz-*m*+Bzl), 7.05 (d, $J=7.8$ Hz, 2H, NH), 6.99 (d, $J=8.4$ Hz, 4H, Tyr-H^δ), 6.81 (d, $J=$ 8.4 Hz, 4H, Tyr-H^δ), 6.68 (d, $J=8.4$ Hz, 4H, Tyr-H^ε), 6.50 (d, $J=8.4$ Hz, 4H, Tyr-H^ε), 5.78 (t, $J=9.7$ Hz, 2H, SAA-H^3), 5.60 (t, $J=9.4$ Hz, 2H, SAA-H^4), 5.09 (s, 4H, Bzl), 5.05 (d, $J=8.1$ Hz, 2H, SAA-H^1), 4.46 (d, $J=9.8$ Hz, 2H, SAA-H^5), 4.43 (dd, $J=14.9$, 7.7 Hz, 2H, Tyr-H^α), 4.34 (m, 2H, Tyr-H^α), 4.15 (dd, $J=10.0$, 8.6 Hz, 2H, SAA-H^2), 3.91 (dd, $J=7.7$, 6.6 Hz, 2H, Glu-H^α), 3.48 (s, 6H, OMe), 3.00 (m, 4H, Tyr-H^β), 2.87 (dd, $J=14.4$, 5.1 Hz, 2H, Tyr-H^β), 2.63 (dd, $J=14.4$, 8.3 Hz, 2H, Tyr-H^β), 2.22 (m, 4H, Glu-H^γ), 1.90 (m, 4H, Glu-H^β); HRMS (FAB) calcd for $C_{102}H_{100}N_8O_{28}$ (M+H): 1907.6545; found 1907.6549.

4.4.12. Cyclo[SAA-Tyr-Glu-Tyr-SAA-Tyr-Glu-Tyr] (1b). Palladium black (75 mg) was suspended in MeOH containing 5% formic acid (3 mL). Compound **19b** (149 mg, 78.8 μmol) was dissolved in MeOH containing 5% formic acid (9 mL) and added to the suspension. After 20 min, the catalyst was filtered off (caution: catalyst may catch fire when filtered to dryness), toluene (5 mL) was added, and the mixture was evaporated. The residue was dissolved in MeOH (30 mL) and NaOMe/MeOH (1 M, 450 μL) was added. The solution was stirred for 22 h, then neutralised with AcOH, and evaporated. The residue was dissolved in water and applied to a C_{18} cartridge. The column was washed with water and the compound was eluted with 30% MeOH in water to afford **1b** (82.3 mg, 81%) as a fluffy white powder after lyophilization. $[\alpha]_D^{22}=-31$ (*c* 0.2, water); ^{1}H NMR (D_2O, 400 MHz) δ 7.03 (d, $J=8.4$ Hz, 8H, Tyr-H^δ), 6.78 (d, $J=8.4$ Hz, 4H, Tyr-H^ε), 6.69 (d, $J=8.4$ Hz, 4H, Tyr-H^ε), 4.58 (dd, $J=10.0$, 4.6 Hz, 2H, Tyr-H^α), 4.51 (t, $J=6.2$ Hz, 2H, Tyr-H^α), 4.45 (d, $J=8.7$ Hz, 2H, SAA-H^1), 3.86 (t, $J=7.1$ Hz, 2H, Glu-H^α), 3.68 (d, $J=10.0$ Hz, 2H, SAA-H^5), 3.66 (t, $J=9.4$ Hz, 2H, SAA-H^2), 3.40 (t, $J=9.5$ Hz, 2H, SAA-H^3), 3.32 (s, 6H, OMe), 3.20 (m, 4H, 2×SAA-H^4+2×Tyr-H^β), 2.92 (m, 4H, Tyr-H^β), 2.16 (m, 4H, 2×Tyr-H^β+2×Glu-H^β), 2.03 (m, 2H, Glu-H^β), 1.88 (m, 4H, Glu-H^γ); HRMS (FAB) calcd for $C_{60}H_{72}N_8O_{24}Na$ (M+Na):1311.4557; found 1311.4581.

4.4.13. Fmoc-SAA(di-OBz)-Tyr-Arg(Mtr)-Tyr-O^{t}Bu (14c). The title compound was prepared from **7** (809 mg, 1.30 mmol) and **10c** (998 mg, 1.30 mmol) using the method described in the synthesis of **14a**. The product was purified with flash chromatography (toluene/MeOH 7:1, $R_f=0.21$) followed by size-exclusion chromatography to give **14c** (837 mg, 1.39 g, 46%) as a white foam. $[\alpha]_D^{24}=-5$ (*c* 0.5, MeOH); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.20 (s, 1H, Tyr-OH), 9.12 (s, 1H, Tyr-OH), 8.15 (m, 2H, NH), 8.11 (d, $J=$ 8.2 Hz, 1H, NH), 7.82 (d, $J=7.0$ Hz, 2H, Fmoc), 7.77 (d, $J=7.3$ Hz, 2H, Bz-o), 7.73 (d, $J=7.3$ Hz, 2H, Bz-*o*), 7.55 (m, 4H, Bz-*p*+1×Fmoc-H+1×NH), 7.37 (m, 7H, 3× Fmoc-H+Bz-*m*), 7.15 (m, 2H, Fmoc), 7.00 (d, $J=8.4$ Hz, 2H, Tyr-H^δ), 6.97 (d, $J=8.5$ Hz, 2H, Tyr-H^δ), 6.66 (s, 1H, Mtr-ArH), 6.66 (d, $J=8.4$ Hz, 2H, Tyr-H^ε), 6.59 (d, $J=$ 8.3 Hz, 2H, Tyr-H^ε), 5.49 (t, $J=9.9$ Hz, 1H, SAA-H^3), 5.34 (t, $J=9.7$ Hz, 1H, SAA-H^4), 4.68 (d, $J=8.3$ Hz, 1H, SAA-H^1), 4.46 (dd, $J=12.5$, 7.6 Hz, 1H, Tyr-H^α), 4.35 (d, $J=$ 9.9 Hz, 1H, SAA-H^5), 4.25 (m, 3H, Arg-H^α+Tyr-H^α+1× Fmoc-H), 4.15 (dd, $J=10.7$ Hz, $J=6.9$ Hz, 1H, Fmoc), 4.02 (t, $J=6.7$ Hz, 1H, Fmoc), 3.76 (s, 3H, Mtr-OMe), 3.73 (q, $J=9.3$ Hz, 1H, SAA-H^2), 3.42 (s, 3H, SAA-OMe), 2.92 (br m, 2H, Arg-H^δ), 2.81 (m, 3H, Tyr-H^β), 2.73 (dd, $J=$ 14.3, 7.7 Hz, 1H, Tyr-H^β), 2.59 (s, 3H, Mtr-Me), ~2.50 (Mtr-Me, obscured by solvent signal), 2.03 (s, 3H, Mtr-Me), 1.52 (m, 1H, Arg-H^β), 1.28 (m, 3H, 1×Arg-H^β+2×Arg-H^γ), 1.28 (s, 9H, O^{t}Bu); HRMS (FAB) calcd for $C_{74}H_{81}N_7O_{18}SNa$ (M+Na): 1410.5257; found 1410.5261.

4.4.14. H-SAA(di-OBz)-Tyr-Arg(Mtr)-Tyr-O^{t}Bu (15c). The title compound was prepared from **14c** (400 mg, 0.288 mmol) using the method described in the synthesis of **15a** to give **15c** (334 mg, 99%) as a yellowish foam. $[\alpha]_D^{24}=-14$ (*c* 0.5, MeOH); ^{1}H NMR (MeOH-d_4, 300 MHz) δ 7.91 (d, $J=8.5$ Hz, 2H, Bz-*o*) 7.79 (d, $J=$ 8.5 Hz, 2H, Bz-*o*), 7.53 (t, $J=7.5$ Hz, 1H, Bz-*p*), 7.45 (t, $J=$ 7.4 Hz, 1H, Bz-*p*), 7.38 (t, $J=7.6$ Hz, 2H, Bz-*m*), 7.29 (t, $J=7.7$ Hz, 2H, Bz-*m*), 7.01 (d, $J=8.4$ Hz, 4H, Tyr-H^δ), 6.69 (d, $J=8.5$ Hz, 2H, Tyr-H^ε), 6.67 (d, $J=8.5$ Hz, 2H, Tyr-H^ε), 6.63 (s, 1H, Mtr-ArH), 5.48 (t, $J=9.6$ Hz, 1H, SAA-H^3), 5.41 (t, $J=9.7$ Hz, 1H, SAA-H^4), 4.46 (t, $J=$ 6.7 Hz, 1H, Tyr-H^α), 4.46 (d, $J=8.0$ Hz, 1H, SAA-H^1), 4.39 (d, $J=7.2$ Hz, 1H, Tyr-H^α), 4.26 (d, $J=9.5$ Hz, 1H, SAA-H^5), 4.19 (dd, $J=8.3$ Hz, $J=5.5$ Hz, 1H, Arg-H^α), 3.79 (s, 3H, Mtr-OMe), 3.56 (s, 3H, SAA-OMe), 3.01 (m, 3H, SAA-H^2+Tyr-H^δ), 2.89 (m, 4H, Tyr-H^β), 2.65 (s, 3H, Mtr-Me), 2.59 (s, 3H, Mtr-Me), 2.09 (s, 3H, Mtr-Me), 1.61 (m, 1H, Arg-H^β), 1.35 (m, 3H, 1×Arg-H^β+2×Arg-H^γ), 1.35 (s, 9H, O^{t}Bu); HRMS (FAB) calcd for $C_{59}H_{71}N_7O_{16}SNa$ (M+ Na): 1188.4576; found 1188.4596.

4.4.15. Fmoc-SAA(di-OBz)-Tyr-Arg(Mtr)-Tyr-SAA(di-OBz)-Tyr-Arg(Mtr)-Tyr-O^{t}Bu (17c). The title compound was prepared from **14c** (315 mg, 0.227 mmol) and **15c** (265 mg, 0.227 mmol) using the method described in the synthesis of **17a**. The product was purified with flash chromatography (CH_2Cl_2/MeOH 12:1, $R_f=0.29$) followed by size-exclusion chromatography to give **17c** (271 mg, 48%) as a white foam. $[\alpha]_D^{22}=+7$ (*c* 0.5, MeOH); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.21 (s. 1H, Tyr-OH), 9.13 (s. 1H, Tyr-OH), 9.10 (s. 1H, Tyr-OH), 9.06 (s, 1H, Tyr-OH), 8.35 (d, $J=8.8$ Hz, 1H, NH), 8.16 (m, 3H, 3×NH), 8.06 (d, $J=$ 8.2 Hz, 1H, NH), 7.82 (d, $J=7.5$ Hz, 2H, Fmoc), 7.78 (m, 5H, 4×Bz-*o*+1×NH), 7.72 (m, 4H, 4×Bz-*o*), 7.55 (m, 6H, 4×Bz-*p*+1×Fmoc-H+1×NH), 7.40 (m, 11H, 3× Fmoc-H+8×Bz-*m*), 7.15 (m, 2H, Fmoc), 7.01 (d, $J=$ 8.5 Hz, 2H, Tyr-H^δ), 6.97 (d, $J=8.6$ Hz, 2H, Tyr-H^δ), 6.97

(d, J=8.6 Hz, 2H, Tyr-H$^{\delta}$), 6.75 (d, J=8.5 Hz, 2H, Tyr-H$^{\delta}$), 6.66 (m, 4H, Tyr-H$^{\varepsilon}$+2×Mtr-ArH), 6.60 (d, J=8.4 Hz, 2H, Tyr-H$^{\varepsilon}$), 6.59 (d, J=8.1 Hz, 2H, Tyr-H$^{\varepsilon}$), 6.47 (d, J=8.4 Hz, 2H, Tyr-H$^{\varepsilon}$), 5.52 (t, J=9.8 Hz, 1H, SAA-H^{3}), 5.48 (t, J=9.7 Hz, 1H, SAA-H^{3}), 5.38 (t, J=9.7 Hz, 1H, SAA-H^{4}), 5.33 (t, J=9.6 Hz, 1H, SAA-H^{4}), 4.72 (d, J=8.4 Hz, 1H, SAA-H^{1}), 4.68 (d, J=8.2 Hz, 1H, SAA-H^{1}), 4.46 (m, 2H, 2×Tyr-H$^{\alpha}$), 4.42 (d, J=10.0 Hz, 1H, SAA-H^{5}), 4.35 (m, 2H, SAA-H^{5}+TyrH$^{\alpha}$), 4.26 (m, 3H, Arg-H$^{\alpha}$+Tyr-H$^{\alpha}$+1×Fmoc-H), 4.15 (m, 1H, Fmoc), 4.08 (m, 2H, SAA-H^{2}+Arg-H$^{\alpha}$), 4.02 (t, J=6.7 Hz, 1H, Fmoc), 3.77 (s, 3H, Mtr-OMe), 3.76 (s, 3H, Mtr-OMe), 3.72 (q, J=9.7 Hz, 1H, SAA-H^{2}), 3.42 (s, 3H, SAA-OMe), 3.41 (s, 3H, SAA-OMe), 2.86 (m, 11H, 4×Arg-H$^{\delta}$+7×Tyr-H$^{\beta}$), 2.59 (s, 6H, Mtr-Me), ~2.50 (2×Mtr-Me, obscured by solvent signal), 2.25 (br dd, J=12.8, 8.7 Hz, 1H, Tyr-H$^{\beta}$), 2.03 (s, 3H, Mtr-Me), 2.01 (s, 3H, Mtr-Me), 1.53 (m, 1H, Arg-H$^{\beta}$), 1.43 (m, 1H, Arg-H$^{\beta}$), 1.29 (s, 9H, O^{t}Bu), 1.28 (m, 6H, 2×Arg-H$^{\beta}$+4×Arg-H$^{\gamma}$); HRMS (FAB) calcd for $C_{129}H_{142}N_{14}O_{33}S_2Na$ (M+Na): 2501.9203; found 2501.9209.

4.4.16. H-SAA(di-OBz)-Tyr-Arg(Mtr)-Tyr-SAA(di-OBz)-Tyr-Arg(Mtr)-Tyr-O^{t}Bu (18c). The title compound was prepared from **17c** (291 mg, 0.117 mmol) using the method described in the synthesis of **15a** to give **18c** (254 mg, 96%) as a yellowish amorphous solid. $[\alpha]_D^{22}$=−16 (*c* 0.5, MeOH); ^{1}H NMR (MeOH-d$_4$, 400 MHz) δ 7.91 (d, J=8.5 Hz, 2H, Bz-*o*) 7.85 (d, J=8.6 Hz, 2H, Bz-*o*), 7.82 (d, J=8.5 Hz, 2H, Bz-*o*), 7.77 (d, J=8.5 Hz, 2H, Bz-*o*), 7.47 (m, 4H, Bz-*p*), 7.31 (m, 8H, Bz-*m*), 7.00 (m, 6H, Tyr-H$^{\delta}$), 6.85 (d, J=8.5 Hz, 2H, Tyr-H$^{\delta}$), 6.67 (m, 6H, Tyr-H$^{\varepsilon}$), 6.62 (s, 1H, Mtr-ArH), 6.61 (s, 1H, Mtr-ArH), 6.56 (d, J=8.5 Hz, 2H, Tyr-H$^{\varepsilon}$), 5.74 (t, J=9.9 Hz, 1H, SAA-H^{3}), 5.47 (t, J=9.7 Hz, 1H, SAA-H^{3}), 5.42 (t, J=9.6 Hz, 1H, SAA-H^{4}), 5.40 (t, J=9.6 Hz, 1H, SAA-H^{4}), ~4.81 (SAA-H^{1}, partially obscured by solvent signal), 4.46 (t, J=6.7 Hz, 1H, Tyr-H$^{\alpha}$), 4.45 (d, J=8.1 Hz, 1H, SAA-H^{1}), 4.40 (m, 3H, 3×Tyr-H$^{\alpha}$), 4.33 (d, J=9.8 Hz, 1H, SAA-H^{5}), 4.25 (d, J=9.7 Hz, 1H, SAA-H^{5}), 4.21 (dd, J=8.4, 5.2 Hz, 1H, Arg-H$^{\alpha}$), 4.14 (m, 2H, Arg-H$^{\alpha}$+SAA-H^{2}), 3.78 (s, 3H, Mtr-OMe), 3.77 (s, 3H, Mtr-OMe), 3.55 (s, 3H, SAA-OMe), 3.47 (s, 3H, SAA-OMe), 3.02 (m, 3H, SAA-H^{2}+Arg-H$^{\delta}$), 2.92 (m, 8H, 2×Arg-H$^{\delta}$+6×Tyr-H$^{\beta}$), 2.77 (dd, J=14.0, 5.1 Hz, 1H, Tyr-H$^{\beta}$), 2.66 (s, 3H, Mtr-Me), 2.65 (s, 3H, Mtr-Me), 2.59 (s, 6H, 2×Mtr-Me), 2.51 (dd, J=14.5 Hz, J=9.7 Hz, 1H, Tyr-H$^{\beta}$), 2.08 (s, 3H, Mtr-Me), 2.06 (s, 3H, Mtr-Me), 1.63 (m, 1H, Arg-H$^{\beta}$), 1.54 (m, 1H, Arg-H$^{\beta}$), 1.44 (m, 1H, Arg-H$^{\beta}$), 1.35 (m, 12H, 1×Arg-H$^{\beta}$+2×Arg-H$^{\gamma}$+O^{t}Bu), 1.19 (m, 2H, Arg-H$^{\gamma}$); HRMS (FAB) calcd for $C_{114}H_{132}N_{14}O_{31}S_2Na$ (M+Na): 2279.8522; found 2279.8530.

4.4.17. Cyclo[SAA(di-OBz)-Tyr-Arg(Mtr)-Tyr-SAA(di-OBz)-Tyr-Arg(Mtr)-Tyr] (19c). The title compound was prepared from **18c** (89.3 mg, 39.5 μmol) using the method described in the synthesis of compound **19a**. The product was purified with flash chromatography (CH_2Cl_2:MeOH 8:1, R_f=0.32) followed by size-exclusion chromatography to give **19c** (29.3 mg, 34%) as a white amorphous solid. $[\alpha]_D^{24}$=−5 (*c* 0.5, MeOH); HRMS (FAB) calcd for $C_{110}H_{122}N_{14}O_{30}S_2Na$ (M+Na): 2205.7790; found 2205.7795. A resolved NMR spectrum could not be obtained.

4.4.18. Cyclo(SAA-Tyr-Arg-Tyr-SAA-Tyr-Arg-Tyr) (1c). Compound **19c** (25.5 mg, 11.7 μmol) was dissolved in TFA containing 5% thioanisole (2.5 mL). After 24 h, toluene (2.5 mL) was added and the mixture was evaporated. The residue was dissolved in MeOH (5 mL) and NaOMe/MeOH (1 M, 75 μL) was added. After 24 h, the mixture was acidified with AcOH and evaporated. The product was purified using preparative HPLC (C_{18} column, 15→20% B in A over 60 min, A: H_2O +0.1% TFA, B: CH_3CN +0.1% TFA, t_R=10 min) to afford **1c** (9.3 mg, 59%) as a fluffy white powder after lyophilization. $[\alpha]_D^{22}$=−15 (*c* 0.2, MeOH); ^{1}H NMR (D_2O, 400 MHz) δ 7.03 (d, J=8.4 Hz, 4H, Tyr-H$^{\delta}$), 7.00 (d, J=8.2 Hz, 4H, Tyr$^{\delta}$), 6.77 (d, J=8.3 Hz, 4H, Tyr$^{\varepsilon}$), 6.68 (d, J=7.8 Hz, 4H, Tyr$^{\varepsilon}$), 4.57 (m, 4H, 4×Tyr-H$^{\alpha}$, partially obscured by solvent signal), 4.43 (d, J=8.6 Hz, 2H, SAA-H^{1}), 3.81 (t, J=7.0 Hz, 2H, Arg-H$^{\alpha}$), 3.66 (m, 4H, SAA-H^{5}+SAA-H^{2}), 3.40 (t, J=9.8 Hz, 2H, SAA-H^{3}), 3.32 (s, 6H, OMe), 3.18 (t, J=9.5 Hz, SAA-H^{4}), 3.11 (dd, J=15.1, 7.4 Hz, 2H, Tyr-H$^{\beta}$), 2.96 (m, 8H, 4×Tyr-H$^{\beta}$+4×Arg-H$^{\delta}$), 2.32 (dd, J=12.3, 11.6 Hz, 2H, Tyr-H$^{\beta}$), 1.66 (m, 2H, Arg-H$^{\beta}$), 1.52 (m, 2H, Arg-H$^{\beta}$), 1.27 (m, 2H, Arg-H$^{\gamma}$), 1.16 (m, 2H, Arg-H$^{\gamma}$); HRMS (FAB) calcd for $C_{62}H_{82}N_{14}O_{20}Na$ (M+Na): 1365.5728; found 1365.5729.

4.5. NMR titrations

All experiments were performed at 400 MHz in a deuterated phosphate buffer (100 mM phosphate, pH 7.2). In the titration experiments, a stock solution with 0.5 mM receptor concentration was prepared. The ligand to be titrated was dissolved in a portion of the stock solution to give a solution with 0.5 mM receptor and 100 mM ligand. These two solutions were mixed in different proportions to give a series of solutions with 0.5 mM receptor and ligand concentrations up to 80 mM. ^{1}H NMR experiments were performed on the solutions and the chemical shifts of receptor signals were fitted to a 1:1 binding isotherm using non-linear regression.[31] Acidic and basic ligands were used as sodium salts and hydrochloride salts, respectively.

Acknowledgements

We thank the Swedish Research Council, the Swedish Strategic Research Foundation, and the program 'Glycoconjugates in Biological Systems' sponsored by the Swedish Strategic Research Foundation for financial support.

References and notes

1. Hartley, J. H.; James, T. D.; Ward, C. J. *J. Chem. Soc., Perkin Trans. 1* **2000**, 3155–3184.
2. Gruner, S. A. W.; Locardi, E.; Lohof, E.; Kessler, H. *Chem. Rev.* **2002**, *102*, 491–514.
3. Schweizer, F. *Angew. Chem., Int. Ed.* **2002**, *41*, 230–253.
4. Chakraborty, T. K.; Ghosh, S.; Jayaprakash, S. *Curr. Med. Chem.* **2002**, *9*, 421–435.

5. Peri, F.; Cipolla, L.; Forni, E.; La Ferla, B.; Nicotra, F. *Chemtracts Org. Chem.* **2001**, *14*, 481–499.
6. Locardi, E.; Stöckle, M.; Gruner, S.; Kessler, H. *J. Am. Chem. Soc.* **2001**, *123*, 8189–8196.
7. Chakraborty, T. K.; Srinivasu, P.; Bikshapathy, E.; Nagaraj, R.; Vairamani, M.; Kiran Kumar, S.; Kunwar, A. C. *J. Org. Chem.* **2003**, *68*, 6257–6263.
8. van Well, R. M.; Marinelli, L.; Erkelens, K.; van der Marel, G. A.; Lavecchia, A.; Overkleeft, H. S.; van Boom, J. H.; Kessler, H.; Overhand, M. *Eur. J. Org. Chem.* **2003**, 2303–2313.
9. Graf von Roedern, E.; Lohof, E.; Hessler, G.; Hoffmann, M.; Kessler, H. *J. Am. Chem. Soc.* **1996**, *118*, 10156–10167.
10. van Well, R. M.; Overkleeft, H. S.; Overhand, M.; Vang Carstenen, E.; van der Marel, G. A.; Van Boom, J. H. *Tetrahedron Lett.* **2000**, *41*, 9331–9335.
11. Lohof, E.; Planker, E.; Mang, C.; Burkhart, F.; Dechantsreiter, M. A.; Haubner, R.; Wester, H.-J.; Schwaiger, M.; Hölzemann, G.; Goodman, S. L.; Kessler, H. *Angew. Chem., Int. Ed.* **2000**, *39*, 2761–2763.
12. Gruner, S. A. W.; Kéri, G.; Schwab, R.; Venetianer, A.; Kessler, H. *Org. Lett.* **2001**, *3*, 3723–3725.
13. Stöckle, M.; Voll, G.; Günther, R.; Lohof, E.; Locardi, E.; Sibylle, G.; Kessler, H. *Org. Lett.* **2002**, *4*, 2501–2504.
14. Gruner, S. A. W.; Truffault, V.; Voll, G.; Locardi, E.; Stöckle, M.; Kessler, H. *Chem. Eur. J.* **2002**, *8*, 4366–4376.
15. van Well, R. M.; Overkleeft, H. S.; van der Marel, G. A.; Bruss, D.; Thibault, G.; de Groot, P. G.; van Boom, J. H.; Overhand, M. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 331–334.
16. Irvine, J. C.; McNicoll, D.; Hynd, A. *J. Chem. Soc.* **1911**, *11*, 250–261.
17. Yamasaki, T.; Kubota, Y.; Tsuchiya, T.; Umezawa, S. *Bull. Chem. Soc. Jpn* **1976**, *49*, 3190–3192.
18. Onodera, K.; Kitaoka, S.; Ochiai, H. *J. Org. Chem.* **1962**, *27*, 156–159.
19. Fugedi P.; Csaba P. U.S. Patent 5,849,709, 1998; *Chem. Abstr.*, **1997**, *126*, 60,291.
20. Mehta, A.; Jaouhari, R.; Benson, T. J.; Douglas, K. T. *Tetrahedron Lett.* **1992**, *33*, 5441–5444.
21. Sheppeck, J. E., II; Kar, H.; Hong, H. *Tetrahedron Lett.* **2000**, *41*, 5329–5333.
22. Quibell, M.; Packman, L. C.; Johnson, T. *J. Chem. Soc., Perkin Trans. 1* **1996**, 1219–1225.
23. Carpino, L. A.; Imazumi, H.; El-Faham, A.; Ferrer, F. J.; Zhang, C.; Lee, Y.; Foxman, B. M.; Henklein, P.; Hanay, C.; Mügge, C.; Wenschuh, H.; Klose, J.; Beyermann, M.; Bienert, M. *Angew. Chem., Int. Ed.* **2002**, *41*, 442–445.
24. Knorr, R.; Trzeciak, A.; Bannwarth, W.; Gillessen, D. *Tetrahedron Lett.* **1989**, *30*, 1927–1930.
25. Bienert, M.; Henklein, P.; Beyermann, M.; Carpino, L. A. In *Synthesis of Peptides and Peptidomimetics*, 4th ed.; Goodman, M., Felix, A., Moroder, L., Toniolo, C., Eds.; Houben–Weyl Methods of Organic Chemistry; Georg Thieme: Stuttgart, 2002; Vol. E22a, Chapter 3.8, p 562.
26. Ehrlich, A.; Heyne, H.-U.; Winter, R.; Beyermann, M.; Haber, H.; Carpino, L. A.; Bienert, M. *J. Org. Chem.* **1996**, *61*, 8831–8838.
27. Carpino, L. A.; El-Faham, A. *J. Org. Chem.* **1994**, *59*, 695–698.
28. ElAmin, B.; Anantharamaiah, G. M.; Royer, G. P.; Means, G. E. *J. Org. Chem.* **1979**, *44*, 3442–3444.
29. Fujino, M.; Wakimasu, M.; Kitada, C. *Chem. Pharm. Bull.* **1981**, *29*, 2825–2831.
30. Horton, D.; Turner, W. N. *J. Org. Chem.* **1965**, *30*, 3387–3394.
31. Wilcox, C. S. In *Frontiers in Supramolecular Organic Chemistry and Photochemistry*; Schneider, H.-J., Dürr, H., Eds.; VCH: Weinheim, 1990; pp 123–143.

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Niobium(V) chloride-catalyzed C–H insertion reactions of α-diazoesters: synthesis of β-keto esters

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Received 5 May 2004; revised 18 October 2004; accepted 12 November 2004

Available online 30 November 2004

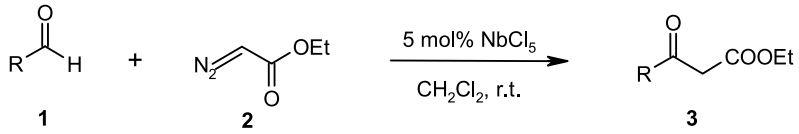
Abstract—Aldehydes react readily with ethyl diazoacetate in the presence of 5 mol% of $NbCl_5$ in dichloromethane to produce the corresponding β-keto esters in good yields with high selectivity. This method is very useful for the preparation of β-keto esters from both electron-rich as well as electron-deficient aromatic aldehydes under mild reaction conditions.

1. Introduction

β-Keto esters are multicoupling reagents having an electrophilic carbonyl group and nucleophilic carbon which make them valuable intermediates for the synthesis of various biologically active compounds such as 3,4-dihydropyrimidinones, 4-alkyl or arylcoumarins, 1,4-dihydropyridines and many others.[1] They are versatile building blocks in the total synthesis of a variety of natural products such as thiolactomycin, trichodiene, polyoxamic acid, chokol, prostaglandin $PGF_{2\alpha}$, psuedotsugonoxide, syncarpic acid, diplodialide, and podophyllotoxin.[2,3] Consequently, numerous methods have been developed for the preparation of β-keto esters.[1] Among them, one of the simple and most straight forward approaches for the synthesis of β-keto esters involves the acid catalyzed C–H insertion of ethyl diazoacetate into aldehydes.[4] These C–H insertion reactions are chemoselective, which allow new carbon–carbon bond formation under mild conditions. Acid catalysts such as boron trifluoride etherate, tin(II) chloride, titanium(IV) chloride, triethyl oxonium tetrafluoroborate, and zinc(II) chloride have been reported for this conversion.[5,6] However, many of these methods involve the use of strongly acidic reagents and harsh reaction conditions and the yields reported are far from satisfactory especially with aromatic aldehydes. Therefore, the development of mild and effiecient alternatives such as niobium(V) chloride would extend the scope and generality of the C–H insertion process of α-diazo esters. Recently, niobium chloride has emerged as an efficient Lewis acid catalyst in promoting various organic transformations such as Diels–Alder reaction, ring-opening of epoxides, Mukaiyama aldol reaction and allylation of aldehydes, imines and *N*-acyliminium ions.[7] In particular, niobium(V) chloride has advantages of low catalyst loading, moisture stability and ease of handling.[8]

In this article, we wish to describe a mild and efficient protocol for the preparation of β-keto esters through C–H insertion of ethyl diazoacetate with a range of aldehydes using niobium(V) chloride as catalyst (Scheme 1).

Accordingly, treatment of benzaldehyde with ethyl diazoacetate in the presence of 5 mol% $NbCl_5$ in dichloromethane afforded ethyl 3-oxo-3-phenylpropanoate **3a** in



R = aryl, alkyl, naphthyl, phenethyl, heterocyclic

Scheme 1.

Keywords: Transition metal catalysis; Carbene insertion; Diazo ester; β-Keto esters.
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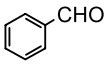
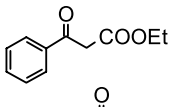
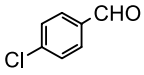
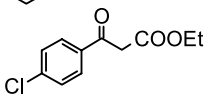
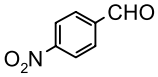
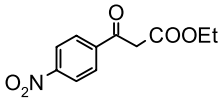
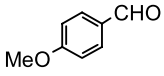
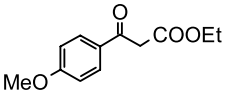
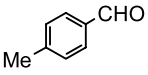
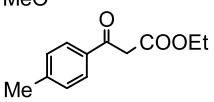
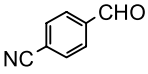
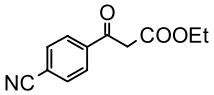
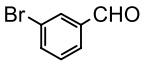
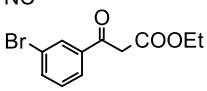
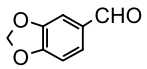
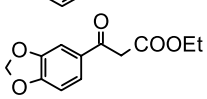
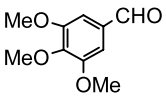
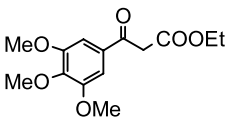
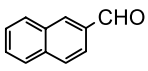
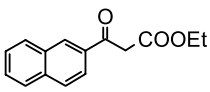
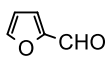
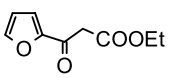
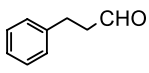
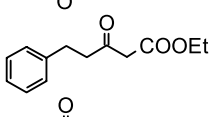
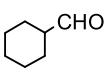
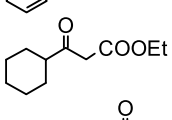
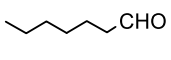
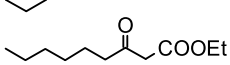
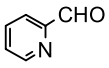
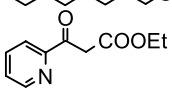
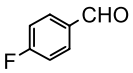
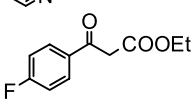
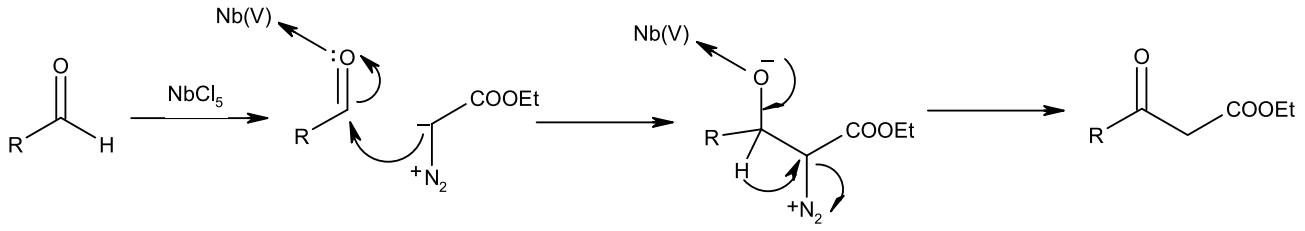
doi:10.1016/j.tet.2004.11.027

85% yield. This remarkable catalytic activity of niobium(V) chloride provided the incentive for further study of reactions with other aromatic aldehydes. Interestingly, various aromatic aldehydes such as chloro-, nitro-, methoxy-, methyl-, cyano-, bromo-, trimethoxy-, naphthyl-derivatives reacted smoothly with ethyl diazoacetate under similar conditions to give the corresponding β-keto esters as the products of C–H insertion (entries **b–j**, Table 1). Aliphatic aldehydes such as *n*-heptanal, cyclohexyl carboxaldehyde, and 3-phenyl propanal afforded the respective β-keto esters in high yields (entries **l**, **m**, and **n**, Table 1). This method also works well with acid sensitive substrates such as furfural (entry **k**, Table 1), which is known to polymerize under most of the reported conditions. In all cases, the reactions proceeded efficiently at room temperature. Both aromatic and aliphatic aldehydes underwent condensation to

Table 1. $NbCl_5$-catalyzed synthesis of β-keto esters from ethyl diazo acetate and aldehydes

Entry	Aldehyde **1**	Product[a] **2**	Time (h)	Yield (%)[b]
a			3.0	85
b			4.0	82
c			4.5	78
d			3.5	76
e			3.0	83
f			3.5	90
g			4.5	86
h			4.0	79
i			4.5	75
j			5.0	82
k			3.0	84
l			3.5	87
m			3.5	85
n			4.0	92
o			4.5	89
p			3.5	80

[a] All the products were characterized by ^1H NMR, IR spectroscopy and mass spectroscopy.
[b] Yield refers to the isolated pure producs after column chromatography.



Scheme 2.

yield the β-keto esters in good yields. The reaction probably proceeds through the activation of the aldehyde by complexation with niobium(V) followed by nucleophilic addition of EDA on the C=O group and subsequent 1,2-hydride shift with loss of N_2 resulting in the formation of β-keto ester **3** (Scheme 2).

The method is clean and the products are obtained in high yields with high selectivity. No side products such as glycidic esters were observed under the reaction conditions. Other side products like diethyl maleate and/or fumarate arising from carbene dimerization were not detected under these conditions. To determine the efficiency of this procedure, we have also performed the reactions with various other Lewis acids such as $InCl_3$, $CeCl_3 \cdot 7H_2O$, $TaCl_5$ and $GdCl_3$. Among these catalysts, $NbCl_5$ was found to be the most effective catalyst for this conversion. In the absence of catalyst, no condensation was observed between aldehyde and ethyl diazoacetate. As solvent, dichloromethane appears to give the best results. Ketones such as acetophenone, cyclohexanone, and tetralone failed to undergo condensation with EDA. This is due to the lower reactivity of ketones in comparison with aldehydes. Other diazoesters such as diethyl diazomalonate and ethyl diazoacetoacetate did not yield any condensation product under the reaction conditions. The scope of this method is illustrated with respect to various aldehydes and ethyl diazoacetate and the results are presented in Table 1.

In summary, we describe a mild, convenient and efficient protocol for the preparation of β-keto esters via the C–H insertion of ethyl diazoacetate into aldehydes using $NbCl_5$ as novel catalyst. This method provides improved yields of β-keto esters including the less reactive aromatic substrates.

2. Experimental

Melting points were recorded on Buchi R-535 apparatus and are uncorrected. IR spectra were recorded on a Perkin–Elmer FT-IR 240-c spectrophotometer using KBr optics. ^{1}H NMR spectra were recorded on Gemini-200 spectrometer in $CDCl_3$ using TMS as internal standard. Mass spectra were recorded on a Finning MAT 1020 mass spectrometer operating at 70 eV.

2.1. General procedure

A mixture of aldehyde (1 mmol), ethyl diazoacetate (1.2 mmol) and $NbCl_5$ (5 mol%) in dichloromethane (10 mL) was stirred at 27 °C for the appropriate time (Table 1). After completion of the reaction, as indicated by TLC, the reaction mixture was extracted with dichloromethane (3×10 mL). The combined organic extracts were dried over anhydrous Na_2SO_4, concentrated in vacuum and purified by column chromatography on silica gel (Merck, 100–200 mesh ethyl acetate–hexane, 1:9) to afford pure β-keto esters.

2.1.1. Ethyl 3-oxo-3-(2-pyridyl) propanoate (3o). Pale yellow liquid, IR (neat): ν 3636, 3058, 2985, 2939, 1741, 1703, 1644, 1582, 1327, 1197, 1032, 791, 746, 648 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.64 (d, J=3.7 Hz, 1H), 8.04 (d, J=8.1 Hz, 1H), 7.84 (t, J=8.1 Hz, 1H), 7.47 (t, J=5.2 Hz, 1H), 4.16 (q, J=7.4, 14.1 Hz, 2H), 4.12 (s, 2H), 1.24 (t, J=7.4 Hz, 3H). EIMS: m/z (%): 193 (M^+, 10), 148 (30), 121 (80), 106 (50), 78 (100), 51 (40). Anal. Calcd for $C_{10}H_{11}NO_3$ (193.201): C, 62.17; H, 5.74; N, 7.25. Found: C, 62.10; H, 5.69; N, 7.31%.

2.1.2. Ethyl 3-(4-flourophenyl)-3-oxopropanoate (3p). Brown oil, IR (neat) ν 3548, 3076, 2985, 1740, 1688, 1600, 1509, 1206, 1032, 845, 768, 569 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.98–7.95 (m, 2H), 7.16–7.11 (m, 2H), 4.18 (q, J=7.4, 14.1 Hz, 2H), 3.89 (s, 2H), 1.26 (t, J=7.4 Hz, 3H). EIMS: m/z (%): 210 (M^+, 10), 166 (5), 124 (100), 96 (20). Anal. Calcd for $C_{11}H_{11}FO_3$ (210.203): C, 62.85; H, 5.27; F, 9.04. Found: C, 62.79; H, 5.32; F, 9.12%.

Acknowledgements

B. V. S. thanks CSIR, New Delhi for the award of fellowship.

References and notes

1. Benetti, S.; Romagnoli, R. *Chem. Rev.* **1995**, *95*, 1065.
2. (a) Hoffmann, R. W.; Helbig, W.; Ladner, W. *Tetrahedron Lett.* **1982**, *23*, 3479. (b) Wang, C. L. J.; Salvino, J. M. *Tetrahedron Lett.* **1984**, *25*, 5243. (c) Gilbert, J. C.; Kelly, T. A. *Tetrahedron Lett.* **1989**, *30*, 4193. (d) Emmer, G. *Tetrahedron* **1992**, *48*, 7165.
3. (a) Ishida, T.; Wada, K. *Chem. Commun.* **1975**, 209. (b) Urban, E.; Knuehl, G.; Helmchen, G. *Tetrahedron* **1995**, *51*, 13031. (c) Magriotis, P. A.; Johnson, F. *J. Org. Chem.* **1984**, *49*, 1460. (d) Ward, R. S. *Synthesis* **1992**, 719.
4. Doyle, M. P.; McKervey, M. A.; Ye, T. *Modern Catalytic Methods for Organic Synthesis with Diazo Compounds*; Wiley: New York, 1998; p 551.
5. (a) Fernandez, V. H.; Herrera, L. F. J.; Perez, G. C. *Carbohydr. Res.* **1983**, *124*, 333. (b) Holmquist, C. R.; Roskamp, E. J.

J. Org. Chem. **1989**, *54*, 3258. (c) Dhavale, D. D.; Patil, P. N.; Mali, R. S. *J. Chem. Res (S)* **1994**, 152.

6. (a) Pellicci, R.; Natalini, B.; Cecchetti, S.; Fringuelli, R. *J. Chem. Soc., Perkin Trans. 1* **1985**, 493. (b) Phukan, P.; Mohan, J. M.; Sudalai, A. *J. Chem. Soc., Perkin Trans. 1* **1999**, 3685. (c) Balaji, B. S.; Chanda, B. M. *Tetrahedron* **1998**, *54*, 13237.
7. (a) Suzuki, K.; Hashimoto, T.; Maeta, H.; Matsumoto, T. *Synlett* **1992**, 125. (b) Andrade, C. K. Z.; Azevedo, N. R.; Oliveira, G. R. *Synthesis* **2002**, 928.
8. (a) Andrade, C. K. Z.; Azevedo, N. R. *Tetrahedron Lett.* **2001**, *42*, 6473. (b) Andrade, C. K. Z.; Oliveira, G. R. *Tetrahedron Lett.* **2002**, *42*, 6473. (c) Andrade, C. K. Z.; Matos, R. A. F. *Synlett* **2003**, 1189.

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Chemoselective allylation of aldehydes using cerium(III) chloride: simple synthesis of homoallylic alcohols

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Received 30 January 2004; revised 15 October 2004; accepted 11 November 2004

Available online 30 November 2004

Abstract—Aldehydes undergo smooth nucleophilic addition with allyltributylstannane in the presence of $CeCl_3 \cdot 7H_2O$ in acetonitrile under extremely mild reaction conditions to afford the corresponding homoallylic alcohols in excellent yields with high chemoselectivity. This method is very useful especially for the allylation of aldehydes bearing acid sensitive functionalities such as TBDMS, THP ethers, acetonides, aryl alkyl ethers and carbamates.

1. Introduction

Lewis acid catalyzed carbon–carbon bond forming reactions are of great importance in organic synthesis because of their high selectivity and mild reaction conditions.[1] In particular, the stereo selective addition of ally metal reagents to aldehydes is one of the most important carbon–carbon bond forming reactions in organic synthesis.[2] Consequently, several methods have been reported for the allylation of aldehydes with allyl metals to produce homoallylic alcohols.[3] One of the most straightforward synthetic procedures involves the nucleophilic addition of allyltin reagents to aldehydes in the presence of Lewis acids.[4–6] However, many of these reagents are expensive, moisture sensitive and difficult to handle, especially on a large scale.

Furthermore, most of these methods involve the use of strongly acidic conditions, which limit their use in the allylation of complex molecules containing acid labile functionalities. Therefore, the development of an efficient and versatile reagent for the allylation of aldehydes is an active ongoing research area and thus there is scope for further improvements towards milder reaction conditions and better yields. Lanthanide salts are unique Lewis acids that are currently of great research interest. Among these catalysts, cerium halides are relatively non-toxic, readily available at low cost and are fairly stable to water. Recently, $CeCl_3 \cdot 7H_2O$ has emerged as an efficient Lewis acid in promoting various organic transformations with high performance.[7]

Cerium(III) is highly oxophilic, and forms strong but labile bonds with oxygen donar ligands. This feature has often allowed the use of cerium(III) chloride as a potential Lewis acid catalyst.[8]

2. Results and discussion

In this article, we describe an efficient and high yielding protocol for the allylation of aldehydes with allyltributylstannane using $CeCl_3 \cdot 7H_2O$ as a novel promoter. Accordingly, treatment of benzaldehyde **1** with allyltributylstannane **2** in the presence of an equimolar amount of cerium(III) chloride afforded 1-phenyl-3-buten-1-ol **3a** in 92% yield (Scheme 1).

The reaction proceeded smoothly at room temperature under extremely mild conditions. Encouraged by the results obtained with benzaldehyde, we turned our attention to various substituted aldehydes. Interestingly, a wide range of substrates including aromatic, aliphatic, heterocyclic and α,β-unsaturated aldehydes reacted efficiently with allyltin under similar reaction conditions to give the corresponding homoallylic alcohols in excellent yields. No bis-allylated products were obtained with methoxy-substituted aryl aldehydes, which are normally observed in the allylation of methoxy-benzaldehydes with allyltrimethylsilane.[3c] Acid sensitive aldehydes such as furfural, and 2-phenyl acetaldehyde (entries **i** and **j** Table 1) were also efficiently converted into their corresponding homoallylic alcohols in

Keywords: Cerium reagents; Aldehydes; Allyltin; Homoallylic alcohols.
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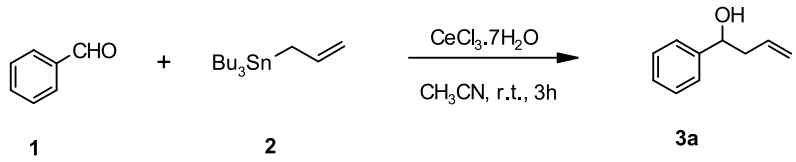
doi:10.1016/j.tet.2004.11.029



Scheme 1.

high yields by using this procedure. In the case of α,β-unsaturated aldehyde (entry **o** Table 1), no 1,4-addition product was obtained. However, 2-phenylpropanal gave a mixture of *syn* and *anti* (3:1 ratio) products (entry **m**, Table 1).[6c] Ketones such as acetophenone, tetralone and cyclohexanone did not react with allyltributylstannane under similar reaction conditions even after a long reaction time and thus this provides a chemo selective allylation of aldehydes without affecting keto functionality. The chemo selectivity of the present method was further studied by using the keto aldehydes (entry **g**, Table 1). It should be noted that chiral aldehydes gave homoallylic alcohols with complete retention of the original configuration (entries **k** and **l**, Table 1). The major advantage of this method is in the selective allylation of aldehydes in the presence of highly acid sensitive acetonide, Boc, TBDMS and THP ethers (entries **b**, **k**, **l** and **p**, Table 1), which do not survive under strongly acidic conditions.[4–6] There are many advantages in the use of cerium(III) chloride for this conversion, which require neither strongly acidic nor harsh conditions. In addition, this method does not require the use of expensive reagents or anhydrous solvents and no precautions need to be taken to exclude moisture from the reaction medium. Furthermore, this method is also useful for the allylation of sugar (*syn*/*anti* products in approximately 1:1 ratio) and Garner's aldehyde without affecting acetonide (entries **k** and **l** Table 1). Thus, the present method is mild to tolerate a wide range of functionalilities. Finally, we have examined the possibility of $CeCl_3 \cdot 7H_2O$ functioning catalytically or at least, in less than stoichiometric amounts. However, best results were obtained with an equimolar ratio of $CeCl_3 \cdot 7H_2O$. Among various Lewis acids such as $CeCl_3 \cdot 7H_2O$, $InCl_3$, $YbCl_3$, $SmCl_3$ and YCl_3 studied for this transformation, cerium(III) chloride was found to be more effective in terms of conversion and selectivity. Reactions were also carried out using 10 mol% of $Ce(OTf)_3$ and the results are summarized in Table 1. The scope and generality of this process is illustrated with respect to various substituted aldehydes and allyltributylstannane.

3. Conclusion

In conclusion, we describe a simple, convenient and efficient protocol for the preparation of homoallylic alcohols from aldehydes and allyltributylstannane using a cheap and readily available reagent ($CeCl_3 \cdot 7H_2O$) that operates under mild conditions thereby leaving acid- and base-labile protecting groups intact. The notable features of this procedure are high functional group compatibility, cleaner reaction profiles, simple operation, and high conversions, which make it a useful and attractive strategy for the synthesis of homoallylic alcohols.

4. Experimental

4.1. General

Melting points were recorded on Buchi R-535 apparatus and are uncorrected. IR spectra were recorded on a Perkin–Elmer FT-IR 240-c spectrophotometer using KBr optics. ^1H and ^{13}C NMR spectra were recorded on Gemini-200 spectrometer in $CDCl_3$ using TMS as internal standard. Mass spectra were recorded on a Finnigan MAT 1020 mass spectrometer operating at 70 eV. All commercially available reagent grade chemicals were purchased from Aldrich Chemical Company and used as received without further purification unless otherwise stated. All the solvents were distilled, dried and stored under nitrogen prior to use. The optical rotations were measured on a Jasco Dip 360 Digital polarimeter.

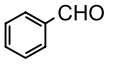
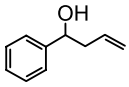
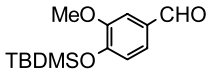
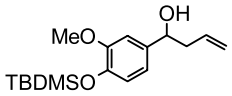
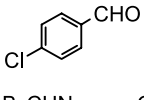
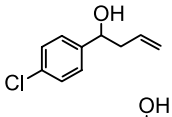
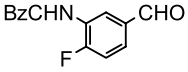
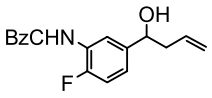
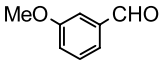
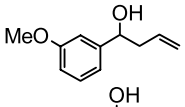
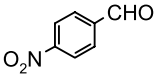
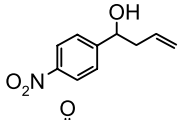
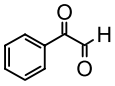
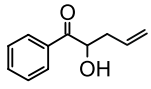
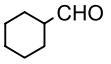
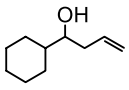
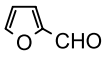
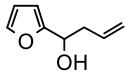
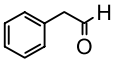
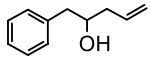
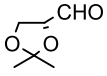
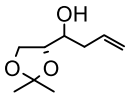
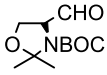
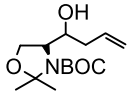
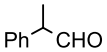
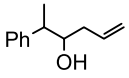
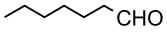
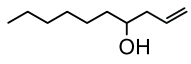
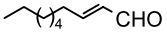
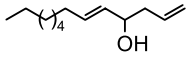
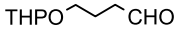
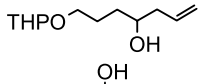
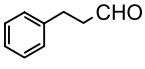
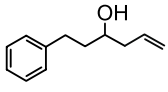
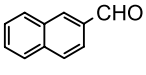
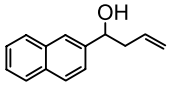
4.1.1. Experimental section. The compounds 1-phenyl-3-buten-1-ol (**3a**),[2c,3c] 1-(4-chlorophenyl)-3-buten-1-ol (**3c**),[3d] 1-(3-methoxyphenyl)-but-3-en-1-ol (**3e**),[3c] 1-(4-nitrophenyl)-3-buten-1-ol (**3f**),[3d] 1-phenyl-1-oxo-4-penten-2-ol (**3g**),[3e] 1-cyclohexyl-3-bute-ne-1-ol (**3h**),[3d] 1-(2-furyl)-3-buten-1-ol (**3i**),[3a] 1-(phenyl)-pent-4-en-2-ol (**3j**),[3c] 1-decen-4-ol (**3n**),[3d] (5*E*)-1,5-trideca-dien-4-ol (**3o**),[3f] 1-phenyl-5-hexen-3-ol (**3q**),[3d] and 1-(2-naphthyl)-but-3-ene-1-ol (**3r**),[3c] all these compounds are known, and their spectroscopic data identical to that reported in the literature.

4.2. General procedure for the synthesis 2,3-dihydro-1,5-benzodiazepines

A mixture of aldehyde (2 mmol), allyltributylstannane (2 mmol) and $CeCl_3 \cdot 7H_2O$ (2 mmol) or $Ce(OTf)_3 \cdot nH_2O$ (10 mol%) in acetonitrile (10 mL) was stirred at 27 °C for the appropriate time (Table 1). After completion of the reaction, as indicated by TLC, the reaction mixture was diluted with water (20 mL) and extracted with ethyl acetate (2×10 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo, and the resulting product was purified by column chromatography on silica gel (Merck, 100–200 mesh, ethyl acetate–hexane, 2:8) to afford pure homoallyl alcohol.

4.2.1. 1-(4-*t*-Butylsilyloxy-3-methoxyphenyl)-3-buten-1-ol (3b). Colorless oil, (536 mg, 87%). IR (KBr): ν_{max}: 3417 (br s), 2933, 2858, 1641, 1589, 1513, 1467,1432, 1354, 1280, 1219, 1156, 1038, 912, 840, 771 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 0.12 (s, 6H), 0.98 (s, 9H), 1.80 (br s, 1H, OH), 2.42–2.50 (m, 2H), 3.82 (s, 3H), 4.60 (t, J= 6.5 Hz, 1H), 5.06–5.18 (m, 2H), 5.72–5.85 (m, 1H), 6.73 (s, 2H), 6.85 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, proton decoupled): δ −4.6, 18.4, 25.7, 43.7, 55.5, 73.3, 107.9, 109.9, 118.2, 120.6, 134.6, 137.5, 144.5, 150.9. EIMS: *m*/*z*:

Table 1. Cerium(III)-promoted synthesis of homoallyl alcohols from aldehydes

Entry	Aldehyde	Homoallyl alcohol	$CeCl_3 \cdot 7H_2O$		$Ce(OTf)_3 \cdot nH_2O$	
			Time (h)	Yield (%)[a]	Time (h)	Yield (%)[a]
a			3.0	92	3.5	87
b			3.5	87	4.5	80
c			3.0	95	4.0	85
d			5.0	91	6.5	82
e			4.0	90	5.0	86
f			3.0	92	3.5	87
g			4.5	88	5.0	85
h			3.5	85	5.0	81
i			3.0	91	4.5	83
j			3.5	89	4.0	86
k			4.0	85[b]	5.0	80[b]
l			5.0	81	6.0	75
m			3.5	90[c]	4.5	85[c]
n			3.5	91	4.0	87
o			2.5	93	3.0	90
p			3.0	87	3.5	82
q			3.5	89	4.0	87
r			3.0	94	4.5	92

All the products were characterized by ^1H NMR, IR and mass spectra.
[a] Isolated and unoptimized yields.
[b] The product was obtained as a mixture of *syn* and *anti* in a 1:1 ratio.
[c] 3:1 ratio of *syn* and *anti* products.

308 (M^+) 291, 267, 251, 195, 167, 136, 73. HRMS Calcd for $C_{17}H_{28}SiO_3$: 308.1807. Found: 308.1836.

4.2.2. 1-(Benzyloxycarbonyl-3-amino-4-fluorophenyl)-3-buten-1-ol (3d). Pale yellow oil, (547 mg, 91%). IR (KBr): ν_{max}: 3500 (br s), 3000, 1650, 910 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 2.00 (br s, OH, 1H), 2.40 (t, J= 6.9 Hz, 2H), 4.70 (t, J=6.9 Hz, 1H), 5.0–5.05 (dd, J=10.2, 17.3 Hz, 2H), 5.10 (s, 2H), 5.70–5.93 (m, 1H), 6.90 (br s, NH, 1H), 7.0 (d, J=7.9 Hz, 2H), 7.20 (s, 5H), 8.12 (d, J= 7.20 Hz, 1H). HRMS Calcd for $C_{18}H_{18}NO_3F$: 315.1270. Found: 315.1214.

4.2.3. *erythro*-1,2-*O*-Isopropylidene hex-5-ene-1,2,3-triol (3k). Viscous oil,[4e] (131 mg, 38%); $[\alpha]_D^{20}=+15.6$ (c, 1.0, $CHCl_3$), {lit.[4e] $[\alpha]_D^{27}=+16.0$ (c, 1.0, $CHCl_3$)}. IR (KBr): ν_{max}: 3468 (OH), 3078, 2888, 1656, 1352, 1201, 913 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 1.33 (s, 3H), 1.40 (s, 3H), 1.80 (br s, 1H, OH), 2.12–2.22 (m, 1H), 2.25–2.38 (m, 1H), 3.70 (t, 1H), 3.85–4.0 (m, 3H), 5.13 (d, J=10.32 Hz, 1H), 5.15 (d, J=16.4 Hz, 1H), 5.90–5.75 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, proton decoupled): δ 25.2, 26.5, 37.6, 65.2, 70.5, 78.1, 109.0, 118.1, 134.0. EIMS: m/z: 157 (M^+ 1), 143, 101, 83, 69, 59, 43.

4.2.4. *threo*-1,2-*O*-Isopropylidene hex-5-ene-1,2,3-triol (3k′). Oil liquid,[4e] (124 mg, 36%); $[\alpha]_D^{27}=+14.2$ (c, 1.0, $CHCl_3$) {lit.[4e] $[\alpha]_D^{27}=+10.2$ (c, 1.0, $CHCl_3$)}. IR (KBr): ν_{max}: 3482, 3129, 2988, 2883, 1695, 1379, 1218, 1070, 771 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 1.35 (s, 3H,), 1.42 (s, 3H), 2.18–2.26 (m, 1H), 2.40–2.60 (m, 1H), 3.38 (br s, 1H, OH), 3.58.3.80 (m, 2H), 3.98–4.20 (m, 2H), 5.04–5.13 (m, 2H), 5.80–6.02 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, proton decoupled): δ 24.9, 26.2, 37.8, 67.6, 71.5, 80.7, 109.0, 117.2, 134.5. EIMS: m/z: 157 ($M^{+1}-15$), 143, 113, 101, 59.

4.2.5. *tert*-Butyl 4-(1-hydroxy-3-butenyl)-2,2-dimethyl-1,3-oxazolane-3-carboxylate (3l). Viscous oil, (438 mg, 81%). IR (KBr): ν_{max}: 3451 (OH), 2979, 1697, 1387, 1254, 1173, 1091, 850, 771 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 1.38 (s, 9H), 1.42 (s, 3H), 1.54 (s, 3H), 2.03–2.21 (m, 2H), 3.62–4.02 (m, 3H), 5.0–5.20 (m, 2H), 5.70–6.05 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, proton decoupled): δ 26.5, 26.7, 27.7, 37.9, 64.5, 71.8, 80.6, 94.1, 117.4, 134.5. FAB: m/z: 272 (M^{+1}), 216, 173, 149, 123, 95, 81, 69, 57. HRMS Calcd for $C_{14}H_{25}NO_4$: 271.1783. Found: 271.1723.

4.2.6. 2-Phenyl-5-hexene-3-ol (3m). Colorless liquid,[3d] (316 mg, 90%). IR (KBr): ν_{max}: 3466 (br s), 2925, 2856, 1736, 1640, 1455, 1329, 1159, 1094, 814, 702, 664 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 1.32 (d, J=7.2 Hz, 3H), 1.57 (br s, OH, 1H), 1.96–2.20 (m, 2H), 2.70–2.80 (m, 1H), 3.62–3.73 (m, 1H), 5.06–5.16 (m, 2H), 5.72–5.85 (m, 1H), 7.16–7.32 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$, proton decoupled): δ 16.3, 39.5, 45.4, 75.0, 117.9, 126.4, 127.7, 128.4, 135.0, 144.4. EIMS: m/z: 176 M^+, 135, 117, 107, 106, 105, 91, 79, 65, 71, 51, 43, 41.

4.2.7. 7-Tetrahydro-2*H*-2-pyranyloxy-1-hepten-4-ol (3p). Colorless oil, (372 mg, 87%). IR (KBr): ν_{max}: 3454 (br s), 2993, 1636, 1218, 1026, 771 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 1.25–1.35 (m, 2H), 1.40–1.60 (m, 6H), 1.65–1.75 (m, 2H), 1.80 (br s, OH, 1H), 2.12–2.30 (m, 2H), 3.35–3.50 (m, 2H), 3.60–3.70 (m, 1H), 3.71–3.86 (m, 1H), 4.60 (t, J=6.5 Hz, 1H), 5.15–5.25 (m, 2H), 5.75–5.90 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, proton decoupled): δ 19.4, 19.5, 25.3, 25.9, 26.1, 30.5, 33.8, 41.8, 62.1, 62.2, 67.5, 134.9. EIMS: m/z: 215 (M^{+1} 215), 203, 191, 178, 167, 154, 137, 107, 95, 85, 69, 57. HRMS Calcd for $C_{12}H_{22}O_3$: 214.1568. Found: 214.1509.

Acknowledgements

B. V. S., G. K., J. S. S. thank CSIR, New Delhi, for the award of fellowships.

References and notes

1. (a) Yamamoto, Y.; Asao, N. *Chem. Rev.* **1993**, *93*, 2207. (b) Marshall, J. A. *Chem. Rev.* **1996**, *96*, 31.
2. (a) Li, C. J.; Chan, T. H. *Tetrahedron* **1999**, *55*, 11149. (b) Hosomi, A.; Sakurai, H. *Tetrahedron Lett.* **1976**, *16*, 1295. (c) Takahiko, A.; Junko, I.; Megumi, S. *Tetrahedron* **1999**, *55*, 7499.
3. (a) Andrade, C. K. Z.; Azevedo, R. *Tetrahedron Lett.* **2001**, *42*, 6473. (b) Yanagisawa, A.; Nakashima, H.; Ishiba, A.; Yamamoto, H. *J. Am. Chem. Soc.* **1996**, *118*, 4723. (c) Aggarwal, V. K.; Vennall, G. P. *Synthesis* **1998**, 1822. (d) Davis, A. P.; Jaspars, M. *J. Chem. Soc., Perkin Trans. 1* **1992**, 2111. (e) Takuwa, A.; Nishigaichi, Y.; Yamashita, K.; Iwamoto, H. *Chem. Lett.* **1990**, 1761. (f) Roush, W. R.; Hoong, L. K.; Palmer, M. A. J.; Park, J. C. *J. Org. Chem.* **1990**, *55*, 4109.
4. (a) Hachiya, I.; Kobayashi, S. *J. Org. Chem.* **1993**, *58*, 6958. (b) Hamasaki, S.; Chounan, Y.; Horino, H.; Yamamoto, Y. *Tetrahedron Lett.* **2000**, *41*, 9883. (c) Ley, S. V.; Cox, L. R. *J. Chem. Soc., Perkin Trans. 1* **1997**, 3315. (d) Shibata, I.; Fukuoka, S.; Yoshimura, N.; Matsuda, H.; Baba, A. *J. Org. Chem.* **1997**, *62*, 3790. (e) Roush, W. S.; Walts, A. E.; Hoong, L. K. *J. Am. Chem. Soc.* **1985**, *107*, 8186.
5. (a) Nagayama, S.; Kobayashi, S. *Angew. Chem., Int. Ed.* **2000**, *93*, 567. (b) Kobayashi, S.; Aoyama, N.; Manabe, K. *Synlett* **2002**, 483. (c) Aspinall, H. C.; Bissett, J. S.; Greeves, N.; Levin, D. *Tetrahedron Lett.* **2002**, *43*, 319.
6. (a) Inoue, K.; Yasuda, M.; Baba, A. *Synlett* **1997**, 699. (b) Naruta, Y.; Ushida, S.; Maruyama, K. *Chem. Lett.* **1979**, 919. (c) Andrade, C. K. Z.; Azevedo, N. R. *Tetrahedron Lett.* **2001**, *42*, 6473. (d) Andrade, C. K. Z.; Azevedo, N. R.; Oliveira, G. R. *Synthesis* **2002**, 928. (e) Choudary, B. M.; Sridhar, Ch.; Sekhar, Ch. V. R. *Synlett* **2002**, 1694–1696.
7. Bartoli, G.; Marcantoni, E.; Sambri, L. *Synlett* **2003**, 2101.
8. (a) Bartoli, G.; Bosco, M.; Marcantoni, E.; Sambri, L.; Torregiani, E. *Synlett* **1998**, 209. (b) Bartoli, G.; Bellucci, M. C.; Bosco, M.; Cappa, A.; Marcantoni, E.; Sambri, L.; Orregiani, E. *J. Org. Chem.* **1999**, *64*, 5696. (c) Marcantoni, E.; Nobili, F.; Bartoli, G.; Bosco, M.; Sambri, L. *J. Org. Chem.* **1997**, *62*, 4183. (d) Yadav, J. S.; Reddy, B. V. S. *Synlett* **2000**, 1275.

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Tetrahedron
Tetrahedron 61 (2005) 883–887

New glycolipid inhibitors of Myt1 kinase

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Received 5 May 2004; revised 9 November 2004; accepted 9 November 2004

Available online 10 December 2004

Abstract—A crude extract of a marine alga showed activity against the enzyme Myt1 kinase. Bioassay-directed fractionation led to the isolation of two bioactive glycoglycerolipids. Lipid **1** was identified as *sn*-1,2-dipalmityl-3-(*N*-palmityl-6-deoxy-6-amino-α-D-glucosyl) glycerol and lipid **2** as *sn*-1-palmityl-2-myristyl-3-(*N*-stearyl-6-deoxy-6-aminoglucosyl)glycerol. Compounds **1** and **2** had IC_{50} values of 0.12 and 0.43 μg/mL, respectively, in the Myt1 kinase inhibitory bioassay, and were inactive against Akt and Chk1 kinases.

1. Introduction

The enzyme Myt1 kinase, which is a Thr-14 and Tyr-15 specific cdc2 kinase, has been shown to be an important regulator of cdc2/cyclin B kinase activity. It has been reported that the inhibitory phosphorylation of cdc2 is important for the timing of entry into mitosis, and studies have shown that premature activation of cdc2 leads to mitotic catastrophe and cell death.[1–3] Inhibition of Myt1 kinase is predicted to cause premature activation of cdc2, and inhibitors of this enzyme would thus be expected to kill rapidly proliferating cells and abrogate normal cell cycle checkpoints. Such inhibitors would thus be attractive for the treatment of cancer, because they could be used in conjunction with conventional chemotherapies to overcome drug resistance and enhance their cytotoxicity by abrogating cell cycle checkpoints.

As a part of our systematic search for potential anti-cancer agents from plants and marine organisms,[4–6] the methanol extract of an alga designated UM 2972M was found to show activity in a bioassay for inhibitors of Myt1 kinase, with an IC_{50} of 4 μg/mL. It was thus selected for fractionation for isolation of its bioactive constituents.

The algae comprise a large group of marine organisms, and have been subjected to intensive chemical studies. Many bioactive compounds, including the neurotoxic amino acids kainic acid and domoic acid[7] and the hypocholesterolemic betain lipids[8] have been isolated from them, as well as carotenoids,[9] steroids,[10] halogenated compounds,[11,12] fatty acids,[13–17] phenolic compounds,[18,19] and terpenoids.[20–22]

2. Results and discussion

The methanol extract (UM 2972 M) from an unknown algal species was subjected to partition between aqueous MeOH and organic solvents, and the aqueous MeOH fraction was then stripped of its MeOH and extracted with *n*-butanol to give a bioactive *n*-butanol fraction ($IC_{50}=1$ μg/mL). Column chromatography of the *n*-butanol fraction on Sephadex LH 20 with elution with CH_2Cl_2–MeOH (3:1), followed by repeated chromatography on RP-18 reversed phase silica gel with MeOH–H_2O (9:1) gave the two bioactive compounds **1** and **2**. Compounds **1** (1.05 mg, 0.025%) and **2** (1.46 mg, 0.033%) had activities of 0.12 and 0.43 μg/mL, respectively, in the Myt1 kinase bioassay.

Compound **1** had a molecular weight of 967, as indicated by a pseudomolecular ion $(M+Na)^+$ at $m/z=990$ in its MALDI TOF mass spectrum, and a composition of $C_{57}H_{109}NO_{10}$, as indicated by FAB-HRMS. Three spin–spin systems were identified from its ^1H NMR and COSY spectra (Table 1). The first spin system indicated the presence of a 1,2 diacylated glyceryl moiety [δ_H 4.51 ppm (1H, dd, $J=11.9$, 3.1 Hz, $H_{sn\text{-}1a}$), 4.18 ppm (1H, dd, $J=12.1$, 7.0 Hz, H_{sn1a}), 5.30 ppm (1H, m, $H_{sn\text{-}2}$), 4.11 ppm

Keywords: Marine; Myt1 kinase; Glycolipid.
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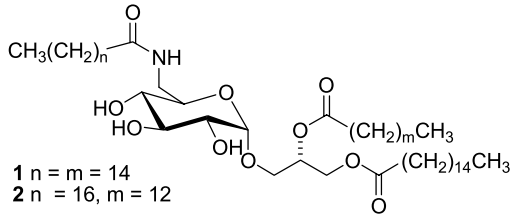
doi:10.1016/j.tet.2004.11.013

Table 1. Partial NMR data of lipids **1** and **2**[a,b]

Position	**1**		**2**	
	δ_H and J	δ_C	δ_H and J	δ_C
sn-1	4.51 (dd, J=12.0, 3.0) 4.18 (dd, J=12.0, 6.9)	64.3	4.50 (dd, J=12.0, 3.0) 4.18 (dd, J=12.0, 6.9)	64.3
sn-2	5.31 (m)	71.7	5.30 (m)	71.7
sn-3	4.10 (dd, J=10.8, 5.2) 3.57 (dd, J=10.8, 6.4)	67.1	4.10 (dd, J=10.7, 5.3) 3.56 (dd, J=10.7, 6.3)	67.1
G-1	4.76 (d, J=3.6)	101.0	4.75 (d, J=3.7)	100.0
G-2	3.40 (dd, J=3.6, 9.5)	73.5	3.39 (dd, J=3.6, 9.6)	73.5
G-3	3.63 (dd, J=9.5, 9.6)	75.0	3.62 (dd, J=9.6, 9.7)	75.0
G-4	3.08 (dd, J=9.6, 9.2)	74.9	3.07 (dd, J=9.7, 9.3)	74.9
G-5	4.07 (ddd, J=9.2, 9.4, 2.0)	69.9	4.06 (ddd, J=9.3, 8.4, 2.0)	68.9
G-6	3.34 (dd, J=14.4, 2.0) 2.91 (dd, J=14.4, 9.4)	54.2	3.34 (dd, J=14.3, 2.0) 2.91 (dd, J=14.3, 8.4)	54.2
CH_3–	0.89 (3×CH_3–, t, J=6.8)	14.5	0.89 (3×CH_3–, t, J=7.0)	14.5
$(–CH_2–)_n$	1.0–1.5		1.0–1.5	
α-CH_2–	2.33 (3×–CH_2–, m)	35.0, 35.2	2.33 (3×–CH_2–, m)	35.0; 35.2
β-CH_2–	1.59 (3×–CH_2–, m)	26.0	1.60 (3×–CH_2–, m)	26.0
–COO–		174.4		174.4
–COO–		175.2		175.2
–COO–		175.2		175.1

[a] δ values are in ppm and J values are in Hz.
[b] Chemical shifts were assigned by DQ COSY, HMQC, and HMBC spectra.

(1H, dd, J=11.8, 5.2 Hz, $H_{sn\text{-}3a}$), and 3.57 ppm (1H, dd, J=11.8, 6.4 Hz, $H_{sn\text{-}3b}$)]. The cross-peaks in the HMBC spectrum [δ_H/δ_C: 5.31 ($H_{sn\text{-}2}$)/174.4 (COO); 2.33 (α-CH_2)/174.4 (COO); 4.51 and 4.18 ($H_{sn\text{-}1}$)/175.2 (COO); 2.33 (α-CH_2)/175.2 (COO)] also indicated the presence of acyl groups on the 1 and 2 positions of a glycerol moiety. The second group of spin systems contained signals for three long chain fatty acids [δ_H 0.89 ppm (9H, t, J=6.83 Hz, 3×CH_3–), 1–1.5 ppm (multi –CH_2–) 1.59 ppm (6H, m, 3×β-CH_2–), 2.33 ppm (6H, m, 3×α-CH_2–). The third spin system indicated the presence of a glycosyl moiety [δ_H 4.76 ppm (1H, d, J=3.8 Hz, $H_{G\text{-}1}$), 3.40 ppm (1H, dd, J=9.5, 3.8 Hz, $H_{G\text{-}2}$), 3.63 ppm (1H, d, J=9.5, 9.1 Hz, $H_{G\text{-}3}$), 3.08 ppm (1H, dd, J=9.1, 9.7 Hz, $H_{G\text{-}4}$), 4.07 ppm (1H, m, $H_{G\text{-}5}$), and 2.91 (1H, dd, J=14.3, 9.3 Hz, $H_{G\text{-}6a}$), and 3.34 ppm (1H, d, J=14.1, 2.1 Hz, $H_{G\text{-}6b}$)]. The HMBC cross-peak δ_H 4.76 ($H_{G\text{-}1}$)/δ_C 67.1 ($C_{sn\text{-}3}$) showed that glycosylation was on the 3 position of the glycerol moiety, and that **1** was a glycoglycerolipid. Its ^{13}C NMR spectrum supported this conclusion. The chemical shifts and the coupling constants of the G-1 to G-4 protons of the glycosyl moiety were very close to those of methyl α-D-glucoside, indicating that these hydroxyl groups were unacylated. The chemical shifts of $H_{G\text{-}6}$ in the glycosyl moiety suggested the presence of an aminoacyl group at this position. The coupling constant of the anomeric proton (J=3.8 Hz) indicated the α configuration, and since glucose has the D-configuration in most cases in nature compound **1** could be assigned as an α-D-6-desoxy-6-aminoglucoside.



When **1** was hydrolyzed under various conditions (1% NaOMe in MeOH, with lipase from *Mucor javanicus*,[23,24] or 0.5% HCl) the only ester that could be detected was methyl palmitate. The polar fraction remaining after acidic hydrolysis was reduced by reaction with $NaBH_4$ and the resulting products acetylated and analyzed by GC–MS. Analysis of the retention times and fragmentation patterns of the two products indicated them to be glycerol triacetate and 6-acetylamino-1,2,3,4,5-penta-acetoxyhexane. This result confirmed that **1** was a glycoglycero lipid. A negative Cotton effect between λ_{max} 200–250 nm indicated that **1** was an *sn*-1, *sn*-2 diacylglycosylglycero-lipid.[25] The difference between the ^{13}C NMR signals for the two carbonyl carbons of the two glyceryl esters (0.6 ppm) indicated that **1** was an *sn*-1, *sn*-2 diacyl ester; had it been an *sn*-1, *sn*-3 diacyl ester these signals would have been closer or even overlapped.[26,27] Thus, compound **1** was assigned the structure *sn*-1,2-di-palmityl-3-(*N*-palmityl-6′-desoxy-6′-amino-α-D-glucosyl)-glycerol.

Compound **2** also had a molecular weight 967 and a composition of $C_{57}H_{109}NO_{10}$, as indicated by its MALDI TOF mass spectrum and FAB-HRMS. Its ^1H NMR and COSY spectra (Table 1) were very similar to those of **1**, and showed the presence of a diacylglyceryl moiety [δ_H 4.50 ppm (1H, dd, J=12.0, 3.0 Hz, $H_{sn\text{-}1a}$), 4.18 ppm (1H, dd, J=12.0, 6.9 Hz, $H_{sn\text{-}1a}$), 5.30 ppm (1H, m, $H_{sn\text{-}2}$), 4.10 ppm (1H, dd, J=10.7, 5.3 Hz, $H_{sn\text{-}3a}$), and 3.56 ppm (1H, dd, J=10.7, 6.3 Hz, $H_{sn\text{-}3b}$)].

The HMBC cross-peaks [δ_H/δ_C: 5.30 ($H_{sn\text{-}2}$)/174.4 (–COO–); 2.33(α-CH_2–)/174.4 (–COOH); 4.50 and 4.18 ($H_{sn\text{-}1}$)/175.2 (–COO–); 2.33 (α-CH_2–)/175.2 (–COO–)] supported this result. Overlapped signals for three long chain fatty acids [δ_H 0.89 ppm (9H, t, J=6.83 Hz, 3×CH_3–), 1–1.5 ppm (multi –CH_2–), 1.59 ppm (6H, m, 3×β-CH_2–), 2.33 ppm (6H, m, 3×α-CH_2–) could also be found in its NMR spectrum. NMR signals for a similar glycosyl moiety to that of **1** [δ_H 4.75 ppm (1H, d, J=3.7 Hz, $H_{G\text{-}1}$), 3.39 ppm (1H, dd, J=9.6, 3.7 Hz, $H_{G\text{-}2}$), 3.62 ppm (1H, d, J=9.6, 9.7 Hz, $H_{G\text{-}3}$), 3.07 ppm (1H, dd, J=9.3, 9.7 Hz, $H_{G\text{-}4}$), 4.06 ppm

(1H, m, H_{G-5}), and 2.91 (1H, dd, $J=14.3$, 8.4 Hz, H_{G-6a}), and 3.34 ppm (1H, d, $J=14.3$, 2.0 Hz, H_{G-6b}) were also observed. All the spectroscopic data for **2** were consistent with its assignment as an α-D-6-desoxy-6-amino-glucoside.

Hydrolysis of **2** with 0.5% HCl followed by 1% NaOMe in methanol and then by treatment with diazomethane yielded approximately equimolar amounts of methyl myristate, methyl palmitate and methyl stearate as determined by GC–MS. The polar fraction remaining after hydrolysis was reduced with $NaBH_4$ and acetylated, and the resulting product analyzed by GC–MS. Analysis of the retention times and fragmentation patterns of the two products indicated than to be glycerol triacetate and 6-acetylamino-1,2,3,4,5-penta-acetoxyhexane. These results indicated that **2** was a glycoglycero lipid with three different acyl groups, so the locations of these groups needed to be determined.

When **2** was hydrolyzed with 1% NaOMe in absolute methanol, only methyl palmitate and methyl myristate were detected by GC–MS. Since amides are hydrolyzed more slowly than esters under alkaline conditions, this result indicated that the stearyl moiety acylated the 6-amino position of glucose, and that the myristyl and palmityl groups both acylated the glyceryl moiety. When **2** was hydrolyzed with lipase from *M. javanicus*[23] and the products treated with diazomethane, methyl stearate and methyl palmitate were both isolated. This indicates that the myristyl group is located at the more hindered *sn*-2 position. Compound **2** was thus assigned as *sn*-1-palmityl-2-myristyl-3-(*N*-stearyl-6′-desoxy-6′-amino-α-D-glucosyl)-glycerol.

Three 6′-desoxy-6′-amino-glucosylglycerolipids have previously been reported in the literature,[28] but the acyl groups in these compounds were different from ours. Compounds **1** and **2** are thus new natural products.

As noted earlier, compounds **1** and **2** had IC_{50} values of 0.12 ± 0.07 and 0.43 ± 0.01 μg/mL, respectively, in the Myt1 kinase bioassay. The bioactivity of compounds of this type as inhibitors of Myt1 kinase is new, although diacylglycerol is well known as an activator of protein kinase C.[29] The inhibition against Myt1 kinase appeared to be selective as we observed no inhibition of two other protein kinases, Chk1 and Akt, with concentrations of compounds **1** and **2** as high as 100 μg/mL.

Although simple acylglycerols are not normally thought of as 'druggable' entities, they can serve as prototypes for compounds with inproved pharmacological properties. Thus, conformationally constrained diacylglycerol-bis-lactones have been investigated as diacylglycerol analogs,[30] and the conversion of an *O*-glycoside to a *C*-glycoside boosted the potency of a ceramide *O*-glycoside by two to three orders of magnitude.[31] In addition, it is worth noting that a sea alga has yielded a sulfoquinovosyl-diacylglycerol which acts as a potent inhibitor of eukaryotic DNA polymerases and HIV reverse transcriptase type 1.[32]

3. Experimental

3.1. General experimental procedures

CD and NMR spectra were obtained as previously described;[33] a 9 Hz optimization was employed for the long-range coupling pathway in HMBC determinations. MALDI spectra were determined on a Kratos Kompact SEQ (Kratos Analytical, Manchester, UK) time of flight mass spectrometer, and FAB mass spectra were obtained on a JEOL JMS-HX-110 instrument. The conditions for GC–MS conditions for fatty acid methyl ester: Hewlett Packard HP5710A Gas Chromatograph with HP-5 column (30 M× 0.32 mM i.d.), oven temperature programmed from 80 to 280 °C at 8 °C/min for **1** and at 4 °C/min for **2**, with He carrier gas at 12 psi. A VG7070E-HF mass spectrometer, scanned from 50 to 500 amu at 1.5 s/scan, was used for detection. Sephadex LH-20 (Sigma) was employed for gel permeation chromatography. Column chromatography was carried out on LRP-2 (RP-18) and reversed-phase TLC on MKC18F silica gel 60 A (RP-18) from Whatman.

3.2. Bioassay methods

The crude extract, fractions, and pure compounds were assayed for inhibitory activity against Myt1 kinase by a modification of a previously described microtiter-based fluorescence polarization assay.[34] Briefly, 10 μg recombinant glutathione-S-transferase (GST) tagged-Myt1 protein was incubated in kinase buffer (50 mM Tris–HCl, pH 7.5, 20 mM $MgCl_2$ and 1 mM DTT) containing 2.5 nM fluorescein-labeled Cdc2-derived peptide (containing Myt1 phosphorylation target sites Thr-14 and Tyr-15) (Molecular Devices, Sunnyvale, CA), 100 μM ATP, and compounds solubilized in dimethyl sulfoxide. The kinase reaction was incubated for 1 h at room temperature after which an anti-phospho-Cdc2 antibody (1 μg/mL) (Cell Signaling Technology, Beverly, MA) was added and incubated for an additional hour. Fluorescence polarization measurements were taken in black 96 well microtiter plates (Costar, Acton, MA) with a Wallac Victor 1420 multilabel microtiter plate reader (Perkin Elmer, Boston, MA). Duplicate samples were subjected to 25 flashes and experiments were performed 2–3 times. Dimethyl sulfoxide was used as the vehicle control; staurosporine (Sigma, St. Louis, MO) was used as the positive control and it had an IC_{50} value of 1.9 μM in this assay.

The effects of **1** and **2** on Akt and Chk1 activity were also studied with 384 well microtiter-based IMAP (Molecular Devices, Sunnyvale, CA) fluorescence polarization assays. Briefly, compounds **1** or **2** (0.1 to 100 μg/mL) were incubated for 30–60 min at room temperature in the kinase buffer (10 mM Tris–HCl, pH 7.2, 10 mM $MgCl_2$, 0.1% bovine serum albumin, and 0.05% NaN_3) with 0.2 U/mL Akt or 0.4 U/mL Chk1, 100 nM fluorescently labeled Akt substrate or Chk1 crosstide substrate peptide, 5 μM ATP in a total volume of 20 μL. IMAP binding solution (15–60 μL) was added to each well and incubated at room temperature for 30 min. Data were collected on an Analyst GT (Molecular Devices) and analyzed using SoftMaxPro software. Triplicate experiments were performed 2–3 times. Staurosporine (Sigma-Aldrich, St. Louis, MO) was used as a

positive control for Akt ($IC_{50}=\sim 60$ nM) and UCN-01 (National Cancer Institute, Bethesda, MD) was the positive control for Chk1 ($IC_{50}=\sim 2$ μM).

3.3. Isolation of bioactive compounds

The crude algal extract UM 2972M[35] (4.04 g) was partitioned between 50% aq MeOH and *n*-hexane, and the aq MeOH layer was then partitioned with CH_2Cl_2. The 50% aq MeOH fraction was then evaporated in vacuo to remove MeOH, and the residual H_2O fraction was extracted with BuOH. The BuOH fraction (1.56 g) was active in the Myt1 kinase bioassay ($IC_{50}=1.0$ μg/mL) and was subjected to column chromatography on Sephadex LH 20 (20 g, eluted with CH_2Cl_2–MeOH (3:1). A total of 45 fractions (5 mL/each fraction) was collected and fractions 8–12 (119.0 mg), which showed the characteristic ^{1}H NMR spectra of a glycoglycerolipid, were the most active ($IC_{50}=0.4$–1.0 μg/mL). The active fractions 9–10 ($IC_{50}=0.4$ μg/mL) were repeatedly purified by chromatography on an RP-18 column with MeOH–H_2O (9:1) as eluant. Compound **1** (1.05 mg, 0.026% based on crude extract) was isolated from fractions 13–28, and compound **2** (1.35 mg, 0.033% based on crude extract) from fraction 11. The inhibitory activities of the isolated compounds were $IC_{50}=0.12$ μg/mL for **1** and $IC_{50}=0.43$ μg/mL for **2**.

3.3.1. Compound 1. MALDI TOF mass spectrum: *m/z* 990 $(M+Na)^+$. HRFABMS *m/z* 968.8059 $[M+H]^+$; (calcd for $C_{57}H_{110}NO_{10}$ 968.8130). $[\alpha]_D^{23}=+41.8$ (*c* 0.12, MeOH). ^{1}H NMR and ^{13}C NMR spectra: see Table 1.

3.3.2. Alkaline hydrolysis of 1. Compound **1** (0.1 mg) in MeOH (0.2 mL) was treated with 1% NaOMe–MeOH solution (0.8 mL) and the solution was stirred at room temperature for 5 h. After partition between *n*-hexane and MeOH, the *n*-hexane extract was subjected on GC–MS. Methyl palmitate was detected with retention time 15′14″ and EIMS *m/z* 270 $(M)^+$, 227, 143, 87, 74, 57, 55. Its retention time and fragmentation peaks were same as the data from a standard sample in the data base.

3.3.3. Enzymatic hydrolysis of 1. Compound **1** (0.1 mg) was mixed with lipase (1 mg) from *M. javanicus* (Fluka Chemie AG, CH-9471, Switzerland, EEC No. 2326199) in dioxane–H_2O (1:1, 1 mL) and the reaction mixture was incubated at 38 °C for 4 h. After removal of the dioxane and H_2O, the residue was dissolved in 2 mL MeOH and partitioned with *n*-hexane. The *n*-hexane extract was treated with diazomethane and analyzed by GC–MS. Methyl palmitate was detected as described above.

3.3.4. Complete hydrolysis of 1. Compound **1** (0.12 mg) was treated with 0.5% HCl at 65 °C overnight. The reaction mixture was evaporated to dryness in vacuo and the residue mixed with 1% NaOMe in MeOH and incubated at room temperature for 6 h. After partitioning with *n*-hexane, the *n*-hexane fraction was evaporated to dryness and treated with diazomethane and analyzed by GC–MS as described above. Methyl palmitate was the only ester detected. The aqueous fraction was evaporated to dryness and treated with $NaBH_4$ in MeOH at room temperature for 4 h with stirring. After evaporation of the solvent, the reaction mixture was acetylated with Ac_2O (0.5 mL) and dry pyridine (20 μL) with stirring overnight. After removal of the reagents with N_2, the reaction mixture was extracted with CH_2Cl_2. The CH_2Cl_2 extract was subjected to GC–MS and glycerol triacetate (retention time: 7′20″, EIMS *m/z* 158 $(M-60)^+$, 145, 116, 115, 183, 74, 73, and 61) and 6-acetylamino-1,2,3,4,5-penta-acetoxyhexane (retention time: 17′47″, and EIMS *m/z* 433 $(M)^+$, 374, 331, 304, 289, 259, 207, and 87) were detected.

3.3.5. Compound 2. MALDI TOF mass spectrum: *m/z*=990 $(M+Na)^+$. HRFABMS *m/z* 968.8076 $[M+H]^+$; (calcd for $C_{57}H_{110}NO_{10}$ 968.8130). $[\alpha]_D^{23}=+31.1$ (*c* 0.10, MeOH). ^{1}H NMR and ^{13}C NMR spectrum data: see Table 1.

3.3.6. Alkaline hydrolysis of 2. Compound **2** (0.11 mg) in MeOH (0.2 mL) was treated with 1% NaOMe in MeOH (1.0 mL) and the solution was stirred at room temperature for 4 h. After extraction with *n*-hexane, the *n*-hexane extract was analyzed by GC–MS. Methyl myristate was detected with retention time 17′46″ and EIMS *m/z* 242 (M^+), 199, 143, 87, 74, and 57 and methyl palmitate with retention time 22″58″ and EIMS *m/z* 270 (M^+), 227, 185, 143, 87, 74, and 57. Their retention times and fragmentation patterns were the same as those of standard samples in the database.

3.3.7. Enzymatic hydrolysis of 2. Compound **2** (0.12 mg) was hydrolyzed with lipase from *M. javanicus* and the *n*-hexane extract treated with diazomethane and analyzed by GC–MS as previously described. Two peaks were detected. One of them was identified as methyl palmitate by its retention time (22′53″) and EIMS (*m/z* 270 (M^+), 239, 227, 185, 171, 143, 129, 115, 99, 87, 74, 57). The second was identified as methyl stearate by its retention time (27′42″) and EIMS (*m/z* 298 (M+), 267, 255, 213, 199, 185, 157, 143, 129, 97, 87, 74, and 57). The retention times and fragmentation patterns of these compounds were the same as those of standard samples in the database.

The MeOH fraction after partition was evaporated to dryness in vacuo. The residue was treated with 1% NaOMe in MeOH at room temperature for 4 h. After extraction with *n*-hexane, the *n*-hexane fraction was concentrated, treated with diazomethane, and analyzed by GC–MS. The major peak was identified as methyl myristate by its retention time of 17′37″ and EIMS *m/z* 242 (M^+), 199, 143, 87, 74, 57, and 55.

3.3.8. Complete hydrolysis of 2. Compound **2** (0.10 mg) was treated with 0.5% HCl solution (1 mL) at 65 °C overnight, the reaction mixture evaporated to dryness, and the residue incubated with 1% NaOMe in MeOH (1 mL) at room temperature for 6 h. After partition with *n*-hexane, the *n*-hexane fraction was evaporated to dryness and treated with diazomethane and analyzed by GC–MS. The first peak was identified as methyl myristate by its retention time of 17′20″ and EIMS *m/z* 242 (M^+), 199, 143, 87, 74, 57, and 55. The second peak was identified as methyl palmitate by its retention time of 22″55″ and EIMS *m/z* 270 (M+), 239, 227, 185, 143, 87, 74, 57. The third peak was detected as methyl stearate by its retention time of 27′46″ and EIMS *m/z* 298 (M+), 267, 255, 213, 199, 143, 87, 74, 57. The aqueous fraction was evaporated to a small volume and reduced with

$NaBH_4$ at room temperature for 4 h with stirring. After evaporation of the solvent and drying under vacuum overnight, the reaction mixture was acetylated with Ac_2O (0.5 mL) and dry pyridine (20 μL) with stirring overnight. After removal of the reagents with N_2, the reaction mixture was extracted with CH_2Cl_2. The CH_2Cl_2 extract was subjected to GC–MS and showed two intense peaks, which were identified as glycerol triacetate and 6-acetylamino-1,2,3,4,5-penta-acetoxyhexane as previously described.

Acknowledgements

This work was supported by a National Cooperative Drug Discovery Group award to the University of Virginia (U19 CA 50771, Dr. S. M. Hecht, Principal Investigator), and this support is gratefully acknowledged. We also thank Mr. Tom Glass and Mr. William R. Bebout, Sr., (Virginia Polytechnic Institute and State University) for spectroscopic support

References and notes

1. Mueller, P. R.; Coleman, T. R.; Kumagai, A.; Dunphy, W. G. *Science* **1995**, *270*, 86–89.
2. Liu, F.; Stanton, J. J.; Wu, Z.; Piwnica-worms, H. *Mol. Cell. Biol.* **1997**, *17*, 571–583.
3. Lago, M. A. Myt1 kinase inhibitors. US patent No. US 6,391,894, May 21, 2002.
4. Zhou, B.-N.; Johnson, R. K.; Mattern, M. R.; Fisher, P. W.; Kingston, D. G. I. *Org. Lett.* **2001**, *3*, 4047–4049.
5. Zhou, B.-N.; Johnson, R. K.; Mattern, M. R.; Wang, X. Y.; Hecht, S. M.; Beck, H. T.; Kingston, D. G. I. *J. Nat. Prod.* **2000**, *63*, 217–221.
6. Zhou, B.-N.; Slebodnick, C.; Johnson, R. K.; Mattern, M. R.; Kingston, D. G. I. *Tetrahedron* **2000**, *56*, 5781–5784.
7. Impellizzeri, G.; Mangiafico, S.; Oriente, G.; Piattelli, M.; Sciuto, S.; Fattorusso, E.; Magno, S.; Santacroce, C.; Sica, D. *Phytochemistry* **1975**, *14*, 1549–1557.
8. Eichenberger, W.; Boschetti, A. *FEBS Lett.* **1978**, *88*, 201–204.
9. Katayama, T.; Yokoyama, H.; Chichester, C. O. *Int. J. Biochem.* **1970**, *1*, 438–444.
10. Combaut, G.; Bruneau, Y.; Codomier, L.; Teste, J. *J. Nat. Prod.* **1979**, *42*, 150–151.
11. Codomier, L.; Bruneau, Y.; Combaut, G.; Teste, J. *C. R. Acad. Sci. Paris Ser. D* **1977**, *284*, 1163–1165.
12. Fenical, W. *Tetrahedron Lett.* **1974**, *15*, 4463–4466.
13. Paul, V. J.; Fenical, W. *Tetrahedron* **1984**, *40*, 2913–2918.
14. Weinheimer, A. J.; Spraggins, R. L. *Tetrahedron Lett.* **1969**, 5185–5188.
15. Radunz, A. *Phytochemistry* **1967**, *6*, 399–406.
16. Klenk, E.; Knipprath, W.; Eberhagen, D.; Koof, H. P. *Hoppe Seyler's Z. Physiol. Chem.* **1963**, *334*, 44–59.
17. Kajiwara, T.; Kashibe, M.; Matsui, K.; Hatanaka, A. *Phytochemistry* **1991**, *30*, 193–195.
18. Glombitza, K. W.; Zieprath, G. *Planta Med.* **1989**, *55*, 171–175.
19. Li, S. M.; l Glombitza, K. W. *Bot. Mar.* **1991**, *34*, 455–457.
20. Amico, V.; Oriente, G.; Piattelli, M.; Tringali, C.; Fattorusso, E.; Magno, S.; Mayol, L. *J. Chem Soc., Chem. Commun.* **1976**, 1024–1025.
21. Enoki, N.; Ishida, R.; Urano, S.; Ochi, M.; Tokoroyama, T.; Matsumoto, T. *Chem. Lett.* **1982**, 1837–1840.
22. Wright, A. D.; König, G. M.; Sticher, O. *Tetrahedron* **1990**, *46*, 3851–3858.
23. Kobayashi, M.; Hayashi, K.; Kawazoe, K.; Kitagawa, I. *Chem. Pharm. Bull.* **1992**, *40*, 1404–1410.
24. Murakami, N.; Morimoto, T.; Imamura, H.; Nagatsu, A.; Sakakibara, J. *Tetrahedron* **1994**, *50*, 1993–2002.
25. Michelsen, P. *Chem. Scr.* **1985**, *25*, 217–218.
26. Diehl, B. W. K.; Herling, H.; Riedl, I.; Heinz, E. *Chem. Phys. Lipids* **1995**, *77*, 147–153.
27. Máñez, S.; Recio, M. d. C.; Gil, I.; Gómez, C.; Giner, R.-M.; Waterman, P. G.; Ríos, J.-L. *J. Nat. Prod.* **1999**, *62*, 601–604.
28. Dai, J. Q.; Zhu, Q. X.; Zhao, C. Y.; Yang, L.; Li, Y. *Phytochemistry* **2001**, *58*, 1305–1309.
29. Newton, A. C. *Curr. Opin. Cell. Biol.* **1997**, *9*, 161–167.
30. Kang, J.-H.; Kim, S. Y.; Lee, J.; Marquez, V. E.; Lewin, N. E.; Pearce, L. V.; Blumberg, P. M. *J. Med. Chem.* **2004**, *47*, 4000–4007.
31. Yang, G.; Schmieg, J.; Tsuji, M.; Franck, R. W. *Angew. Chem., Int. Ed.* **2004**, *43*, 3818–3822.
32. Ohta, K.; Mizushina, Y.; Hirata, N.; Takemura, M.; Sugawara, F.; Matsukage, A.; Yoshida, S.; Sakaguchi, K. *Chem. Pharm. Bull.* **1998**, *46*, 684–686.
33. Zhou, B.-N.; Mattern, M. R.; Johnson, R. K.; Kingston, D. G. I. *Tetrahedron* **2001**, *57*, 9549–9554.
34. Kristiansdottir, K.; Rudolph, J. *Anal. Biochem.* **2003**, *316*, 41–49.
35. The algal material was provided by GlaxoSmithKline Pharmaceuticals, but regrettably its taxonomy was not fully determined and cannot now be ascertained. Glyceroglycolipids are distributed among the red, brown, and green algae,[36] and so chemotaxonomy cannot be applied to the determination of the type of algal material that was extracted.
36. Khotimchenko, S. V. *Chem. Nat. Comp.* **2002**, *38*, 223–229.

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Tetrahedron 61 (2005) 889–895

Relationship between local electrophilicity and rate coefficients for the hydrolysis of carbenium ions

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Received 28 September 2004; revised 8 November 2004; accepted 9 November 2004

Available online 10 December 2004

Abstract—The local electrophilicity of a series of 28 carbenium ions has been ranked within a theoretical absolute scale. New substituent constants are introduced to account for the responses of the electrophilicity pattern induced by multiple substitutions at the carbocation site. The model is used to predict rate coefficients ordering in terms of the experimental hierarchy of electrophilicity established for these systems [Minegishi, S.; Mayr, H. *J. Am. Chem. Soc.* **2003**, *125*, 286].

1. Introduction

After the introduction of the concepts of electrophilicity and nucleophilicity by Ingold[1] in the 1930s, there has been a growing interest in classifying atoms and molecules within empirical scales of electrophilicity/nucleophilicity. The main idea behind this objective has been the search of absolute scales that could be independent on the reactivity of the nucleophile/electrophile partners. This objective is ambitious if one considers that a universal scale should accommodate a wide diversity of chemical species presenting quite different structural and bonding properties. For instance, one of the first attempts to classify electron donors within a single nucleophilicity scale was reported by Swain and Scott,[2] who defined a nucleophilicity number as an intrinsic property of nucleophiles, using rate coefficients for a series of S_N2 reactions. Other attempts to quantitatively rank the nucleophilic power of molecules were proposed by Edwards, using a four parameter scheme,[3] and by Edwards and Pearson[4] using the hard and soft acids and bases (HSAB) empirical rule.

Recently, Mayr et al. in a series of articles persuasively argued in favour of nucleophilicity and electrophilicity parameters that are independent of the reaction partner.[5–10] They proposed that the rate coefficients for the reactions of carbocations with uncharged nucleophiles obey the linear free energy relationship:

$$\log k = s(E + N) \tag{1}$$

where E and N are the electrophilicity and nucleophilicity parameters, respectively, and s is the nucleophilic-specific slope parameter. This sensitivity parameter is usually close to unity, so that it may be neglected for the purpose of qualitative comparisons. These authors have clearly emphasized and illustrated the usefulness of the nucleophilicity/electrophilicity scales to quantitatively discuss reactivity as well as inter molecular selectivity.[5–10]

From a theoretical point of view, the electrophilicity concept has attracted the attention of several authors.[11–15] Most of the proposed definitions of electrophilicity are framed on reactivity indexes. By construction, the theoretical scales of electrophilicity, based on descriptors of the electronic structure defined at the ground state of molecules (i.e., static reactivity indexes) are absolute scales, in the sense that they are independent on the nucleophile partners. On the other hand, a quantitative definition of nucleophilicity in terms of electronic reactivity indexes turned out to be a more difficult task, and few attempts to quantitatively define nucleophilicity within this framework have been reported to date. The Fukui function based *philicity* index introduced by Chattaraj et al.[16] and the nucleophilicity index defined from vertical ionization potentials[17] are among the few efforts devoted to this subject.

Keywords: Hammett substituent constants; Local electrophilicity scale; Rate coefficients from local electrophilicity index.
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0040–4020/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2004.11.014

Validated theoretical scales of electrophilicity/nucleophilicity are highly desirable, as they can further provide valuable information about the intramolecular selectivity. In this article, we present a quantitative classification of the electrophilicity pattern for a series of 28 carbenium ions, using the global electrophilicity index introduced by Parr et al.,[12] and a local extension condensed to atom or functional groups.[18] The local contribution incorporates the electrophilic Fukui function to regionally project the global electrophilicity of these charged electron acceptors. From the knowledge of the electrophilicity index at the carbocation site, accurate rate coefficients may be predicted.

2. The model

The concept of electrophilicity viewed as a reactivity index was introduced rather recently by Parr et al.[12] It is based on a second order expansion of the electronic energy with respect to the charge transfer ΔN at fixed geometry. Since electrophiles are species that stabilize upon receiving an additional amount of electronic charge from the environment, there exist a minimum of energy for a particular ΔN^{*} value. Using this simple idea, Parr et al. performed a variational calculation that led to the definition of the global electrophilicity index as $\omega = -\Delta E(\Delta N^{*})$, which may be recast into the more familiar form:[12]

$$\omega = \frac{\mu^2}{2\eta}; \tag{2}$$

in terms of the electronic chemical potential μ and the chemical hardness η. The ω index establishes an absolute scale of electrophilicity in the sense that the hierarchy of electrophilicity is built up from the electronic structure of molecules, independent of the nucleophilic partner, which is replaced by an unspecified environment viewed as sea of electrons.[12]

Beside the global electrophilicity index, it is possible to define its local (or regional) counterpart condensed to atoms. The local electrophilicity index ω_k condensed to atom k is easily obtained by projecting the global quantity onto any atomic center k in the molecule by using the electrophilic Fukui function (i.e., the Fukui function for nucleophilic attack, f_k^+). There results:[18]

$$\omega_k = f_k^+ \omega \tag{3}$$

The regional or condensed to atom electrophilicity index has been shown to correctly assess the regioselectivity in a number of cases.[18–20] In summary, while the global electrophilicity index categorizes within a unique scale the electron acceptor ability of molecules, its local or regional counterpart plays a key role in the elucidation of the intramolecular selectivity of the same systems. Note that site electrophilic activation may also be assessed as the variation in local electrophilicity induced for instance by chemical substitution or any source of external perturbation to the molecular system.

3. Computational details

All the structures included in this study were optimized at the B3LYP/6-31G(d) level of theory using the Gaussian98 package of programs.[21] The values of the electronic chemical potential and the chemical hardness were obtained from the expressions $\mu \approx (\varepsilon_H + \varepsilon_L)/2$ and $\eta \approx \varepsilon_L - \varepsilon_H$, in terms of the one electron energies of the HOMO and LUMO frontier molecular orbitals, ε_H and ε_L, respectively.[22] With these quantities at hand, the global electrophilicity was obtained using Eq. (2). The local electrophilicity values were obtained from the global electrophilicity index and the electrophilic Fukui function using Eq. (3). The electrophilic Fukui function was evaluated from a single point calculation in terms of the molecular orbital coefficients and the overlap matrix using a procedure described elsewhere.[23,24]

4. Results and discussion

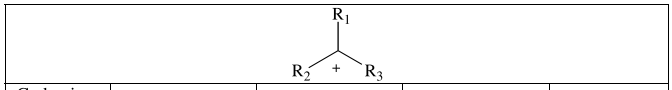
The systems considered in the present study are depicted in Chart 1. Included in these series are the tritylium, benzhydrylium, and benzylium ions. In order to set up the appropriate scenario to discuss the electrophilicity pattern of these systems, let us mention that, for instance, the neutral electrophilic species participating in Diels–Alder reactions (i.e., the dienophiles) present local electrophilicity numbers in the range 1.14 eV (acrolein–BH_3 complex) to 0.10 eV (dimethylvinylamine) within the theoretical scale.[18] As expected, the electron deficient carbenium ions display an even higher local electrophilicity range comprised between [2.0–6.0] eV (see fourth column of Table 1 and Chart 2).

Figure 1 summarizes the comparison between the local electrophilicity index evaluated at the carbocation site, ω_C, and the logarithm of the rate coefficient for the hydrolysis for the whole series of 28 carbenium ions depicted in Chart 1. The resulting regression equation is:

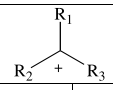
$$\text{Log } k_w = 4.714\ \omega_C - 13.827. \tag{4}$$

The linear relationship between both variables is qualitatively acceptable (regression coefficient $R \approx 0.94$) if one considers that this series of carbenium ions comprises a large variety of different structures, thereby suggesting that the regional electrophilicity patterns at a carbocation site, imbedded in different chemical environments may be used to correctly assess the effect of chemical substitution on the electrophilic potential of molecules, and therefore it can be further considered as a reliable descriptor of reactivity. The deviations of compounds 21, 22, 23 that show the highest values of electrophilicity at the carbocation site may be at least partially due to the fact that the rate constants of these compounds with water are close to the diffusion control limit. For this reason the increase of ω_C cannot be reflected in these rate constants.[25]

Despite the deviations observed, we may still validate the linear relationship given in Eq. (4) by using it to predict the rate coefficient for the neutral hydrolysis of other carbenium ions not included in the present data base. An immediate application of Eq. (4) is the evaluation of the rate coefficient for the p-CH_3 tritylium ion not included in the regression



Carbenium ions	R_1	R_2	R_3	$\sigma_p(\omega_C)$
1				0.01
2	OCH_3			-0.34
3	OCH_3	OCH_3		-0.68
4	$N(CH_3)_2$			-0.81
5	$N(CH_3)_2$	OCH_3		-1.05
6	OCH_3	OCH_3	OCH_3	-1.07
7	$N(CH_3)_2$	$N(CH_3)_2$		-1.35
8	OCH_3	OCH_3	$N(CH_3)_2$	-1.31
9	$N(CH_3)_2$	$N(CH_3)_2$	$N(CH_3)_2$	-1.92
10	H			1.14
11	H	CH_3		0.82
12	H	CH_3	CH_3	0.56
13	H	OCH_3		0.35
14	H	OCH_3	CH_3	0.16



Carbenium ions	R_1	R_2	R_3	$\sigma_p(\omega_C)$
15	H	OCH_3	OCH_3	-0.11
16	H	$N(CH_3)CH_2CF_3$	$N(CH_3)CH_2CF_3$	-0.81
17	H			-1.13
18	H	$N(CH_3)_2$	$N(CH_3)_2$	-1.15
19	H			-1.36
20	H			-1.36
21	CH_3		H	2.89
22	CH_3		CH_3	2.35
23	CH_3	CH_3	H	1.90
24	H	OCH_3	H	1.35
25	CH_3	OCH_3	H	1.08
26	H		OCH_3	1.17
27	CH_3		OCH_3	0.64
28	CH_3	OCH_3	OCH_3	-0.21

Chart 1. Carbenium ions considered in the present study and substituent constants for multiple substitutions.

Table 1. Global electrophilicity (ω), electrophilic Fukui function at the carbocation site (f_C^+), local electrophilicity at the carbocation site (ω_C) and log of the rate coefficients for hydrolysis of carbenium ions, k_w [a,b]

Carbenium ions	ω [eV]	f_C^+	ω_C [eV]	Log k_w [s^{-1}]
1	11.1	0.3363	3.72	5.18
2	10.7	0.3207	3.44	3.00
3	10.2	0.3105	3.17	1.98
4	10.1	0.3015	3.07	−2.02
5	9.7	0.2956	2.88	−2.34
6	9.4	0.3044	2.86	1.17
7	9.3	0.2853	2.64	−3.68
8	9.1	0.2924	2.67	−2.64
9	7.9	0.2781	2.19	−4.71
10	13.0	0.3550	4.61	8.48
11	12.6	0.3456	4.36	8.08
12	12.3	0.3376	4.15	7.51
13	12.1	0.3311	3.99	6.32
14	11.8	0.3252	3.84	5.96
15	11.5	0.3161	3.62	5.11
16	10.6	0.2903	3.07	0.58[b]
17	10.1	0.2790	2.81	−0.48[b]
18	9.9	0.2845	2.80	−1.59[b]
19	9.4	0.2845	2.63	−2.25[b]
20	9.4	0.2799	2.63	−2.66[b]
21	13.7	0.4376	6.00	11.00
22	12.8	0.4358	5.57	10.23
23	12.5	0.4172	5.22	9.60
24	12.2	0.3925	4.78	8.30
25	11.6	0.3948	4.57	7.70
26	11.2	0.4163	4.64	9.30
27	10.4	0.4054	4.22	7.70
28	9.5	0.3743	3.54	6.85

[a] Log k_w values from Refs. 5, 7 and 26.
[b] Log k_w values from Ref. 9.

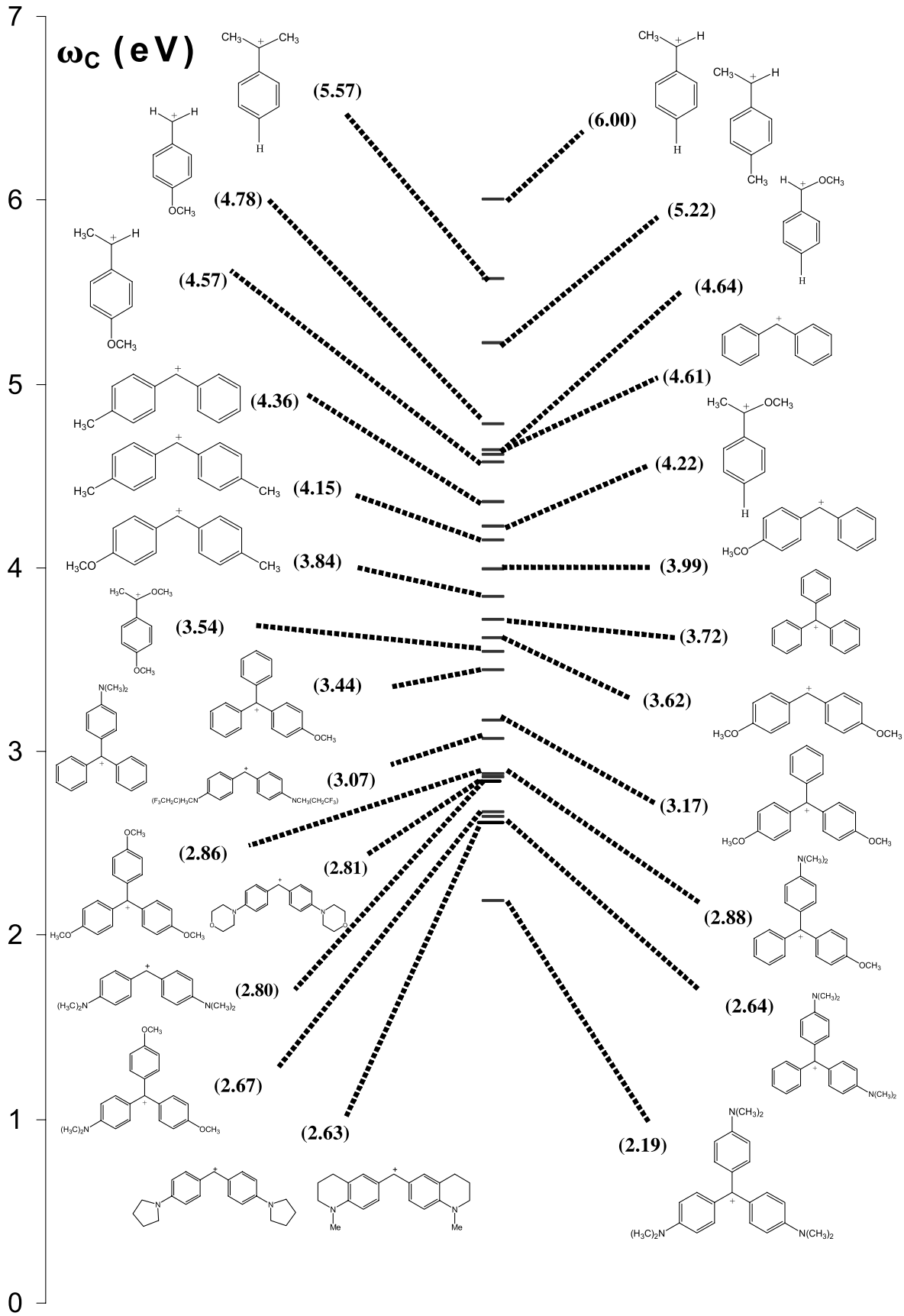


Chart 2. Local electrophilicity scale for carbenium ions.

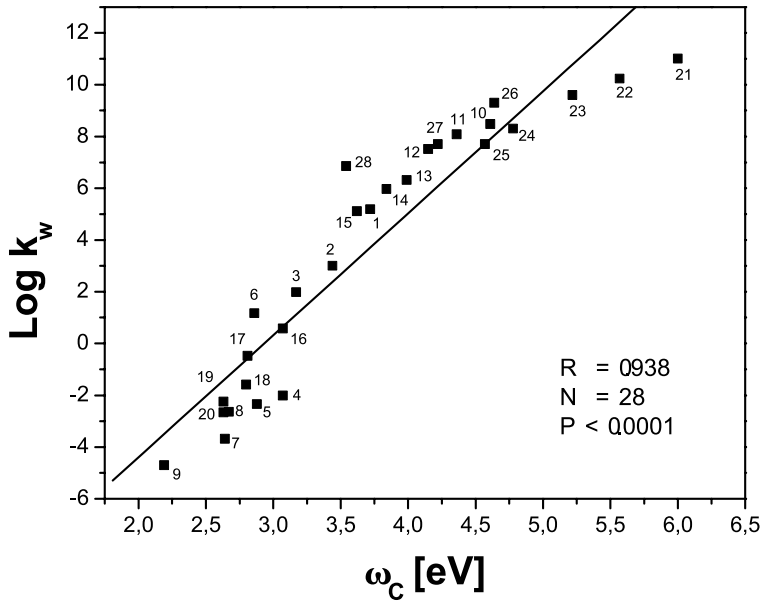


Figure 1. Comparison between experimental log k_w for the neutral hydrolysis of 28 carbenium ions and the local electrophilicity index at the carbocation site, ω_C. *R* is the regression coefficient; *N* is the number of points and *P* is the probability that the observed correlation was randomly obtained.

analysis shown in Figure 1, for which $\omega_C = 3.60$ eV. Application of Eq. (4) to this compound yields a predicted log $k_w = 3.14$. The experimental electrophilicity parameter ($E = -0.13$) for this compound has been recently reported by Mayr and Minegishi.[9] It is upper bounded by the *E* parameter for tritylium ion ($E = 0.51$, log $k_w = 5.18$, compound **1** in Table 1), and lower bounded by (p-OCH_3 tritylium ion ($E = -1.87$, log $k_w = 3.00$, compound **2** in Table 1). Since the *E* parameter shows a linear relationship with log k_w,[8] the order relationship log k_w (p-OCH_3 tritylium ion) $<$log k_w (p-CH_3 tritylium ion) $<$log k_w (tritylium ion) is also satisfied. Another pertinent prediction concerns the rate coefficient for the hydrolysis of the tris-(o,p-$(OCH_3)_2$), tritylium ion experimentally evaluated by Ritchie[26] and not included in the present database. Using its local electrophilicity value $\omega_C = 2.65$ eV, the predicted rate coefficient obtained from Eq. (4) yields log $k_w = -1.33$ which is again correctly upper and lower bounded by those of tritylium ion (log $k_w = 5.18$, compound **1** in Table 1) and (p-$N(Me)_2$, p-OCH_3) tritylium ion (log $k_w = -2.34$, compound **5** in Table 1).

The analysis of the effect that the different substituents may have on the rate coefficients in this case is particularly hard to perform by using simple inductive effects, as described for instance by the Hammett substituent constants. Note that, despite the fact that most of the 28 carbenium ions exhibit *para*-substitution, the general structure on top of Chart 1, shows a complex substitution pattern at the carbocation centre. In order to asses the effect of multiple

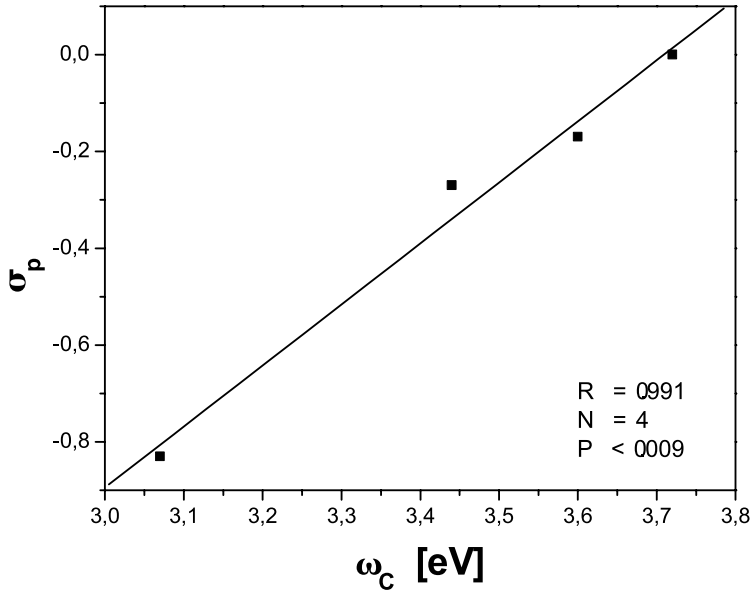


Figure 2. Comparison between Hammett substituent σ_p constants for singly substituted carbenium ions and the local electrophilicity index ω_C. *R* is the regression coefficient; *N* is the number of points and *P* is the probability that the observed correlation was randomly obtained.

substitution at this site, we first considered those compounds that have two fixed hydrogen atoms at the *p*-position of the phenyl group, which according to Hammett classification have $\sigma_p(H)=0.0$, and the third phenyl group substituted at *p*-position with H (compound **1** in Chart 1), OCH_3 (compound **2** in Chart 1), $N(Me)_2$ (compound **4** in Chart 1), plus the *p*-CH_3 substituted compound not shown in Chart 1 (for which $\omega_C=3.60$ eV). The comparison between the σ_p values and the local electrophilicity index ω_C is shown in Figure 2. It may be seen that for this short series the σ_p increases linearly with the local electrophilicity index ω_C (regression coefficient $R\approx0.99$). The resulting empirical equation is:

$$\sigma_p = 1.261\omega_C - 4.676. \quad (5)$$

From this equation, we define a new substituent constant $\sigma_p(\omega_C)$ which is uniquely determined by the knowledge of the ω_C index. The $\sigma_p(\omega_C)$ values for the whole series of carbenium ions considered in this work are shown in Chart 1, last column. The following results are relevant: in the tritylium subseries (compounds **1–9** in Chart 1), multiple substitution at the carbocation site with *p*-OCH_3 and *p*-$N(Me)_2$ substituted phenyl groups results in an electrophilic deactivation ($\sigma_p(\omega_C)<0$), thereby indicating that the net effect of these groups is to act as electron releasing substituents. Note that the rate coefficients are consistently predicted to be less than the reference compound **1** (see Table 1). While the –OCH_3 group seems to make an approximately additive contribution to $\sigma_p(\omega_C)$ of *c.a.* 0.34 units, in the case of increasing substitution with $N(Me)_2$ this rule is less clear.

However, a more important result is found in the subseries of benzhydrylium ions (compounds **10–20** in Chart 1). For this series, the $\sigma_p(\omega_C)$ values indicate that while for the double substitution with phenyl groups at R_2 and R_3 and some combination of *p*-CH_3 and *p*-OCH_3, the global effect is activating ($\sigma_p(\omega_C)>0$ for compounds **10–14** in Chart 1), some other combinations involving *p*-OCH_3, *p*-$N(Me)_2$, $(mfa)_2$, $(mor)_2$, $(thq)_2$ and $(pyr)_2$,[9] result in a global substituent effect that becomes deactivating ($\sigma_p(\omega_C)<0$ for compounds **15–20** in Chart 1). Note that the highest activating effect is shown by the Ph_2CH^+ ion ($\sigma_p(\omega_C)=1.14$, compound **10** in Chart 1), thereby suggesting that in this compound, two adjacent Ph groups cooperatively stabilize the carbocation by resonance. Increasing substitution by one and two methyl groups at *p*-position significantly attenuate this activation pattern (see compounds **11** and **12** in Chart 1). Other combination including mixed substitution with (Ph and *p*-OCH_3) and (*p*-OCH_3 and *p*-CH_3) result in a marginal electrophilic activation at the carbocation site (see for instance compounds **13** and **14** in Chart 1). Any combination of substitution involving *p*-OCH_3, *p*-$N(Me)_2$ and $(mfa)_2$, $(mor)_2$, $(thq)_2$ and $(pyr)_2$,[9] on the other hand systematically result in increasing electrophilic deactivation at the carbocation site ($\sigma_p(\omega_C)<0$, for compounds **15** and **20** in Chart 1). Finally, for the subseries of substituted benzyl ions (compounds **21–28** in Chart 1) we may observe that for some combination of *p*-CH_3 or *p*-OCH_3 with CH_3 and OCH_3 groups at R_1 and R_3, the global effect is activating ($\sigma_p(\omega_C)>0$ for compounds **21–27** in Chart 1), with the only exception of compound **28**, for which $\sigma_p(\omega_C)<0$. Note that the only difference with respect to compound **27** for which $\sigma_p(\omega_C)>0$, is the *p*-OCH_3 substitution at R_2. The effect observed in the cases of *p*-OCH_3 in compounds **24** and **28** may be again traced to resonance effects. For instance, while in compound **24** there is the possibility to form one oxonium structure at the *p*-OCH_3 position by resonance, structure **28** offers an additional oxonium resonant structure at R_3.

In summary, the reactivity pattern of the series of **28** carbenium ions considered in the present study may be rationalized in terms of a complex substituent constant $\sigma_p(\omega_C)$ assessing the inductive effect for multiple substitution. This index is derived from the standard Hammett σ_p constant and the local electrophilicity index at the carbocation site for singly substitution using a four point equation. The $\sigma_p(\omega_C)$ index not only assesses multiple inductive effects but some additional substituent effects like resonance seems to be incorporated in this new electronic descriptor of reactivity.

5. Concluding remarks

The local electrophilicity of a series of 28 carbenium ions has been ranked within an absolute theoretical scale. New substituent constants are introduced to account for the responses of the electrophilicity pattern induced by multiple substitutions at the carbocation site. The model correctly explains the experimental electrophilicity ordering established in terms of experimental scales.

Acknowledgements

Work was supported by Fondecyt, grants 1030548 and 1020069, DI-12-02 from Universidad Andrés Bello, project No. 13.04.23 from Universidad Técnica Federico Santa Maria and Millennium Nucleus for Applied Quantum Mechanics and Computational Chemistry, grant No. P02-004-F.

References and notes

1. Ingold, C. K. *Recl. Trav. Chim.* **1929**, *48*, 797.
2. Swain, C. G.; Scott, C. B. *J. Am. Chem. Soc.* **1953**, *75*, 141.
3. Edwards, J. O. *J. Am. Chem. Soc.* **1954**, *76*, 1540.
4. Edwards, J. O.; Pearson, R. G. *J. Am. Chem. Soc.* **1962**, *84*, 16.
5. Mayr, H.; Patz, M. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 938.
6. Mayr, H.; Müller, K.-H.; Ofial, A. R.; Buhl, M. *J. Am. Chem. Soc.* **1999**, *121*, 2418.
7. Mayr, H.; Bug, T.; Gotta, M. F.; Hering, N.; Irrgang, B.; Janker, B.; Kempf, B.; Loos, R.; Ofial, A. R.; Remennikov, G.; Schimmel, H. *J. Am. Chem. Soc.* **2001**, *123*, 9500.
8. Mayr, H.; Kempf, B.; Ofial, A. R. *Acc. Chem. Res.* **2003**, *36*, 66.
9. Minegishi, S.; Mayr, H. *J. Am. Chem. Soc.* **2003**, *125*, 286.
10. Minegishi, S.; Kobayashi, S.; Mayr, H. *J. Am. Chem. Soc.* **2004**, *126*, 5174.

11. Maynard, A. T.; Huang, M.; Rice, W. G.; Covell, D. G. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 11578.
12. Parr, R. G.; Szentpály, L. v.; Liu, S. *J. Am. Chem. Soc.* **1999**, *121*, 1922.
13. Pérez, P.; Domingo, L. R.; Aurell, M. J.; Contreras, R. *Tetrahedron* **2003**, *59*, 3117.
14. Roy, R. K.; Krishnamurti, S.; Geerlings, P.; Pal, S. *J. Phys. Chem. A* **1998**, *102*, 3746.
15. Pérez, P.; Toro-Labbé, A.; Aizman, A.; Contreras, R. *J. Org. Chem.* **2002**, *67*, 4747.
16. Chattaraj, P. K.; Maiti, B.; Sarkar, U. *J. Phys. Chem. A* **2003**, *107*, 4973.
17. Contreras, R.; Andrés, J.; Safont, V. S.; Campodonico, P.; Santos, J. G. *J. Phys. Chem. A* **2003**, *107*, 5588.
18. Domingo, L. R.; Aurell, M. J.; Pérez, P.; Contreras, R. *J. Phys. Chem. A* **2003**, *106*, 6871.
19. Pérez, P. *J. Phys. Chem. A* **2003**, *107*, 522.
20. Pérez, P. *J. Org. Chem.* **2003**, *68*, 5886.
21. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. J. A.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; W. Gill, P. M.; Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. GAUSSIAN98, Revision A.6 Gaussian, Inc.: Pittsburgh, PA, 1998.
22. Parr, R. G.; Yang, W. *Density Functional Theory of Atoms and Molecules*; Oxford Science: Oxford, 1994.
23. Contreras, R.; Fuentealba, P.; Galván, M.; Pérez, P. *Chem. Phys. Lett.* **1999**, *304*, 405.
24. Fuentealba, P.; Pérez, P.; Contreras, R. *J. Chem. Phys.* **2000**, *113*, 2544.
25. Lucius, R.; Loos, R.; Mayr, H. *Angew. Chem., Int. Ed.* **2002**, *41*, 91.
26. Ritchie, C. D. *Can. J. Chem.* **1986**, *64*, 2239.

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Tetrahedron
Tetrahedron 61 (2005) 897–902

The synthesis of calix[4]crown based dendrimer

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Received 28 July 2004; revised 4 November 2004; accepted 5 November 2004

Available online 8 December 2004

Abstract—A second generation of dendrimer with calixcrown as repeat unit was first synthesized. Its structure and conformation was determined by ^{1}H NMR and MALDI-TOF mass spectra.

1. Introduction

Dendrimers are macromolecules that radiate out from a central core and have at least one branch at each repeat unit. A typical of dendrimer contains three different regions: core, branches, and periphery, and all of them can be modified to exhibit certain function. Due to their unique physical property and structure, such as, highly branched structure, monodispersed molecular weight, globular and symmetrical conformation, and high density of peripheral functionalities, the researches based on them has expanded exponentially in recent years.[1]

There are two basic approaches for the stepwise synthesis of dendrimers: The divergent approach[2] and convergent approach.[3] In the divergent approach, the synthetic sequence is from the core to periphery; while the convergent approach is from the periphery to core.

Calixarene are well-defined macrocyclic molecules, which are readily available in large quantities and easily modified by chemical reaction to bind various kinds of anions, cations, and neutral molecules.[4] It is an original idea to integrate calixarenes and dendrimers as well as their unique properties to form new structural host, and there have several examples about dendrimers with calixarene as core.[5] However, it may be due to the serious steric problem caused by the large sized calixarenes, there are only a few examples on dendrimers with calixarenes as branched units reported,[6] and in most of these works, only the first-generation dendrimers was obtained, except one example of second-generation dendrimer with seven calixarene moieties.[6e] Therefore, the research on this field is still largely unexplored.

If the 1,3-calix[4]crown, an excellent ionphore could be introduced to the dendrimers as repeat unit, a 'multi-metal recognition central' dendrimer will be obtained. It may be helpful to mimic the ion channel, a very important biological structure. Here, we report the synthesis and conformation of a second generation of dendrimer with 1,3-calix-[4]-benzocrown-6 as repeat unit.

2. Results and discussion

2.1. Synthesis of monomer

In order to obtain a dendrimer with calixcrown as repeat unit, we needed to synthesize a calixcrown monomer with three functional groups in two different types. For a 1,3-calix[4]crown, the positions where functional group could be introduced included the four upper rim carbon atoms and two low rim oxygen atoms. If the monomer was a calixazacrown or calixbenzocrown, the nitrogen atom or the phenyl unit could be another position. However, the upper rim was not a good choice for introducing functional groups, because of much harsh synthetic conditions and low yield. At the same time, a rather large group at the upper rim facing the ether crown ring would hinder the ether crown to recognize the guest.[7] Therefore, a calixbenzocrown **4** (Scheme 1), which had two ester groups at lower rim and one aldehyde group at the phenyl unit, was chosen as the monomer.

There were several advantages for choosing **4** as the monomer: (1) it was easy to synthesize; (2) the ester groups and aldehyde group located at each end of the molecule, which could reduce the steric repulsion to a very low

Keywords: Calix[4]crown; Dendrimer; Convergent approach.
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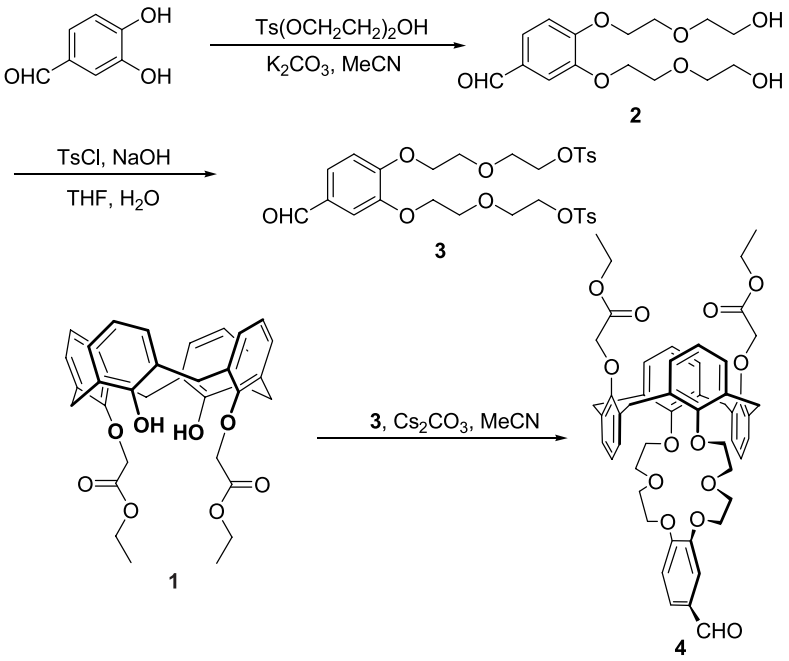
doi:10.1016/j.tet.2004.11.043



Scheme 1.

degree; (3) because the ester group and aldehyde group did not react each other under common conditions, we did not need to use protection and deprotection strategy during the synthesis of dendrimer. On the other hand, they were easy to be converted to carboxyl group and benzyl alcohol group, and then linked together under mild condition with high yield; (4) the three functional groups had little effect on the recognition ability of the ether crown ring.

Monomer **4** could be obtained using the method developed by Reinhoudt et al.,[8] so we needed to synthesize the corresponding diethylene glycol ditosylate **3**, first. This compound could be prepared from 3,4-dihydroxybenzaldehyde through two steps in a fairly good yield (Scheme 1). In the presence of Cs_2CO_3, **3** reacted with 25,27-diethoxycarbonylmethoxycalix[4]arene (**1**) in MeCN under argon affording the monomer **4** in 55% yield. The signal at 37.6 ppm in its ^{13}C NMR spectrum indicated it fixed in 1,3-alternative conformation (Schemes 2–4).[9]

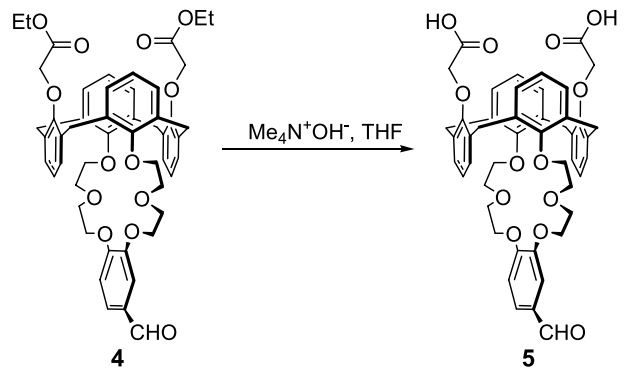
2.2. The 1st generation of dendrimer

Because the ester and aldehyde groups could not react each other, we needed to activate them respectively before preparing 1st generation of dendrimer.

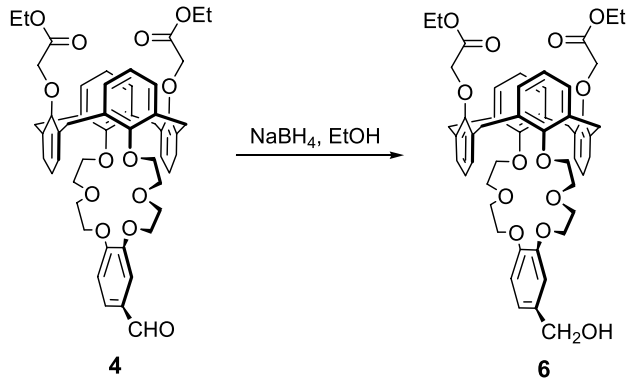
Hydrolysis of **4** using 10% tetramethylammonium hydroxide aqueous solution under room temperature afforded the diacid **5**, in nearly quantitative yield.

Reduction of **4** with $NaBH_4$ under ice bath, the aldehyde group could be converted to benzyl alcohol group in 60% yield. The relatively low yield was contributed to that the ester group at the low rim could be reduced to ethylene glycol under similar condition. To prevent this side reaction, the quantity of $NaBH_4$ was not allowed to exceed 2 equiv of the compound **4**, the reaction temperature must be low, and reaction time must be short. However, under this condition, the reaction could not acquire completion. The exceeding reactant could be retrieved by chromatography.

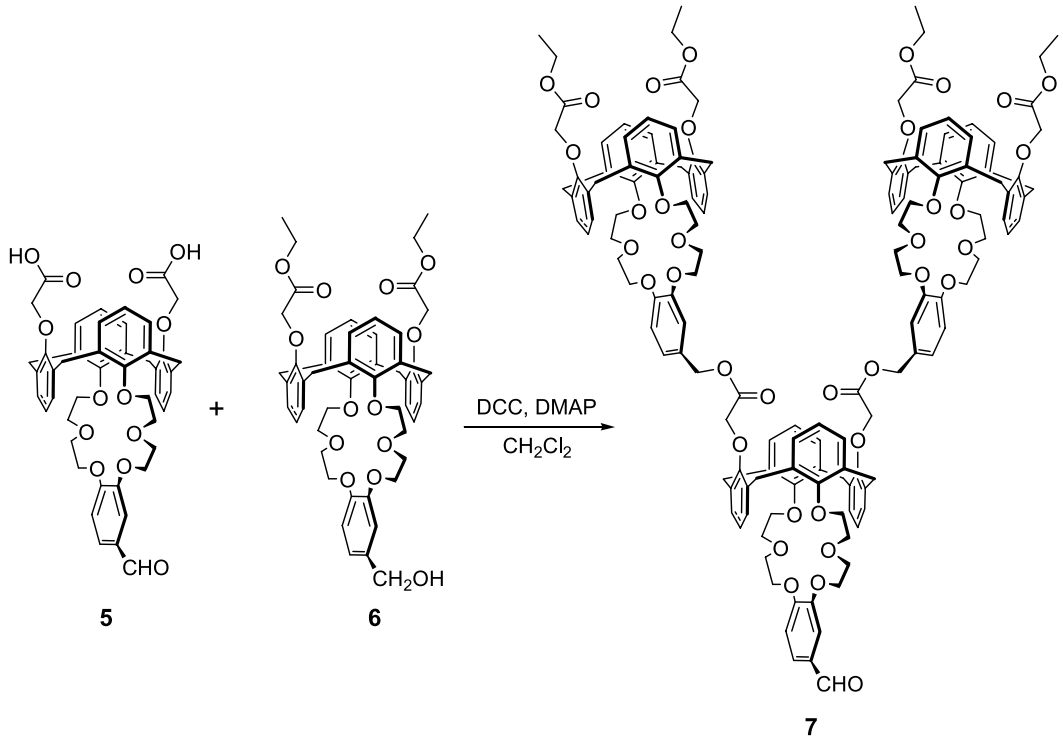
With the two blocks in hand, the 1st generation of dendrimer could be obtained by the condensation of 1 equiv of **5** and 2 equiv of **6** under the treatment of DCC/DMAP in high yields (91%).



Scheme 2.



Scheme 3.



Scheme 4.

The two carbon signals at 37.7 and 37.5 in its ^{13}C NMR spectra indicated that all of the calix[4]crown were fixed in 1,3-alteralternative conformation.[9]

2.3. The 2nd generation of dendrimer

There are two ways to prepare the 2nd generation of dendrimer: the divergent approach and the convergent approach.

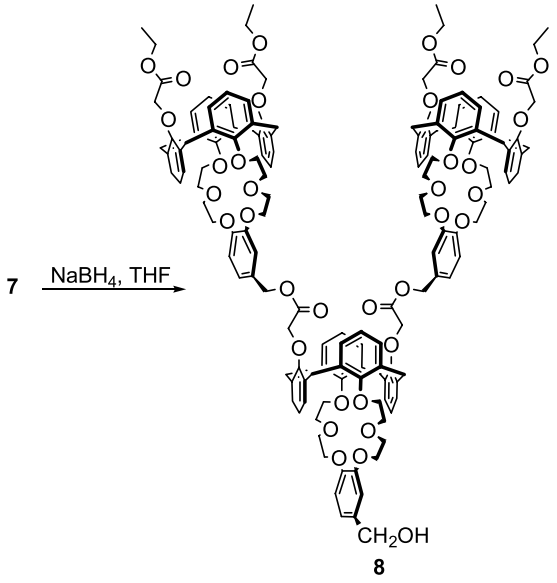
If the convergent approach were chosen, we would face the same problem of over reduction we had encountered in the synthesis of **6**, and the much larger 1st generation dendrimer would cause more serious steric problem during the synthesis of 2nd generation of dendrimer. But if the divergent way were chosen, we would face much more difficult problems: (1) because there were six ester groups, it would be very difficult to selectively saponify the four peripheric ones; (2) even the saponification could acquire success, the total yield would be low since the next condensation step needed the relative low-yield compound **6** as reactant; (3) the divergent way required four reaction positions during the process of the condensation, but the convergent way only need two.

Due to the inherent shortcomings of the divergent way, we chose convergent way for the preparation of the 2nd generation of dendrimer.

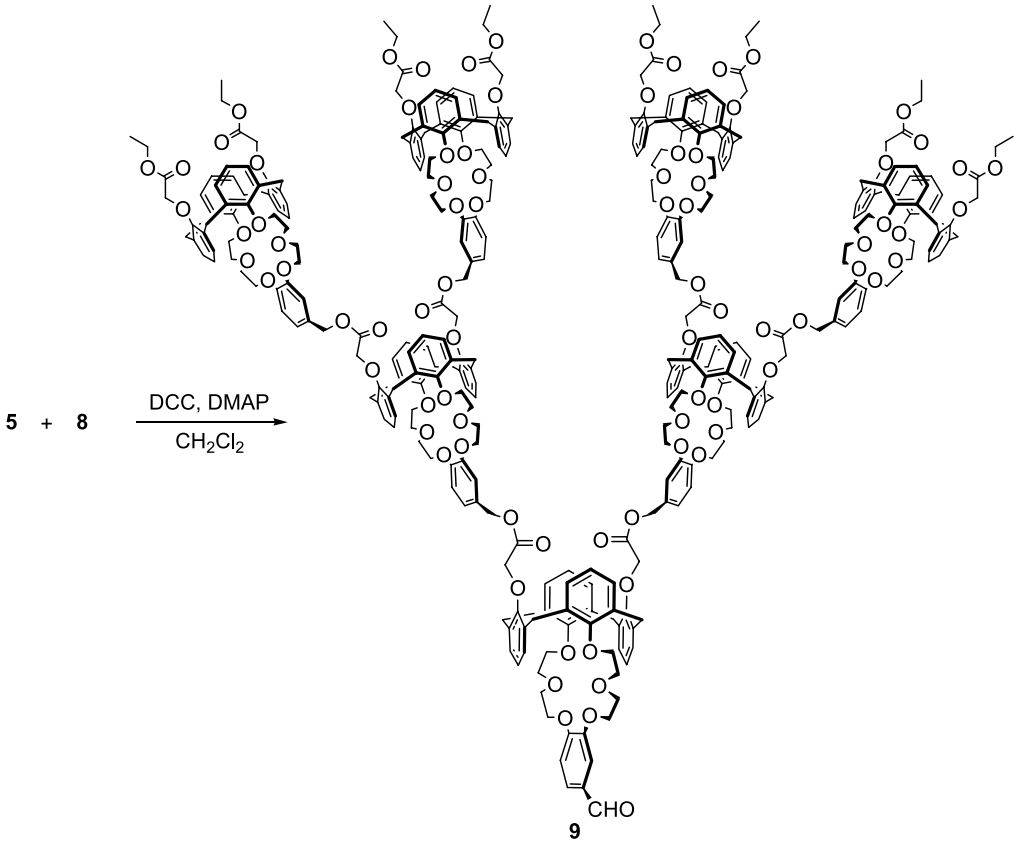
First, we intended to obtain the corresponding benzyl alcohol compound **8** from **7** by the same method in the synthesis of compound **6**, but failed. The reason may be that both **7** and **8** were hardly dissolved in EtOH. So we used THF instead of EtOH as solvent, and the **8** was obtained in a fairly good yield (Scheme 5).

The 2nd generation of dendrimer **9** can be obtained by the condensation of 2 equiv of **8** and 1 equiv of **5** in high yields using the same method for the preparation of 1st generation dendrimer (Scheme 6).

In conclusion, we have synthesized the first 2nd generation of dendrimer based on calixcrown. Its structure and conformation were confirmed by ^{1}H NMR and MALDI-TOF mass spectra. The recognition properties of the dendrimers are under investigation.



Scheme 5.



Scheme 6.

3. Experimental

3.1. General

Melting points were determined on an electrothermal melting point apparatus and uncorrected. ^{1}H and ^{13}C NMR spectra were obtained at 300 and 75 MHz ($CDCl_3$, TMS as internal standard) respectively on a Bruker DMX300 NMR. MALDI-TOF MS was recorded on a Bruker BIFLEXIII mass spectrometer with CCA (2-cyano-4′-hydroxycinnamic acid) as the matrix. Elemental analyses were performed by the Analytical Laboratory of the Institute. IR Spectra were recorded on a JASCO 480 spectrometer. Preparative column chromatography was performed with silica gel (200–300 mesh). Petroleum ether for column chromatography refers to that of 60–90 °C boiling range. MeCN was dried over 4 Å molecular sieve. THF was freshly distilled over Na before used. All other chemicals were reagent grade and were used without further purification. Compound **1** was prepared according to literature procedures.[10]

3.1.1. Compound 2. 3,4-Dihydroxy-benzaldehyde (690 mg, 5 mmol) was dissolved in MeCN (50 mL), then diethylene glycol mono-tosylate (2.6 g, 10 mmol) and potassium carbonate (1.38 g, 10 mmol) were added. The mixture was refluxed under argon for 36 h. After cooling to room temperature, the mixture was filtered, and the solvent was removed under reduced pressure. The crude product was purified on a silica column using petroleum ether/acetone = 2/1. The product was obtained as colorless oil: yield 86%; IR (KBr): 3378, 2755, 1682, 1586 cm^{-1}; ^{1}H NMR: δ 9.84 (s, 1H, ArC*H*O), 7.47–7.41 (m, 2H, Ar*H*), 6.98 (d, 1H, J = 8.1 Hz, Ar*H*), 4.26–4.21 (m, 4H, Ar–OCH_2CH_2O–), 3.97–3.92 (m, 4H, Ar–OCH_2CH_2O–), 3.77–3.75 (m, 4H, –OCH_2CH_2OH), 3.70–3.68 (m, 4H, –OCH_2CH_2OH), 3.52 (s, 2H, CH_2O*H*); ^{13}C NMR: δ 190.8 (Ar*C*HO), 153.8, 148.8, 130.2, 126.9, 111.7, 110.6 (Ar*C*), 72.8, 72.7, 69.0, 68.8, 68.5, 68.4, 61.6 (–O*C*H_2*C*H_2O–); MS (EI): 314 (27), 164 (32), 89 (23), 45 (100). Anal. Calcd for $C_{15}H_{22}O_7 \cdot 0.25H_2O$ C, 56.51; H, 7.11. Found, C, 56.66; H, 7.06.

3.1.2. Compound 3. Compound **2** (950 mg, 3 mmol) was dissolved in THF (15 mL), and a solution of NaOH (360 mg, 9 mmol) in H_2O (10 mL) was added. The mixture was cooled to 0 °C, then a solution of *p*-toluenesulfonyl chloride (1.2 g, 6.3 mmol) in THF (15 mL) was added dropwise during a period of 2 h. The mixture was stirred under ice bath for another 2 h, and then 1 mol L^{-1} of HCl was added until the pH value of the solution reach to 3. THF was removed under reduced pressure, and the residue was extracted with CH_2Cl_2 (30 mL × 2). The combined organic phase was dried with Na_2SO_4. Then purified on silica column, using ether/acetone = 3/1 as eluant to afford a colorless oil: yield 75%; IR (KBr): 3559, 1686, 1593, 1509, 1437, 1354, 1270 cm^{-1}; ^{1}H NMR: δ 9.84 (s, 1H, ArC*H*O), 7.78 (d, 4H, J = 8.1 Hz, Ar*H*), 7.46 (d, 1H, J = 8.2 Hz, Ar*H*), 7.38 (s, 1H, Ar*H*), 7.30 (d, 4H, J = 8.1 Hz, Ar*H*), 6.97 (d, 1H, J = 8.2 Hz, Ar*H*), 4.19–4.10 (m, 8H, Ar–OCH_2CH_2O–), 3.86–3.75 (m, 8H, –OCH_2CH_2O–), 2.41 (s, 6H, ArCH_3); ^{13}C NMR: δ 190.8 (Ar*C*HO), 154.1, 149.0, 144.8, 132.9, 130.3, 129.8, 127.9, 126.8, 112.4, 111.5 (Ar*C*), 69.6, 69.5,

69.3, 69.0, 68.9, 68.6 (–O*C*H$_2$*C*H$_2$O–), 21.6 (Ar*C*H$_3$); MS (MALDI-TOF): 622.9 (M+H$^+$), 644.9 (M+Na$^+$), 660.8 (M+K$^+$). Anal. Calcd for: $C_{29}H_{34}O_{11}S_2$ C, 55.94; H, 5.50. Found, C, 55.83; H 5.72.

3.1.3. Compound 4. 25,27-Diethoxycarbonylmethoxycalix[4]arene (**1**) (596 mg, 1 mmol) was dissolved in MeCN (250 mL), and **3** (653 mg, 1.05 mmol), cesium carbonate (652 mg, 2 mmol) was added under argon. The mixture was refluxed for 30 h, and then cooled to room temperature. The solvent was removed under reduced pressure, and the residue was dissolved in CH_2Cl_2 (30 mL) and 1 mol L^{-1} HCl (30 mL). The organic layer was separated, and washed with water (30 mL×2), dried with Na_2SO_4. Then the crude product was purified on silica column, using ether/acetone=5/1 as eluant to afford a white solid: yield 55%; mp 65–67 °C; IR (KBr): 2730, 1752 cm^{-1}; ^{1}H NMR: δ 9.88 (s, 1H, ArC*H*O), 7.53–7.51 (m, 2H, Ar*H*), 7.15–7.09 (m, 9H, Ar*H*), 6.80 (t, 2H, J=7.5 Hz, Ar*H*), 6.68 (t, 2H, J=7.5 Hz, Ar*H*), 4.26–4.21 (m, 4H, Ar–O–C*H*$_2$CH$_2$–), 4.09 (q, 4H, J=7.2 Hz, CH$_3$C*H*$_2$O–), 4.05 (d, 4H, J=14.1 Hz, ArC*H*$_2$Ar), 3.87–3.80 (m, 6H, Ar–OCH$_2$-C*H*$_2$O–, ArC*H*$_2$Ar), 3.74–3.67 (m, 6H, –OCH$_2$C*H*$_2$O–), 3.60–3.45 (m, 4H, –OCH$_2$C*H*$_2$O–), 3.40 (s, 4H, –OC*H*$_2$–CO–), 1.22 (t, 6H, J=7.2 Hz, C*H*$_3$CH$_2$–); ^{13}C NMR: δ 190.8 (Ar*C*HO), 170.0 (–O*C*O–), 156.8, 155.3, 154.4, 149.2, 134.5, 133.9, 133.8, 130.6, 130.5, 130.4, 127.0, 123.1, 122.5, 112.9, 112.4 (Ar*C*), 70.4, 70.3, 70.2, 70.0, 69.9, 69.4, 69.3, 68.4, 60.3 (–O*C*H$_2$*C*H$_2$O–, –O*C*H$_2$CO–, –*C*H$_2$CH$_3$), 37.6 (Ar*C*H$_2$Ar), 14.1 (–CH$_2$*C*H$_3$); MS (MALDI-TOF): 897.5 (M+Na$^+$), 913.5 (M+K$^+$). Anal. Calcd for: $C_{51}H_{54}O_{13}$ C, 70.01; H, 6.22. Found, C, 69.61; H, 6.19.

3.1.4. Compound 5. Compound **4** (175 mg, 0.2 mmol) was dissolved in THF (5 mL), and then 1 mL of 10% tetramethylammonium hydroxide was added. The mixture was stirred under argon for 4 h, then acidified with 1 mol L^{-1} HCl until white precipitation was occurred, filtered to afford white solid, yield 95%; mp 211–213 °C; IR (KBr): 3420, 1778, 1758, 1732 cm^{-1}; ^{1}H NMR: δ 9.89 (s, 1H, ArC*H*O), 7.56–7.50 (m, 2H, Ar*H*), 7.13–7.04 (m, 9H, Ar*H*), 6.90 (t, 2H, J=7.5 Hz, Ar*H*), 6.80 (t, 2H, J=7.5 Hz, Ar*H*), 4.18 (t, 2H, J=4.8 Hz, Ar–O–C*H*$_2$CH$_2$–), 4.14 (t, 2H, J=4.8 Hz, Ar–O–C*H*$_2$CH$_2$–), 4.11 (s, 4H, –OC*H*$_2$–CO–), 3.94 and 3.80 (AB, 8H, J=16.2 Hz, ArC*H*$_2$Ar), 3.70 (t, 2H, J=4.8 Hz, Ar–O–C*H*$_2$CH$_2$–), 3.65–3.57 (m, 6H, Ar–O–CH$_2$C*H*$_2$–), 3.48 (t, 4H, J=5.7 Hz, Ar–O–CH$_2$C*H*$_2$–); ^{13}C NMR: δ 190.9 (Ar*C*HO), 168.4 (–O*C*O–), 156.1, 154.5, 153.4, 149.3, 133.7, 133.6, 130.7, 130.2, 130.1, 129.6, 127.1, 124.4, 113.2, 112.8 (Ar*C*), 70.4, 70.2, 70.1, 70.0, 69.9, 69.8, 67.1 (–O*C*H$_2$*C*H$_2$O–, –O*C*H$_2$CO–), 37.5 (Ar*C*H$_2$Ar); MS (MALDI-TOF): 840.8 (M+Na$^+$), 856.7 (M+K$^+$). Anal. Calcd for: $C_{47}H_{46}O_{13}\cdot H_2O$ C, 67.45; H, 5.78. Found, C, 67.61; H, 5.56.

3.1.5. Compound 6. Compound **4** (175 mg, 0.2 mmol) was suspended in EtOH (5 mL) under ice bath, and then $NaBH_4$ (17 mg, 0.45 mmol) was added. After stirred for 10 min, 5 mL of water was added. The solution was extracted with CH_2Cl_2 (10 mL×2), and the combined organic layer was washed successively with 1 mol L^{-1} HCl (10 mL×2), and water (10 mL×2), dried with Na_2SO_4. The pure product was obtained as white solid by chromatography (silica column, petroleum ether/acetone=5/1): yield: 63%; mp 57–59 °C; IR (KBr): 3441, 1753 cm^{-1}; ^{1}H NMR: δ 7.13 (d, 4H, J=7.5 Hz, Ar*H*), 7.09 (d, 4H, J=7.5 Hz, Ar*H*), 7.04 (s, 1H, Ar*H*), 6.97 (s, 2H, Ar*H*), 6.79 (t, 2H, J=7.5 Hz, Ar*H*), 6.70 (t, 2H, J=7.5 Hz, Ar*H*), 4.65 (s, 2H, ArC*H*$_2$OH), 4.17–4.12 (m, 4H, Ar–O–C*H*$_2$CH$_2$–), 4.08 (q, 4H, J=7.2 Hz, CH$_3$C*H*$_2$–), 4.04 (d, 4H, J=15.9 Hz, ArC*H*$_2$Ar), 3.80–3.74 (m, 8H, ArC*H*$_2$Ar, Ar–O–C*H*$_2$CH$_2$–), 3.70 (t, 4H, J=6.0 Hz, Ar–O–CH$_2$C*H*$_2$–), 3.50 (t, 4H, J=6.0 Hz, Ar–O–CH$_2$C*H*$_2$–), 3.35 (s, 4H, –OC*H*$_2$–CO–), 1.21 (t, 3H, J=7.2 Hz, C*H*$_3$CH$_2$–); ^{13}C NMR: δ 170.1 (–O*C*O–), 157.0, 155.3, 149.4, 148.6, 134.9, 134.7, 133.8, 130.6, 130.5, 123.1, 122.7, 120.5, 115.6, 114.3 (Ar*C*), 70.6, 70.5, 70.3, 69.9, 69.7, 68.4, 65.2 (–O*C*H$_2$*C*H$_2$O–, –O*C*H$_2$CO–, Ar*C*H$_2$-OH), 60.3 (–O*C*H$_2$CH$_3$), 37.7 (Ar*C*H$_2$Ar), 14.1 (–OCH$_2$*C*H$_3$); MS (MALDI-TOF): 899.4 (M+Na$^+$), 915.4 (M+K$^+$). Anal. Calcd for: $C_{51}H_{56}O_{13}$ C, 69.85; H, 6.44. Found, C, 69.59; H, 6.50.

3.1.6. Compound 7. Compound **5** (82 mg, 0.1 mmol) and **6** (175 mg, 0.2 mmol) was dissolved in 3 mL of dried CH_2Cl_2, then DCC (42 mg, 0.2 mmol) and DMAP (5 mg, 0.04 mmol) was added under argon. After the mixture was stirred for 12 h, DCU was filtered. The organic layer was washed successively with 1 mol L^{-1} HCl (5 mL×2) and water (10 mL×2), dried with Na_2SO_4. The crude product was purified on a silica column, using petroleum ether/acetone=3/1 as eluant to afford white solid: yield 91%; mp 102–104 °C; IR (KBr): 1755, 1734, 1687 cm^{-1}; ^{1}H NMR: δ 9.88 (s, 1H, ArC*H*O), 7.51 (s, 2H, Ar*H*), 7.12 (d, 8H, J=7.5 Hz, Ar*H*), 7.08–7.06 (m, 17H, Ar*H*), 6.99 (s, 2H, Ar*H*), 6.96 (s, 4H, Ar*H*), 6.79 (t, 4H, J=7.5 Hz, Ar*H*), 6.71–6.61 (m, 8H, Ar*H*), 5.02 (s, 4H, ArC*H*$_2$–O–CO–), 4.23–4.03 (m, 24H, Ar–O–C*H*$_2$CH$_2$–, ArC*H*$_2$Ar, CH$_3$C*H*$_2$–), 3.97 (AB, J=16.6 Hz, 12H, ArC*H*$_2$Ar) 3.84–3.61 (m, 32H, Ar–O–C*H*$_2$CH$_2$–, Ar–O–CH$_2$C*H*$_2$–), 3.59–3.47 (m, 12H, Ar–O–CH$_2$C*H*$_2$–), 3.40 (s, 4H, Ar–O–C*H*$_2$CO–), 3.35 (s, 8H, Ar–O–C*H*$_2$CO–), 1.21 (t, 12H, J=7.2 Hz, C*H*$_3$CH$_2$–); ^{13}C NMR: δ 190.8 (Ar*C*HO), 170.0, 169.8 (–O*C*O–), 156.9, 155.3, 154.4, 149.4, 149.3, 149.1, 134.6, 134.5, 133.8, 130.7, 130.5, 130.4, 129.3, 123.1, 122.6, 122.5, 116.2, 115.2, 112.9, 112.4 (Ar*C*), 70.5, 70.2, 70.1, 69.9, 69.8, 69.7, 69.4, 69.3, 68.5, 68.3 (–O*C*H$_2$*C*H$_2$O–, –O*C*H$_2$CO–), 60.3 (–O*C*H$_2$CH$_3$), 37.7, 37.5 (Ar*C*H$_2$Ar), 14.1 (–OCH$_2$*C*H$_3$); MS: (MALDI-TOF): 2557.1 (M+Na$^+$). Anal. Calcd for: $C_{149}H_{154}O_{37}$ C, 70.55; H, 6.12. Found, C, 70.67; H, 6.64.

3.1.7. Compound 8. Compound **7** (126 mg, 0.05 mmol) was suspended in 5 mL of THF under ice bath, and then $NaBH_4$ (4 mg, 0.10 mmol) was added. After stirred for 10 min, 5 mL of water was added. The solution was extracted with CH_2Cl_2 (10 mL×2), and the combined organic layer was washed successively with 1 mol L^{-1} HCl (10 mL×2), then water (10 mL×2), dried with Na_2SO_4. The pure product was obtained as white solid by chromatography (silica column, petroleum ether/acetone=5/1): yield: 60%; mp 105–107 °C; IR (KBr): 3441, 1753, 1734 cm^{-1}; ^{1}H NMR: δ 7.12 (d, 8H, J=7.5 Hz, Ar*H*), 7.07 (d, 16H, J=7.5 Hz, Ar*H*), 7.04–6.92 (m, 9H, Ar*H*) 6.79 (t, 4H, J=7.5 Hz, Ar*H*), 6.69 (t, 6H, J=7.5 Hz, Ar*H*), 6.30 (t, 2H, J=7.5 Hz, Ar*H*), 5.01 (s, 4H, Ar–O–C*H*$_2$CO–), 4.65 (s, 2H Ar–O–C*H*$_2$CO–), 4.20–4.12 (m, 12H, Ar–C*H*$_2$–Ar, Ar–O–C*H*$_2$–), 4.08 (q, 8H,

$J=7.2$ Hz, CH_3CH_2–), 4.09–4.05 (m, 4H, Ar–CH_2–Ar), 3.98 (d, 8H, $J=15.2$ Hz, Ar–CH_2–Ar), 3.84–3.61 (m, 36H, Ar–CH_2–Ar, Ar–O–CH_2CH_2–, Ar–O–CH_2CH_2–), 3.59–3.43 (m, 12H, Ar–O–CH_2CH_2–), 3.35 (s, 8H, Ar–OCH_2CO–), 3.33 (s, 4H, Ar–OCH_2CO–), 1.21 (t, 12H, $J=7.2$ Hz, CH_3CH_2–); ^{13}C NMR: δ 170.1, 170.0 (–O*C*O–), 157.0, 155.3, 155.2, 149.4, 149.2, 148.6, 134.7, 134.6, 133.9, 133.8, 130.7, 130.6, 130.5, 129.4, 129.3, 123.2, 122.7, 116.2, 115.5, 115.3 (Ar*C*), 70.5, 70.3, 70.0, 69.9, 68.4, 66.0, 65.2 (–O*C*H_2*C*H_2O–, –O*C*H_2CO–, Ar*C*H_2OH), 60.3 (–O*C*H_2*C*H_3), 37.7 (Ar*C*H_2Ar), 14.1 (–OCH$_2$*C*H_3); MS (MALDI-TOF): 2560.1 ($M+Na^+$), 2576.1 ($M+K^+$). Anal. Calcd for: $C_{149}H_{156}O_{37}$ C, 70.49; H, 6.19. Found, C, 70.69; H, 6.56.

3.1.8. Compound 9. Compound **5** (8 mg, 0.01 mmol) and **8** (51 mg, 0.02 mmol) was treated in the same way as described in the synthesis of **7**: yield: 88%; mp 115–117 °C; IR (KBr): 1755, 1734 cm^{-1}; ^{1}H NMR: δ 9.88 (s, 1H, ArC*H*O), 7.52–7.50 (m, 2H, Ar*H*), 7.13–7.06 (m, 56H, ArH), 6.99–6.96 (m, 19H, ArH), 6.78 (t, $J=7.4$ Hz, 10H, ArH), 6.69–6.58 (m, 18H, ArH), 5.00 (s, 12H, Ar–CH_2–O–), 4.30–3.69 (m, 152H, Ar–CH_2–Ar, Ar–O–CH_2–CH_2–), 3.59–3.42 (m, 32H, 3.35 (s, 28H, ArO–CH_2–CO–), 1.21 (t, $J=7.2$ Hz, 24H, CH_3CH_2–); MS: (MALDI-TOF): 5879.1 ($M+Na^+$). Anal. Calcd for: $C_{345}H_{354}O_{85}$ C, 70.71; H, 6.09. Found, C, 70.41; H, 6.51.

Acknowledgements

We thank the National Natural Science Foundation of China, the Major State Basic Research Development Program of China (Grant No. G2000078100) and the Chinese Academy of Sciences for financial support.

References and notes

1. For reviews about dendrimers, see: (a) Newkome, G. R.; Moorefield, C. N.; Vögtle, F. *Dendrimers and Dendrons: Concepts, Syntheses, Applications*, 2nd ed.; Wiley-VCH: New York, 2001. (b) Bosman, A. W.; Janssen, H. M.; Meijer, E. W. *Chem. Rev.* **1999**, *99*, 1665–1688. (c) Fischer, M.; Vögtle, F. *Angew. Chem. Int. Ed.* **1999**, *38*, 884–905. (d) Matthews, O. A.; Shipway, A. N.; Stoddart, J. F. *Prog. Polym. Sci.* **1998**, *23*, 1–56. (e) Tomalia, D. A. *Adv. Mater.* **1994**, *6*, 529–539. (f) Zeng, F.; Zimmerman, S. C. *Chem. Rev.* **1997**, *97*, 1681–1712.
2. (a) Buhleier, E.; Wehner, W.; Vögtle, F. *Synthesis* **1978**, 155–158. (b) Tomalia, D. A.; Baker, H.; Dewald, J. R.; Hall, M.; Kallos, G.; Martin, S.; Roeck, J.; Ryder, J.; Smith, P. *Polym. J.* **1985**, *17*, 117–132. (c) Newkome, G. R.; Yao, Z.-Q.; Baker, G. R.; Gupta, V. K. *J. Org. Chem.* **1985**, *50*, 2003–2004.
3. (a) Fréchet, J. M. J.; Jiang, Y.; Hawker, C. J.; Philippides, A. E. *Proc. IUPAC Int. Symp., Macromol. (Seoul)* **1989**, 19–20. (b) Hawker, C. J.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **1990**, *112*, 7638–7647. (c) Hawker, C.; Fréchet, J. M. J. *Chem. Commun.* **1990**, 1010–1013.
4. Gutsche, C. D. Calixarene Revisited. In *Monographs in Supramolecular Chemistry*; Stoddart, J. F., Ed.; Royal Society of Chemistry: London, 1998.
5. (a) Ferguson, G.; Gallagher, J. F.; McKervery, M. A.; Madigan, E. *J. Chem. Soc. Perkin Trans. 1* **1996**, 599–602. (b) Nagasaki, T.; Tamagaki, S.; Ogino, K. *Chem. Lett.* **1997**, 717–718. (c) Yamakawa, Y.; Ueda, M.; Nagahata, R.; Takeuchi, K.; Asai, M. *J. Chem. Soc., Perkin Trans. 1* **1998**, 4135–4139. (d) Roy, R.; Kim, J. M. *Angew. Chem., Int. Ed.* **1999**, *38*, 369–372.
6. (a) Mogck, O.; Parzuchowski, P.; Nissinen, M.; Bohmer, V.; Rokicki, G.; Rissanen, K. *Tetrahedron* **1998**, *54*, 10053–10068. (b) Wang, J. S.; Gutsche, C. D. *J. Org. Chem.* **2002**, *67*, 4423–4429. (c) Budka, J.; Dudi, M.; Lhothk, P.; Stibor, I. *Tetrahedron* **1999**, *55*, 12647–12654. (d) Liu, J.-M.; Zheng, Y.-S.; Zheng, Q.-Y.; Xie, J.; Wang, M.-X.; Huang, Z.-T. *Tetrahedron* **2002**, *58*, 3729–3736. (e) Szemes, F.; Drewb, M. G. B.; Beer, P. D. *Chem. Commun.* **2002**, 1228–1229.
7. Casnati, A.; Pochini, A.; Ungaro, R.; Bocchi, C.; Ugozzoli, F.; Egberink, R. J. M.; Struijk, H.; Lugtenberg, R.; de Jong, F.; Reinhoudt, D. N. *Chem. Eur. J.* **1996**, *2*, 436–445.
8. Casnati, A.; Pochini, A.; Ungaro, R.; Ugozzoli, J. F.; Arnaud, F.; Fanni, S.; Schwing, M.-J.; Egberink, R. J. M.; de Jong, F.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **1995**, *117*, 2767–2777.
9. Jaime, C.; de Mendoza, J.; Prados, P.; Nieto, P. M.; Shched, C. *J. Org. Chem.* **1991**, *56*, 3372–3376.
10. Van Loon, J.-D.; Arduini, A.; Coppi, L.; Verboom, W.; Pochini, A.; Ungaro, R.; Harkema, S.; Reinhoudt, D. N. *J. Org. Chem.* **1990**, *55*, 5639–5646.

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Tetrahedron 61 (2005) 903–918

A mild and practical copper catalyzed amination of halothiophenes☆

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Received 23 April 2004; revised 4 November 2004; accepted 5 November 2004

Available online 8 December 2004

Abstract—We have found that *N*,*N*-dimethylethanolamine (deanol) is a useful solvent and ligand for copper catalyzed amination of a variety of unactivated and activated 2- or 3-halothiophenes. Primary amines, acyclic secondary amines, cyclic secondary amines and acyclic secondary amines with 2-hydroxyethyl functionality react with halothiophenes in moderate to excellent yields. The mildly basic conditions utilized are compatible with many functional groups. The amination of halobithiophenes has also been examined. The aminothiophenes produced by this method are important intermediates in a variety of electronic and optoelectronic materials.

1. Introduction

The thiophene ring is often substituted for benzene in many materials and systems so as to deliver some enhanced function, as in the case of pharmaceuticals[1–3] and optoelectronic materials.[4,5] More specifically, and in spite of an often relatively difficult synthesis, aminothiophenes have been widely compared to anilines due to their exceptional electronic properties.[6] However, little in the way of general or straightforward synthetic methodology exists for amine-substituted thiophenes, especially those that are electron-neutral or electron-rich. The methods available for the synthesis of aminothiophenes include these categories: (1) creation of the thiophene ring by conversion of acyclic starting materials;[7–10] (2) reactions of halothiophenes with metal amide, reduction of nitrothiophenes and rearrangement reactions that are limited to the parent aminothiophenes without alkyl substitution;[11,12] (3) alkylation of one aminothiophene to create another;[13] (4) nucleophilic aromatic substitution of halogenated substrates bearing one or more electron withdrawing and activating substituents;[14] (5) substitution reactions of mercaptothiophenes;[15] (6) electrophilic substitution of thienyl cuprates with *N*-alkylhydroxylamines;[16] (7) oxidative intramolecular coupling of amidocuprates[17] and, finally, (8) transition metal mediated amination reactions of halothiophenes.[18–20]

Some of these preparative methods are generally limited by either the availability of starting materials or by the compatibility of sensitive functional groups in the substrates. Multi-step synthesis may often be required to obtain even the starting materials required for thiophene ring building. Since the parent aminothiophenes are themselves generally unavailable and unstable, an alkylation approach to make *N*-substituted aminothiophenes is impractical.[11,21] 2-Methylaminothiophene and 2-isopropylaminothiophene were made by electrophilic amination of cuprates with *N*-alkyl-*O*-(trimethylsilyl) hydroxylamines but this method is not compatible with other base sensitive functionality.[16] Nucleophilic aromatic substitution (S_NAr) is an established and straightforward method, but it is limited to the thiophenes with the appropriate type, number and location of electron deficient-substituents. For example, Prim demonstrated that some weakly activated bromothiophenes such as 5-bromothiophene-2-carboxaldehyde react efficiently with a variety of secondary aliphatic amines in aqueous media by S_NAr reaction and no transition metal catalyst was required.[14] However, 4-bromothiophene-2-carboxaldehyde did not give any amination product with cycloaliphatic amines under the same conditions. Aminothiophenes bearing a secondary amine group have been prepared from 2-mercaptothiophene and the corresponding aliphatic secondary amines.[15] This method is still used to prepare this family of compounds although it 'requires several unattractive steps with respect to yields and substituent variations'.[22] The oxidative intramolecular coupling may be described as a special case of copper mediated amination process, but it often requires sensitive organometallic reagents that are not compatible with some

☆ Presented in part at the 227th ACS National Meeting, Anaheim, CA, United States, March 28, April 1, 2004.

Keywords: Amination; Copper; Catalysis.
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0040–4020/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2004.11.017

functional groups, although in certain cases it has clear-cut advantages for the application to bulky alkylamines and arylamines.[23,24]

Transition metal mediated substitution reactions have been extensively explored in recent years and have been successfully applied to the amination of a range of halothiophenes.[25,26] Watanabe reported the amination of unactivated bromothiophenes with diarylamines under strongly basic conditions with *t*-BuONa and a $Pd(OAc)_2$/P-*t*-Bu_3 catalyst. No aliphatic amines were examined in this report.[18] Rasmussen expanded the use of the same catalyst system to reactions of 3-bromothiophene with primary and secondary aliphatic amines.[27] When primary amines were used under these reaction conditions a mixture of 3-(*N*,*N*-dialkyl) and 3-(*N*-alkyl)-aminothiophenes was obtained in moderate yield. Luker described an amination reaction with a palladium/BINAP catalyst and Cs_2CO_3 base for halothiophenes with a halogen activated by an electron-deficient group. This method failed when applied to non-conjugated activated halothiophenes such as the 2,4 isomer and electron neutral or rich substrates.[28] In a recent systematic report Hartwig showed that unfunctionalized bromothiophenes and other five membered haloheterocycles reacted smoothly with *N*-methylaniline in the presence of $Pd(dba)_2$/P^{t}Bu$_3$.[20] However, the reactions of hexylamine, dibutylamine, piperidine, or morpholine with most 2-halothiophenes afforded 'little or no amination products'. Secondary amines such as morpholine and 4-phenylpiperazine reacted with 3-bromothiophene in moderate to good yields but the reactions of primary amines with 3-bromothiophene under these conditions were less successful. For example, *n*-hexylamine reacted with 3-bromothiophene in only 20–30% yield.

The copper-catalyzed Ullmann condensation is a very attractive approach because of economic considerations for large-scale industrial applications.[29] In some cases, stoichiometric amounts of copper and a polar aprotic solvent such as HMPT were required for this method.[30,31] Padwa recently reported copper-catalyzed amidations of bromine substituted furans and thiophenes in the presence of *N*,*N*′-dimethylethylenediamine as ligand.[19,32] Buchwald disclosed an example of copper catalyzed amination of 3-bromobenzothiophene with primary amines.[33] In contrast, copper catalyzed amination of the readily available electron neutral 2-halo or 3-halo thiophenes still remains unexplored. We have recently described a mild copper catalyzed amination reaction with *N*,*N*-dimethylethanolamine (deanol) as solvent and found that 2-iodo and 2-bromothiophene could be successfully coupled with a variety of primary amines and cyclic secondary amines.[34] Subsequently, deanol was examined as a ligand for copper-catalyzed amidation of vinyl halides, but no accelerating effect was observed in this case.[35] The palladium catalyzed amination of electron rich thiophenes with aliphatic amine are not successful. It has been observed that copper and palladium catalyzed amination processes often provide complementary results.[36] We now disclose the details of the copper-catalyzed amination reactions of 2-halothiophenes in deanol and extend the scope of this method to the amination of 3-bromothiophene and halobithiophenes with a more diverse selection of amines. The method described here provides an alternative and sometimes complementary or superior approach to palladium-mediated aminations.

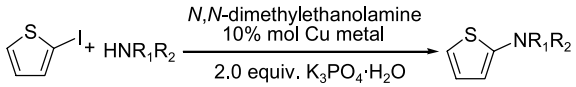
2. Results and discussion

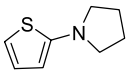
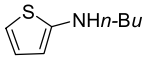
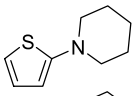
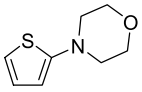
In our initial communication, we reported a mild copper-catalyzed amination reaction of aromatic iodides with unhindered primary amines and cyclic secondary amines by using *N*,*N*-dimethylethanolamine (deanol) as ligand and solvent. While aryl bromides were generally not good amination substrates we found that 2-bromothiophenes to be exceptionally reactive. This observation prompted us to explore further additional copper-mediated reactions of bromothiophenes and amines. We have since investigated the coupling of a larger variety of amines with 2-bromothiophene and we have also found that 3-bromothiophene is a good substrate for this reaction, even with primary amines. This latter finding is in contrast to the case of some palladium-catalyzed reactions of primary amines that have been reported to poison reactions with 2-bromothiophenes and are also not very successful for 3-bromothiophenes.[20] We have also found that reaction of acyclic secondary amines with bromothiophenes are only moderately successful but acyclic secondary amines with β-hydroxyl functionality are exceptionally reactive towards bromothiophenes and give very good amination yields.

The amination reaction conditions described here were modified somewhat since our initial report.[34] Copper metal was originally used as the catalyst for 2-iodothiophenes and copper metal alone has proven very successful for iodide substrates. However, in the case of bromothiophenes a mixture of copper metal and CuI (1:1 to 3:1) is employed as an induction period is observed when copper metal alone is used. The reaction temperatures and reaction times have been chosen depending on the reaction progress monitored by GC and TLC.

The amination reactions of 2-iodothiophene with a range of amines are found in Table 1. The reactions of 2-iodothiophene with both primary amines and cyclic secondary amines proceed smoothly. Compared to iodobenzene, 2-iodothiophene is more reactive and shorter reaction times and lower temperatures are required. The coupling products between primary amine 2-iodothiophene are somewhat unstable. Since *N*-monoalkylaminothiophenes are fairly unstable to oxidation and acidic conditions due to tautomerization,[11,37,38] we have employed Kugelrohr distillation to purify these products. Nevertheless, once purified they rapidly turn red upon exposure to air and/or light. The reason for some of the relatively low yields here is probably due at least in part to this poor stability and the related mechanical losses during workup and purification. We have found that 2-pyrrolidinothiophene is unstable on silica gel unless the column is pretreated with 5% triethylamine in hexane to neutralize the most active acidic sites. However, 2-pyrrolidinothiophene is easily purified by vacuum distillation. Thiophene derivatives with six-membered ring amines are generally more stable than analogues containing five-membered ring amines and primary amines.

Table 1. Copper metal catalyzed amination of 2-iodothiophene



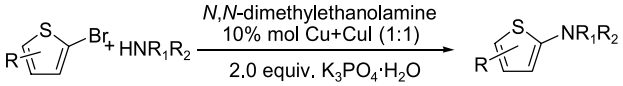
Entry	Amine	Product	Temperature/time (°C/h)	Yield (%)[a]
1			60/10	91
2	$H_2Nn\text{-}Bu$		60/11	62
3			70/10	87
4			75/24	62

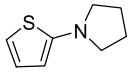
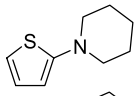
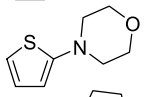
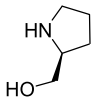
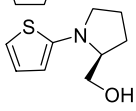
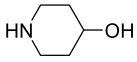
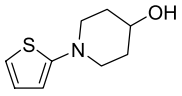
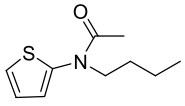
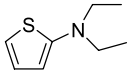
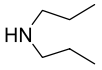
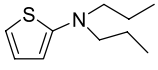
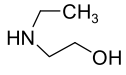
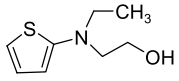
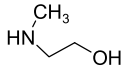
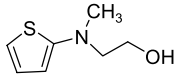
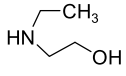
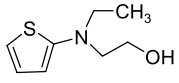
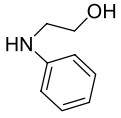
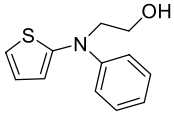
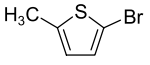
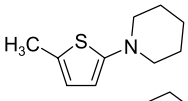
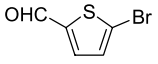
[a] Isolated yield, with 10 mmol 2-iodothiophene and 1.5 equiv of amine.

Since 2-bromothiophene is more accessible than 2-iodothiophene, and encouraged by our early results reacting this bromide with pyrrolidine, we have extended this amination reaction to other amines (Table 2). Cyclic six-member ring amines also afforded very good yields of amination products (Table 2, entries 2 and 3). Cyclic amines with hydroxyl functionality were also examined. The reactivity of (*S*)-(+)-pyrrolidinemethanol and 4-hydroxypiperidine are quite similar to the amines without additional hydroxyl functionality (Table 2, entries 4 and 5). However, the reaction between 2-bromothiophene and primary amines is not very satisfactory. For example, when *n*-butylamine is coupled with 2-bromothiophene, the reaction mixture turned black quickly and the reaction did not proceed to completion. Longer reaction times, higher catalyst loading and higher reaction temperatures also resulted in incomplete conversion of 2-bromothiophene. The reasons for the difficulty in conversion of 2-bromothiophene are not straightforward to elucidate. Hartwig observed an immediate poisoning effect of hexylamine in the palladium-catalyzed coupling of 2-bromothiophene with *N*-methylaniline while the poisoning effect was not observed for the reaction of bromobenzene with *N*-methylaniline.[20] It appears unlikely here that the primary amine alone would poison the catalyst because primary amines are coupled to 3-bromothiophenes very successfully (Table 3, entries 1–3). In another experiment, the reaction started with 10 mmol of 2-bromothiophene and a 1:1 ratio of pyrrolidine (1.2 equiv) and *n*-butylamine (1.2 equiv) under the typical reaction conditions. Incomplete conversion of 2-bromothiophene was observed (~15% remained) and additional reaction time and catalyst did not help. Interestingly, the ratio of the two amination products was 1:1, the same mole ratio as the starting amines, indicating similar reactivity for the two amines. We assumed that the unstable 2-(*n*-butylamino)thiophene product might interact with the active catalytic species and inhibit the catalytic process. In order to test this hypotheses, 30% mol of 2-(*n*-butylamino)thiophene was added to the reaction of 2-bromothiophene and pyrrolidine before the reaction started. The 2-(*n*-butylamino)thiophene additive almost completely stopped this reaction as less than 5% conversion of 2-pyrrolidinothiophene was observed after 48 h at 80 °C. Here again, additional catalyst and reaction time did not further improve the conversion. These experiments suggest that it is the amination product of 2-bromothiophene or possibly some degradation product of that amine product, rather than the primary amine itself, that inhibits this copper-catalyzed amination reaction. Due to the instability of 2-(*n*-butylamino)thiophene, for characterization purposes we chose to convert it to a more stable amide by reacting with acetic anhydride in pyridine. The stable *N*-butyl-*N*-2-thienylacetamide can be separated easily by flash chromatography on silica gel (Table 2, entry 6).

In the case of halobenzenes, a limitation of this copper-catalyzed deanol method was already observed in its inapplicability to coupling with ordinary acyclic secondary amines.[34] For example, only 2% of *N*,*N*-diethylaniline was isolated from the reaction of iodobenzene with diethylamine with deanol/copper catalyst. It has been reported that the palladium-catalyzed amination of 2-bromothiophene with most secondary aliphatic amines is very challenging. For example, no amination product was obtained from the reaction of dibutylamine with 2-bromothiophene.[20] However, we have found moderate success for the reaction between an acyclic secondary amine with 2-bromothiophene (Table 2, entries 7 and 8). 2-*N*,*N*-Diethylaminothiophene was isolated in 39% yield and 2-*N*,*N*-dipropylaminothiophene was isolated in 35% yield from the respective amines and 2-bromothiophene with a high loading of both catalyst (30–40%) and amine (4 equiv). No advantage was observed for the use of 2-iodothiophene instead of 2-bromothiophene in these reactions as a higher amount of deanol ether byproduct was obtained (~20% yield) using iodothiophene. The main reason for the low yields here was that a slow poisoning effect quite similar to the reaction of primary amine with 2-bromothiophene was also observed in the reaction of diethylamine and dipropylamine with 2-bromothiophene. However, the stability of 2-*N*,*N*-dialkylaminothiophenes appear to be higher than 2-*N*-monoalkylaminothiophenes and so a high catalyst and amine loading could push the reaction to high conversion. As yet another complication, the stability of 2-*N*,*N*-dialkylaminothiophenes on silica gel is far less than

Table 2. Copper metal–cuprous iodide catalyzed amination of 2-bromothiophenes



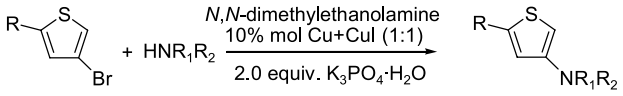
Entry	Thiophene	Amine	Product	Temperature/time (°C/h)	Yield (%)
1				80/50	81[a]
2				80/50	73[a]
3				80/72	65[a]
4				80/50	71[a,b]
5				80/50	64[a]
6		H_2Nn-Bu		Two steps	23[c]
7				80/48	39[d]
8				80/48	35[d]
9				80/72	31[a]
10				80/24	81[e]
11				80/24	81[e]
12				80/48	15[e]
13				80/24	71[a]
14				65/48	77[a]

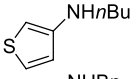
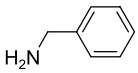
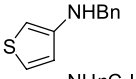
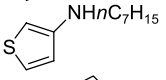
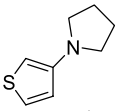
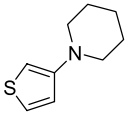
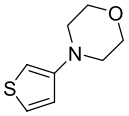
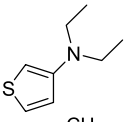
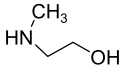
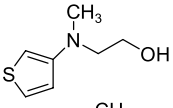
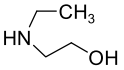
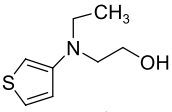
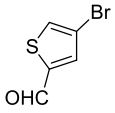
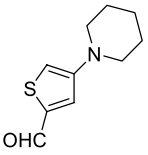
[a] Deanol was used as solvent, 10 or 20 mmol of bromothiophene and 1.5 equiv of amine.
[b] 10 mmol of amine and 15 mmol of 2-bromothiophene.
[c] Copper-catalyzed amination followed by acylation using acetic anhydride and pyridine.
[d] 40% mol of Cu and 4.0 equiv of amine.
[e] 2-Alkylaminoethanol was used as solvent.

2-pyrrolidinothiophene and so they were purified using a neutral alumina column.

In contrast to the ordinary acyclic secondary amines, acyclic secondary amines with 2-hydroxyethyl functionality possess extraordinary reactivity (Table 2, entries 9–12). Such amines are frequently introduced into opto-electronic materials and polymers by nucleophilic aromatic substitution reaction.[39] With our standard method, in which deanol was used as solvent and 2-(*N*-ethylamino)ethanol was used as reactant, a low yield (31%, Table 2, entry 9) was obtained in coupling with 2-bromothiophene. However, when deanol was omitted and the amino alcohol was used as the solvent, the reactions of 2-(*N*-methylamino)ethanol and

Table 3. Copper-catalyzed amination of 3-bromothiophenes

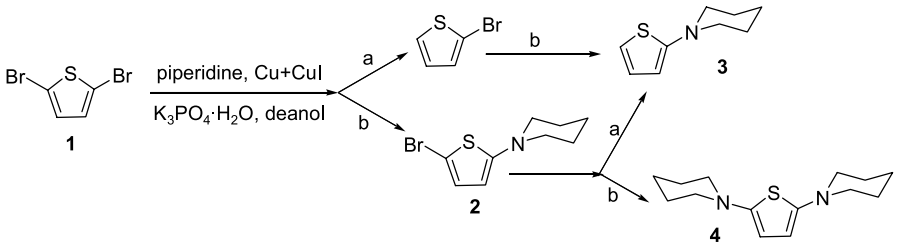


Entry	Thiophene	Amine	Product	Temperature/time (°C/h)	Yield (%)
1		H_2Nn-B*u*		80/36	85[a]
2				80/40	86[a]
3		$H_2NnC_7H_{15}$		80/45	83[a]
4				80/45	83[a]
5				80/40	73[a]
6				80/70	23[a]
7				85/60	15[b]
8				80/24	90[c]
9				80/45	45[c]
10				65–75/70	53[a]

[a] Deanol as solvent, 10 or 20 mmol of bromothiophene and 1.5 equiv of amine.
[b] 35% of Cu and CuI (4:1) and 4 equiv of diethylamine.
[c] Alkylaminoalcohol as solvent.

2-(*N*-ethylamino)ethanol with 2-bromothiophene both afforded an 81% yield of the respective amination product. No reaction was observed between 2-bromothiophene and the aromatic amines aniline, diphenylamine and carbazole under the typical reaction conditions. In contrast, the reaction between 2-anilinoethanol and 2-bromothiophene afforded the amination product in 15% yield when 2-anilinoethanol was used a solvent at 90 °C for 48 h. Attempts to enhance this yield with higher temperature (up to 120 °C), longer reaction time and higher catalyst loading were all unsuccessful. In spite of the low yield, this additional case substantiates the enhanced reactivity of β-aminoethanols in copper-catalyzed aminations.

We have also examined the amination of some other functionalized bromothiophenes. Coupling of 2-bromo-5-methylthiophene with piperidine afforded almost the same amination yield as the reaction of 2-bromothiophene, but the product with methyl substitution is more stable (Table 2, entry 13).[40] The 5-proton may play a role in the instability of 2-piperidinothiophene and the methyl group may inhibit reaction at this site in the tautomer of 2-piperidino-5-methylthiophene. This copper-mediated method in deanol also works well with thiophenes bearing conjugated electron-withdrawing groups. 5-Bromo-2-thiophenecarboxaldehyde was successfully reacted with piperidine at 65 °C and the corresponding 2-piperidinothiophene-5-carboxaldehyde was isolated in 77% yield (Table 2, entry 14). The lower yield here compared to that obtained from the corresponding aromatic nucleophilic substitution reaction may be due to competitive C–Br reduction.[14] The reaction temperature employed here is lower compared to conditions reported for the uncatalyzed aromatic nucleophilic substitution reaction (110 °C).[41] A similar reaction under the same



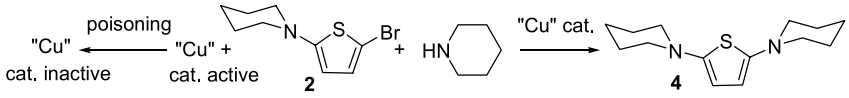
Scheme 1. Amination of 2,5-dibromothiophene (**1**) with piperidine.

conditions but without copper catalyst was also examined. Only 19% yield of amination product was separated and the major byproduct was 2-thiophenecarboxaldehyde accompanied by some decarbonylation product 2-bromothiophene. This dehalogenation is often observed during transition metal-catalyzed coupling or reduction.[42,43] We are still uncertain how the decarbonylation reaction happened here, nevertheless the experiments prove that copper played a catalytic role.

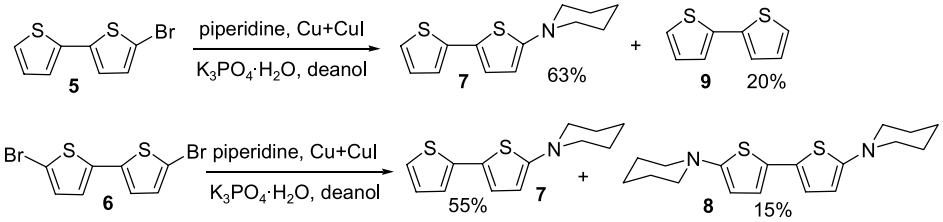
We have also explored the amination of 2,5-dibromothiophene with the intended goal to prepare 2-bromo-5-dialkylaminothiophenes (Scheme 1). However, we found that the reaction of **1** with 1.2–3.0 equiv piperidine stopped within 24 h and most of **1** was recovered. Analysis of the reaction mixture by GC–MS indicates that the concentration of the component assigned as 2-bromo-5-piperidinothiophene was low at all times during the whole reaction process. Significant dehalogenation was also evident in this reaction. For instance, after 48 h reaction between 2,5-dibromothiophene and piperidine (3.0 equiv) at 80 °C, the major products were isolated 2-piperidinothiophene (31%) and 2,5-dipiperidinothiophene (20%) along with a smaller amount of 2-bromothiophene. Although the 2-bromo-5-piperidinothiophene was detected by GC–MS all attempts to isolate a pure sample by silica gel chromatography failed. This result is similar to the reaction of 2-bromothiophene with primary amines wherein the reaction gradually came to a halt prior to completion. The use of less amine (1.2 or 1.5 equiv) resulted in earlier termination of the reaction and more (>50%) of 2,5-dibromothiophene remaining. After aqueous work-up and extraction with diethyl ether, a significant amount of black precipitate was observed in the aqueous layer. This result may be due to the formation of a labile 2-bromo-5-piperidinothiophene (**2**) that would react with the catalytic copper species and precipitate out from the solution. However, and to our surprise, when more piperidine (6 equiv) and copper catalyst (20% mol, Cu/CuI is 3:1) were used, all the 2,5-bromothiophene (**1**) was consumed, the half reduced product 2-piperidinothiophene (**3**) was isolated in 26% yield and 2,5-dipiperidinothiophene (**4**) was now isolated in 46% yield.

Since intermediate **2** is present as a low concentration species in all cases (with 1.2–6.0 equiv of piperidine and at low or high catalyst loading), we assume that the intermediate **2** is more reactive than other bromothiophenes towards this copper-catalyzed amination reaction to form products of reduction or amination. Scheme 2 describes a competition between the desired catalytic reaction and the 'Cu' poisoning reaction. If the concentration of piperidine and catalyst loading were both low, the amination reaction of intermediate **2** would be slow. In this case, the reaction of intermediate **2** with the active copper catalyst species becomes a significant one and therefore the copper catalytic species would precipitate out from the solution. This process would slow down and eventually stop the catalytic amination reaction; if the piperidine concentration and the catalyst load are increased, intermediate **2** would react with piperidine faster than reacting with copper species towards the poisoning direction, and therefore the whole catalytic reaction moves to the amination product and all the starting material could be converted. However, the poisoning reaction could not be excluded even in the later case because a small amount of brown precipitate was also observed.

The amination of bromobithiophenes was also briefly



Scheme 2. Competition between the catalytic reaction and the 'Cu' poisoning reaction.



Scheme 3. Amination of 5-bromo-2,2-bithiophene (**5**) and 5,5-dibromo-2,2-bithiophene (**6**).

explored as is illustrated in Scheme 3. Reaction of 5-bromo-2,2′-bithiophene (**5**) with piperidine appeared slightly more facile than the reaction of 2-bromothiophene with piperidine, although the yield of 5-piperidino-2,2′-bithiophene (**7**) (63%) was less than the reaction giving 2-piperidinothiophene (**3**, Table 2, entry 2, 73%). It appears that 5-bromo-2,2′-bithiophene (**5**) has a higher tendency to be reduced than 2-bromothiophene as about 20% of 2,2′-bithiophene (**9**) was isolated from this reaction. Although poisoning effects were noticeable for the amination of 2,5-dibromothiophene, no obvious poisoning was observed in the case of 5,5′-dibromo-2,2′-bithiophene (**6**). To our surprise, the major product (54%) from the reaction of 5,5′-dibromo-2,2′-bithiophene (**6**) with piperidine was the half reduced amination product 5-piperidino-2,2′-bithiophene (**7**). The 5,5′-dipiperidino-2,2′-bithiophene (**8**) was isolated in a substantially lower yield (14%) and the doubly reduced product, 2,2′-bithiophene (**9**) was also isolated in 9% yield.

Based on these very preliminary results the copper-deanol method appears to be quite useful for the amination of dihalothiophenes and halooligothiophenes. However, studies with these substrates do point out the role that reduction plays in the diminution of yield of the desired amination products. The use of more amine in these reactions may be beneficial in minimizing the amount of reduction.

In order to expand and elucidate the scope of this mild amination reaction for other electron-neutral and electron-rich thiophenes, we have also performed reactions of 3-bromothiophene with a range of aliphatic amines. In contrast to the unsuccessful or complicated product distributions reported for palladium-catalyzed amination reactions of primary amines with 3-bromothiophene,[20,27] we found that the copper-mediated deanol method is a mild and effective process even for primary amines.

Table 3 shows the copper-catalyzed amination results of 3-bromothiophenes. All the primary amines examined afforded very good yields of monoarylation products in contrast to palladium-catalyzed aminations that also gave diarylation byproducts as well as monoarylation product.[27] The successful amination of 3-bromothiophene with primary amines may be related to the better stability of 3-(*N*-alkylamino)thiophenes compared with 2-(*N*-alkylamino)thiophenes. Unlike 2-(*N*-alkylamino)thiophene, a longer reaction time is less prone to decompose 3-(*N*-alkylamino)thiophenes in the reaction system and no poisoning effect was observed for 3-(*N*-alkylamino)thiophenes. Amines with longer alkyl chains required a longer reaction time (entry 3, Table 3). In addition, the 3-aminothiophenes are fairly stable to air for short periods and the products can be easily purified by flash chromatography on silica gel, however, they are not stable for long term storage even at low temperature. The 3-bromothiophene is less reactive to acyclic secondary amines than 2-bromothiophene but the product is more stable and can be isolated by silica gel chromatography. For instance, after 60 h reaction at high catalyst and amine loading, only 15% of 3-*N*,*N*-diethylaminothiophene was isolated. Similar to 2-bromothiophene, most cyclic secondary amines also afforded good yields (Table 3, entries 4–6) and the lack of reactivity of 3-bromothiophenes towards acyclic secondary amines is quite similar to that of 2-bromothiophene. The ordinary acyclic secondary amines are again not reactive under these conditions, but amines with 2-hydroxyethyl functionality also possess significantly enhanced reactivity for 3-bromothiophene. For instance, when the 2-(*N*-methylamino)ethanol and 2-(*N*-ethylamino)ethanol were used as solvent, both successfully coupled with 3-bromothiophene and afforded 90 and 45% yields of the respective amination products (Table 3, entries 8 and 9).

It is particularly interesting to apply this process to the amination of 4-bromo-2-thiophenecarboxaldehyde since this substrate failed to give any amination product by the nucleophilic aromatic substitution method.[14] We have found that the present method does provide a moderate yield of amination product when 4-bromo-2-thiophenecarboxaldehyde reacts with piperidine at 65–75 °C (Table 3, entry 10). The product of reductive debromination (2-thiophenecarboxaldehyde) was isolated as the major byproduct and the decarbonylation product (2-piperidinothiophene) was also observed as another byproduct. It is worth noting that if the reaction was run at higher reaction temperature (>85 °C), the amination product yield was not improved, probably due to the instability of the aminothiophenecarboxaldehyde or the starting aldehyde.

A usually minor but ubiquitous competing side reaction of the copper-catalyzed amination reaction of aryl halides in deanol is aryl ether formation involving the alcohol group of deanol. In our earlier study with aryl halide substrates the aryl ether byproducts were isolated.[34] In the case of the thiophenes studied here no systematic effort was made to isolate and characterize these deanol ether byproducts except those from 2-bromothiophene and 3-bromothiophene (see supporting information). The thiophene ethers from deanol are significantly more polar than the desired amine derivatives and were not isolated by chromatography. The yield of these ether byproducts is estimated at 2–10% depending on the reactivity of the specific halothiophenes and amines. Larger amounts of aryl ethers were isolated when acyclic secondary amines were used as reactants. The more reactive iodothiophene gave less ether byproduct. Another side reaction is the reduction of the halo group in halothiophene. Bithiophenes and thiophenes with electron-withdrawing substitution seem to afford more reduced product. We found that this method could not be extended to a useful synthesis of ether-substituted thiophenes. For example, treatment of 2-iodothiophene with 3 equiv of 1-propanol and 15% mol Cu and CuI (2:1) catalyst for 24 h at 80 °C (standard amination conditions), produced mainly the deanol ether of 2-iodothiophene ($\sim$40% isolated) and only a trace of 2-propoxythiophene was detected.

All the workups and purifications described here were carried out in the air and noticeable color changes of many of these aminothiophenes were observed. Even refrigeration will not prevent the degradation of neat samples of these aminothiophenes, particularly the mono-alkylated aminothiophenes, and so immediate subsequent follow-up reaction or use is suggested.

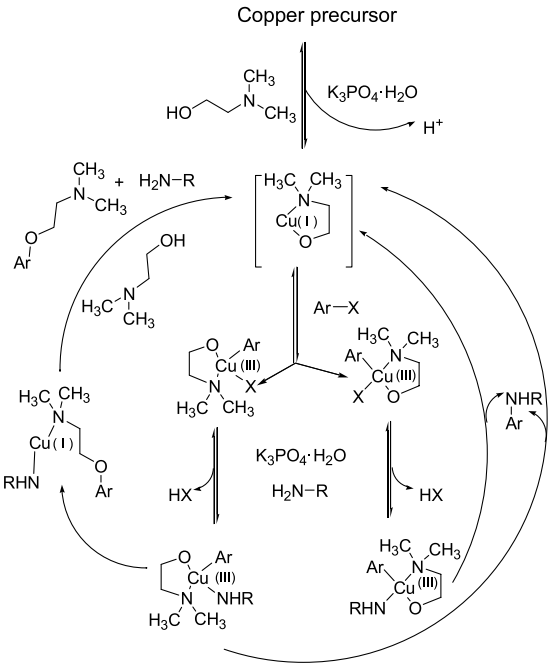


Figure 1. Proposed mechanism for copper-catalyzed amination in deanol.

The proposed mechanism is shown in Figure 1 and uses aromatic halides and a primary amine as an example. The reaction proceeds via a pathway involving copper (III) intermediate(s) similar to the recently developed copper catalyzed coupling reactions of pentavalent bismuth, trimethylsiloxanes, aryboronic acids and arylstannanes.[44–47] In the past, copper (III) intermediates were sometimes described as elusive high-energy species that were unlikely intermediates even though Cu(III) intermediate has often been proposed for copper mediated coupling reactions.[48,49] However, recently more and more copper (III) compounds have been prepared and characterized with X-ray crystallography and spectroscopic methods.[50,51] Reductive elimination of a copper (III) peralkyl intermediate has been proposed for the 1,4-addition in organocuprate reactions.[52] In some cases, oxygen was used to convert a copper (II) intermediate to a copper (III) intermediate to facilitate reductive elimination.[45] The catalytic cycle starts with a Cu(I) deanol complex. The mechanism includes mainly three steps: (a) oxidative addition of ArX occurs first to generate two aromatic and deanol coordinated Cu(III) species; (b) ligand exchange between X and amine; and (c) reductive elimination which lead to the final amination product. Reductive elimination of coordinated deanol and aryl group leads to aryl deanol byproduct.

In conclusion, we have demonstrated that copper-catalyzed amination in *N*,*N*-dimethylethanolamine is a mild and practical method to prepare amine derivatives from a variety of 2 and 3-halothiophenes, 2,5-dihalothiophenes and halobithiophenes. To our knowledge, this is the first report of copper catalyzed reaction of unactivated halothiophenes with aliphatic amines under such mild conditions. Primary, secondary acyclic, and cyclic amines, and acyclic secondary amines with hydroxyethyl functionality, provided moderate to excellent yields of the corresponding aminated thiophenes. The application of this new copper mediated amination in deanol, in combination with other existing methods, provides a means from wide range of aromatic halide to aromatic amine transformation.

3. Experimental

3.1. General methods

Unless otherwise noted, all chemicals were used as received from commercial suppliers. Copper metal (~45 μm powder) and CuI were purchased from ACROS. 5-Bromo-2,2′-bithiophene and 5,5′-dibromo-2,2′-bithiophene were prepared according to literature procedures.[53] ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra were obtained as solutions in $CDCl_3$, and chemical shifts are reported in parts per million (ppm) downfield from internal standard Me_4Si (TMS) for ^{1}H and ^{13}C coupling constants are reported in hertz (Hz). Spectral splitting patterns are designated as: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; comp, complex multiplet; and br, broad. The infrared spectra were recorded neat on Bruker Vector 33 and are reported in wave numbers (cm^{-1}). Melting points were measured on Olympus BH-2 polarized light microscope equipped with a Mettler FP5 temperature controller and FP52 hot stage. Low-resolution mass spectra were obtained on a Thermo Finnigan Polaris Q Ion Trap GC(Trace GC ultra)/MS with Electron Ionization or Chemical Ionization. High-resolution mass spectra were obtained for selected compounds on a Micromass Q-TOF II Mass Spectrometer at the Mass Spectrometry and Proteomics Facility, Ohio State University. Candidate samples for HRMS were chosen on the basis of stability and as representative of the type of amines prepared. The purity of all the isolated compounds was higher than 95% which was examined by TLC, GC and ^{1}H NMR. Copies of ^{1}H and ^{13}C NMR of all new compounds and some of the known compounds are available in supporting materials.

In most cases, aminothiophenes are sensitive to air, light and acid but they are sufficiently stable for manipulation neat or in solution. However, immediate use rather than protracted storage (even at low temperature) is advised. For example, severe decomposition was observed for 3-(*N*-benzylamino)thiophene (Table 3, entry 2) and 3-*N*-(heptylamino)thiophene (Table 3, entry 1) after two months of storage in the freezer (−16 °C). Chromatography columns employed for purification of sensitive compounds by gravity or flash methods were prepared from a slurry of silica gel in hexane/triethylamine (97:3).

3.1.1. 2-Pyrrolidinothiophene.[54] (Table 1, entry 1) 2-Iodothiophene (2.1 g, 10 mmol), pyrrolidine (1.1 g, 15 mmol), Cu metal (~45 μm powder, 64 mg, 1 mmol), $K_3PO_4 \cdot H_2O$ (4.6 g, 20 mmol) and deanol (10 ml) were added to a flask fitted with a magnetic stirbar, a condenser and sealed with a septum. The reaction mixture was stirred at 60 °C for 11 h under nitrogen positive pressure. After the reaction cooled to room temperature, 10 ml of water was added and the mixture was extracted with diethyl ether (3× 100 ml). The combined organic layers were then washed with brine and dried over anhydrous $MgSO_4$. Solvent was removed by rotary evaporation and the residue was purified

by Kugelrohr distillation (75 °C, 0.05 mmHg). The product was obtained as a yellow liquid that turns blue on exposure to air and is unstable on silica gel. (1.40 g, 91% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.83 (dd, J=5.4, 3.9 Hz, 1H), 6.42 (dd, J=5.4, 1.2 Hz, 1H), 5.79 (dd, J=5.4, 1.2 Hz, 1H), 3.32–3.28 (m, 4H), 2.07–2.02 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.97, 126.94, 108.35, 100.08, 51.25, 25.90. GC–MS (CI) 154.1 (M+H, 47), 52.9 (100), 153.1 (62), 152.1 (56), 55 (37), 91 (34), 120.1 (30).

3.1.2. *N*-Butylthiophen-2-amine (Table 1, entry 2). 2-Iodothiophene (2.1 g, 10 mmol), *n*-butylamine (1.1 g, 15 mmol), Cu metal (~45 μm powder, 64 mg, 1 mmol), $K_3PO_4\cdot H_2O$ (4.6 g, 20 mmol) and deanol (10 ml) were added to a flask fitted with a magnetic stirbar, a condenser and sealed with a septum. Air was removed from the reaction system and replaced with nitrogen. The reaction mixture was stirred at 60 °C for 11 h under nitrogen positive pressure. After the reaction cooled to room temperature, 10 ml of water was added and the black mixture was quickly extracted with diethyl ether (3×100 ml). The combined organic layers were then washed with brine and dried over anhydrous $MgSO_4$ for 3 min. Solvent was removed in vacuo, and the residue was purified by Kugelrohr distillation (75 °C, 0.05 mmHg). The product was obtained as a light yellow liquid, but quickly turned red on exposure to air (0.96 g, 62% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.73 (dd, J=3.57, 5.50 Hz, 1H), 6.46 (dd, J=1.38, 5.50 Hz, 1H), 6.02 (dd, J=1.38, 3.57 Hz, 1H), 3.78 (s, br, 1H), 3.13 (t, J=7.0 Hz, 2H), 1.67–1.60 (m, 2H), 1.47–1.42 (m, 2H), 0.97 (t, J=7.3 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.70, 126.39, 110.19, 103.80, 48.14, 31.85, 20.39, 14.04. GC–MS (CI) 156.1 (M+H, 100), 112.1 (65), 155.1 (58), 100.1 (54), 67.1 (15), 157.1 (13).

3.1.3. 2-Piperidinothiophene.[15] (Table 1, entry 3) *From 2-iodothiophene (10 mmol).* 2-Iodothiophene (2.1 g, 10 mmol), piperidine (1.3 g, 15 mmol), Cu metal (~45 μm powder, 64 mg, 1 mmol), $K_3PO_4\cdot H_2O$ (4.6 g, 20 mmol) and deanol (10 ml) were added to a flask fitted with a magnetic stirbar, a condenser and sealed with a septum. The reaction mixture was stirred at 70 °C for 10 h under nitrogen positive pressure. After the reaction cooled to room temperature, 10 ml of water was added and the mixture was extracted with diethyl ether (100 ml, 3×). The combined organic layers were then washed with brine and dried over anhydrous $MgSO_4$. Solvent was removed in vacuo, and the residue was purified by flash chromatography on silica gel eluted with hexane and then hexane/EtOAc (10:1). The product was obtained as a liquid (1.45 g, 87% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.79 (dd, J=3.85, 5.50 Hz, 1H), 6.58 (dd, J=1.38, 5.50 Hz, 1H), 6.11 (dd, J=1.38, 3.85 Hz, 1H), 3.13 (t, J=5.6 Hz, 4H), 1.77–1.69 (m, 4H), 1.61–1.52 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.51, 126.24, 111.86, 105.02, 53.10, 25.59, 23.99. GC–MS (CI) 167.1 (M+H, 87), 168.1 (100), 166.1 (46), 134.1 (24), 169.1 (13), 84.1 (11).

3.1.4. 2-(Morpholino)thiophene.[15] (Table 1, entry 4) *From 2-iodothiophene (10 mmol).* 2-Iodothiophene (2.1 g, 10 mmol), morpholine (1.75 g, 20 mmol), Cu metal (~45 μm powder, 64 mg, 1 mmol), $K_3PO_4\cdot H_2O$ (4.6 g, 20 mmol) and deanol (10 ml) were added to a flask fitted with a magnetic stirbar, a condenser and sealed with a septum. The reaction mixture was stirred at 75 °C for 24 h under nitrogen positive pressure. After the reaction cooled to room temperature, 10 ml of water was added and the mixture was extracted with diethyl ether (3×100 ml). The combined organic layers were washed with brine and dried over anhydrous $MgSO_4$. Solvent was removed in vacuo, and the residue was purified by flash chromatography on silica gel eluted with hexane and then hexane/EtOAc (4:1). The product was obtained as a liquid, 1.04 g, 62% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.80 (dd, J=5.5, 3.9 Hz, 1H), 6.64 (dd, J=5.5, 1.4 Hz, 1H), 6.16 (dd, J=3.9, 1.4 Hz, 1H), 3.86–3.83 (m, 4H), 3.14–3.11 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.5, 126.24, 112.87, 105.76, 66.56, 52.08. GC–MS (CI) 170.01 (M+H, 100), 169.1 (67), 171.7 (15), 168.1 (13).

3.1.5. 2-Pyrrolidinothiophene.[54] (Table 2, entry 1) *From 2-bromothiophene (160 mmol).* 2-Bromothiophene (26.2 g, 160 mmol), pyrrolidine (17.2 g, 240 mmol), Cu metal (~45 μm powder, 1.0 g, 16 mmol), $K_3PO_4\cdot H_2O$ (73 g, 320 mmol) and deanol (160 ml) were added to a flask fitted with a magnetic stirbar, a condenser and sealed with a septum. The reaction mixture was stirred at 80 °C for 50 h under nitrogen positive pressure. After the reaction cooled to room temperature, 100 ml of water was added and the mixture was extracted with diethyl ether (3×300 ml). The combined organic layers were then washed with brine and dried over anhydrous $MgSO_4$. Solvent was removed by rotary evaporation and the residue was purified by distillation under vacuum (67–69 °C, 0.05 mmHg). The product was obtained as a colorless liquid, 20.1 g, 81% yield.

3.1.6. 2-Piperidinothiophene.[15] (Table 2, entry 2) *From 2-bromothiophene (20 mmol).* 2-Bromothiophene (3.3 g, 20 mmol), piperidine (2.6 g, 30 mmol), Cu metal (~45 μm powder, 64 mg, 1 mmol), CuI (190 mg, 1 mmol), $K_3PO_4\cdot H_2O$ (9.2 g, 40 mmol) and deanol (20 ml) were added to a flask with a magnetic stirbar, fitted with a condenser and sealed with a septum. The reaction mixture was stirred at 80 °C for 48 h under nitrogen positive pressure. After the reaction cooled to room temperature 20 ml of water was added and the mixture was extracted with diethyl ether (3×100 ml). The combined organic layers were then washed with brine and dried over anhydrous $MgSO_4$. Solvent was removed in vacuo, and the residue was purified by flash chromatography on silica gel eluted with hexane and then hexane/EtOAc (10:1). The product was obtained as a liquid (2.43 g, 73% yield).

A larger scale reaction with 100 mmol of 2-bromothiophene was also run. After 50 h the reaction was worked up by extraction with diethylether and this ether solution was dried over anhydrous $MgSO_4$. Solvent was removed and the product was further purified by vacuum fractional distillation with a Vigreux column. Bp 71–73 °C, 0.15 mmHg. 12.7 g, 76% yield.

3.1.7. 2-(Morpholino)thiophene.[15] (Table 2, entry 3) *From 2-bromothiophene (10 mmol).* 2-Bromothiophene (3.3 g, 20 mmol), morpholine (3.5 g, 40 mmol), Cu metal (~45 μm powder) (64 mg, 1 mmol), CuI (190 mg,

1 mmol), $K_3PO_4 \cdot H_2O$ (9.2 g, 40 mmol) and deanol (20 ml) were added to a flask with a magnetic stirbar, a condenser and sealed with a septum. The reaction mixture was stirred at 80 °C for 72 h under nitrogen positive pressure. After the reaction cooled to room temperature, 20 ml of water was added and the mixture was extracted with diethyl ether (3× 100 ml). The combined organic layers were then washed with brine and dried over anhydrous $MgSO_4$. Solvent was removed in vacuo, and the residue was purified by flash chromatography on silica gel eluted with hexane and then hexane/EtOAc (4:1). The product was obtained as a liquid (2.18 g, 65% yield).

3.1.8. (*S*)-2-(2-Hydroxymethylpyrrolidino)thiophene (Table 2, entry 4). 2-Bromothiophene (2.45 g, 15 mmol), (*S*)-(+)-pyrrolidinemethanol (1.01 g, 10 mmol), Cu metal (~45 μm powder, 64 mg, 1 mmol), CuI (190 mg, 1 mmol), $K_3PO_4 \cdot H_2O$ (9.2 g, 40 mmol) and deanol (10 ml) were added to a flask with a magnetic stirbar, fitted with a condenser and sealed with a septum. The flask was cooled to −78 °C, air was evacuated and nitrogen was backfilled. The reaction mixture was stirred at 80 °C for 48 h under nitrogen positive pressure. After the reaction cooled to room temperature 20 ml of water was added and the mixture was extracted with diethyl ether (3×100 ml). The combined organic layers were then washed with brine and dried over anhydrous $MgSO_4$. Solvent was removed in vacuo, and the residue was purified by column chromatography on silica gel pretreated with 4% of triethylamine. The product was eluted with hexane/EtOAc (4:1) and was obtained as an oil (1.3 g, 71% % yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.80 (dd, J=3.8, 5.5 Hz, 1H), 6.45 (dd, J=1.38, 5.50 Hz, 1H), 5.94 (dd, J=1.38, 3.85 Hz, 1H), 3.79–3.72 (m, 1H), 3.65–3.58 (m, 2H), 3.53–3.47 (m, 1H), 3.20–3.11 (m, 1H), 2.21 (t, br. J=5.6 Hz, 1H), 2.08–1.97 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.30, 126.74, 109.50, 102.08, 64.93, 63.80, 53.75, 29.20, 24.86. MS (EI) 183.1 (M^+, 32), 152.1 (100), 110.2 (33), 153.1 (18). HRMS (EI) calcd for $C_9H_{14}NOS$: 184.0796 ($M+H^+$), found: 184.0795. IR (neat, cm^{-1}) 3356, 3107, 3067, 2948, 2874, 2833, 1531, 1468, 1367, 1351, 1311, 1280, 1250, 1230, 1180, 1148, 1078, 1033, 1002, 982, 836, 754, 651.

3.1.9. 2-(4-Hydroxypiperidino)thiophene (Table 2, entry 5). 2-Bromothiophene (1.65 g, 10 mmol), 4-hydroxypiperidine (1.35 g, 13 mmol) Cu (32 mg, 0.5 mmol), CuI (100 mg, 0.5 mmol), and deanol (10 ml) were added to a flask with a magnetic stirbar, fitted with a condenser and sealed with a septum. The flask was cooled to −78 °C, air was evacuated and nitrogen was introduced. The reaction mixture was stirred at 80 °C for 72 h under nitrogen positive pressure. After the reaction cooled to room temperature 20 ml of water was added and the mixture was extracted with diethyl ether (3×100 ml). The combined organic layers were then washed with brine and dried over anhydrous $MgSO_4$. Solvent was removed in vacuo, and the residue was purified by column chromatography on silica gel pretreated with 3% of triethylamine and eluted with hexane/EtOAc (3:1). The product was obtained as an oil (1.18 g, 64.5% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.77 (dd, J=3.85, 5.50 Hz, 1H), 6.59 (dd, J=1.38, 5.50 Hz, 1H), 6.12 (dd, J=1.38, 3.85 Hz, 1H), 3.88–3.79 (m, 1H), 3.49–3.42 (m, 2H), 2.98–2.90 (m, 2H), 2.04–1.95 (m, 2H), 1.78–1.69 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.38, 126.35, 112.50, 105.98, 67.40, 49.94, 33.82. GC–MS (EI) 183.1 (M^+, 100), 164.0 (43), 110.0 (37), 182.1 (19), 138.1 (19), 111.0 (15), 184.2 (14), 112.1 (14), 165.1 (10). IR (neat, cm^{-1}) 3344, 3106, 3069, 2943, 2819, 1528, 1450, 1380, 1336, 1258, 1222, 1197, 1142, 1112, 1069, 1055, 1011, 978, 878, 838, 774, 662.

3.1.10. *n*-Butyl-*N*-2-thienylacetamide (Table 2, entry 6). 2-Bromothiophene (3.3 g, 20 mmol), *n*-butylamine (3.0 g, 40 mmol), Cu metal (~45 μm powder, 64 mg, 1 mmol), CuI (190 mg, 1 mmol), $K_3PO_4 \cdot H_2O$ (9.2 g, 40 mmol) and deanol (20 ml) were added to a flask with a magnetic stirbar, fitted with a condenser and sealed with a septum. Air was removed from the reaction system and replaced with nitrogen. The reaction mixture was stirred at 75 °C under nitrogen positive pressure until almost all the 2-bromothiophene was consumed (usually about 24 h). After the reaction cooled to room temperature, 20 ml of water was added and the black mixture was quickly extracted with diethyl ether (3×100 ml). The combined organic layers were then washed with brine and dried over anhydrous $MgSO_4$. Solvent was removed by rotary evaporation and the residue was Kugelrohr distilled (75 °C, 0.05 mmHg). The distillate was quickly added to a premixed mixture of pyridine (31 mmol) and acetic anhydride (30 mmol) and stirred at room temperature for 3 h. EtOAc (200 ml) was added and washed with water (40 ml, 3×). The organic layer was dried over anhydrous $MgSO_4$ and solvent was removed. The residue was further purified by flash chromatography on silica gel, eluted with hexane/ethyl acetate (3:1). The product was obtained as liquid (0.90 g, 23%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.18 (dd, J=1.38, 5.50 Hz, 1H), 6.91 (dd, J=3.57, 5.50 Hz, 1H), 6.77 (dd, J=1.38, 3.57 Hz, 1H), 3.63 (t, J=7.56 Hz, 2H), 1.94 (s, 3H), 1.57–1.47 (m, 2H), 1.36–1.23 (m, 2H), 0.88 (t, J=7.3 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.33, 146.01, 125.90, 125.36, 124.68, 49.86, 29.97, 22.57, 20.02, 13.92. GC–MS (CI) 198.1 (M+H, 100), 156.1 (96), 197.0 (15), 100.1 (14), 155.1 (12), 157.1 (12), 112.1 (12), 199.1 (10), 196.1 (9). HRMS (EI) calcd for $C_{10}H_{15}NOSNa$: 220.0772 (M+Na), found: 220.0768.

3.1.11. 2-*N*,*N*-Diethylaminothiophene.[15] (Table 2, entry 7) 2-Bromothiophene (1.63 g, 10 mmol), diethyamine (2.95 g, 40 mmol), Cu (190 mg, 3 mmol), CuI (100 mg, 0.5 mmol), and $K_3PO_4 \cdot H_2O$ (4.6 g, 20 mmol) and deanol (10 ml) were added to a flask fitted with a magnetic stirbar, a condenser and sealed with a septum. Air was removed and replaced with nitrogen. The reaction mixture was stirred at 80 °C under nitrogen positive pressure for 48 h. After the reaction cooled to room temperature, 150 ml of water was added and the mixture was extracted with diethyl ether (3× 150 ml). The combined organic layers were then washed with brine. Solvent was removed in vacuum, and the residue was added to a short silica gel (pretreated with triethylamine) column and eluted with hexane. A crude product containing about 6% of 2-bromothiophene was obtained by removing the solvent. The crude product was further purified by column chromatography on neutral alumina and the product was obtained as a liquid, 0.60 g, 39% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.77 (dd, J=3.8, 5.5 Hz, 1H), 6.46 (dd, J=1.4, 5.5 Hz, 1H), 5.91 (dd, J=1.4, 3.8 Hz,

1H), 3.28 (q, J=7.1 Hz, 4H), 1.18 (t, J=7.1 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.58, 126.65, 109.48, 102.70, 47.53, 12.33. GC–MS (EI) 155.1 (M^+, 57), 140.0 (100), 112.0 (85), 85.0 (34), 98.0 (29), 54.0 (27), 110.1 (21), 78.0 (19), 97.0 (17). IR (neat, cm^{-1}) 3108, 3070, 2971, 2932, 2870, 1534, 1483, 1462, 1444, 1375, 1359, 1302, 1243, 1198, 1180, 1127, 1077, 1053, 834, 785, 753, 650, 564.

3.1.12. 2-*N*,*N*-Dipropylaminothiophene (Table 2, entry 8). 2-Bromothiophene (1.63 g, 10 mmol), dipropylamine (4.0 g, 40 mmol), Cu (190 mg, 3 mmol), CuI (100 mg, 0.5 mmol), and $K_3PO_4 \cdot H_2O$ (4.6 g, 20 mmol) and deanol (10 ml) were added to a flask fitted with a magnetic stirbar, a condenser and sealed with a septum. Air was removed and replaced with nitrogen. The reaction mixture was stirred at 80 °C under nitrogen positive pressure for 48 h. After the reaction cooled to room temperature, 100 ml of water was added and the mixture was extracted with diethyl ether (3× 150 ml). The combined organic layers were then washed with brine. Solvent was removed in vacuum, and the residue was added to a short silica gel (pretreated with triethylamine) column and eluted with hexane. A crude product containing about 8% of 2-bromothiophene was obtained by removing the solvent. The crude product was further purified by column chromatography on neutral alumina and the product was obtained as a liquid, 0.65 g, 35% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.76 (dd, J=3.8, 5.5 Hz, 1H), 6.41 (dd, J=1.4, 5.5 Hz, 1H), 5.85 (dd, J=1.4, 3.8 Hz, 1H), 3.20–3.15 (m, 4H), 1.71–1.58 (m, 4H), 0.94 (t, J= 7.4 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.26, 126.67, 108.67, 101.66, 56.06, 20.47, 11.67. GC–MS (EI) 183.1 (M^+, 85), 126.2 (100), 154.1 (87), 112.2 (57), 184.1 (32), 85.3 (16), 78.2 (15), 127.1 (10). HRMS (EI) calcd for $C_{10}H_{18}NS$: 184.1160 ($M+H^+$), found: 184.1163. IR (neat, cm^{-1}) 3109, 3070, 2960, 2933, 2872, 1535, 1482, 1466, 1452, 1379, 1368, 1346, 1304, 1271, 1221, 1179, 1133, 1102, 1077, 1053, 960, 946, 882, 837, 749, 688, 649, 580, 571, 562.

3.1.13. 2-[Ethyl(2-thienyl)amino]ethanol (deanol was used as a solvent) (Table 2, entry 9). 2-Bromothiophene (3.3 g, 20 mmol), 2-(ethylamino)ethanol (3.6 g, 40 mmol), Cu metal (~45 μm powder, 64 mg, 1 mmol), CuI (190 mg, 1 mmol), $K_3PO_4 \cdot H_2O$ (9.2 g, 40 mmol) and deanol (20 ml) were added to a flask fitted with a magnetic stirbar, a condenser and sealed with a septum. The reaction mixture was stirred at 80 °C for 72 h under nitrogen positive pressure. After the reaction cooled to room temperature, 20 ml of water was added and the mixture was extracted with diethyl ether (3×100 ml). The combined organic layers were then washed with brine and dried over anhydrous $MgSO_4$. Solvent was removed in vacuo and the residue was purified by flash chromatography on silica gel eluted with hexane/EtOAc (4:1). The product was obtained as a liquid, 1.06 g, 31% yield.

3.1.14. 2-(*N*-Methyl-*N*−2-hydroxyethylamino)thiophene (Table 2, entry 10). 2-Bromothiophene (3.3 g, 20 mmol), 2-(methylamino)ethanol 15 ml (also solvent), Cu metal (−45 μm powder, 64 mg, 1 mmol), CuI (190 mg, 1 mmol), and $K_3PO_4 \cdot H_2O$ (9.2 g, 40 mmol) were added to a flask with a magnetic stirbar, a condenser and sealed with a septum. The reaction mixture was stirred at 80 °C for 24 h under nitrogen positive pressure. After the reaction cooled to room temperature, 20 ml of water was added and the mixture was extracted with diethyl ether (3×150 ml). The combined organic layers were then washed with brine and dried over anhydrous $MgSO_4$. Solvent was removed in vacuo, and the residue was purified by flash chromatography on silica gel eluted with hexane/EtOAc (1:1). The product was obtained as a liquid that easily changed to blue color, 2.54 g, 81% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.77 (dd, J=5.5, 3.8 Hz, 1H), 6.50 (dd, J=5.5, 1.4 Hz, 1H), 5.98 (dd, J=3.8, 1.4 Hz, 1H), 3.78 (t, J=5.6 Hz, 2H), 3.35 (t, J= 5.6 Hz, 2H), 2.95 (s, 3H), 2.42 (s, br, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.05, 126.75, 110.74, 103.65, 59.85, 58.75, 41.64. MS (EI) 157.0 (25), 126.0 (100), 85.0 (14), 110 (11), 898.1 (9). IR (neat, cm^{-1}) 3355, 3109, 3068, 2944, 2875, 2805, 1536, 1483, 1437, 1418, 1356, 1265, 1112, 1073, 1045, 836, 756, 655.

3.1.15. 2-[Ethyl(2-thienyl)amino]ethanol (Table 2, entry 9 and 11). 2-Bromothiophene (3.3 g, 20 mmol), 2-(ethylamino) ethanol 20 ml (also solvent), Cu metal (~45 μm powder, 64 mg, 1 mmol), CuI (190 mg, 1 mmol), and $K_3PO_4 \cdot H_2O$ (9.2 g, 40 mmol) were added to a flask fitted with a magnetic stirbar, a condenser and sealed with a septum. The reaction mixture was stirred at 80 °C for 24 h under nitrogen positive pressure. After the reaction cooled to room temperature, 20 ml of water was added and the mixture was extracted with diethyl ether (3×150 ml). The combined organic layers were then washed with brine and dried over anhydrous $MgSO_4$. Solvent was removed in vacuum, and the residue was purified by flash chromatography on silica gel eluted with hexane/EtOAc (1:1). The product was obtained as a liquid, very easily changed to blue color, 2.77 g, 81% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.76 (dd, J=5.5, 3.6 Hz, 1H), 6.52 (dd, J=5.5, 1.4 Hz, 1H), 6.02 (dd, J=3.6, 1.4 Hz, 1H), 3.74 (t, J=5.6 Hz, 2H), 3.34–3.28 (m, 4H), 1.16 (t, J=7.2 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.40, 126.56, 111.24, 105.18, 59.83, 55.98, 49.82, 12.01. GC–MS (EI) 171.1 (24), 140.0 (100), 112.1 (52), 98 (16), 85 (21), 78 (16). IR (neat, cm^{-1}) 3362, 3108, 3068, 2969, 2931, 2871, 1532, 1482, 1460, 1443, 1373, 1361, 1298, 1244, 1073, 1047, 835, 755, 652.

3.1.16. 2-[Phenyl(2-thienyl)amino]ethanol (Table 2, entry 12). 2-Bromothiophene (3.3 g, 20 mmol), 2-anilinoethanol as solvent 20 ml (also solvent), Cu metal (~45 μm powder, 64 mg, 1 mmol), CuI (190 mg, 1 mmol), and $K_3PO_4 \cdot H_2O$ (9.2 g, 40 mmol) were added to a flask with a magnetic stirbar, a condenser and sealed with a septum. The reaction mixture was stirred at 85 °C for 48 h under nitrogen positive pressure. After the reaction cooled to room temperature, 20 ml of water was added and the mixture was extracted with diethyl ether (3×100 ml). The combined organic layers were then washed with water and brine. Diethylether was removed in vacuo, and the anilinoethanol was removed by Kugelrohr distillation. The residue was purified by column chromatography on silica gel eluted with hexane and ethyl acetate (9:1 to 3:1). The product was obtained as a liquid, 0.65 g, 15% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.28–7.22 (m, 2H), 7.03–6.89 (m, 5H), 6.72–6.70 (dd, J=3.0, 1.2 Hz, 1H), 3.86 (s, 4H), 2.19 (s, br, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.15, 149.11, 129.32, 126.13, 120.84, 120.69, 120.48, 117.04, 60.05, 56.30. GC–MS (EI)

219.0 (M+, 65), 188.1 (100), 91.1 (97), 77.0 (37), 175.0 (21), 189.1 (14), 104.1 (13), 220.1 (11), 65.1 (11), 173.1 (10). IR (neat, cm^{-1}) 3364, 3066, 3037, 2929, 2880, 1596, 1537, 1496, 1460, 1436, 1365, 1339, 1281, 1241, 1208, 1178, 1089, 1034, 843, 748, 690.

3.1.17. 2-Piperidino-5-methylthiophene (Table 2, entry 13). 2-Bromo-5-methylthiophene (0.95 g, 5.4 mmol), piperidine (1.0 g, 12 mmol), Cu (32 mg, 0.5 mmol), CuI (50 mg, 0.25 mmol), $K_3PO_4 \cdot H_2O$ (3.9 g, 15 mmol) and deanol (10 ml) were added to a flask fitted with a magnetic stirbar, a condenser and sealed with a septum. The reaction mixture was stirred at 80 °C for 24 h under nitrogen positive pressure. After the reaction cooled to room temperature, 20 ml of water was added and the mixture was extracted with diethyl ether (3×80 ml). The combined organic layers were then washed with brine. Solvent was removed in vacuo, and the residue was purified by flash chromatography on silica gel eluted with hexane. The product was obtained as a white solid, 0.67 g, 71% yield. Mp 31.2–32.0 °C (hexane). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.41–6.39 (m, 1H), 5.90 (d, J=3.6 Hz, 1H), 3.05 (t, J=5.5 Hz, 4H), 2.36 (d, J=0.8 Hz, 3H), 1.75–1.66 (m, 4H), 1.58–1.50 (m, 2). ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.35, 126.72, 123.46, 105.29, 53.39, 25.64, 24.01, 15.47. GC–MS (EI) 181.1 (M+, 100), 180.1 (75), 97.0 (20), 124.1 (17), 112.1 (15), 182.1 (15), 111.1 (15), 166.1 (14). HRMS (EI) calcd for $C_{10}H_{16}NS$: 182.1003 ($M+H^+$), found: 182.1001. IR (neat, cm^{-1}) 3089, 3033, 2933, 2854, 2823, 1559, 1506, 1444, 1381, 1233, 1195, 1158, 1126, 1015, 886, 862, 840, 832, 758.

3.1.18. 5-(Piperidino)thiophene-2-carboxaldehyde (Table 2, entry 14). 5-Bromo-2-thiophenecarboxaldehyde (0.96 g, 5 mmol), piperidine (1.7 g, 20 mmol), Cu (32 mg, 0.5 mmol), CuI (50 mg, 0.25 mmol), and $K_3PO_4 \cdot H_2O$ (2.3 g, 10 mmol) and deanol (10 ml) were added to a flask fitted with a magnetic stirbar, a condenser and sealed with a septum. Air was removed and replaced with nitrogen. The reaction mixture was stirred at 65 °C under nitrogen positive pressure until the 5-bromo-2-thiophenecarboxaldehyde was consumed (up to 48 h). After the reaction cooled to room temperature, 100 ml of water was added and the mixture was extracted with diethyl ether (3×100 ml). The combined organic layers were then washed with brine. Solvent was removed in vacuum, and the residue was purified by flash chromatography on silica gel eluted with hexane/ethyl acetate (3:1). The product was obtained as orange crystals, 0.75 g, 77% yield. Mp 92.8–93.5 °C (hexane). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.51 (s, 1H), 7.47 (d, J=4.4 Hz, 1H), 6.06 (d, J=4.4 Hz, 1H), 3.34 (t, J=5.5 Hz, 4H), 1.72–1.63 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 180.53, 168.63, 140.45, 126.75, 104.06, 51.07, 25.08, 23.68. GC–MS (EI) 195.1 (M+, 100), 194.1 (85), 138.0 (22), 196.1 (16), 166.2 (12), 154.1 (10), 111.1 (15), 180.1 (10). IR (neat, cm^{-1}) 3050, 3032, 2937, 2855, 2728, 1619, 1536, 1474, 1442, 1380, 1264, 1129, 1121, 1063, 1039, 1011, 890, 857, 763, 752, 742.

3.1.19. 2,5-Dipiperidinothiophene (compound 4). 2,5-Dibromothiophene (2.90 g, 12 mmol), piperidine (6.0 g, 70 mmol), Cu (100 mg, 1.56 mmol), CuI (100 mg, 0.50 mmol), and $K_3PO_4 \cdot H_2O$ (11.0 g, 48 mmol) and deanol (16 ml) were added to a flask fitted with a magnetic stirbar, a condenser and sealed with a septum. Air was removed and replaced with nitrogen. The reaction mixture was stirred at 75–80 °C under nitrogen positive pressure until the 2,5-dibromothiophene was consumed (usually 48 h, additional 50 mg Cu metal may be added if the conversion is not completed). After the reaction cooled to room temperature, 50 ml of water was added and the mixture was extracted with diethyl ether (3×120 ml). The combined organic layers were then washed with brine and dried over anhydrous $MgSO_4$. Solvent was removed in vacuum, and the residue was purified by flash chromatography on silica gel pretreated with 3% triethylamine in hexane. The column was first eluted with hexane and then hexane/ethyl acetate (97:3). 2-Piperidinothiophene was eluented first (0.53 g, 26%). The 2,5-dipiperidinothiophene was obtained as yellow crystals, 1.38 g, 46% yield. Mp 47.8–48.8 °C (hexane). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.86 (s, 2H), 2.98 (t, J=5.6 Hz, 8H), 1.72–1.64 (m, 8H), 1.55–1.49 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 150.52, 105.90, 54.27, 25.83, 24.02. GC–MS (EI) 250.1 (M^+, 100), 180.3 (64), 207.2 (53), 208.2 (47), 181.2 (35), 84.2 (25), 251.2 (16), 194.3 (11). Anal. Calcd $C_{14}H_{22}N_2S_2$: C, 67.15; H, 8.86; N, 11.19; S, 12.81. Found: C, 66.87; H, 9.02; N, 11.27; S, 12.63. HRMS (EI) calcd for $C_{14}H_{22}N_2SNa$: 273.1401 (M+Na), found: 273.1400. IR (neat, cm^{-1}) 2927, 2850, 2818, 1556, 1528, 1463, 1446, 1381, 1324, 1230, 1189, 1126, 1120, 1019, 891, 858, 740, 717, 606.

3.1.20. 5-Piperidino-2,2′-bithiophene (compound 7).[10] 5-Bromo-2,2′-bithiophene (1.25 g, 5 mmol), piperidine (0.65 g, 8 mmol), Cu (32 mg, 0.5 mmol), CuI (50 mg, 0.25 mmol), and $K_3PO_4 \cdot H_2O$ (2.3 g, 10 mmol) and deanol (7 ml) were added to a flask fitted with a magnetic stirbar, a condenser and sealed with a septum. Air was removed and replaced with nitrogen. The reaction mixture was stirred at 80 °C under nitrogen positive pressure until the 5-bromo-2,2′-bithiophene was consumed (36 h). After the reaction cooled to room temperature, 50 ml of water was added and the mixture was extracted with diethyl ether (3×60 ml). The combined organic layers were then washed with brine and dried over anhydrous $MgSO_4$. Solvent was removed in vacuum, and the residue was purified by flash chromatography on silica gel first eluted with hexane and then hexane/ethyl acetate (97:3). The product was obtained as yellow crystals, 0.78 g, 63% yield. Mp 59.8–60.0 °C (hexane). Lit 59–60 °C.[10] ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.10–7.07 (m, 1H), 6.97–6.95 (m, 1H), 6.87 (d, J=3.9 Hz, 1H), 5.98 (d, J=3.9 Hz, 1H), 3.15 (t, J=5.6 Hz, 4H), 1.76–1.68 (m, 4H), 1.61–1.53 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.21, 138.80, 127.74, 123.31, 122.55, 121.51, 105.16, 52.58, 25.38, 23.88. GC–MS (EI) 249.1 (M^+, 100), 179.0 (25), 248.2 (24), 96.0 (24), 207.0 (20), 194.1 (20), 180.0 (19), 250.1 (18), 193.1 (18), 121.0 (16), 69.0 (14). HRMS (EI) calcd for $C_{13}H_{16}NS_2$: 250.0724 ($M+H^+$), found: 250.0708. IR (neat, cm^{-1}) 3104, 3068, 2939, 2853, 2830, 1510, 1485, 1459, 1444, 1380, 1256, 1242, 1219, 1192, 1127, 1067, 1012, 860, 824, 809, 758, 692, 679.

3.1.21. 5,5′-Diperidino-2,2′-bithiophene (compound 8). 5,5′-Dibromo-2,2′-bithiophene (1.62 g, 5 mmol), piperidine (1.62 g, 20 mmol), Cu (32 mg, 0.5 mmol), CuI (50 mg, 0.25 mmol), and $K_3PO_4 \cdot H_2O$ (4.6 g, 20 mmol) and deanol (8 ml) were added to a flask fitted with a magnetic stirbar, a

condenser and sealed with a septum. Air was removed and replaced with nitrogen. The reaction mixture was stirred at 80 °C under nitrogen positive pressure until all the 5,5′-dibromo-2,2′-bithiophene (48 h) was consumed. After the reaction cooled to room temperature, 50 ml of water was added and the mixture was extracted with diethyl ether (3× 150 ml). The combined organic layers were then washed with brine and dried over anhydrous $MgSO_4$. Solvent was removed in vacuum, and the residue was purified by flash chromatography on silica gel first eluted with hexane and then hexane/ethyl acetate (50:1 to 10:1). 5-Piperidino-2,2′-bithiophene was isolated as a major product. It was obtained as yellow crystals, 0.68 g, 54.5% yield. The targeted 5,5′-dipiperidino-2,2′-bithiophene was also isolated as orange crystals. Mp 189.6–190.0 °C (ethyl acetate). 0.24 g, 14.5% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.60 (s, br, 2H), 5.95 (s, br, 2H), 3.11 (s, br, 8H), 1.74–1.67 (m, 8H), 1.59–1.53 (m, 4H). (Note: The ^{1}H NMR resonances of the aromatic protons and the protons on the carbons connected to nitrogen of the piperidine ring were either very broad or sometimes disappeared entirely. The $CDCl_3$ solution turns red and eventually purple. The ^{13}C NMR spectrum was normal). ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.06, 124.90, 120.91, 105.24, 52.76, 25.47, 23.93. MS (ASCI) 333.2 (M+1). Anal. Calcd $C_{18}H_{24}N_2S_2$: C, 65.02; H, 7.27; N, 8.42; S, 19.29. Found: C, 64.66; H, 7.40; N, 8.47; S, 19.33. HRMS (EI) calcd for $C_{18}H_{24}N_2S_2Na$: 355.1279 (M+Na), found: 355.1284. IR (neat, cm^{-1}) 3084, 3023, 2933, 2853, 2819, 1527, 1477, 1459, 1442, 1377, 1297, 1232, 1188, 1120, 1065, 1041, 1013, 892, 858, 822, 753.

3.1.22. 3-*N*-(Butylamino)thiophene (Table 3, entry 1). 2-Bromothiophene (3.3 g, 20 mmol), *n*-butylamine (2.2 g, 30 mmol), Cu (64 mg, 1 mmol), CuI (190 mg, 1 mmol), $K_3PO_4\cdot H_2O$ (9.2 g, 40 mmol) and deanol (30 ml) were added to a flask fitted with a magnetic stirbar, a condenser and sealed with a septum. Air was removed from the reaction system and replaced with nitrogen. The reaction mixture was stirred at 80 °C for 36 h under nitrogen positive pressure. After the reaction cooled to room temperature, 60 ml of water was added and the mixture was extracted with diethyl ether (3×100 ml). The combined organic layers were then washed with brine and dried over anhydrous $MgSO_4$. Solvent was removed in vacuo, and the residue was purified by flash chromatography on silica gel, eluted with hexane/triethylamine (98:2). The product was obtained as a yellow liquid (2.54 g, 84.5% yield) that turns red quickly on exposure to air. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.16 (dd, J=5.2, 3.0 Hz, 1H), 6.62 (dd, J=5.2, 1.7 Hz, 1H), 5.95 (dd, J=3.0, 1.7 Hz, 1H), 3.56 (s, br, 1H), 3.08 (t, J=7.0 Hz, 2H), 1.67–1.57 (m, 2H), 1.50–1.38 (m, 2H), 0.97 (t, J=7.2 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.07, 125.18, 120.07, 95.39, 46.24, 31.90, 20.50, 14.08. MS (EI) (%) 155.1 (M^+, 26), 112.0 (100), 85.0 (27), 50.8 (14), 113.1 (11). IR (neat) 3390, 3101, 2957, 2928, 2861, 1560, 1478, 1425, 1366, 1229, 1191, 1077, 868, 834, 743, 623.

3.1.23. 3-(*N*-Benzylamino)thiophene (Table 3, entry 2). 2-Bromothiophene (3.3 g, 20 mmol), benzylamine (3.2 g, 30 mmol), Cu (64 mg, 1 mmol), CuI (190 mg, 1 mmol), $K_3PO_4\cdot H_2O$ (9.2 g, 40 mmol) and deanol (30 ml) were added to a flask fitted with a magnetic stirbar, a condenser and sealed with a septum. Air was removed from the reaction system and filled with nitrogen. The reaction mixture was stirred at 80 °C for 40 h under nitrogen positive pressure. After the reaction cooled to room temperature, 60 ml of water was added and the mixture was extracted with diethyl ether (3×100 ml). The combined organic layers were then washed with brine and dried over anhydrous $MgSO_4$. Solvent was removed in vacuo, and the residue was purified by flash chromatography on silica gel, eluted with hexane/triethylamine/EtOAc (94:2:4). The product was obtained as a red-brown liquid (3.16 g, 86% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47–7.37 (m, 5H), 7.20 (dd, J=5.2, 3.0 Hz, 1H), 6.68 (dd, J=5.2, 1.7 Hz, 1H), 6.02 (dd, J=3.0, 1.7 Hz, 1H), 4.31 (s, 2H), 4.03 (s, br, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 148.70, 139.50, 128.82, 127.94, 127.53, 125.41, 120.14, 96.26, 50.83. MS (EI) (%) 189.1 (M^+, 57), 91 (100), 188.1 (25), 65.0 (25), 50.7 (17). IR (neat, cm^{-1}) 3384, 3101, 3061, 3028, 2923, 2836, 1558, 1493, 1452, 1424, 1232, 1186, 1076, 1028, 864, 840, 800, 745, 697.

3.1.24. 3-*N*-(Heptylamino)thiophene (Table 3, entry 3). 2-Bromothiophene (1.65 g, 10 mmol), *n*-heptylamine (1.73 g, 15 mmol), Cu (32 mg, 0.5 mmol), CuI (100 mg, 0.5 mmol), $K_3PO_4\cdot H_2O$ (4.6 g, 20 mmol) and deanol (10 ml) were added to a flask fitted with a magnetic stirbar, a condenser and sealed with a septum. Air was removed from the reaction system and replaced with nitrogen. The reaction mixture was stirred at 80 °C for 45 h under nitrogen positive pressure. After the reaction cooled to room temperature, 60 ml of water was added and the mixture was extracted with diethyl ether (3×100 ml). The combined organic layers were then washed with brine and dried over anhydrous $MgSO_4$. Solvent was removed in vacuo, and the residue was purified by flash chromatography on silica gel, eluted with hexane/triethylamine (98:2). The product was obtained as a yellow liquid (1.6 g, 83% yield) that turns red quickly on exposure to air. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.15 (dd, J=5.2, 3.0 Hz, 1H), 6.62 (dd, J=5.2, 1.7 Hz, 1H), 5.96 (dd, J=3.0, 1.7 Hz, 1H), 3.59 (s, br, 1H), 3.08 (t, J=7.0 Hz, 2H), 1.66–1.59 (m, 2H), 1.44–1.29 (m, 8H), 0.92 (t, J=7.0 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.07, 125.18, 120.11, 95.36, 46.58, 32.03, 29.81, 29.36, 27.40, 22.82, 14.30. MS (EI) (%) 197.1 (M^+, 27), 112.0 (100), 85.0 (23), 133.0 (13), 154.1 (8).

3.1.25. 3-(Pyrrolidino)thiophene (Table 3, entry 4).[15] 3-Bromothiophene (1.65 g, 10 mmol), pyrrolidine (1.1 g, 150 mmol), Cu (32 mg, 0.5 mmol), CuI (100 mg, 0.5 mmol), $K_3PO_4\cdot H_2O$ (4.6 g, 20 mmol) and deanol (15 ml) were added to a flask fitted with a magnetic stirbar, a condenser and sealed with a septum. Air was removed from the reaction system and replaced with nitrogen. The reaction mixture was stirred at 80 °C for 48 h under nitrogen positive pressure. After the reaction cooled to room temperature, 60 ml of water was added and the mixture was extracted with diethyl ether (3×100 ml). The combined organic layers were then washed with brine and dried over anhydrous $MgSO_4$. Solvent was removed in vacuo, and the residue was purified by flash chromatography on silica gel, eluted with hexane/ethylacetate (99:1). The product was obtained as a yellow liquid (1.3 g, 85% yield) that turns red on exposure to air. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.22 (dd, J=5.2, 3.0 Hz, 1H), 6.72 (dd, J=3.0, 1.7 Hz, 1H), 5.81 (dd, J=5.2, 1.7 Hz, 1H), 3.28–3.24 (m, 4H), 2.01–1.97 (m, 4H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 149.99, 125.20, 118.66, 93.88, 49.99, 25.47. MS (EI) 153.1 (M^+, 63), 152.1 (100), 97.0 (31), 110.1 (27), 154.0 (11). IR (neat, cm^{-1}) 3106, 2967, 2884, 2823, 1552, 1487, 1460, 1426, 1398, 1356, 1286, 1217, 1171, 1150, 1085, 997, 846, 737, 616.

3.1.26. 3-(Piperidino)thiophene (Table 3, entry 5).[55] 2-Bromothiophene (3.3 g, 20 mmol), piperidine (2.6 g, 30 mmol), Cu (64 mg, 1 mmol), CuI (190 mg, 1 mmol), $K_3PO_4 \cdot H_2O$ (9.2 g, 40 mmol) and deanol (30 ml) were added to a flask with a magnetic stirbar, a condenser and sealed with a septum. Air was removed from the reaction system and replaced with nitrogen. The reaction mixture was stirred at 80 °C for 40 h under nitrogen positive pressure. After the reaction cooled to room temperature, 60 ml of water was added and the mixture was extracted with diethyl ether (3×100 ml). The combined organic layers were then washed with brine and dried over anhydrous $MgSO_4$. Solvent was removed in vacuo, and the residue was purified by flash chromatography on silica gel, eluted with hexane/triethylamine/EtOAc (92:2:6). The product was obtained as a yellow liquid (2.38 g, 73.3% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.22 (dd, J=5.2, 3.0 Hz, 1H), 6.89 (dd, J=5.2, 1.7 Hz, 1H), 6.18 (dd, J=3.0, 1.7 Hz, 1H), 3.07 (t, J=7.0 Hz, 4H), 1.76–1.68 (m, 4H), 1.60–1.52 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.53, 125.13, 120.70, 100.14, 51.93, 25.82, 24.27. MS (EI) (%) 167.1 (M^+, 65), 166.1 (100), 110.0 (36), 138.1 (18), 152.1 (14), 111.1 (13), 168.1 (12). IR (neat) 3112, 2933, 2853, 2802, 1538, 1450, 1421, 1385, 1255, 1216, 1190, 1132, 1088, 959, 882, 866, 837, 799, 749, 677.

3.1.27. 3-(Morpholino)thiophene (Table 3, entry 6).[20] 3-Bromothiophene (1.65 g, 10 mmol), morpholine (1.5 g, 30 mmol), Cu (32 mg, 0.5 mmol), CuI (100 mg, 0.5 mmol), $K_3PO_4 \cdot H_2O$ (4.6 g, 20 mmol) and deanol (15 ml) were added to a flask fitted with a magnetic stirbar, a condenser and sealed with a septum. Air was removed from the reaction system and replaced with nitrogen. The reaction mixture was stirred at 80 °C for 40 h under nitrogen positive pressure. After the reaction cooled to room temperature, 60 ml of water was added and the mixture was extracted with diethyl ether (3×100 ml). The combined organic layers were then washed with brine and dried over anhydrous $MgSO_4$. Solvent was removed in vacuo, and the residue was purified by flash chromatography on silica gel, eluted with hexane/triethylamine/EtOAc (92:2:6). The product was obtained as a yellow liquid (0.4 g, 23% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.26 (dd, J=5.2, 3.0 Hz, 1H), 6.86 (dd, J=5.2, 1.7 Hz, 1H), 6.20 (dd, J=3.0, 1.7 Hz, 1H), 3.84 (t, J=5.0 Hz, 4H), 3.09 (t, J=5.0 Hz 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.59, 125.74, 119.77, 100.58, 66.82, 50.90. MS (EI) (%) 169.1 (M^+, 90), 100.0 (100), 111.0 (94), 154.0 (63), 84 (20), 112.1 (17), 170.1 (10). HRMS (EI) calcd for $C_8H_{12}NOS$: 170.0639 ($M+H^+$), found: 170.0639. IR (neat, cm^{-1}) 3112, 2933, 2853, 2802, 1538, 1450, 1421, 1385, 1255, 1216, 1190, 1132, 1088, 959, 882, 866, 837, 799, 749, 677.

3.1.28. 3-Diethylaminothiophene (Table 3, entry 7).[13] 3-Bromothiophene (1.63 g, 10 mmol), diethylamine (2.95 g, 40 mmol), Cu (190 mg, 3 mmol), CuI (100 mg, 0.5 mmol), and $K_3PO_4 \cdot H_2O$ (4.6 g, 20 mmol) and deanol (10 ml) were added to a flask fitted with a magnetic stirbar, a condenser and sealed with a septum. Air was removed and replaced with nitrogen. The reaction mixture was stirred at 85 °C under nitrogen positive pressure for 60 h. After the reaction cooled to room temperature, 150 ml of water was added and the mixture was extracted with diethyl ether (3× 150 ml). The combined organic layers were then washed with brine. Solvent was removed in vacuum, and the residue was purified by silica gel column and eluted with hexane and hexane: ethyl acetate (50:1). The product was obtained as a liquid, 0.24 g, 15% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ. ^{13}C NMR (75 MHz, $CDCl_3$) δ 7.20 (dd, J=3.0, 5.2 Hz, 1H), 6.77 (dd, J=1.7, 5.2 Hz, 1H), 5.90 (dd, J=1.7, 3.0 Hz, 1H), 3.25 (q, J=7.0 Hz, 4H), 1.13 (t, J=1.13 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 150.12, 124.00, 119.33, 95.95, 45.73, 12.48. GC–MS (EI) 155.1 (M+, 50), 140.1 (100), 112.0 (68), 85.0 (25), 110.0 (25), 97.1 (9). IR (neat, cm^{-1}). 3112, 2969, 2930, 2897, 2870, 1547, 1460, 1447, 1427, 1375, 1362, 1347, 1290, 1255, 1228, 1177, 1137, 1115, 1090, 1076, 1017, 950, 849, 781, 738, 640.

3.1.29. 3-[*N*-Methyl-(*N*-2-hydroxy)amino]thiophene (Table 3, entry 8). 3-Bromothiophene (1.65 g, 10 mmol), 2-(methylamino)ethanol 15 ml (also solvent), Cu metal (−45 μm powder, 32 mg, 0.5 mmol), CuI (100 mg, 0.5 mmol), and $K_3PO_4 \cdot H_2O$ (4.6 g, 20 mmol) were added to a flask with a magnetic stirbar, a condenser and sealed with a septum. The reaction mixture was stirred at 80 °C for 24 h under nitrogen positive pressure. After the reaction cooled to room temperature, 20 ml of water was added and the mixture was extracted with diethyl ether (3×150 ml). The combined organic layers were then washed with brine and dried over anhydrous $MgSO_4$. Solvent was removed in vacuo, and the residue was purified by flash chromatography on silica gel eluted with hexane/EtOAc (1:1). The product was obtained as a liquid, 1.40 g, 90% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.21 (dd, J=5.2, 3.0 Hz, 1H), 6.81 (dd, J=5.2, 1.7 Hz, 1H), 6.02 (dd, J=3.0, 1.7 Hz, 1H), 3.74 (t, J=5.5 Hz, 2H), 3.31 (t, J=5.5 Hz, 2H), 2.87 (s, 3H), 2.36 (s, br, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.26, 125.54, 119.62, 97.67, 59.97, 57.24, 40.06. MS (EI) 157.0 (22), 126.1 (100), 97.2 (14), 93.2 (13), 93.1 (12), 110.1 (12), 65.2 (11). IR (neat, cm^{-1}) 3355, 3110, 2946, 2873, 2797, 1548, 1448, 1431, 1413, 1393, 1261, 1169, 1121, 1086, 1066, 1042, 986, 939, 864, 845, 830, 805, 742, 629.

3.1.30. 3-[*N*-Ethyl-(*N*−2-hydroxy)amino]thiophene (Table 3, entry 9). 3-Bromothiophene (1.65 g, 10 mmol), 2-(ethylamino)ethanol 15 ml (also solvent), Cu metal (−45 μm powder, 32 mg, 0.5 mmol), CuI (100 mg, 0.5 mmol), and $K_3PO_4 \cdot H_2O$ (4.6 g, 20 mmol) were added to a flask with a magnetic stirbar, a condenser and sealed with a septum. The reaction mixture was stirred at 80 °C for 45 h under nitrogen positive pressure. After the reaction cooled to room temperature, 20 ml of water was added and the mixture was extracted with diethyl ether (3×150 ml). The combined organic layers were then washed with brine and dried over anhydrous $MgSO_4$. Solvent was removed in vacuo, and the residue was purified by flash chromatography on silica gel eluted with hexane/EtOAc (1:1). The product was obtained as a yellow liquid (0.75 g, 45% yield) that turns brown quickly on exposure to air. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.20 (dd, J=5.2, 3.0 Hz, 1H), 6.79 (dd, J=5.2,

1.7 Hz, 1H), 5.99 (dd, $J=3.0$, 1.7 Hz, 1H), 3.73 (t, $J=5.5$ Hz, 2H), 3.33–3.27 (m, 4H), 2.33 (s, br, 1H), 1.12 (t, $J=7.0$ Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 150.14, 125.34, 119.71, 97.54, 60.08, 54.23, 47.55, 11.97. IR (neat, cm^{-1}) 3357, 3115, 2968, 2931, 2872, 1547, 1425, 1374, 1257, 1166, 1129, 1072, 1045, 1009, 845, 784, 739, 634. MS (EI) (%) 171.1 (M^+, 19.5), 140.0 (100), 112.0 (53), 50.8 (39.0), 85.1 (23.1) 110.0 (17.5).

3.1.31. 4-(Piperidino)thiophene-2-carboxaldehyde (Table 3, entry 10). 4-Bromo-2-thiophenecarboxaldehyde (0.96 g, 5 mmol), piperidine (1.7 g, 20 mmol), Cu metal (32 mg, 0.5 mmol), CuI (50 mg, 0.25 mmol), and $K_3PO_4 \cdot H_2O$ (2.3 g, 10 mmol) and deanol (10 ml) were added to a flask fitted with a magnetic stirbar, a condenser and sealed with a septum. Air was removed and replaced with nitrogen. The reaction mixture was stirred at 65–75 °C under nitrogen positive pressure until 4-bromo-2-thiophenecarboxaldehyde was consumed (up to 72 h). After the reaction cooled to room temperature, 100 ml of water was added and the mixture was extracted with diethyl ether (3×100 ml). The combined organic layers were then washed with brine. Solvent was removed in vacuo, and the residue was purified by flash chromatography on silica gel eluted with hexane/ethyl acetate (3:1). The product was obtained as a liquid, 0.50 g, 53% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.81 (d, $J=1$, 2 Hz, 1H), 7.51 (d, $J=1.9$ Hz, 1H), 6.62–6.61 (m, 1H), 3.09 (t, $J=5.5$ Hz, 4H), 1.75–1.66 (m, 4H), 1.60–1.52 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 183.17, 154.03, 142.33, 127.79, 110.40, 51.41, 25.56, 23.98. GC–MS (EI) 195.1 (M+, 86), 194.1 (100), 138.1 (30), 139.0 (16), 166.1 (16), 196.1 (12). IR (neat, cm^{-1}) 3320, 3103, 2935, 2852, 2809, 1663, 1547, 1439, 1386, 1365, 1248, 1227, 1177, 1120, 1045, 1028, 976, 885, 861, 831, 803, 728, 667.

Acknowledgements

Z.K.L. thanks Dr. S. D. Huang for support and Kent State University for a University Fellowship. The assistance with some NMR, MS and elemental analysis experiments provided by Dr. M. Gangoda is greatly appreciated.

Supplementary data

Copies of ^{1}H and ^{13}C NMR spectra for all new and some known compounds are provided.

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tet.2004.11.017

References and notes

1. Matsuno, K.; Nakajima, T.; Ichimura, M.; Giese, N. A.; Yu, J. C.; Lokker, N. A.; Ushiki, J.; Ide, S. I.; Oda, S.; Nomoto, Y. *J. Med. Chem.* **2002**, *45*, 4513–4523.
2. Beers, S. A.; Malloy, E. A.; Wu, W.; Wachter, M.; Ansell, J.; Singer, M.; Steber, M.; Barbone, A.; Kirchner, T.; Ritchie, D.; Argentieri, D. *Bioorg. Med. Chem.* **1997**, *5*, 779–786.
3. Fevig, T. L.; Phillips, W. G.; Lau, P. H. *J. Org. Chem.* **2001**, *66*, 2493–2497.
4. Würthner, F.; Yao, S.; Debaerdemaeker, T.; Wortmann, R. *J. Am. Chem. Soc.* **2002**, *124*, 9431–9447.
5. Kiryanov, A. A.; Seed, A. J.; Sampson, P. *Tetrahedron Lett.* **2001**, *42*, 8797–8800.
6. Jen, A. K. Y.; Rao, V. P.; Wong, K. Y.; Drost, K. J. *J. Chem. Soc., Chem. Commun.* **1993**, 90–92.
7. Heyde, C.; Zug, I.; Hartmann, H. *Eur. J. Org. Chem.* **2000**, 3273–3278.
8. Tarasova, O. A.; Klyba, L. V.; Vvedensky, V. Y.; Nedolya, N. A.; Trofimov, B. A.; Brandsma, L.; Verkruijsse, H. D. *Eur. J. Org. Chem.* **1998**, 253–256.
9. Pinto, I. L.; Jarvest, R. L.; Serafinowska, H. T. *Tetrahedron Lett.* **2000**, *41*, 1597–1600.
10. Manuela, M.; Raposo, M.; Kirsch, G. *Heterocycles* **2001**, *55*, 1487–1498.
11. Norris, R. K. Aminothiophenes and their Derivatives in Thiophene and its Derivatives. In Gronowitz, S., Ed.; The Chemistry of Heterocyclic Compounds; Wiley: New York, 1985; Vol. 44, pp 631–799; part 2.
12. Reinecke, M. G.; Adickes, H. W. *J. Am. Chem. Soc.* **1968**, *90*, 511–513.
13. Outurquin, F.; Lerouge, P.; Paulmier, C. *Bull. Soc. Chim. Fr.* **1986**, 259–266.
14. Prim, D.; Kirsch, G. *Tetrahedron* **1999**, *55*, 6511–6526.
15. Hartmann, H.; Scheithauer, S. *J. für Prakt. Chem. (Leipzig)* **1969**, *311*, 827–843.
16. Bernardi, P.; Dembech, P.; Fabbri, G.; Ricci, A.; Seconi, G. *J. Org. Chem.* **1999**, *64*, 641–643.
17. Cane, F.; Brancaleoni, D.; Dembech, P.; Ricci, A.; Seconi, G. *Synthesis* **1997**, 545–548.
18. Watanabe, M.; Yamamoto, T.; Nishiyama, M. *Chem. Commun.* **2000**, 133–134.
19. Crawford, K. R.; Padwa, A. *Tetrahedron Lett.* **2002**, *43*, 7365–7368.
20. Hooper, M. W.; Utsunomiya, M.; Hartwig, J. F. *J. Org. Chem.* **2003**, *68*, 2861–2873.
21. Binder, D.; Habison, G.; Noe, C. R. *Synthesis* **1977**, 255–256.
22. Würthner, F. *Synthesis* **1999**, 2103–2113.
23. Yamamoto, H.; Maruoka, K. *J. Org. Chem.* **1980**, *45*, 2739–2740.
24. Whitesides, G. M.; San Filippo, J. Jr.; Casey, C. P.; Panek, E. J. *J. Am. Chem. Soc.* **1967**, *89*, 5302–5303.
25. Hartwig, J. F. In *Handbook of Organopalladium Chemistry for Organic Synthesis*, 2002; Vol. 1, pp 1051–1096.
26. Muci, A. R.; Buchwald, S. L. In *Cross-Coupling Reactions*; Springer: Berlin, 2002; Vol. 219; pp 131–209.
27. Ogawa, K.; Radke, K. R.; Rothstein, S. D.; Rasmussen, S. C. *J. Org. Chem.* **2001**, *66*, 9067–9070.
28. Luker, T. J.; Beaton, H. G.; Whiting, M.; Mete, A.; Cheshire, D. R. *Tetrahedron Lett.* **2000**, *41*, 7731–7735.
29. For recent reviews, see: (a) Kunz, K.; Scholz, U.; Ganzer, D. *Synlett* **2003**, 2428–2439. (b) Ley, S. V.; Thomas, A. W. *Angew. Chem. Int. Ed.* **2003**, *42*, 5400–5449.
30. Bedworth, P. V.; Cai, Y. M.; Jen, A.; Marder, S. R. *J. Org. Chem.* **1996**, *61*, 2242–2246.
31. King, F. D.; Walton, D. R. M. *J. Chem. Soc., Chem. Commun.* **1974**, 256–257.
32. Padwa, A.; Crawford, K. R.; Rashatasakhon, P.; Rose, M. *J. Org. Chem.* **2003**, *68*, 2609–2617.
33. Kwong, F. Y.; Buchwald, S. L. *Org. Lett.* **2003**, *5*, 793–796.

34. Lu, Z.; Twieg, R. J.; Huang, S. D. *Tetrahedron Lett.* **2003**, *44*, 6289–6292.
35. Jiang, L.; Job, G. E.; Klapars, A.; Buchwald, S. L. *Org. Lett.* **2003**, *5*, 3667–3669.
36. Huang, X. H.; Anderson, K. W.; Zim, D.; Jiang, L.; Klapars, A.; Buchwald, S. L. *J. Am. Chem. Soc.* **2003**, *125*, 6653–6655.
37. Brandsma, L.; Vvedensky, V. Y.; Nedolya, N. A.; Tarasova, O. A.; Trofimov, B. A. *Tetrahedron Lett.* **1998**, *39*, 2433–2436.
38. Eck, D. L.; Stacy, G. W. *J. Heterocycl. Chem.* **1969**, *6*, 147–151.
39. Lupo, D.; Ringsdorf, H.; Schuster, A.; Seitz, M. *J. Am. Chem. Soc.* **1994**, *116*, 10498–10506.
40. Götz, G.; Scheib, S.; Klose, R.; Heinze, J.; Bäuerle, P. *Adv. Funct. Mater.* **2002**, *12*, 723–728.
41. Chun, H.; Moon, I. K.; Shin, D. H.; Song, S.; Kim, N. *J. Mater. Chem.* **2002**, *12*, 858–862.
42. Dapperheld, S.; Feldhues, M.; Litterer, H.; Sistig, F.; Wegener, P. *Synthesis* **1990**, 403–405.
43. Hassan, J.; Lavenot, L.; Gozzi, C.; Lemaire, M. *Tetrahedron Lett.* **1999**, *40*, 857–858.
44. Arnauld, T.; Barton, D. H. R.; Doris, E. *Tetrahedron* **1997**, *53*, 4137–4144.
45. Lam, P. Y. S.; Deudon, S.; Averill, K. M.; Li, R. H.; He, M. Y.; DeShong, P.; Clark, C. G. *J. Am. Chem. Soc.* **2000**, *122*, 7600–7601.
46. Lam, P. Y. S.; Clark, C. G.; Saubern, S.; Adams, J.; Averill, K. M.; Chan, D. M. T.; Combs, A. *Synlett* **2000**, 674–676.
47. Collman, J. P.; Zhong, M.; Zhang, C.; Costanzo, S. *J. Org. Chem.* **2001**, *66*, 7892–7897.
48. Cohen, T.; Cristea, I. *J. Am. Chem. Soc.* **1976**, *98*, 748–753.
49. Lockhart, T. P. *J. Am. Chem. Soc.* **1983**, *105*, 1940–1946.
50. Ribas, X.; Jackson, D. A.; Donnadieu, B.; Mahia, J.; Parella, T.; Xifra, R.; Hedman, B.; Hodgson, K. O.; Llobet, A.; Stack, T. D. P. *Angew. Chem. Int. Ed.* **2002**, *41*, 2991–2994.
51. DuBois, J. L.; Mukherjee, P.; Stack, T. D. P.; Hedman, B.; Solomon, E. I.; Hodgson, K. O. *J. Am. Chem. Soc.* **2000**, *122*, 5775–5787.
52. Woodward, S. *Chem. Soc. Rev.* **2000**, *29*, 393–401.
53. Bäuerle, P.; Würthner, F.; Götz, G.; Effenberger, F. *Synthesis* **1993**, 1099–1103.
54. Ikemoto, N.; Estevez, I.; Nakanishi, K.; Berova, N. *Heterocycles* **1997**, *46*, 489–501.
55. Scheithauer, S.; Hartmann, H.; Mayer, R. *Zeitschrift füer Chemie* **1968**, *8*, 181–183.

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Cyclodextrin assisted enantiomeric recognition of benzo[*de*]isoquinoline-1,3-dione derived amino acids

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Received 20 July 2004; revised 3 November 2004; accepted 5 November 2004

Available online 30 November 2004

Abstract—Spectroscopic evidence for enantiomeric recognition of properly modified amino acids through the cyclodextrin assisted formation of polymer like self-assemblies is presented. The requirements for the formation of these assemblies through aromatic π–π stacking are discussed. It is suggested that this approach can be used for enantiomeric separation of chiral compounds that contain both electron-rich and electron-poor moieties.

1. Introduction

It is now well accepted that marketing only one enantiomer of a chiral drug is crucial for the pharmaceutical industry.[1] There are two commonly used approaches for preparing the enantiomerically pure compounds needed for chiral drug marketing. These approaches include; (a) development of preparation procedures that yield only the desirable enantiomer[2] and (b) using preparation procedures that generate both enantiomers and then purifying the racemic mixture using expensive chiral separation techniques. The first approach is very expensive because it is necessary to use either chiral intermediates or chiral catalysts to obtain the desired enantiomerically pure product, while the second approach, which results in the production of racemates, causes problems with waste management and chiral separations. Therefore, it would be ideal to develop new chemistry that can address both of these problems by applying inexpensive preparation procedures for the production of racemic products, which can then be transferred from a racemic mixture to the desired enantiomerically pure targeted compound.

There are many drugs that have aromatic moieties and are currently marketed as racemic mixtures. These drugs include Prozac, Escitalopram, and Clopidogrel to name a few.[1d] It is also known that cyclodextrins are capable of forming stable complexes with aromatic compounds.[3] Therefore, it is reasonable to propose that cyclodextrins can form diasteriomeric inclusion complexes with racemic aromatic compounds. Based on the knowledge that diasteriomers have different physical properties, it is reasonable to expect that one of the diasteriomeric inclusion complexes will crystallize out from the racemic aqueous cyclodextrin solution more readily.

It has also been well established that stronger enantiomeric recognition can be accomplished if two enantiomers compete to bind to a multi-chiral resolving molecule.[4] The same effect should be present if two enantiomers are competing to bind to a homochiral self-assembly polymer made from one of the enantiomers. There are many nonbonding interactions (such as hydrogen bonding, electrostatic interactions, π–π-aromatic stacking. dipole–dipole, etc.) responsible for the formation of molecular associates.[5] Aromatic π–π stacking interactions are crucial for formation of molecular associates, interactions between substrate and enzymes, biomolecular associates, organic molecule conformation, etc.[6] Furthermore, multi-chirality and multi-point interactions are very important for enantiomeric recognition.[7]

The ideal cyclodextrin assisted molecular associate, which has all of these requirements, is presented in Figure 1. Molecule **M** has four moieties that should be essential for the formation of homochiral polymer-like molecular associates. The two aromatic moieties are complementary to one another, and are the electron-donor and the electron-acceptor. This aromatic complementarity enables the molecule to form molecular associates through π–π stacking. In general, the π–π stacking of aromatic complexes are very weak, therefore these complexes should

Keywords: Enantiomers; Enantiomeric recognition; Cyclodextrin complexation.
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doi:10.1016/j.tet.2004.11.021

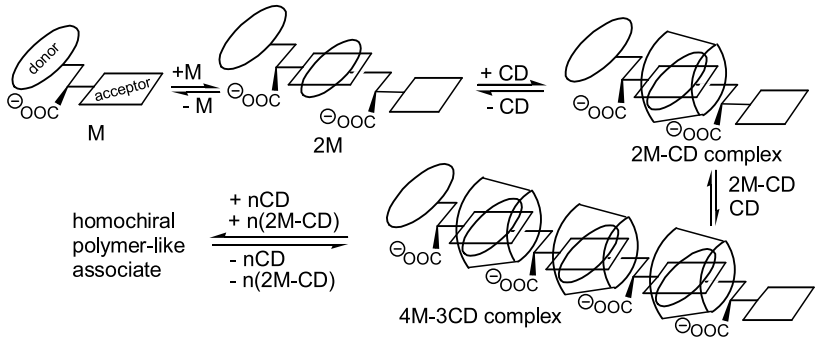


Figure 1. Possible pathway to cyclodextrin assisted formation of homochiral polymer-like associate.

be stabilized through their binding into the cyclodextrin cavity (Fig. 1). During the formation of this cyclodextrin assisted molecular associate, two aromatic moieties, each from different **M** molecules, are now ready to form a new cyclodextrin inclusion complex and molecular associate with a new **M** molecule. In this way, the polymer-like molecular associate would be formed.

The presence of polar groups, such as carboxylates, enable this polymer-like associate to interact with water surroundings. On the other hand, chirality of **M** makes the molecular associate diasteriomeric and stereoselective for incorporating only one enantiomer of **M** into the homochiral molecular associate.

To test the validity of the proposed cyclodextrin assisted self-assembly approach to enantiomeric recognition, the proper molecular system must be selected. Amino acids seem to be the best molecular choice for this study, provided that the amino acids have both electron-rich and electron-poor aromatic moieties. None of the natural amino acids satisfy this requirement; therefore, through simple chemical modifications of natural amino acids this requirement can be achieved. For instance, simple aromatic molecules such as methylindole, toluene, and methylbenzoisoquinoline-1,3-dione (Fig. 2) have a noticeable difference in aromatic electronic properties (Fig. 2). The AM1[11] computed frontier molecular orbital (HOMO–LUMO) energies suggest that toluene is electronically neutral, indole is electron rich and, benzoisoquinoline-1,3-dione is electron poor (Fig. 2). If these molecular moieties are incorporated into amino acids, then the requirement for molecule **M** has been accomplished.

Three amino acids were selected to test the validity of this hypothesis: alanine (without aromatic moiety), phenylalanine (neutral aromatic electronic properties), and trypthophan (electron-rich electronic properties). Through amino group replacement with benzo[*de*]isoquinoline-1,3-dione, new molecular systems **1a**, **1b**, and **1c** were generated. As expected, the LUMO orbital energy for all these three molecules is similar (because each has the same electron poor aromatic moiety) while the HOMO energies vary dramatically (Fig. 3) with the expected best complementarily of HOMO–LUMO interactions with **1c**. The AM1 estimated LUMO–HOMO energy difference as measured for π–π aromatic stacking capabilities are 8.00, 7.85, and 7.69 eV for **1a**, **1b**, and **1c**, respectively (the lower LUMO–HOMO energy gap the higher capability to form molecular associates through π–π molecular stacking[8]).

The synthetic conditions for the preparation of these amino

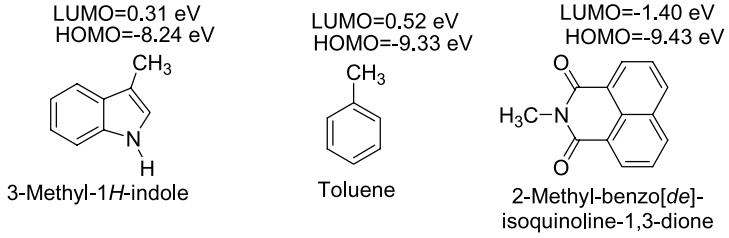


Figure 2. The AM1 estimated HOMO–LUMO energies for the aromatic moieties.

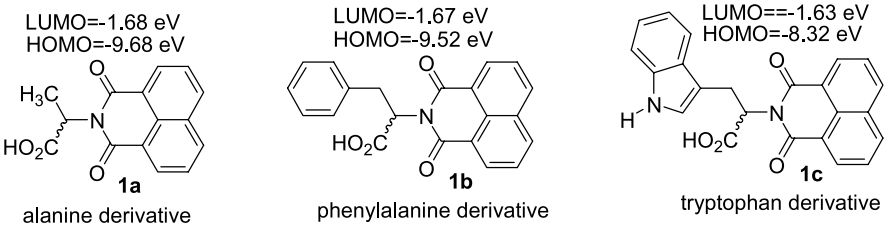
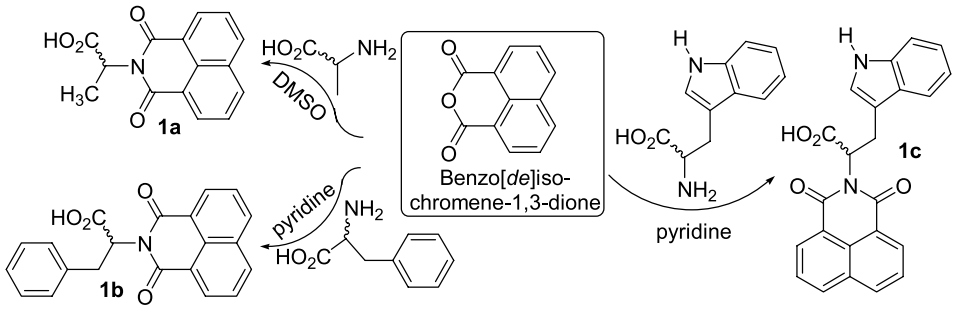


Figure 3. The AM1 estimated HOMO–LUMO energies for three modified amino acids.



Scheme 1. Preparation of electron-deficient alanine, phenylalanine, and tryptophan.

acid derivatives are presented in Scheme 1. Reactions are performed with the amino acid and benzo[*de*]isochromene-1,3-dione in DMSO for alanine and pyridine for phenylalanine and tryptophan (Scheme 1). The isolated yields range between 80 and 95%.

To demonstrate the existence of weak nonbonding interactions, as well as enantiomeric recognition when one of the enantiomers acts as resolving agent,[9] spectroscopic studies of racemic and non-racemic highly concentrated solutions of **1a**, **1b**, and **1c** were studied. These compounds are soluble in DMSO, therefore this was our solvent of choice. Both concentration (0.0001–0.3 M) and molar ratio (1:10, 1:5, 1:1, 5:1, and 10:1) of the *S* and *R* enantiomers were varied. In none of our NMR studies with all three compounds were we able to observe any NMR evidence for enantiomeric recognition.

For the study of the cyclodextrin assisted molecular complexes, water is a much better solvent of choice. However, none of the studied compounds is sufficiently soluble in water to perform reliable NMR studies. Therefore, sodium salts of **1a**, **1b**, and **1c** were used for the NMR spectroscopic studies. Neither **1a** nor **1b** non-racemic mixtures at any concentration range up to 0.1 M and any enantiomeric ratio show spectroscopic recognition. On the other hand, there is NMR enantiomeric recognition of non-racemic **1c** (Fig. 4). The spectroscopic recognition is not due to the formation of molecular associates through hydrogen bonding, nor through electrostatic interactions between the carboxylate group and sodium, because these interactions are also present in **1a** and **1b**. The only reasonable explanation is that molecule **1c** can form molecular associates through π–π stacking between the indole and benzo[*de*]isochromene-1,3-dione moieties.[10]

If this nonbonding π–π stacking is responsible for enantiomeric recognition, then the presence of cyclodextrin will enhance NMR spectroscopic recognition. This is due to the fact that both aromatic moieties tend to bind into the cyclodextrin cavity, and then orient themselves in the proper way for the aromatic stacking and for strong homochiral molecular associates. This is perfectly demonstrated in Figure 5 for γ-cyclodextrin assisted enantiomeric recognition of the racemic **1c**. The best results are obtained in γ-cyclodextrin in comparison with β-cyclodextrin, while no enantiomeric recognition was observed in α-cyclodextrin. This finding is not surprising considering that the cyclodextrin cavity must be large enough to accommodate two aromatic rings.

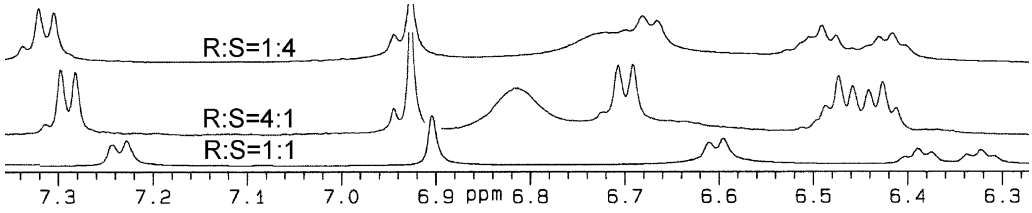


Figure 4. The NMR spectra of aromatic portion of **1c** (0.1 M) in aqueous $NaHCO_3$ (0.3 M) with different enantiomer ratios.

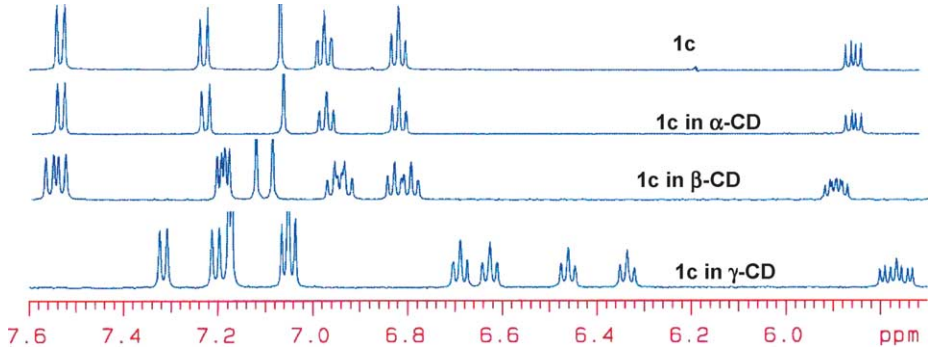


Figure 5. A portion of NMR spectra of racemic **1c** (0.001 M) in aqueous $NaHCO_3$ (0.003 M) with α, β, and γ-CD (0.01 M), respectively.

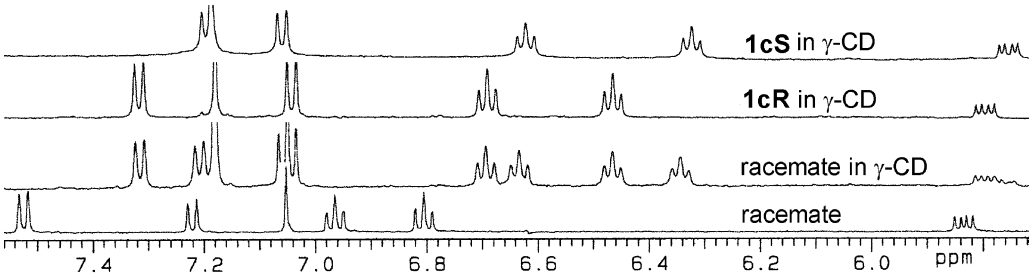


Figure 6. A portion of NMR spectra of **1c** (0.001 M) in aqueous $NaHCO_3$ (0.003 M) and γ-CD (0.01 M).

There are two sets of peaks in racemate NMR in presence of γ-CD. It is also possible to easily recognize each enantiomer peak if individual enantiomers NMR in γ-CD compared with the racemate NMR in γ-CD (Fig. 6).

Enantiomeric recognition can be observed even if there is no formation of the molecular associates described in Figure 1. The enantiomer binding into the cyclodextrin cavity can initiate the chiral environment necessary to observe spectroscopic differences. In α-cyclodextrin no NMR spectroscopic recognition was observed due to fact that neither the indole nor benzo[*de*]isochromene-1,3-dione aromatic moieties can bind into the α-cyclodextrin cavity. The β-cyclodextrin cavity is sufficiently large to accommodate the indole ring. If there is formation of molecular associates higher than the 1:1 cyclodextrin complex with respect to **1c**, we should observe this in electrospray mass spectroscopy. By performing this experiment it is clearly demonstrated that the cyclodextrin inclusion complex with **1c** was formed (Fig. 7) but no higher order (1:2 or 2:1) complex between β-CD and **1c** was observed.

Now we turn our attention to the molecular system that shows the best NMR enantiomeric recognition; γ-CD complexes with **1c** (Fig. 5). The electrospray mass spectra clearly shows the formation of 2:1 and 1:2 γ-CD complexes with **1c** (signals at 1488 and 1032, Fig. 8). All the other molecular complexes are present as well.

These are very encouraging results, but there is no experimental evidence for the formation of polymer-like cyclodextrin assisted molecular assemblies presented in Figure 1. For this reason, we performed MALDI MS spectra analysis of the aqueous γ-cyclodextrin solution of **1c**. If the structural patterns presented in Figure 1 are present, then typical fragmentation similar to the fragmentation of polymers should be observed in the MALDI MS spectra. All possible fragments were detected with lower intensity for larger molecular fragments (Fig. 9).

To better understand the binding of these guest compounds with host cyclodextrins, association constants (K, M^{-1}) were measured by ^{1}H NMR (500 MHz) at different temperature. In Table 1 are listed association constant K, standard free energy ΔG°, standard entropy term $T\Delta S^\circ$ and standard enthalpy ΔH° of guest **1c** with β and γ cyclodextrin. The chemical shift change of NMR signal of guest **1c** in presence of α cyclodextrine was too small to measure (as shown in Fig. 5). So the association constant is too small to measure practically. The solution of **1c**

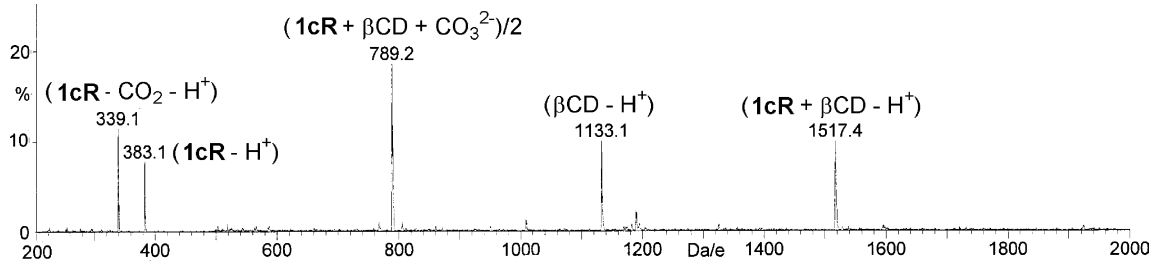


Figure 7. Negative electrospray mass spectrophotometry of **1c***R* (0.001 mol) in aqueous $NaHCO_3$ (0.003 M) and β-cyclodextrin (β-CD, 0.01 M).

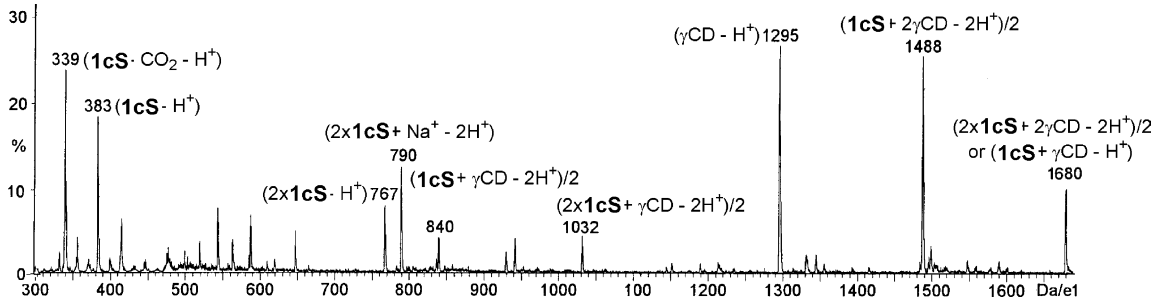


Figure 8. Negative electrospray mass spectra of **1c***S* (0.001 M) in aqueous $NaHCO_3$ (3×10^{-3} M) and γ-cyclodextrin (γ-CD, 10^{-2} M).

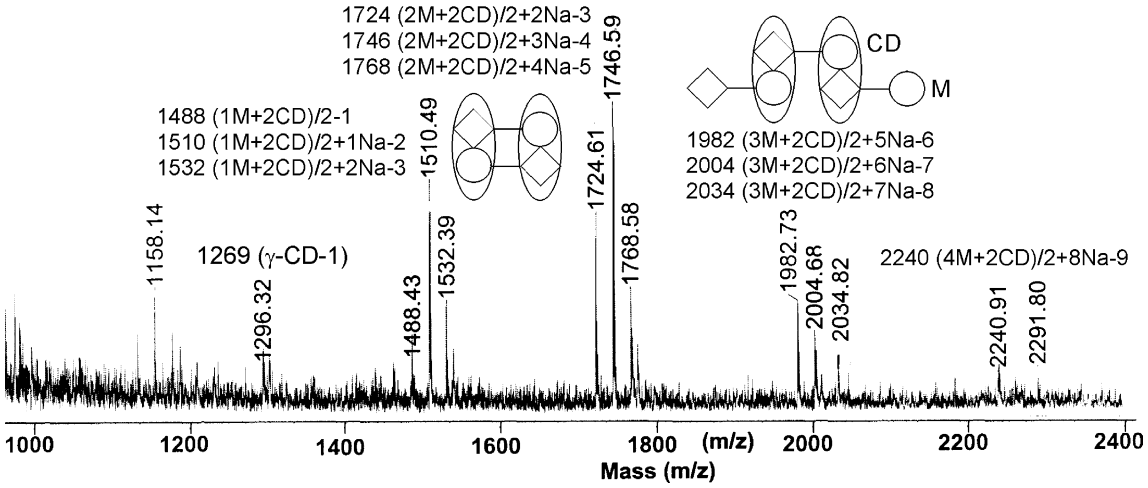


Figure 9. Positive MALDI-MS spectra of aqueous γ-CD and **1c**.

Table 1. Optical rotations of guest compounds **1a**, **1b** and **1c**

Guest compound	Optical rotation	
	L-enantiomer	D-enantiomer
1a	−19.5 (*c* 0.1, MeOH)	+18.0 (*c* 0.1, MeOH)
1b	−31.6 (*c* 1, MeOH)	+28.4 (*c* 1, MeOH)
1c	−17.0 (*c* 0.1, H_2O)	+16.2 (*c* 0.1, H_2O)

(0.001 M) in aqueous $NaHCO_3$ (3×10^{-3} M) was titrated with host solution at different temperature. Each time the change in chemical shift was measured. Non-linear regression analysis using origin 6.1 (Aston Scientific Ltd) was used to generate the association constant K_a according to the equation[12]:

$$\Delta = \frac{\Delta_{max} K_a[H]}{1 + K_a[H]}$$

where Δ, the peak shift in ppm, Δ_{max}, the maximum peak shift in ppm, and [H], the concentration of host. At least two experiments were performed for each system. Standard free energy was calculated using $\Delta G^\circ = -RT \ln K_a$ equation. Standard enthalpy ΔH° was determined according to the Van't Hoff equation: $d \ln K/d(1/T) = -\Delta H^\circ/R$ where $R = 8.3144\ J\ K^{-1}\ mol^{-1}$ and ΔH° in $J\ mol^{-1}$. $T\Delta S^\circ$ was calculated using $\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ$ equation.

As shown in Table 2, the association constant of guest **1c** is more than seven times higher for γ-CD compare to the association constant for β-CD. These results are consistent with what we understand from Figure 5. The cyclodextrin cavity must be large enough to accommodate two aromatic rings which γ-CD have. Also the association constant for L-enantiomer is higher than that of D-enantiomer.

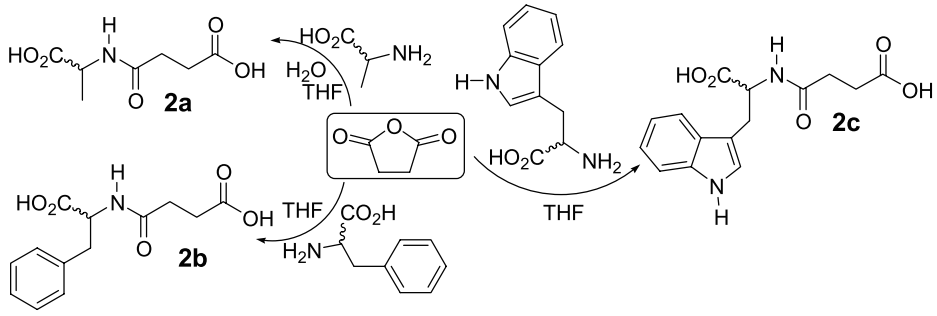
To show that the presence of the electron-poor aromatic moiety is crucial for the formation of the molecular assemblies discussed above, as well as make the amino acid derivative very soluble in water, mono amino acid amides with succinic acid were prepared (Scheme 2). Each and every of these reactions requires specific conditions, which were optimized by monitoring the reaction by NMR spectroscopic studies. After all reaction conditions were optimized, the isolated yields were higher than 85% (Table 3).

All of these compounds are very soluble in water, which makes it possible to perform the NMR spectroscopic studies in DMSO, $CDCl_3$, and water. We did not observe NMR enantiomeric recognition in DMSO and water, as well as in aqueous cyclodextrins. We were able to detect through electrospray MS spectroscopic study the formation of β-CD inclusion complexes with both **2b** and **2c**. It seems obvious that these cyclodextrin inclusion complexes cannot produce the different NMR environment to cause the NMR spectroscopic recognition. When two aromatic rings are together in the γ-CD cavity, as is the case with **1c**, the currency of one

Table 2. ^1H NMR derived thermodynamic parameters for the binding of guest molecule **1c** to host β and γ-CD

Temperature (K)	L-enantiomer		D-enantiomer	
	β-CD	γ-CD	β-CD	γ-CD
298	$K=63.02\pm19.63$	$K=413.03\pm21.45$	$K=47.26\pm6.72$	$K=320.93\pm19.5$
	$\Delta G^\circ=-10.27\pm0.7$	$\Delta G^\circ=-14.93\pm0.13$	$\Delta G^\circ=-9.56\pm0.3$	$\Delta G^\circ=-14.31\pm0.15$
	$T\Delta S^\circ=-4.84\pm2.9$	$T\Delta S^\circ=-9.7\pm0.12$	$T\Delta S^\circ=-28.89\pm1.5$	$T\Delta S^\circ$ -18.71 ± 3.5
313	$K=42.57\pm10.34$	$K=262.26\pm11.95$	$K=19.88\pm3.61$	$K=210.7\pm5.73$
	$\Delta G^\circ=-9.22\pm0.6$	$\Delta G^\circ=-13.81\pm0.11$	$\Delta G^\circ=-7.41\pm0.4$	$\Delta G^\circ=-13.26\pm0.07$
	$T\Delta S^\circ=-5.76\pm3.1$	$T\Delta S^\circ=-10.83\pm0.11$	$T\Delta S^\circ=-31.05\pm1.6$	$T\Delta S^\circ=-19.76\pm3.4$
343	$K=26.94\pm3.39$	$K=113.35\pm4.86$	$K=6.12\pm1.26$	$K=59.26\pm12.93$
	$\Delta G^\circ=-8.16\pm0.3$	$\Delta G^\circ=-11.73\pm0.11$	$\Delta G^\circ=-4.49\pm0.5$	$\Delta G^\circ=-10.12\pm0.49$
	$T\Delta S^\circ=-6.84\pm3.4$	$T\Delta S^\circ=-12.9\pm0.1$	$T\Delta S^\circ=-33.95\pm1.7$	$T\Delta S^\circ=-22.9\pm3.8$
	$\Delta H^\circ=-15\pm3.7$	$\Delta H^\circ=-24.63\pm0.01$	$\Delta H^\circ=-38.4\pm1.2$	$\Delta H^\circ=-33\pm3.4$

All K, ΔG°, $T\Delta S^\circ$, ΔH° values are in kJ/mol unit.



Scheme 2. Preparation of acid-amides of alanine, phenylalanine, and tryptophan.

Table 3. Optical rotations of guest compounds **2a**, **2b**, and **2c**

Guest compound	Optical rotation	
	L-enantiomer	D-enantiomer
2a	+22.0 (*c* 1, MeOH)	−21.6 (*c* 1, MeOH)
2b	−14.9 (*c* 1, MeOH)	+13.4 (*c* 1, MeOH)
2c	+37.1 (*c* 1, MeOH)	−32.8 (*c* 1, MeOH)

ring causes a change of magnetic properties on the other ring, which is easily detected through NMR spectroscopic studies, resulting in spectroscopic recognition of two enantiomers.

2. Conclusion

In conclusion we can state that there are spectroscopic evidences for the formation of chiral cyclodextrin assisted polymer-like aggregates with γ-cyclodextrin that accommodate two aromatic rings and modified chiral amino acids. For successful cyclodextrin assistance in the formation of these kinds of aggregates, modified amino acids must contain both electron-poor and electron-rich aromatic moieties. Cyclodextrin with a sufficiently large cavity, such as γ-cyclodextrin must be used to be able to bind both aromatic rings involved in aromatic π–π stacking.

3. Experimental

3.1. General

Melting points were taken on an Electrothermal IA 9000 Digital Melting Point Apparatus and are uncorrected. The ^{1}H and ^{13}C NMR spectra were run on Varian Unity 400 and Varian INOVA 500 MHz spectrophotometer with DMSO-d_6 as a solvent and internal standard (2.50 and 35.91 ppm for ^{1}H and ^{13}C NMR, respectively). The mass spectra were recorded on a Micromass Quattro 2 Triple Quadropole Mass Spectrometer; Optical rotations of guest compounds were detected at 25 °C with the light of sodium D-line (589 nm) using Autopo III automatic polarimeter Rudolph Research, Flanders, New Jersy. Elemental Analysis was performed by Atlantic Microlab, Inc., Norcross, GA.

3.1.1. Preparation of (*S*)-2-(1,3-dioxo-1*H*,3*H*-benzo[*de*]-isoquinolin-2-yl)propionic acid (1a*S*). Dimethyl sulfoxide (30 mL) solution of benzo[*de*]isochromene-1,3-dione (1.98 g; 0.01 mol) and alanine (0.89 g; 0.01 mol) was refluxed for 1 h. Reaction mixture was cooled to room temperature and slowly added into stirring water (200 mL). Formed precipitate was separated by filtration, washed with water (3×30 mL) and dried at 110 °C for a few hours to afford 2.15 g (80%) pure product. Mp 257–261 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ ppm: 8.51 (dd, 4H, J_1=10.5 Hz; J_2=7.5 Hz), 7.89 (t, 2H, J=7.5 Hz), 5.54 (q, 1H, J=7 Hz), and 1.53 (d, 3H, J=6.5 Hz). ^{13}C NMR (DMSO-d_6, 500 MHz) δ ppm: 171.4, 162.9 (carbonyls), 134.7, 131.3, 131.1, 127.4, 121.7 (aromatic carbons), 48.5 (chiral carbon CH), 14.5 (methylene carbon). MS-ES$^+$ (CH_3OH) *m/z* 292.2 (65%, M+Na$^+$), 324.1 (85%, M+CH_3OH+Na$^+$), 561.3 (100%. 2M+Na$^+$), and 829.8 (50%, 3M+Na$^+$). Anal. Calcd for $C_{15}H_{11}NO_4$ (269.07): C, 66.91; H, 4.12; N, 5.20 Found: C, 66.85; H, 4.21; N, 5.11.

3.1.2. Preparation of (*R*)-2-(1,3-dioxo-1*H*,3*H*-benzo[*de*]-isoquinolin-2-yl)propionic acid (1a*R*). The stereoisomer *R* was prepared in 87% yield by following procedure for preparation of *S* isomer. The NMR and MS-ES spectra of *R* and *S* stereoisomers are identical.

3.1.3. Preparation of (*S*)-2-(1,3-dioxo-1*H*,3*H*-benzo[*de*]-isoquinolin-2-yl)-3-phenyl-propionic acid (1b*S*). Pyridine solution (150 mL) of benzo[*de*]isochromene-1,3-dione (0.99 g; 5 mmol) and L-phenylalanine (0.825 g; 5 mmol) was refluxed for 6 h. Volume of the reaction mixture was reduced to ~5 mL and hot reaction mixture was added to ice cooled aqueous hydrochloric acid (150 mL water and 50 concd HCl). Formed solid precipitate was separated by filtration, washed with water (3×20 mL) and dried at 110 °C for a few hours to afford 1.6 g (93%) pure product. Mp 250–252 °C ^{1}H NMR (DMSO-d_6, 500 MHz) δ ppm: 8.41 (d, 2H naphthalene ring, J=7 Hz), 8.37 (d, 2H naphthalene ring, J=7 Hz), 7.78 (t, 2H naphthalene ring, J=7.5 Hz,), 7.14 (d, 2H, J=7.5 Hz), 7.07 (2H, t, J=7.5 Hz) 7.00 (t, 1H, J=7.5 Hz) 5.92 (dd,1H, J_1=10 Hz; J_2=5.5 Hz) 3.58 (dd, 1H, J_1=14 Hz; J_2=5.5 Hz), and 3.39 (dd,1H, J_1=14.5 Hz; J_2=10 Hz). ^{13}C NMR (DMSO-d_6, 500 MHz) δ ppm: 171.8, 163.0 (carbonyls), 137.9, 134.7, 131.2, 129.0, 128.1, 127.5, 127.3, 127.2, 126.3, 121.2 (10 aromatic carbons), 53.9 (chiral carbon CH), and 24.3 (methylene carbon). MS-ES$^-$ *m/z* 344 (100% M−H$^+$). Anal. Calcd for $C_{21}H_{15}NO_4$ (345.10): C, 70.03; H, 4.38; N, 4.06. Found: C, 69.91; H, 4.42; N, 3.95.

3.1.4. Preparation of (*R*)-2-(1,3-dioxo-1*H*,3*H*-benzo[*de*]-isoquinolin-2-yl)-3-phenyl-propionic acid (1b*R*). Stereoisomer *R* was prepared in 91% yield and has same spectroscopic characteristics as stereoisomer *S*.

3.1.5. Preparation of (*S*)-2-(1,3-dioxo-1*H*,3*H*-benzo[*de*]-isoquinolin-2-yl)-3-(1*H*-indol-3-yl)-propionic acid (1c*S*). Dimethyl sulfoxide (30 mL) solution of benzo[*de*]isochromene-1,3-dione (0.99 g; 5 mmol) and L-tryptophan (1.04 g; 5 mmol) was heated at 150 °C for 30 min. Red colored reaction mixture was slowly added into stirring water (200 mL). Formed yellow precipitate was separated by filtration, washed with water (3×20 mL) and dried at 110 °C to give 1.6 g (83%) of pure product. Mp 235–239 °C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 12.80 (br s, 1H), 10.63 (1H, s), 8.44 (dd, 4H, J_1=4.4 Hz, J_2=7.2 Hz), 7.85 (t, 2H, J=7.6 Hz), 7.46 (d, 1H, J=8.0 Hz), 7.17 (d, 1H, J=7.6 Hz), 6.99 (s, 1H,), 6.92 (t, 1H, J=7.6 Hz), 6.79 (t, 1H, J=7.2 Hz), 5.88 (dd, 1H, J_1=9.6 Hz, J_2=5.6 Hz), 3.65 (dd, 1H, J_1=14.8 Hz, J_2=5.6 Hz), 5.54 (dd, 1H, J_1=14.8 Hz, J_2=9.6 Hz), ^{13}C NMR (DMSO-d_6, 500 MHz) δ ppm: 171.0, 163.1, 135.9, 134.7, 131.2, 127.4, 127.2, 123.5, 121.4, 120.8, 118.2, 118.0, 111.3, 110.3, 53.7, and 24.0 ppm. ESI$^+$ (CH_3CO_2H) *m*/*z* 385 (100, M+H$^+$) and 769 (45%, 2M+H$^+$). Anal. Calcd for $C_{23}H_{16}N_2O_4$ (384.38): C, 71.87; H, 4.20; N, 7.29 Found: C, 71.55; H, 4.31; N, 7.18.

3.1.6. Preparation of (*R*)-2-(1,3-dioxo-1*H*,3*H*-benzo[*de*]-isoquinolin-2-yl)-3-(1*H*-indol-3-yl)-propionic acid (1c*R*). The *R* stereoisomer has same spectroscopic characteristics as *S* isomer and is prepared in 87% isolated yield by following the *S* preparation procedure.

3.1.7. Preparation of (*S*)-*N*-(1-carboxyethyl)succinamic acid (2a*S*). Water solution (5 mL) of *S*-alanine (1.5 g; 0.017 mol) was slowly added into stirring tetrahydrofuran solution (200 mL) of succinic anhydride (1 g; 0.01 mol). Resulting suspension was stirred at 30 °C for 1 h. Solid was separated by filtration and filtrate was evaporated under reduced pressure. Liquid residue was mixed with ethyl acetate and the resulting mixture was dried over anhydrous sodium sulfate. Drying reagent was removed by filtration and filtrate was evaporated to solid residue and solid residue was slurred in petroleum ether. Product was isolated by filtration of white suspension in 79% yield. Mp 152–155 °C. ^{1}H NMR (D_2O, 500 MHz) δ ppm: 4.35 (q, 1H, chiral CH; J=7 Hz), 2.69 (s, 4H, two CH_2 groups of the succinic acid moiety) and 1.42 (d, 3H, J=7.5 Hz). ^{13}C NMR (D_2O, drop of DMSO-d_6 added for reference, 500 MHz) δ ppm: 177.5, 177.4, 175.2 (carbonyl carbon), 49.4, 30.5, 29.8, 29.5 (four aliphatic carbon). ES-MS$^-$ *m*/*z* 188.1 (85%, M−H$^+$), 377.3 (100%, 2M−1H$^+$), and 566.0 (30%, 3M−H$^+$). Anal. Calcd for $C_7H_{11}NO_5$ (189.06): C, 44.45; H, 5.86; N, 7.40 Found: C, 44.37; H, 5.98; N, 7.33.

3.1.8. Preparation of (*R*)-*N*-(1-carboxyethyl)succinamic acid (2a*R*). The *R* isomer was prepared by following the same synthetic procedure with 83% isolated yield. The spectroscopic characteristics are identical to the *S* isomer.

3.1.9. Preparation of (*S*)-*N*-(1-carboxy-2-phenylethyl)-succinamic acid (2b*S*). Tetrahydrofuran (500 mL) suspension of phenylalanine (1.65 g; 0.01 mol) and succinic anhydride (1.0 g; 0.01 mol) was refluxed until the suspension becomes clear solution (approximately 30 h). Solvent was evaporated under reduced pressure. Solid residue was scurried in petroleum ether (100 mL), separated by filtration, and washed with petroleum ether (3×20 mL) and dried at 60 °C for a few hours. The isolated yield is 2.5 g (95%) of pure product. Mp 127–129 °C. ^{1}H NMR (DMSO-d_6, 500 MHz) δ ppm: 8.15 (d, 1H, amide NH, J=8 Hz) 7.25 (m, 5H benzene ring), 4.50 (ddd, 1H chiral), 3.05 (dd, 1H, J_1=13.5 Hz; J_2=5 Hz), 2.86 (dd, 1H, J_1=13.5 Hz; J_2=9.5 Hz), and 2.35 (s, 4H aliphatic two CH_2 group). ^{13}C NMR (DMSO-d_6, 500 MHz) δ ppm: 73.8, 173.1, 171.1 (carbonyls), 129.2, 128.2, 126.4 (aromatic carbons), 53.6, 37.0, 29.9, and 29.1 (four aliphatic carbon). MS-ES$^+$ *m*/*z* 266.1 (22%, M+H$^+$) and 288.1 (100%, M+Na$^+$). Anal. Calcd for $C_{13}H_{15}NO_5$ (265.10): C, 58.86; H, 5.70; N, 5.28 Found: C, 58.92; H, 5.77; N, 5.15.

3.1.10. Preparation of (*R*)-*N*-(1-carboxy-2-phenylethyl)-succinamic acid (2b*R*). The *R* stereoisomer was prepared in 93% isolated yield by following the procedure described for the *S* stereoisomer. The spectral characteristics for this compound are identical to the spectral characteristics of the *S* stereoisomer.

3.1.11. Preparation of (*S*)-*N*-[1-carboxy-2-(1*H*-indol-3-yl)ethyl]succinamic acid (2c*S*). Tetrahydrofuran (1 L) suspension of tryptophan (2 g; 0.01 mol) and succinic anhydride (1 g; 0.01 mol) was refluxed until it became solution (approximately 40 h). Solvent was evaporated and solid residue was mixed with petroleum ether (200 mL). Solid material was separated by filtration, washed with petroleum ether (3×20 mL), and dried at 60 °C for several hours to afford 2.8 g (92%) of pure product. Mp (hydroscopic). ^{1}H NMR (DMSO-d_6, 500 MHz) δ ppm: 10.82 (s, 1H the pyrrole ring NH) 8.15 (d, 1H amide hydrogen, J=7.5 Hz), 7.52 (d, 1H benzene ring H, J=7.5 Hz), 7.32 (d, 1H, J=8 Hz), 7.14 (s, 1H the pyrrole ring CH) 7.05 (t, 1H, J=7 Hz) 6.97 (t, 1H, J=7 Hz) 4.46 (ddd, 1H) 3.14 (dd, 1H, J_1=14 Hz; J_2=5.5 Hz) 3.00 (dd, 1H, J_1=14 Hz; J_2=8 Hz), 2.35 ppm (s, 4H aliphatic two CH_2 group hydrogen). ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 173.8, 173.5, 171.1 (carbonyls), 136.1, 127.3, 123.6, 121.0, 118.4, 118.2, 111.4, 109.9 (eight aromatic carbons), 53.1, 29.9, 29.1, and 27.2 ppm (four aliphatic carbon). MS-ES$^-$ *m*/*z* 303.1 (35%, M−H$^+$), 431.3 (15%, 3M−CO_2−2H$^+$), and 607.1 (100%, 2M−H$^+$). Anal. Calcd for $C_{15}H_{16}N_2O_5$ (304.30): C, 59.21; H, 5.30; N, 9.21 Found: C, 59.15; H, 5.38; N, 9.16.

3.1.12. Preparation of (*R*)-*N*-[1-carboxy-2-(1*H*-indol-3-yl)ethyl]succinamic acid (2c*R*). The *R* stereoisomer was prepared in 96% yield by following same procedure outlined for the *S* stereoisomer. All spectra of *R* stereoisomer are identical to the *S* stereoisomer spectra.

Acknowledgements

We thank the Louisiana Board of Reagents for their financial support (LEQSF (2001-04)-RD-B-12) for this work.

References and notes

1. For instance see (a) *Chiral Drugs*; Challener, C. A., Ed.; Gower: New York, 2002. (b) *The Impact of Stereochemistry on Drug Development and Use*; Aboul-Enein, H. Y., Wainer, I. W., Eds.; Wiley: New York, 1997. (c) Rouhi, A. M. 'Chiral Business', Chemical and Engineering News; 2003. May 5, pp 45–55. (d) Rouhi, A. M. 'Chirality at Work', Chemical and Engineering News; 2003, May 5, pp 55–61.
2. For definition of stereochemistry see: Eliel, E. L.; Wilen, S. H. *Stereochemistry of Organic Compounds*; Wiley: New York, 1994.
3. For more information regarding cyclodextrin complexes see: (a) Schneider, H.-J.; Hacket, F.; Rüdiger, V. *Chem. Rev.* **1998**, *98*, 1755–1785. (b) Rekharsky, M. V.; Inoue, Y. *Chem. Rev.* **1998**, *98*, 1875–1917. (c) Song, L.; Purdy, W. C. *Anal. Chem.* **1992**, *64*, 1405–1412.
4. *Chirality in Natural and Applied Sciences*; Lough, W. J., Wainer, I. W., Eds.; CRC: Boca Raton, FL, 2002.
5. (a) Boeyens, J. C. A. Intermolecular bonding In *Inermolecular Interactions*; Plenum: New York, NY, 1998. (b) Soltis, R. D.; Hasz, D. Studies on the nature of intermolecular bonding in antigen–antibody complexes *Immunology* **1982**, *46*, 175–181.
6. (a) Guan, L.; Hu, Y.; Kaback, H. R. *Biochemistry* **2003**, *42*, 1377–1382. (b) Lawrence, A. J. *Chem. Rev.* **1954**, *54*, 713–776. (c) Cubberley, M. S.; Iverson, B. L. *J. Am. Chem. Soc.* **2001**, *123*, 7560–7563. (d) Guckian, K. M.; Schweitzer, B. A.; Ren, R. X.-F.; Sheils, C. J.; Tahmassebi, D. C.; Kool, E. T. *J. Am. Chem. Soc.* **2000**, *122*, 2213–2222. (e) Ilhan, F.; Gray, M.; Blanchette, K.; Rotello, V. M. *Mocromolecules* **1999**, *32*, 6159–6162.
7. Bentley, R. *Arch. Biochem. Biophys.* **2003**, *414*, 1–12.
8. For frontier orbital interactions see: Fleming, I. *Frontier Orbitals and Organic Chemical Reactions*; Wiley: London, 1976.
9. For similar enantiomeric recognition through hydrogen bonding interactions in highly concentrated chloroform solutions see: (a) Jursic, B. S.; Goldberg, S. I. *J. Org. Chem.* **1992**, *57*, 7172–7174. (b) Jursic, B. S.; Goldberg, S. I. *J. Org. Chem.* **1992**, 7270–7372. (c) Jursic, B. S.; Zdravkovski, Z. *J. Org. Chem.* **1993**, *58*, 5245–5250. (d) Jursic, B. S.; Zdravkovski, Z.; Zuanic, M. *Tetrahedron: Asymmetry* **1994**, *5*, 1711–1716.
10. For π–π aromatic stacking in enantiomeric recognition see: Jursic, B. S. *J. Chem. Soc. Perkin Trans. 2* **1994**, 961–969. For enantiomeric recognition in molecular aggregates such as micelles see: Jursic, B. S. *Tetrahedron Lett.* **1993**, *34*, 963–966.
11. Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902–3909.
12. Connors, K. A. *Binding Constants*; Wiley: New York, 1987.

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Tetrahedron 61 (2005) 927–939

RuO_4-catalyzed oxidative polycyclization of squalene. Determination of the configuration of the penta-tetrahydrofuranyl diol product

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Received 1 July 2004; revised 12 October 2004; accepted 5 November 2004

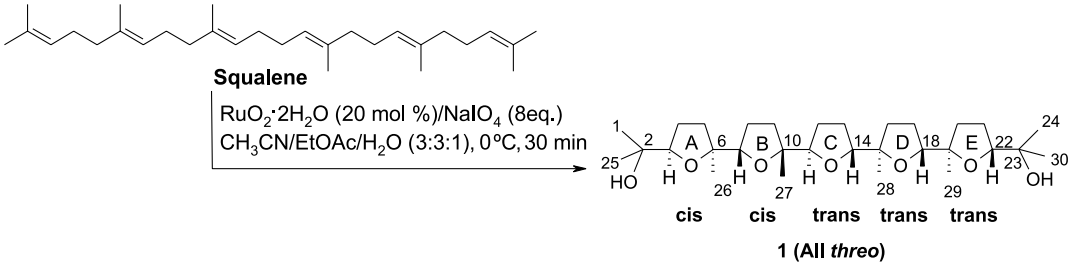
Available online 8 December 2004

Abstract—The configuration of the penta-tetrahydrofuranyl diol (penta-THF) product obtained by a single-step, RuO_4-catalyzed oxidative polycyclization of squalene, has been determined as *cis–threo–cis–threo–trans–threo–trans–threo–trans*. The *cis–cis–trans–trans–trans* sequence for the five contiguous THF rings has been established through extensive 2D-NMR spectroscopic studies carried out both on the intact molecule and on some of its derivatives, including the oxidative cleavage products obtained by degradation of the penta-THF with PCC/AcOH. Four different chemical approaches were devised to determine each of the four *threo* relationships within each carbon pair connecting adjacent THF rings in the molecule. To this aim, studies have been carried out either on some intermediates of the process leading to penta-THF, obtained by stopping the oxidation of squalene prior to completion, or on a degradation product of the penta-THF, obtained from the latter through a bidirectional double oxidative degradation with PCC.

1. Introduction

2,5-Bonded polytetrahydrofurans (poly-THF) are key structural fragments in natural[1–3] and non-natural[4] products. They are embodied, for example, in the structure of bioactive squalene-derived natural products such as glabrescol,[1] longilene peroxide[2] and teurilene,[3] in polyether antibiotics such as monensin and ionomicin as well as in many *Annonaceous* acetogenins[5] a class of natural products with remarkable antitumoral and pesticide activity that has stimulated synthetic efforts from many research groups.[6]

Recently, we have discovered a novel, RuO_4-catalyzed, oxidative process that allows, in a single step, the stereoselective synthesis of adjacently linked poly-tetrahydrofuranyl diol products starting from isoprenoid polyenes.[7] Among the studied cases, the oxidation of squalene appears to be very intriguing since it furnishes the penta-THF **1** (Scheme 1; configuration as determined in this work), as a single diastereomer, in a remarkable 50% yield. This is the sole example, so far known, of a single-step *quintuple* oxidative polycyclization leading to a poly-THF product.



Scheme 1. Oxidative polycyclization of squalene with cat. RuO_4.

Keywords: RuO4; Squalene; Oxidative polycyclization; Penta-THF configuration; PCC; Oxidative cleavage.
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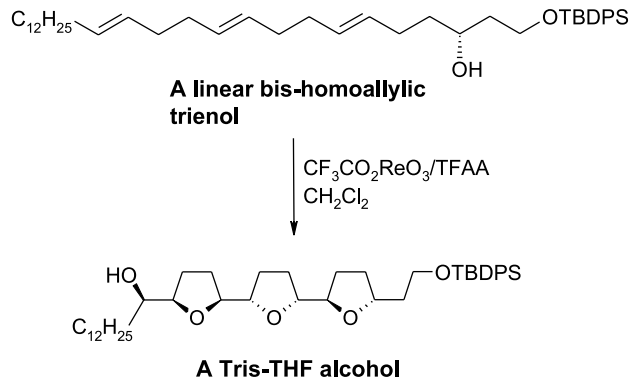
doi:10.1016/j.tet.2004.11.018

The new process appears to be related to the polycyclization of bis-homoallylic hydroxypolyenes mediated by Re(VII) oxo species, mostly Re_2O_7 and $CF_3CO_2ReO_3$, that allows the assembly of up to three contiguous THF rings in a single step or in a sequential way. As an example, the synthesis of an advanced intermediate in the synthesis of 17,18-*bisepi*-goniocin, through a single-step triple oxidative cyclization reaction in the presence of a Re(VII) reagent, is shown in Scheme 2.[8] A notable difference between the two processes stands in the catalytic nature of the Ru-mediated process; also to be noted is the structure of the poly-THF product: a poly-THF alcohol for the process induced by rhenium, a poly-THF diol for the Ru-mediated one.



Scheme 2. An example of rhenium(VII)-mediated polycyclization of a bis-homoallylic trienol.

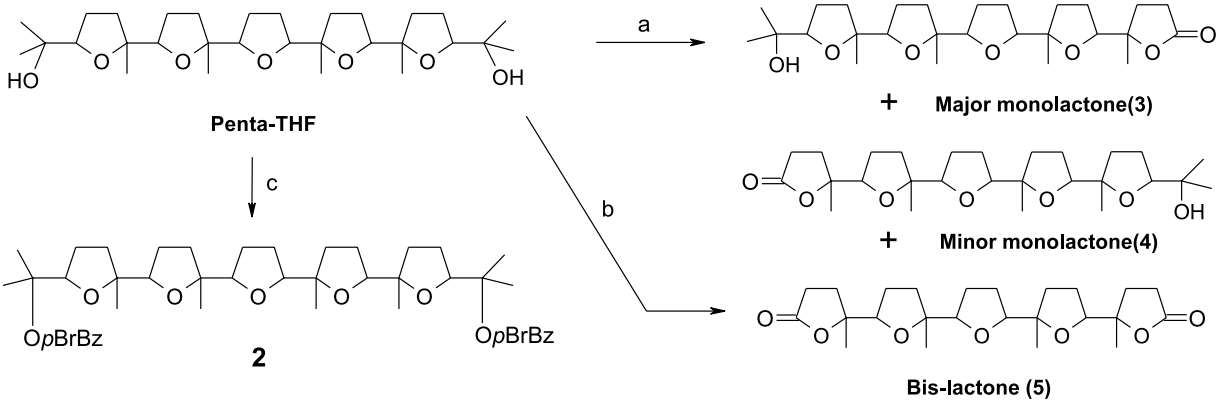
While for the Re-mediated process Sinha and co-workers have proposed simple stereoselectivity rules capable to predict its stereochemical outcome, mostly for linear polyenes,[8] the factors affecting the stereochemical course of the RuO_4-induced process are still unclear mainly due to the scarcity of available data, a fact that strongly hampers a close comparison of the two related processes. Therefore, with the aim of gaining further insight into the above Ru-mediated process the determination of the configuration of penta-THF **1** was accomplished as described in this paper; this proved to be a challenging task that required extensive 2D-NMR and chemical work, carried out both on **1** and on some of its derivatives.

2. Results and discussion

As a first step towards this goal we attempted the crystallization of **1**. Unfortunately, this product could not be induced to crystallize in a variety of organic solvents or solvent mixtures. Nor the bis-*p*-bromobenzoyl derivative of **1** (**2**, 80%, Scheme 3) obtained by reaction with *p*-bromobenzoyl chloride/DMAP in CH_2Cl_2, gave crystals suitable for X-ray analysis. Our attention was then turned to NMR studies. However, the full NMR assignment on compound **1** was intrinsically complicated by the high degree of superimposition of ^1H and ^{13}C resonances which occurred even in the two-dimensional experiments. For instance, all the methines belonging to THF rings A–E showed a severe overlapping and, in particular, all the tetrhydrofuran protons possess ^1H chemical shift values falling in the very narrow range of 0.13 ppm. Due to the high repetitive character of this structure, the same situation applies to most nuclei belonging to the five THF rings.

This particularly unfavourable situation required, together with the routine 2D-COSY, TOCSY, HSQC, and HMBC experiments, the acquisition of a set of three experiments which turned to be crucial for the complete assignment of **1**. In particular, the 2D-HSQC-TOCSY allowed to extrapolate the ^1H and the ^{13}C resonances for each of the five THF rings in **1**; subsequently 2D-INEPT-INADEQUATE and 2D-INADEQUATE experiments were employed in the resonance specific assignment of all ^1H and the ^{13}C signals, respectively.

However, the relative configuration of **1** could not be assigned just by using NMR data collected for **1**, due to the presence of some ambiguous cross-peaks in its ROESY spectrum. This prompted us to seek suitable derivatives of **1**, that could be obtained with the minimum stereochemical sacrifice, to be used for further NMR studies. In doing so, we were attracted by the PCC-mediated oxidative degradation of some bis-THF compounds, sharing with **1** a 2-hydroxypropyl terminus, previously carried out by McDonald and Towne.[9] We were pleased to find that treatment of **1** in the reported conditions [PCC (5 equiv), AcOH (70 equiv), Celite in CH_2Cl_2] cleanly gave a 6:1:6 mixture (52% overall yield) of the two mono-lactones **3** and



Scheme 3. Some derivates of penta-THF 1. Reagents and conditions: (a) PCC (5 equiv), AcOH (70 equiv), celite (10×weight of **1**), CH_2Cl_2, 24 h (**3**: 22%; **4**: 4%; **5**: 26%); (b) PCC (10 equiv), AcOH (150 equiv), celite (10×weight of **1**), CH_2Cl_2, 60 h (70%); (c) *p*BrBzCl (15 equiv), DMAP (30 equiv), CH_2Cl_2 (80%).

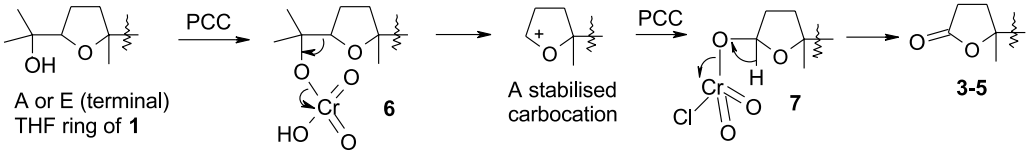
4 and the bis-lactone **5** (Scheme 4), derived from the oxidative cleavage of one or both the three-carbon 2-hydroxypropyl termini. Under forcing conditions (PCC: 10 equiv, AcOH 150 equiv, 60 h) bis-lactone **5** was obtained as the sole reaction product in a 70% yield.

^{1}H NMR spectra of derivatives **3**–**5** were simplified by the lack of one THF proton and two methyl signals in **3** and **4**, and two THF protons and four methyl signals in **5**. Collection of a new set of NMR data for these compounds finally allowed to unambiguously assign a *cis–cis–trans–trans–trans* sequence to the five adjacent THF rings in compound **1**.

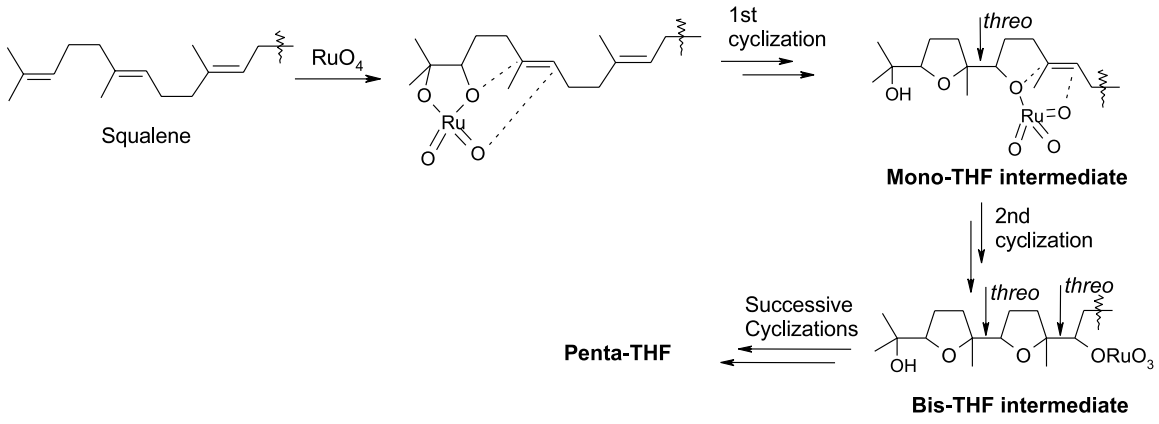
In particular, the application of the same assignment strategy employed for **1** afforded the sequence specific attribution of all crucial methine and methyl resonances belonging to rings A–E of compounds **2**–**5**. The comparative analysis of the ROESY spectra of compounds **1**–**5** allowed to establish the relative configuration indicated in Scheme 1. The *cis*-configuration of ring A was suggested by a strong ROESY correlation between H-3 and Me-26 present in the spectra of compounds **1**–**3** (**4** and **5** lack H-3). Likewise, ROESY correlations between proton H-7 and Me-27 were detected in the spectra of all compounds **1**–**5**, allowing to determine a *cis*-arrangement for B ring as well. Ring C is characterized by a *trans*-arrangement suggested primarily by the lack of ROESY contacts between H-11 and H-14 in the spectra of **1**–**5**. Further support came from key ROESY correlations, across ring functions, between H-11 and Me-28 of ring D, and H-14 and Me-27 of ring B. On the basis of a similar analysis, the configuration of rings D and E was also established. Particularly, diagnostic for determining the *trans*-arrangement for both rings D and E were the expected lack of ROESY correlations between Me-28 and H-18, and between Me-29 and H-22. Again the latter configurational assignment was corroborated by a dipolar coupling between Me-28 and Me-29, observed in the ROESY spectrum of compound **2**.

A plausible mechanism explaining the oxidative cleavage of the two terminal isopropyl portions in **1** is shown in Scheme 4 that also agrees with related PCC chemistry.[10] Thus, the initially formed chromium (VI) ester **6** fragments to form a stabilised carbocation species that is then intercepted by another PCC molecule to form **7**. Subsequent elimination of a chromium species generates the lactone function found in compounds **3**–**5**. The markedly different yields of monolactones **3** (22%) and **4** (4%) is evidence of a greater propensity of the side chain next to the *trans*-THF ring to undergo cleavage. This is a relatively unknown process.

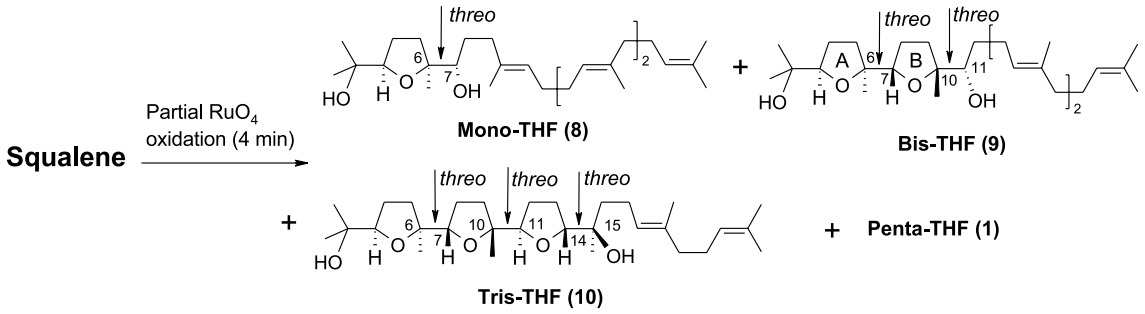
In the next step towards the elucidation of the configuration of **1**, we had to establish the stereochemical relationship within each of the four carbon pairs connecting the adjacent THF rings in **1**. As previously experienced in similar studies from our own group[7] and of other researchers,[11] this could not be accomplished by NMR spectroscopic analysis alone. In fact, the pattern of dipolar contacts across adjacent THF rings can be hardly converted into useful information on their relative orientation, as a simple inspection of molecular models would confirm. Though, in principle, both an *erythro* and *threo* relationship could subsist for each THF pair, closure of each THF ring likely takes place by *syn*-addition[12] of two oxygens through the [3+2] cycloaddition of a RO-Ru=O portion on each of the six double bonds of the polyene, as shown in Scheme 5 for the first two ring-closing steps. This would agree with previous results from the cyclization of farnesyl acetate (FA) and geranyl geranyl acetate (GGA)[7] and related Re(VII) chemistry,[8] and would imply that an all-*threo* penta-THF should be obtained from the *all-trans* squalene. A definitive confirmation of this appeared, nevertheless, necessary to put our understanding of the process on a firm basis.



Scheme 4. Proposed mechanism for the PCC-mediated oxidative cleavage of **1**.



Scheme 5. Mechanistic hypothesis for the oxidative polyciclization of squalene with cat. RuO_4.



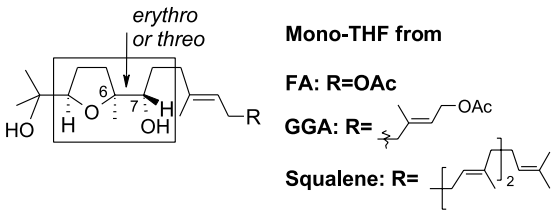
Scheme 6. Partial oxidation of squalene with cat. RuO_4.

Proof for the all-*threo* arrangement of **1** was gained through extensive chemical work carried out on the intermediates of the polycyclization process, obtained by partial oxidation of squalene, as well as on some degradation products of **1**. In particular, when the oxidation of squalene was stopped prior to completion (4 min), the mono-, bis-, and tris-THF compounds **8**–**10** (Scheme 6), derived from the partial cyclization of squalene, could be isolated by HPLC, along with penta-THF **1** and the unreacted polyene. Rather surprisingly, the tetra-THF diol intermediate, possessing the A/B/C/D ring sequence (see structure **22** in Scheme 11), could not be detected among the reaction products even after a careful HPLC analysis of the reaction mixture.

Based on the assumption that compounds **8**–**10** possess an all-*threo* arrangement, as the penta-THF to which they eventually give rise, a determination of the C_6–C_7 and C_{10}–C_{11} stereorelationships in **1** was made on compounds **8** and **9**. Though this furnishes an indirect evidence of the above configurations in **1**, we are confident that compounds **8**–**10** are indeed intermediates of the process, leading to the penta-THF formation based on the following considerations. Firstly, they are the sole mono-, bis- and tris-THF diol products isolated during the partial oxidation of squalene obtained as main products of the reaction mixture; secondly, we have previously demonstrated that the mono- to tris-THF diols isolated from the partial oxidation of FA and GGA with RuO_4 possess configuration that matched that of the poly-THF product of which they are precursors. Determination of the relationship between the carbon pairs connecting B/C and C/D rings (C_{14}–C_{15}, and C_{18}–C_{19} carbon pairs, respectively) was carried out on two products derived from degradation of **1** as depicted in Schemes 9 and 11 (later in the discussion), also taking into account evidence collected on compounds **8**–**10** and the above assumption.

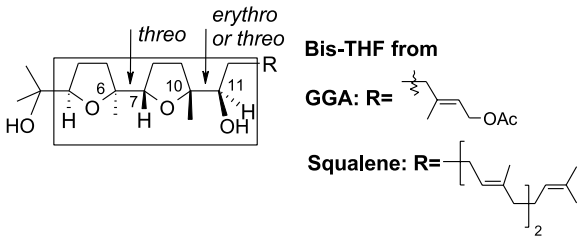
NMR evidence from the proton spectra of compounds **8** and **9** pointed to an all-*threo* arrangement for both. In fact, chemical shift values of the C*H*–O and Me proton resonances belonging to the mono- or bis-THF alcohol portions in these compounds (Tables 1 and 2, see the structural portions in the boxes) showed a very good agreement with those observed for the corresponding protons in the structurally related *cis–threo* mono-THF and *cis–threo–cis–threo* bis-THF analogues, respectively, previously isolated from the partial oxidation of FA and GGA.[7] In particular, diagnostic for the configuration at C-7 (squalene numeration) in **8** is the H-7 proton resonance that in the *threo*-isomers falls in the narrow range δ 3.38–3.39 (Table 1) while is downfield shifted at δ 3.54–3.56 in the *erythro*-isomers. Analogously, in the bis-THF series (Table 2) the H-11 resonance is a probe for the configuration at the C-11 centre. This proton exhibited a chemical shift value in the range δ 3.38–3.41 (virtually the same observed for H-7 in the mono-THF series) for the C_{10}–C_{11} *threo* compounds while it is once again downshifted, at δ 3.52–3.54, in the C_{10}–C_{11} *erythro* isomers. Also noticeable, on passing from *threo* to *erythro* (C_6–C_7 and C_{10}–C_{11}) isomers, is the small but diagnostic downfield shift (from 1.24–1.25

Table 1. Comparison of diagnostic ^{1}H NMR data of some isoprenoidic mono-THF diols (squalene numeration)



Proton	Mono-THF from squalene (**8**) (*cis–threo*)	Mono-THF from FA (*cis–threo*)	Mono-THF from GGA (*cis–threo*)	Mono-THF from squalene (*cis–erythro*)	Mono-THF from FA (*cis–erythro*)
C*H*-O(THF)	3.85	3.85	3.85	3.82	3.82
C*H*-OH	3.39	3.38	3.38	3.56	3.54
3×Me	1.12	1.12	1.13	1.13	1.14
	1.16	1.16	1.16	1.15	1.16
	1.24	1.24	1.24	1.26	1.26

Table 2. Comparison of diagnostic ^{1}H NMR data of some isoprenoidic bis-THF diols (squalene numeration)



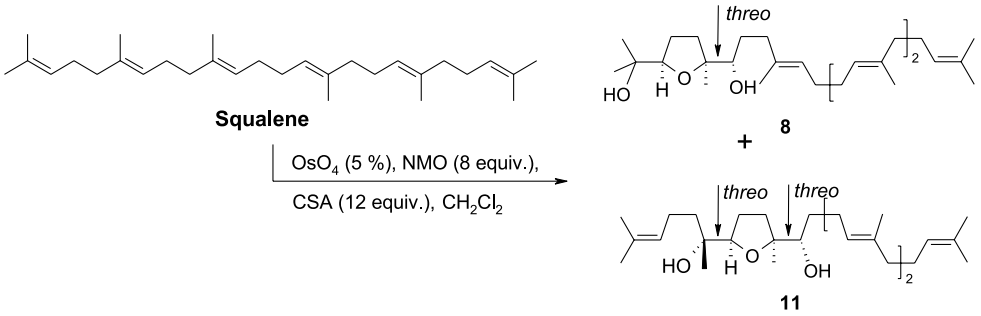
Proton	Bis-THF from squalene (**9**) (*cis–threo–cis–threo*)	Bis-THF from GGA (*cis–threo–cis–threo*)	Bis-THF from squalene (*cis–threo–cis–erhytro*)	Bis-THF from GGA (*cis–threo–cis–erhytro*)
2×C*H*-O(THF)	3.82	3.81	3.82	3.82
	3.92	3.93	3.91	3.92
C*H*-OH	3.41	3.38	3.54	3.52
4×Me	1.09	1.09	1.11	1.11
	1.13	1.13	1.13	1.14
	1.13	1.15	1.14	1.14
	1.25	1.24	1.26	1.26

to 1.26 ppm), observed in both series, of one of the methyl signals.

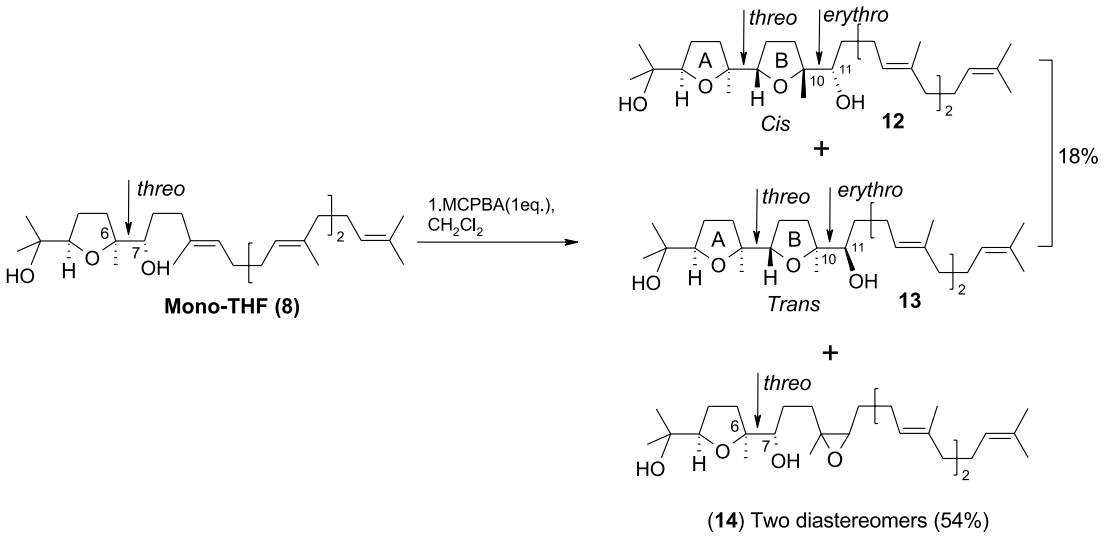
Confirmation of the *cis–threo* configuration of **8** was obtained by synthesis via *cis*-streoselective OsO_4-mediated cyclization of squalene. This reaction has previously been carried out in high yields on 1,5-dienes.[13] Extension of the process to a polyene possessing more than two double bonds has never been carried out before and was expected to give lower cyclization yields of the expected mono-THF **8**. In fact, in principle, three isomeric mono-THF diols could be obtained from the process involving the three different 1,5-dienic substructures present in the polyene; in addition, once formed these products could likely undergo further attack of the oxidant to the other olefinic double bonds of the molecule. Thus, treatment of squalene with catalytic OsO_4 (10 mol%) in the presence of NMO (8 equiv) and CSA (12 equiv) (Scheme 7) afforded only two out of the three expected isomers namely mono-THF **8** and **11**, in a ca. 1:1 ratio (**8**: 7%; **11**: 6% respect to reacted squalene) along with unreacted squalene (ca. 10%). The low yields of these two products were mainly due to the presence of some more polar substances (not further studied), probably obtained, as above anticipated, from the further evolution of the first-formed monocyclized species. It is also to be noted that compounds **8** and **11** are the sole noticeable mono-THF products obtained from the process and the HPLC profile of the reaction mixture showed no peaks other than those relative to these products in the region corresponding to the polarity expected for squalene-derived mono-THF diols.

Unambiguous evidence of the all-*threo* configuration for bis-THF **9** was gained by comparison with the two isomeric C_{10}–C_{11} *erythro* bicyclic tetrahydrofuranyl diol products (*cis–threo–cis–erythro* **12** and *cis–threo–trans–erythro* **13**) obtained from mono-THF **8** by MCPBA epoxidations (1 equiv in CH_2Cl_2, Scheme 8). In particular, this reaction afforded a mixture of the two diastereomeric hydroxy-epoxides **14** (54%) along with the expected two bis-THF compounds **12** and **13** (18% overall), derived from the acid-catalyzed intramolecular *anti*-opening of the hydroxy-epoxides **14**. As expected spectral data and chromatographic (HPLC) properties of these compounds were different from those exhibited by the bis-THF **9** confirming it to possess a C_{10}–C_{11} *threo* relationship.

As for the *threo* relationship within the C_{14}–C_{15} carbon pair it was demonstrated by symmetry considerations on a degradation product obtained from **1** as shown in Scheme 9. In particular, LAH reduction of the pentacyclic bis-lactone **5**, followed by acetylation gave the tris-THF **15**. This compound possesses two tertiary alcohol functions flanking the B and D THF rings, a structural feature also found in **1** that appears to be responsible for the observed reactivity of this compound towards PCC. When compound **15** was subjected to the same PCC degradation conditions employed for the conversion **1**→**5**, the



Scheme 7. Oxidative cyclization of squalene with OsO_4.

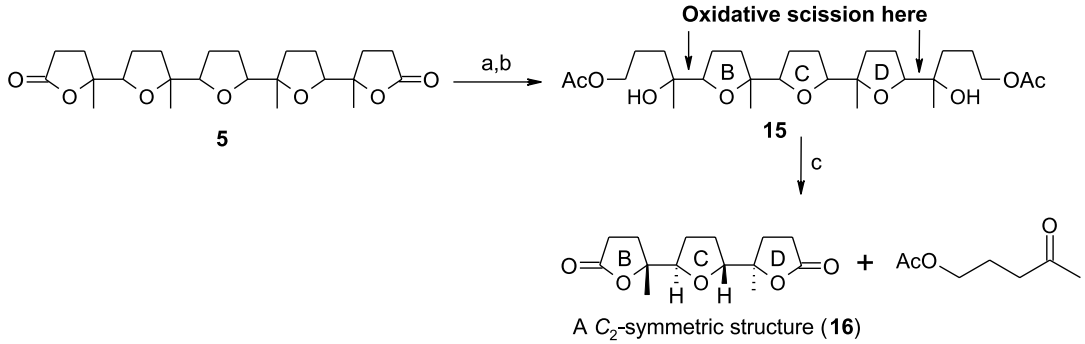


Scheme 8. Synthesis of squalene-derived, C_{10}–C_{11} *erythro*, bis-THF diols **12** and **13**.

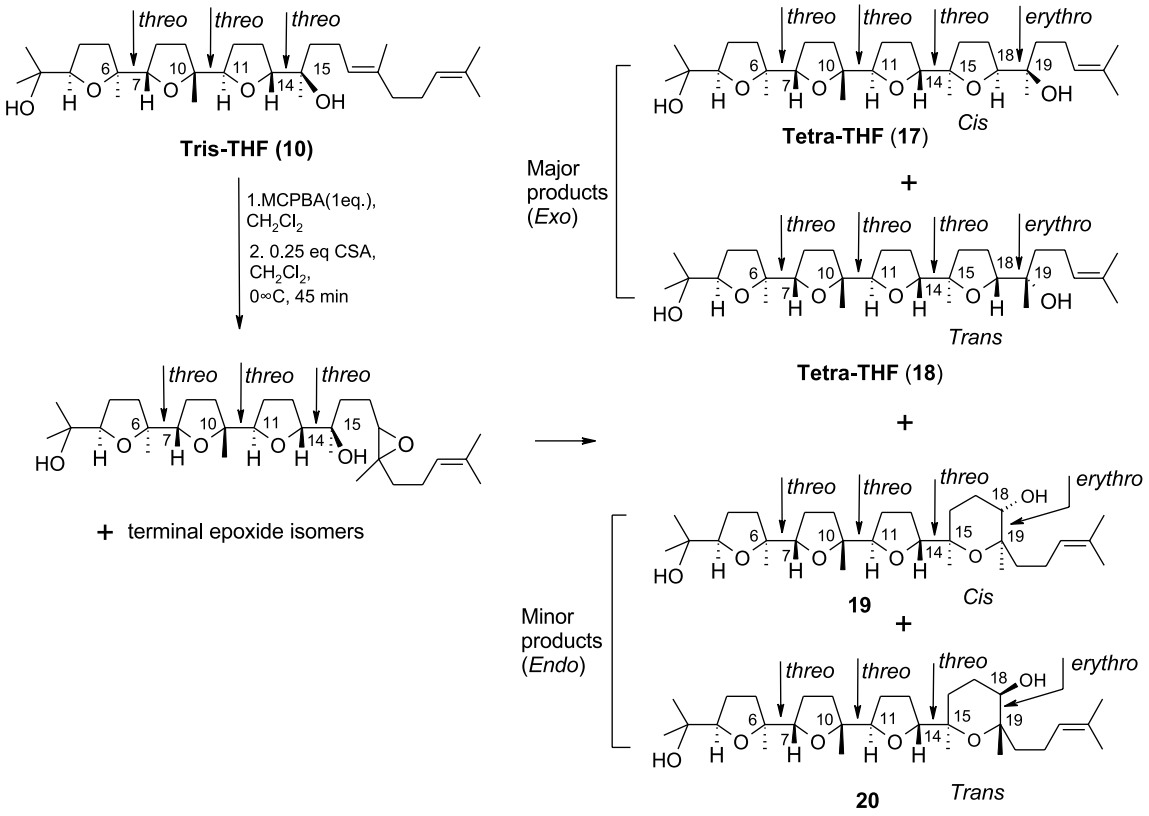
C_2-symmetric bis-lactone **16** was cleanly obtained along with 5-acetoxypentan-2-one.[14] The isolation of the latter compound, derived from the oxidative cleavage of the two side chains bonded to rings B and D, adds further support to the mechanism proposed in Scheme 4, for the PCC-mediated oxidative cleavage of **1**. As required for a C_2-symmetric, *threo–trans–threo*, structure (remind that the C_{10}–C_{11} *threo* had already been established), compound **16** displayed half of the expected NMR signals in both the ^{1}H and ^{13}C NMR spectra.

Finally, determination of the C_{18}–C_{19} *threo* relationship was based on the same reasoning employed to establish the C_{10}–C_{11} relationship. Therefore, tris-THF **10** (Scheme 10), was transformed into the isomeric C_{18}–C_{19} *erythro* tetra-THF **17** and **18** via MCPBA epoxidation, followed by acid-catalyzed (CSA) cyclization. In this case, the acidic treatment was necessary because the first-formed hydroxyepoxides did not spontaneously cyclize to THF. This reaction afforded, besides expected **17** and **18** (major products; *exo*-oxacyclizations) the two ring-D tetrahydropyrane isomers **19** and **20**, derived from *endo*-oxacyclization processes. Their structure was in agreement with the presence of an acetylatable OH group. In addition, the chemical shift and *J*-values for the H-18 proton in the *trans–erythro* isomer **20** (J=10.8, 5.2 Hz; axial proton in a chair conformation) well agree with those reported for a recently synthesized product embodying a 2-hydroxy-*trans*-THP ring in the structure.[15] This proton in the *cis–erythro* isomer **19** displayed *J* values (3 and 4 Hz) in accordance with those expected for an equatorial proton in the chair conformation.

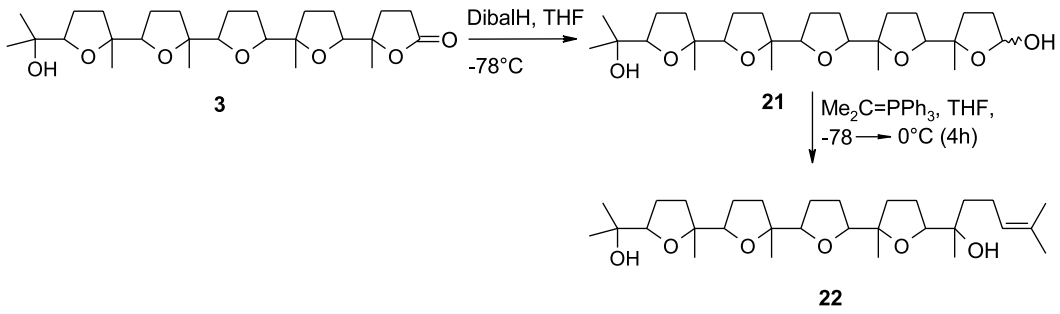
As above mentioned, the partial RuO_4 oxidation of squalene did not furnish the tetra-THF intermediate of the process, even stopping the process at different reaction times (1.5, 3, 6, 7.5 and 10 min). However, this compound (**22**) could be obtained from mono-lactone **3**, in turn derived from penta-THF **1** (Scheme 3), as shown in Scheme 11. In particular, reduction of **3** with DIBALH, followed by Wittig olefination reaction of lactol **21**, in the presence of excess isopropyltripehenylphosphonium iodide, afforded the desired tetra-THF **22**, though in a low 8–10% yield (HPLC). This compound displayed spectral and chromatographyc properties different from those exhibited by the pair **17**/**18**, indicating it to be the all-*threo*-isomer. A double-Wittig reaction of the above type has recently been carried out in high yields on the bis-lactol possessing a gross structure corresponding to bis-lactone **5**, a compound strictly similar to **21**.[16] The low yield in our case is probably ascribable to the presence in **21** of an unprotected alcohol function in the



Scheme 9. Bidirectional PCC oxidative degradation of bis-lactone **5**. Reagents and conditions: (a) $LiAlH_4$, Et_2O, rt, 1 h; (b) Ac_2O, pyridine (**15**, 72% for two steps); (c) PCC (1 equiv), AcOH (150 equiv), celite, CH_2Cl_2 (**16**, 47%).



Scheme 10. Synthesis of squalene-derived, C_{18}–C_{19} *erythro*, tetra-THF diols **17** and **18**.



Scheme 11. Synthesis of squalene derived tetra-THF **22**.

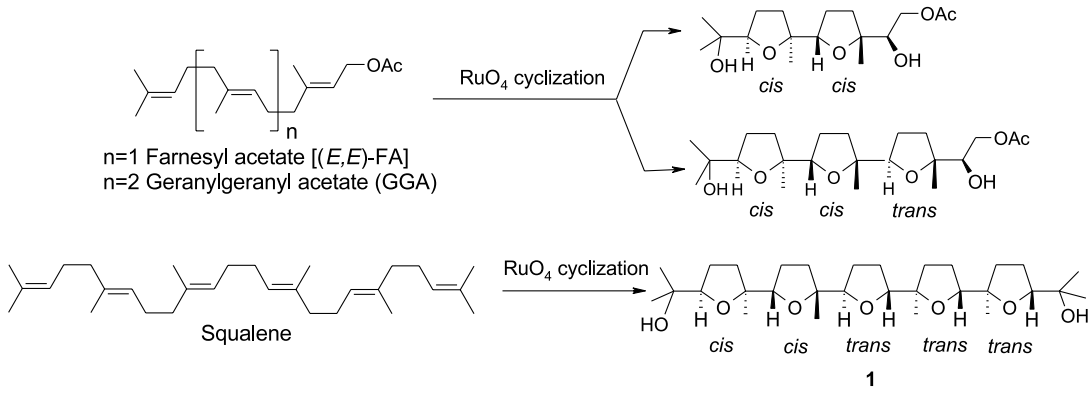
structure, as previously experienced by others in similar cases.[17]

Previous studies on the synthesis of squalene-derived penta-THF products have been reported[18] mostly addressed to the synthesis of glabrescol, a naturally occurring isomer of **1** isolated from *Spatelia glabrescens.*[1] An approach based on exo-selective oxacyclizations of polyepoxides was generally used in these syntheses allowing to obtain all-*erythro* penta-THF materials. The easy access to poly-THF compounds **1**, **8**–**10** and **22** from the low-price, commercially available, squalene opens the way to the preparation of other penta-THF materials, isomeric with **1**, through mixed RuO_4-based and epoxidation/acid-catalysed *anti* cyclization, chemistry, as well as through synthetic strategies previously reported by others.[19] At least some of these materials are expected to possess ionoforic activity[20] or could be used in other studies.

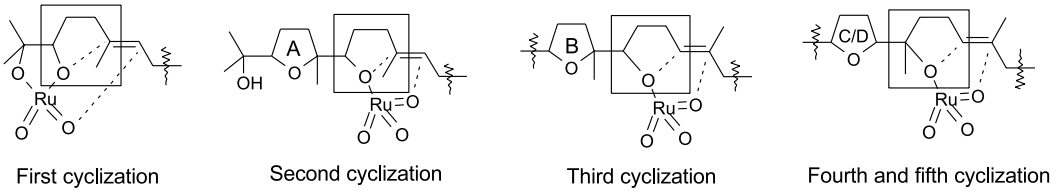
3. Conclusion

The elucidation of the configuration of the above penta-THF gives a further insight into the stereocontrol of the oxidative polycyclization process mediated by RuO_4. However, at the present level of knowledge it seems still hazardous trying to rationalize, either the stereochemical outcome for the oxidation of squalene, or to trace out general rules (if any) to predict the streochemical course of this kind of process.

Some observation should, nevertheless, be made. With reference to **1**, we can notice that the first two cyclizations lead to *cis*-THF rings as it happens for the oxidation of the structurally related polyenes FA and GGA (Scheme 12).[7] Thus, it appears that three consecutive isoprene units bonded head-to-tail invariably give rise to two consecutive *cis*-THF rings. As for the third cyclization step leading to the central cycle in **1**, it proceeds in a *trans* manner as it



Scheme 12. Summary of the poly-THF diol products obtained from the oxidation of isoprenoid polyenes.



Scheme 13. Supposed cyclizing species in the oxidation of squalene.

occurs in the third cyclization of GGA, though a structural difference exists between this compound and squalene (head-to-tail versus tail-to-tail bond of the fourth isoprene unit).

When considering the supposed cyclizing species for each ring-closing step, it should be noted that, apart from the first cyclization, three different, but strictly similar, structural situations subsist which refer to the second, third and fourth/fifth cyclizations (Scheme 13, see boxes). Can only the structural change in the cyclizing portion be responsible for the observed sterochemical control? The formation of *trans*-THF rings from the last two cyclizations, proceeding through similar cyclizing substructures, would seem to back up this hypothesis. However, if this is the case, it remains to be explained why the third cyclization in GGA proceeds with the formation of a *trans*-THF ring, despite the cyclizing portion being similar to that involved in the second cyclizations of FA, GGA and squalene, where a *cis*-ring is formed. Therefore, other factors should be considered such as the THF-*O*-coordination to ruthenium[21] of the THF ring adjacent to the cyclizing portion, as well as its configuration which could arrange the molecule in a conformation facilitating either of the two competing cyclization routes.

4. Experimental

4.1. General methods

All reagents and anhydrous solvents were purchased (Aldrich and Fluka) at the highest commercial quality and used without further purification. Where necessary, flame-dried and argon-charged glassware was used. Reactions were monitored by thin-layer chromatography carried out on precoated silica gel plates (Merck 60, F_{254}, 0.25 mm thick). Merck silica gel (Kieselgel 40, particle size 0.063–0.200 mm) was used for column chromatography. HPLC separations were carried out on a Varian 2510 apparatus equipped with a Waters R403 dual cell differential refractometer using phenomenex 250×10 mm and 250×4.6 mm (both 5μ) columns. NMR experiments were performed on Bruker DRX-600, Bruker WM 400, Varian 300, and Gemini 200 spectrometers in $CDCl_3$. All the 2D-NMR spectra were acquired at 600 MHz in the phase-sensitive mode with the transmitter set at the solvent resonance and TPPI (Time Proportional Phase Increment) used to achieve frequency discrimination in the ω_1 dimension. Standard pulse sequence and phase cycling were used for DQF-COSY, 2D-TOCSY, HSQC, 2D-HSQC-TOCSY, HMBC, 2D-INEPT-INADEQUATE, 2D-INEDAQUATE and ROESY spectra. The NMR data were processed on a Silicon Graphic Indigo2 Workstation using UXNMR software. Proton chemical shifts were referenced to the residual $CHCl_3$ signal (7.26 ppm); ^{13}C NMR chemical shifts were referenced to the solvent (77.0 ppm). Abbreviations for signal coupling are as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. IR spectra were collected on a Jasco FT-IR-430 spectrometer. ESI mass spectrometric analyses were recorded on a Waters Micromass ZQ mass spectrometer equipped with an Electrospray source used in the positive mode. HRMS spectra were recorded on a Voyager DE-PRO mass spectrometer using MALDI-TOF ionization.

For all the reported products the numeration of penta-THF **1** is used.

4.2. Oxidation of squalene. Synthesis of penta-THF 1 and isolation of the mono-, bis-, tris-THF diols intermediates of the process

Squalene (500 mg, 1.22 mmol) was dissolved in the biphasic mixture EtOAc/CH_3CN/H_2O (3:3:1) (140 mL),

then $NaIO_4$ (2.09 g, 9.76 mmol) and a catalytic amount of $RuO_2 \cdot 2H_2O$ (32.5 mg, 0.244 mmol, 20 mol%) were added in sequence under vigorously mechanic stirring at 0 °C. After 30 min excess of a saturated $Na_2S_2O_3 \cdot 5H_2O$ solution was added (50 mL) until the mixture turned to black; then the mixture was filtered and extracted with EtOAc (3× 150 mL) and the combined organic phase was dried over Na_2SO_4 and evaporated. The oily residue was purified by silica gel column chromatography (eluent $CHCl_3$/MeOH, 97:3) followed by HPLC (hexane/ethyl acetate 1:1) to yield **1** (320 mg, 50%, $t_R = 11.5$ min).

When the oxidation of squalene (1.49 g, 3.6 mmol) was stopped after 4 min, the crude reaction mixture was separated by column chromatography (silica, gradient elution from 9:1 hexanes/ethyl ether to ethyl ether) to yield, in the first-eluted fractions, unreacted squalene (450 mg, 30%), the C-7 ketone corresponding to mono-THF **8** (40 mg, 4%) and the C-11 ketone corresponding to bis-THF **9** (55 mg, 5%). A fraction at an intermediate polarity (330 mg) contained mono-THF diol **8**, bis-THF diol **9** and tris-THF diol **10**. A slightly more polar fraction gave pure penta-THF **1** (320 mg, 28%). Compounds **8–10** were separated by HPLC (hexane/EtOAc, 75:25) to yield **8** (45 mg, 5%; $t_R = 21$ min), **9** (52 mg, 4.5%; $t_R = 17.5$ min) and **10** (50 mg, 4.3%; $t_R = 14$ min).

4.2.1. Compound 8. IR (neat): ν_{max} 3396 (br) cm^{-1}. ^1H NMR (200 MHz): δ 5.20 (1H, m, olefinic proton), 5.12 (3H, m, olefinic protons), 3.85 (1H, t, $J = 6.8$ Hz, H-3), 3.39 (1H, dd, $J = 7.7$, 3.6 Hz, H-7), 1.68 (3H, s, Me), 1.59 (12H, s, 4× Me), 1.24, 1.16, 1.12 (3H each, s's, 3×C(O)Me). ^{13}C NMR (100 MHz): δ 135.2, 135.0, 134. 9, 131.2, 124.8, 124.4, 124.25, 124.19, 85.8, 85.2, 71.5, 39.7, 36.6, 35.1, 30.4, 28.3, 28.2, 27.3, 26.8, 26.7, 26.6, 25.6, 24.8, 21.0, 17.6, 16.0. LRMS *m/z* 499 (78, $[M+K]^+$), 483 (100, $[M+Na]^+$). HRMS Calcd for $C_{30}H_{52}O_3Na$ 483.3816, found 483.3831.

4.2.2. Compound 9. IR (neat): ν_{max} 3447 (br) cm^{-1}. ^1H NMR (200 MHz): δ 5.10 (3H, m, olefinic protons), 3.92, 3.82 (1H each, both t, $J = 7.2$ Hz for both, H-3 and H-7), 3.41 (1H, dd, $J = 9.2$, 2.6 Hz, H-11), 1.67 (3H, s, Me), 1.62 (3H, s, Me), 1.59 (6H, s, 2×Me), 1.25 (3H, s, C(O)Me), 1.13 (6H, s, 2×C(O)Me), 1.09 (3H, s, C(O)Me). ^{13}C NMR (100 MHz): δ 135.7, 134.9, 131.3, 124.4, 124.3, 124.1, 86.0, 85.1, 83.4, 83.0, 76.1, 71.6, 39.8, 39.7, 35.1, 34.4, 32.3, 27.9, 27.2, 26.8, 26.7, 25.8, 25.7, 25.0, 23.9, 20.1, 17.7, 16.1, 16.0. LRMS *m/z* 515 (100, $[M+K]^+$), 499 (77, $[M+Na]^+$). HRMS Calcd for $C_{30}H_{52}O_4Na$ 499.3765, found 499.3778.

4.2.3. Compound 10. IR (neat): ν_{max} 3420 (br) cm^{-1}. ^1H NMR (200 MHz): δ 5.16, 5.10 (1H each, both bt, $J = 6.9$ Hz for both, olefinic protons), 3.93–3.77 (4H, overlapped multiplets, H-3, H-7, H-11 and H-14), 1.68 (3H, s, Me), 1.60 (6H, s, 2×Me), 1.24, 1.18, 1.12, 1.05, 0.98 (3H each, s's, 5×C(O)Me). ^{13}C NMR (100 MHz): δ 134.3, 131.4, 125.1, 124.5, 85.5, 85.4, 85.1, 84.7, 83.2, 83.1, 72.9, 71.7, 40.0, 39.7, 35.3, 34.8, 28.98, 28.1, 27.0, 26.8, 26.6, 25.6, 25.2, 24.6, 22.4, 20.9, 19.7, 17.6, 15.9. LRMS *m/z* 531 (100, $[M+K]^+$), 515 (45, $[M+Na]^+$). HRMS Calcd for $C_{30}H_{52}O_5Na$ 515.3714, found 515.3725.

4.2.4. Compound 1. IR (neat): ν_{max} 3466 (br) cm^{-1}. ^1H NMR (600 MHz, attribution by 2D-NMR): δ 3.93 (1H, t, $J = 6.5$ Hz, H-11), 3.87 (1H, t, $J = 7.5$ Hz, H-7), 3.83 (1H, dd, $J = 8.0$, 2.5 Hz, H-18), 3.81, 3.80, (3H, overlapped m's, H-3, H-14, H-22), 2.20, 1.56 (2H, m's, H_2-16), 2.19, 1.51, (2H, m's, H_2-5), 2.10, 1.58 (2H, m's, H_2-20), 1.76, 1.70 (2H, m's, H_2-21), 2.00, 1.56, (2H, m's, H_2-9), 1.90, 1.85 (2H, m's, H_2-17), 1.90, 1.70 (2H, m's, H_2-13), 1.94, 1.92 (2H, m's, H_2-4), 1.88 (4H, m, H_2-8, H_2-12), 1.22 (3H, s, Me-25), 1.20 (3H, s, Me-30), 1.15 (3H, s, Me-26), 1.14 (3H, s, Me-27), 1.11 (3H, s, Me-29), 1.10 (3H, s, Me-24), 1.07 (6H, s, Me-28, Me-1). ^{13}C NMR (150 MHz): δ ^{13}C NMR (150 MHz, attribution by 2D-NMR): δ 87.4 (CH-22), 86.4 (CH-18), 85.4 (CH-3), 85.1 (C-15, CH-11), 84.7 (CH-14), 84.1 (C-6), 83.9 (C-19), 83.7 (C-10), 82.6 (CH-7), 72.0 (CH-3), 70.7 (C-23), 34.9 (CH_2-20), 34.8 (CH_2-16), 34.6 (CH_2-9), 34.1 (CH_2-5), 28.1 (CH_3-30, CH_3-25), 28.2 (CH_2-13), 27.4 (CH_2-17), 27.3 (CH_2-8), 27.2 (CH_2-12), 26.7 (CH_2-21), 26.1 (CH_2-4), 25.1 (CH_3-28), 24.7 (CH_3-29), 24.4 (CH_3-24, CH_3-1), 24.1 (CH_3-26), 23.2 (CH_3-27). LRMS: *m/z* 563 (18, $[M+K]^+$), 547 (24, $[M+Na]^+$), 525 (100, $[M+H]^+$). HRMS Calcd for $C_{30}H_{52}O_7Na$ 547.3612, found 547.3621.

4.3. Bis-*p*-bromobenzoate ester (2)

To a solution of **1** (30 mg, 0.06 mmol) in CH_2Cl_2 (0.5 mL) was added DMAP (221.7 mg, 1.81 mmol) and *p*-bromobenzoyl chloride (219.5 mg, 1.00 mmol) dissolved in CH_2Cl_2 (2 mL) and the resulting solution was stirred at room temperature for 60 h. The mixture was diluted with $CHCl_3$, filtered through a 4 cm silica gel pad. Elution with ethyl ether and evaporation gave a residue that was purified by preparative TLC (hexanes/ethyl ether 75:25) to yield **2** (43 mg, 80%) as a colorless oil.

4.3.1. Compound 2. IR (neat): ν_{max} 1716 cm^{-1}. ^1H NMR (600 MHz, attributions by 2D-NMR): δ 7.83, 7.82, (2H each, d's, $J = 8.8$ Hz for both, aromatic protons), 7.53 (4H, d, $J = 8.4$ Hz, aromatic protons), 4.22 (1H, dd, $J = 7.0$, 7.0 Hz, H-3), 4.18 (1H, dd, $J = 9.2$, 5.8 Hz, H-22), 3.93 (1H, dd, $J = 8.5$, 6.6 Hz, H-7), 3.85, 3.83, 3.73 (3H, overlapped m's, H-11, H-14, H-18), 2.22, 2.01, 1.99, 1.97, 1.94, 1.89, 1.87, 1.84, 1.74, 1.59 (20H, overlapped m's, 10×CH_2), 1.62 (3H, s, Me-30), 1.59 (3H, s, Me-1), 1.58 (3H, s, Me-25), 1.57 (3H, s, Me-24), 1.22 (3H, s, Me-26), 1.15 (3H, s, Me-27), 1.14 (3H, s, Me-29), 1.09 (3H, s, Me-28). ^{13}C NMR (150 MHz, attributions by 2D-NMR): δ 166.4 (C), 165.3 (C), 131.6–131.2 (10 CH, aromatic methines), 128.2 (2 C, aromatic quaternary), 87.0 (C) 86.7 (CH-18), 86.1 (CH-22), 85.6 (CH-11), 85.4 (CH-14), 85.1 (C), 84.8 (C), 84.4 (C), 83.9 (C), 83.7 (C), 82.8 (CH-3), 82.6 (CH-7), 72.2 (C), 70.8 (C), 34.8 (CH_2), 34.7 (CH_2), 33.1 (CH_2), 27.9 (CH_2), 27.3 (2×CH_2), 27.2 (CH_2), 27.1 (CH_2), 26.9 (CH_2), 25.1 (CH_3-29), 24.4 (CH_3-28), 23.7 (CH_3-27), 23.4 (CH_3-26), 23.3 (CH_3-30), 23.0 (CH_3-1), 21.8 (CH_3-25), 21.7 (CH_3-24). LRMS *m/z* 931/929/927 (18, $[M+K]^+$), 915/913/911 (30, $[M+Na]^+$). HRMS Calcd for $C_{44}H_{58}Br_2O_9Na$ 911.2346, found 911.2328.

4.4. Bis-lactone 5 and monolactones 3 and 4

To a solution of **1** (200 mg, 0.38 mmol) in CH_2Cl_2 (8 mL)

was added celite (2 g), PCC (1.9 mmol, 409.5 mg) and AcOH (26.6 mmol, 1.5 mL) and the resulting heterogeneous mixture was stirred at room temperature for 24 h and then loaded on a silica gel column chromatography. Elution with $CHCl_3$/MeOH 9:1 afforded a colourless oil (150 mg). HPLC separation (hexane/EtOAc 60:40) gave unreacted **1** (28 mg, 14%, $t_R=15$ min), monolactones **3** (40 mg, 22%, $t_R=$ 18 min) and **4** (7.5 mg, 4%, $t_R=17$ min), and bis-lactone **5** (43 mg, 26%, $t_R=20.5$ min). When the reaction was carried out with 10 equiv PCC and 150 equiv AcOH, the yield of bis-lactone **5** raised to 70%, yields for compounds **3** and **4** were 6 and 2%, respectively, and a 2% amount of unreacted **1** was recovered.

4.4.1. Compound 3. IR (neat): ν_{max} 3467 (br), 1772 cm^{-1}. ^1H NMR (600 MHz, attributions by 2D-NMR): δ 3.85, 3.87, 3.89 (3H, overlapped, m's, H-7, H-11, H-18), 3.78 (1H, dd, $J=7.6$, 5.4 Hz, H-3), 3.74 (1H, dd, $J=9.7$, 5.5 Hz, H-14), 2.75 (1H, ddd, $J=17.2$, 10.8, 10.8 Hz, H_a-21), 2.40, 2.38, 2.20, 2.16, 1.94, 1.90, 1.87, 1.66, 1.58, 1.52, (19H, overlapped m's, $9\times CH_2$ and H_b-21), 1.30 (3H, s, Me-29), 1.19 (3H, s, Me-25), 1.12 (3H, s, Me-26), 1.11 (3H, s, Me-27), 1.03 (3H, s, Me-1), 1.01 (3H, s, Me-28). ^{13}C NMR (150 MHz, attributions by 2D-NMR): δ 178.2 (C), 86.6 (C) 86.3 (CH-18), 86.2 ((C), 85.3 (CH-3), 85.2 (C), 85.1 (CH-11), 84.8 (CH-14), 84.1 (C), 82.3 (CH-7), 71.8 (C), 34.5 (CH_2), 34.4 (CH_2), 34.3 (CH_2), 32.6 (CH_2), 30.2 (CH_2), 28.3 (CH_2), 28.1(CH_3-25), 27.0 (CH_2), 26.8 (CH_2), 26.6 (CH_2), 26.0 (CH_2), 24.7 (CH_3-1), 23.9 (CH_3-29), 23.8 (CH_3-26), 23.6 (CH_3-28), 22.3 (CH_3-27). LRMS *m/z* 519 (22, $[M+K]^+$), 503 (48, $[M+Na]^+$), 481 (30, $[M+H]^+$). HRMS Calcd for $C_{27}H_{44}O_7Na$ 503.2986, found 503.2971.

4.4.2. Compound 4. IR (neat): ν_{max} 3497 (br), 1772 cm^{-1}. ^1H NMR (600 MHz, attributions by 2D-NMR): δ 3.75, 3.80, 3.83, 3.87, 3.91 (5H, overlapped m's, H-7, H-11, H-14, H-18, H-22), 2.80 (1H, ddd, $J=17.4$, 10.8, 10.8 Hz, H_a-4), 2.45, 2.42, 2.17, 1.95, 1.91, 1.89, 1.88, 1.79, 1.61, 1.59, 1.56, (19H, overlapped m's, $9\times CH_2$ and H_b-4), 1.35 (3H, s, Me-26), 1.20 (3H, s, Me-30), 1.14 (3H, s, Me-27), 1.11 (3H, s, Me-29), 1.10 (3H, s, Me-24), 1.07 (3H, s, Me-28). ^{13}C NMR (150 MHz, attributions by 2D-NMR): δ 178.2 (C), 87.7 (C) 87.4 (CH, C-22), 86.6 (CH-18), 86.0 (C), 85.7 (CH-11), 85.3 (C), 85.1 (CH-14), 84.3 (C), 84.1 (CH-7), 71.1 (C), 34.8 ($2\times CH_2$), 34.6 (CH_2), 32.2 (CH_2), 30.2 (CH_2), 28.1 (CH_2), 27.2 ($2\times CH_2$), 26.6 ($2\times CH_2$), 28.0 (CH_3-30), 24.7 (CH_3-26), 24.7 (CH_3-29), 24.4 (CH_3-24), 24.3 (CH_3-28), 22.2 (CH_3-27). LRMS *m/z* 519 (23, $[M+K]^+$), 503 (47, $[M+Na]^+$), 481 (12, $[M+H]^+$). HRMS Calcd for $C_{27}H_{44}O_7Na$ 503.2986, found 503.2988.

4.4.3. Compound 5. IR (neat): ν_{max} 1770 cm^{-1}. ^1H NMR (600 MHz, attributions by 2D-NMR): 3.85, 3.89, 3.93 (3H, overlapped m's, H-7, H-11, H-18), 3.72 (1H, dd, $J=9.6$, 5.8 Hz, H-14), 2.90, 2.77, 2.43, 2.40, 1.95, 1.89, 1.87, 1.57 1.55, (20H, overlapped m's, $10\times CH_2$),.1.02 (3H, s, Me-28), 1.11 (3H, s, Me-27), 1.33 (6H, s, Me-26, Me-29), ^{13}C NMR (150 MHz, attributions by 2D-NMR): δ 179.6 (C), 178.7 (C), 87.3 (C) 87.1 (C), 86.8 (C), 86.7 (C), 86.3 (CH-18), 85.6 (CH-14), 85.5 (CH-11), 84.3 (CH-7), 34.2 ($2\times CH_2$), 32.5 ($2\times CH_2$), 30.3 ($2\times CH_2$), 28.2 (CH_2), 27.0 (CH_2), 26.7 (CH_2), 26.3 (CH_2), 24.2 (CH_3-29, CH_3-26), 23.7 (CH_3-28), 20.7 (CH_3-27). LRMS *m/z* 475 (100, $[M+K]^+$), 459 (88, $[M+Na]^+$). HRMS Calcd for $C_{24}H_{36}O_7Na$ 459.2359, found 459.2358.

4.5. Mono-THF diols 8 and 11

A solution of squalene (160 mg, 0.39 mmol) in CH_2Cl_2 (40 mL) was treated with *N*-methylmorpholine *N*-oxide monohydrate (420 mg, 3.12 mmol) and CSA (1.09 g, 4.7 mmol) followed by osmium tetroxide (10 mg, 0.039 mmol, 10%) and the solution was stirred at room temperature for 24 h. The reaction was quenched with saturated aqueous sodium thiosolfate and $NaHCO_3$ and the biphasic solution was extracted with CH_2Cl_2 (3×20 mL), then the organic phase was dried with Na_2SO_4 and evaporated. The oily residue was purified by HPLC (hexane/EtOAc 70:30) to give unreacted squalene (16 mg, 10%, $t_R=6$ min), mono-THF diol **8** (13 mg, 7%, $t_R=$ 15.4 min) and mono-THF diol **11** (11 mg, 6%, $t_R=9.6$ min).

4.5.1. Compound 11. IR (neat): ν_{max} 3412 (br) cm^{-1}. ^1H NMR (200 MHz): δ 5.11 (4H, m, olefinic protons), 3.88 (1H, t, $J=7.5$ Hz, H-7), 3.39 (1H, dd, $J=8.7$, 2.7 Hz, H-11), 1.68 (6H, s, $2\times$Me), 1.63, 1.62 (3H each, s's, $2\times$Me), 1.60 (6H, s, $2\times$Me), 1.14, 1.08, (3H each, s's, $2\times$C(O)Me). LRMS *m/z* 499 (97, $[M+K]^+$), 483 (100, $[M+Na]^+$). HRMS Calcd for $C_{30}H_{52}O_3Na$ 483.3816, found 483.3822.

4.6. Bis-THF diols 12 and 13

To a solution of **8** (25 mg, 0.054 mmol) in CH_2Cl_2 (2.5 mL) was added MCPBA (10.2 mg, 0.059 mmol) at 0 °C and the mixture was stirred at this temperature for 1.5 h. Then the reaction was quenched with saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2 (3×10 mL). The organic phase was dried over Na_2SO_4 and evaporated. The residue was purified by HPLC (hexane/EtOAc 65:35) to yield unreacted **8** (6.2 mg, 25%, $t_R=12.5$ min), the two diasteroisomeric C_{10}–C_{11} epoxides **14** (major isomer: 7.7 mg, 30%, $t_R=18.2$ min; minor isomer: 6.1 mg, 24%, $t_R=15.7$ min) and bis-THF diols **12** (2.3 mg, 9%, $t_R=24.6$ min) and **13** (2.3 mg, 9%, $t_R=26.8$ min).

4.6.1. Major epoxide. IR (neat): ν_{max} 3420 (br) cm^{-1}. ^1H NMR (200 MHz): δ 5.12 (3H, m, olefinic protons), 3.85 (1H, t, $J=7.0$ Hz, H-3), 3.38 (1H, dd, $J=5.8$, 4.4 Hz, H-7), 2.70 (1H, t, $J=5.8$ Hz, H-11), 1.67 (3H, s, Me), 1.60, 1.59 (overall 9H, s's, Me), 1.25, 1.24, 1.15, 1.11 (3H each, s's, $4\times$C(O)Me). LRMS *m/z* 515 (48, $[M+K]^+$), 499 (100, $[M+Na]^+$).

4.6.2. Minor epoxide. IR (neat): ν_{max} 3407 (br) cm^{-1}. ^1H NMR (200 MHz): δ 5.18 (2H, m, olefinic protons), 5.07 (1H, t, $J=7.0$ Hz, olefinic proton), 3.85 (1H, t, $J=7.0$ Hz, H-3), 3.38 (1H, dd, $J=5.8$, 4.4 Hz, H-7), 2.70 (1H, t, $J=$ 5.8 Hz, H-11), 1.68 (3H, s, Me), 1.60 (9H, s, $3\times$Me), 1.24 (6H, s, $2\times$C(O)Me), 1.16, 1.12 (3H each, s's, $2\times$C(O)Me). LRMS *m/z* 515 (59, $[M+K]^+$), 499 (100, $[M+Na]^+$).

4.6.3. Compound 12. IR (neat): ν_{max} 3447 (br) cm^{-1}. ^1H NMR (200 MHz): δ 5.12 (3H, m, olefinic protons), 3.85 (2H, m, H-3 and H-7), 3.52 (1H, dd, $J=7.7$, 1.2 Hz, H-11), 1.67, 1.62 (3H each, s's, $2\times$Me), 1.59 (6H, s, $2\times$Me), 1.25, 1.19, 1.12, 1.08 (3H each, s's, $4\times$C(O)Me). LRMS *m/z* 515

(69, $[M+K]^+$), 499 (100, $[M+Na]^+$), 477 (27, $[M+H]^+$).HRMS Calcd for $C_{30}H_{52}O_4Na$ 499.3765, found 499.3771.

4.6.4. Compound 13. IR (neat): ν_{max} 3446 (br) cm^{-1}. ^1H NMR (200 MHz): δ 5.09 (3H, m, olefinic protons), 3.91, 3.82 (1H each, t's, $J=7.4$, 7.0 Hz, respectively, H-3 and H-7), 3.54 (1H, dd, $J=9.6$, 2.8 Hz, H-11), 1.67, 1.62 (3H each, s's, 2×Me), 1.59 (6H, s, 2×Me), 1.26, 1.14, 1.13, 1.11 (3H each, s's, 4×C(O)Me). LRMS *m/z* 515 (49, $[M+K]^+$), 499 (100, $[M+Na]^+$), 477 (12, $[M+H]^+$). HRMS Calcd for $C_{30}H_{52}O_4Na$ 499.3765, found 499.3752.

4.7. Diacetate 15

$LiAlH_4$ (45.5 mg, 1.20 mmol) was added in portions to a solution of bis-lactone **5** (240 mg, 0.55 mmol) in dry ethyl ether (5 mL) at 0 °C. The mixture was allowed to warm to room temperature over 1 h and then quenched by dropwise addition of wet ethyl ether and water. After all inorganic materials were precipitated, the solid was filtered and washed with EtOAc and the organic phase was evaporated to give an oily products that was treated with Ac_2O in pyridine at room temperature for 16 h. Then the mixture was partitioned between EtOAc and 0.1 M HCl (20 mL) and the organic phase was washed with saturated aqueous $NaHCO_3$ (20 mL), dried over Na_2SO_4 and evaporated to give a colorless oil. HPLC purification (hexane/EtOAc 35:65) yielded diacetate **15** (209 mg, 72% for two steps, $t_R=$ 11.0 min).

4.7.1. Compound 15. IR (neat): ν_{max} 3403 (br), 1733 cm^{-1}. ^1H NMR (400 MHz): δ 4.02 (4H, m, 2×CH_2OAc), 3.90 (1H, dd, $J=8.1$, 3.9 Hz, THF proton), 3.87 (1H, dd, $J=7.9$, 5.1 Hz, THF proton), 3.78, (1H, m, THF proton), 3.74 (1H, dd, $J=8.8$, 5.9 Hz, THF proton), 2.05 (6H, s, 2×OAc), 1.12, 1.11, 1.06, 1.02, (3H each, s's, 4×C(O)Me). ^{13}C NMR (100 MHz): δ 171.1, 171.1, 85.8, 85.13, 85.09, 84.7, 84.1, 83.2, 73.6, 71.9, 65.3, 65.0, 36.5, 36.3, 35.1, 34.7, 27.9, 27.2, 26.4, 25.7, 24.7, 24.0, 23.3, 23.1, 21.8, 21.1, 21.0, 20.9. LRMS *m/z* 567 (50, $[M+K]^+$), 551 (100, $[M+Na]^+$), 477 (26, $[M+H]^+$). HRMS Calcd for $C_{28}H_{48}O_9Na$ 551.3197, found 551.3200.

4.8. Bis lactone 16

To a solution of **15** (30 mg, 0.056 mmol) in CH_2Cl_2 (1.5 mL) was added celite (300 mg), PCC (120.7 mg, 0.56 mmol) and AcOH (480 μL, 8.4 mmol) and the resulting heterogeneous mixture was stirred at room temperature for 24 h and the loaded on a silica gel column. Elution with $CHCl_3$/MeOH 9:1 afforded a colorless oil. HPLC purification gave **16** (6.7 mg, 47%, $t_R=$23.6 min), 2-oxo-penten-1-yl acetate (5.6 mg, 70%, $t_R=$11.3 min)[15] along with a monolactone acetate product derived from the cleavage of only one of the two side chains (1.7 mg, 7.5%, $t_R=$27.8 min).

4.8.1. Compound 16. IR (neat): ν_{max} 3527 (br), 1770 cm^{-1}. ^1H NMR (200 MHz): δ 3.88 (2H, ddd, $J=9.3$, 5.2, 3.2 Hz, H_{11} and H_{14}), 2.68 (2H, ddd, $J=17.8$, 10.3, 9.3 Hz, H_a-8 and H_a-17), 2.50 (2H, ddd, $J=17.7$, 10.8, 3.7 Hz, H_b-8 and H_b-17), 2.36 (2H, ddd, $J=16.5$, 9.9, 3.7 Hz, H_a-9 and H_a-16), 1.97 (6H, m, H_b-9 and H_b-16, H_2-12 and H_2-13), 1.31 (6H, s, 2×C(O)Me). ^{13}C NMR (50 MHz): δ 177.2, 85.9, 85.7, 32.4, 29.7, 26.5, 23.5. LRMS *m/z* 307 (43, $[M+K]^+$), 291 (100, $[M+Na]^+$), 269 (60, $[M+H]^+$). HRMS Calcd for $C_{14}H_{20}O_5Na$ 291.1209, found 291.1210.

4.8.2. Monolactone acetate. IR (neat): ν_{max} 3412 (br), 1772, 1737 cm^{-1}. ^1H NMR (200 MHz): δ 4.08 (2H, m, CH_2OAc), 3.96–3.78 (3H, m, THF protons), 2.04 (s, acetate), 1.33, 1.09, 1.02 (3H each, s's, 3×C(O)Me). LRMS *m/z* 437 (43, $[M+K]^+$), 421 (100, $[M+Na]^+$), 399 (35, $[M+H]^+$).

4.9. Tetra-THF diols 17 and 18

To a solution of tris-THF **10** (25 mg, 0.051 mmol) in CH_2Cl_2 (2.5 mL) was added MCPBA (10 mg, 0.056 mmol) at 0 °C and the mixture was stirred at the same temperature for 1.5 h. After the reaction was quenched with saturated aqueous $NaHCO_3$ the mixture was extracted with CH_2Cl_2 (3×10 mL), the organic phase was dried over Na_2SO_4 and evaporated. The residue was purified by HPLC (hexane/EtOAc 80:20) to give unreacted **10** (7.5 mg, 30%, $t_R=$ 10.2 min), a mixture of unseparable C_{18}–C_{19} diastereomeric epoxides contaminated by a 20% amount of terminal epoxides (7.3 mg, 47%, $t_R=30.5$ min) along with a mixture of the two diastereomeric terminal epoxides (3.6 mg, 25%, $t_R=29$ min).

4.9.1. Mixture of C_{18}–C_{19} epoxides. IR (neat): ν_{max} 3423 (br) cm^{-1}. ^1H NMR (200 MHz, data from the mixture contaminated by terminal epoxides): δ 5.07 (t, $J=7.0$ Hz, olefinic protons), 3.95–3.70 (m, H-3, H-7, H-11 and H-14), 2.70 (t, $J=6.2$ Hz, H-18), 1.67, 1.61, (s's, 2×Me), 1.27, 1.24, 1.17, 1.12 1.02, 0.97 (s,s, 6×C(O)Me). LRMS: *m/z* 547 (43, $[M+K]^+$), 531 (100, $[M+Na]^+$).

4.9.2. Mixture of terminal epoxides. IR (neat): ν_{max} 3420 (br) cm^{-1}. ^1H NMR (200 MHz): δ 5.20 (bt, $J=7.0$ Hz, olefinic protons), 4.09 (bs, OH), 3.94–3.78 (m, H-3, H-7, H-11 and H-14), 2.70 (t, $J=6.4$ Hz, H-22), 1.62, (s, Me), 1.29, 1.25, 1.24, 1.17, 1.12 1.04, 0.98 (3H each, s's, 7×C(O)Me). LRMS: *m/z* 547 (32, $[M+K]^+$), 531 (100, $[M+Na]^+$).

To the mixture of tris-THF epoxides obtained as above (7 mg, 0.013 mmol) in CH_2Cl_2 (1 mL) was added CSA (0.75 mg, 0.0032 mmol) at 0 °C and the resulting solution was stirred at the same temperature for 45 min. Then the reaction was quenched with saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2 (3×5 mL). The organic phase was dried over Na_2SO_4 and evaporated. The residue was purified by HPLC (hexane/EtOAc 75:25) to give two diastereomeric tetra-THF diols (**17**/**18**, *exo* products) (major isomer: 2.9 mg, 45%, $t_R=14.5$ min; minor isomer: 1.5 mg, 23%, $t_R=16.5$ min) along with the tetrahydropyrane-containing isomers **19** and **20** *(endo products)* (major isomer: 1.2 mg, 18%, $t_R=17.7$ min; minor isomer: 0.9 mg, 14%, $t_R=$ 19.5 min).

4.9.3. Major tetra-THF diol. IR (neat): ν_{max} 3403 (br) cm^{-1}. ^1H NMR (600 MHz): δ 5.12 (1H, bt, $J=6.7$ Hz, olefinic proton), 3.95 (1H, dd, $J=7.0$, 7.0 Hz, THF proton),

3.88 (1H, dd, $J=7.5$, 7.5 Hz, THF proton), 3.80, 3.77, 3.75 (3H, overlapped m's, 3×THF protons), 1.69, 1.62 (3H each, s's, 2×Me), 1.23, 1.18, 1.15, 1.14, 1.08 1.07 (3H each, s's, 6×C(O)Me). ^{13}C NMR (150 MHz): δ 131.6 (C), 124.9 (CH), 86.7 (CH), 85.2 (C), 85.1 (CH), 84.8 (CH), 84.6 (CH), 84.2 (C), 84.0 (C), 82.3 (CH), 72.1 (C), 71.7 (C), 34.5 (CH_2), 33.8 (CH_2), 29.7 (CH_2), 29.5 (CH_2), 29.1 (CH_2), 28.0 (Me), 27.4 (CH_2), 26.8 (CH_2), 26.7 (CH_2), 26.0 (CH_2), 25.6 (CH_3), 25.1 (CH_3), 24.2 (CH_3), 24.0 (CH_2), 23.9 (2×CH_3), 22.7 (CH_3), 17.6 (CH_3). LRMS: *m/z* 547 (40, $[M+K]^+$), 531 (100, $[M+Na]^+$), 509 (15, $[M+H]^+$). HRMS Calcd for $C_{30}H_{52}O_6Na$ 531.3663, found 531.3673.

4.9.4. Minor tetra-THF diol. IR (neat): ν_{max} 3395 (br) cm^{-1}. ^{1}H NMR (600 MHz): δ 5.11 (1H, bt, $J=7.9$ Hz, olefinic proton), 3.98 (1H, bdd, $J=8.4$, 8.4 Hz, THF proton), 3.89–3.71 (4H, overlapped m's, 4×THF protons), 1.68, 1.61 (3H each, s's, 2×Me), 1.20, 1.19, 1.17, 1.13, 1.08, 1.06 (3H each, s's, 6×C(O)Me). ^{13}C NMR (150 MHz): δ 131.1 (C), 124.6 (CH), 84.2 (C), 83.9 (C), 83.7 (C), 72.7 (C), 72.2 (C), 35.2 (CH_2), 34.7 (CH_2), 29.9 (2×CH_2), 27.7 (2×CH_2), 27.5 (2×CH_2), 26.8 (2×CH_2), 85.5 (CH), 85.4(CH), 85.1 (CH), 84.9 (CH), 82.9 (CH), 28.3 (CH_3), 24.9 (CH_3) 24.6 (CH_3), 24.5 (CH_3), 24.0 (2×CH_3), 20.9 (CH_3), 17.4 (CH_3). LRMS: *m/z* 547 (20, $[M+K]^+$), 531 (100, $[M+Na]^+$), 509 (78, $[M+H]^+$). HRMS Calcd for $C_{30}H_{52}O_6Na$ 531.3663, found 531.3649.

4.9.5. Major THP-containing isomer (19). IR (neat): ν_{max} 3412 (br) cm^{-1}. ^{1}H NMR (400 MHz): δ 5.08 (1H, bt, $J=$ 7.0 Hz, olefinic proton), 4.03–3.68 (4H, overlapped m's, 4×THF protons), 3.39 (1H, dd, $J=4.0$, 3.0 Hz, H-19), 1.68, 1.61 (3H each, s's, 2×Me), 1.24, 1.21, 1.16, 1.12, 1.09, 1.08 (3H each, s's, 6×C(O)Me). LRMS: *m/z* 547 (27, $[M+K]^+$), 531 (100, $[M+Na]^+$), 509 (56, $[M+H]^+$). HRMS Calcd for $C_{30}H_{52}O_6Na$ 531.3663, found 531.3674.

4.9.6. Minor THP-containing isomer (20). IR (neat): ν_{max} 3407 (br) cm^{-1}. ^{1}H NMR (400 MHz): δ 5.09 (1H, bt, $J=$ 7.0 Hz, olefinic proton), 3.94–3.70 (4H, overlapped m's, 4×THF protons), 3.47 (1H, dd, $J=10.8$, 5.2 Hz, H-19), 1.67, 1.60 (3H each, s's, 2×Me), 1.22, 1.18, 1.14, 1.13, 1.10, 1.06 (3H each, s's, 6×C(O)Me). LRMS: *m/z* 547 (14, $[M+K]^+$), 531 (100, $[M+Na]^+$), 509 (55, $[M+H]^+$). HRMS Calcd for $C_{30}H_{52}O_6Na$ 531.3663, found 531.3667.

4.10. Synthesis of tetra-THF diol 22

To a solution of monolactone **3** (100 mg, 0.21 mmol) in dry THF (1.5 mL) DIBALH (1.0 M in THF, 0.42 mmol, 450 μL) was added at −78 °C and the solution was stirred at the same temperature for 1 h. Saturated aqueous NH_4Cl was added and the mixture was extracted with $CHCl_3$ (3× 10 mL) then dried over Na_2SO_4 and evaporated to give crude lactol **21** (70 mg, 70%). Further HPLC purification ($CHCl_3$, $t_R=22$ min) afforded pure **21**.

4.10.1. Compound 21. IR (neat): ν_{max} 3420 (br) cm^{-1}. ^{1}H NMR (400 MHz): δ 5.33 (1H, d, $J=3.8$ Hz, H-22), 3.98–3.74 (5H, overlapped m's, 5×THF protons), 1.22 (3H, s, C(O)Me), 1.14, 1.13 (6H each, s's, 4×C(O)Me), 1.06 (3H, s, C(O)Me). ^{13}C NMR (100 MHz): δ 98.8, 85.0, 84.9, 84.9, 84.6, 83.9, 83.7, 83.6, 82.4, 71.6, 34.5, 34.3, 34.2, 33.9, 31.3, 28.0, 27.8, 27.0, 27.0, 26.6, 25.8, 24.7, 24.2, 24.0, 23.8, 23.7, 22.6. LRMS: *m/z* 521 (12, $[M+K]^+$), 505 (16, $[M+Na]^+$). HRMS Calcd for $C_{27}H_{46}O_7Na$ 505.3142, found 505.3129.

n-Butyllithium (1.6 M in hexane, 0.46 mmol) was dropwise added through syringe to a solution of isopropyltriphenilphosphonium iodide (200 mg, 0.46 mmol) in THF (2 mL) at 0 °C. After stirring at this temperature for 20 min, the deep red solution was brought to −78 °C and then transferred via cannula to a solution of **21** (63 mg, 0.13 mmol) in THF (2 mL) at −78 °C. The reaction mixture was allowed to warm to room temperature over 4 h. Then aqueous NH_4Cl was added and the mixture was extracted with ethyl ether (3×30 mL), the organic phase was dried over Na_2SO_4 and evaporated. The residue was purified by HPLC (hexane/EtOAc 30:70) to give unreacted **21** (19 mg, 30%, $t_R=$ 36 min) and terta-THF **22** (6 mg, 8–10% respect to reacted **21**, $t_R=16.5$ min).

4.10.2. Compound 22. IR (neat): ν_{max} 3461 (br) cm^{-1}. ^{1}H NMR (600 MHz): δ 5.11 (1H, bt, $J=7.0$ Hz, olefinic proton), 3.95 (1H, t, $J=7.1$ Hz, THF proton), 3.88 (1H, t, $J=7.6$ Hz, THF proton), 3.82–3.80 (3H, overlapped m's, 3×THF protons), 1.68, 1.61 (3H each, s's, 2×Me), 1.22, 1.15, 1.12, 1.08, 1.07, 1.06 (3H each, s's, 6×C(O)Me). ^{13}C NMR (150 MHz) δ 131.1 (C), 124.6 (CH), 86.2 (C), 85.3 (CH), 85.0 (C), 84.7 (CH), 84.6 (2×CH), 84.5 (C), 82.2 (CH), 71.7 (C), 71.2 (C), 34.3 (CH_2), 34.2 (CH_2), 33.7 (CH_2), 29.1 (CH_2), 27.7 (CH_3), 27.6 (CH_2), 26.7 (2×CH_2), 26.3 (CH_2), 26.2 (CH_2), 25.6 (CH_3), 25.5 (CH_2), 24.4 (CH_3), 24.2 (CH_3), 23.9 (CH_3), 23.7 (CH_3), 20.9 (CH_3), 17.4 (CH_3). LRMS: *m/z* 547 (15, $[M+K]^+$), 531 (20, $[M+Na]^+$), 509 (16, $[M+H]^+$). HRMS Calcd for $C_{30}H_{52}O_6Na$ 531.3663, found 531.3675.

Acknowledgements

We are grateful to MURST, Italy (PRIN 2003), for financial support in favour of this investigation and to the 'Centro di Metodologie Chimico-Fisiche dell'Università di Napoli 'Federico II' ' for NMR and MS facilities.

References and notes

1. Harding, W. W.; Lewis, P. A.; Jacobs, H.; McLean, S.; Reynolds, W. F.; Tay, L.-L.; Yang, J.-P. *Tetrahedron Lett.* **1995**, *36*, 9137–9140.
2. Itokawa, H.; Kishi, E.; Morita, H.; Takeya, K.; Iitaka, Y. *Chem. Lett.* **1991**, 2221–2222.
3. Suzuki, T.; Suzuki, M.; Kurusaki, A.; Matsumoto, T.; Kato, A.; Imanaka, Y.; Kurosawa, E. *Tetrahedron Lett.* **1985**, *38*, 1329–1332.
4. (a) Koert, U.; Stein, M.; Harms, K. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1180–1182. (b) Koert, U.; Stein, M.; Wagner, H. *Liebigs Ann.* **1995**, 1415. (c) Wagner, H.; Harms, K.; Koert, U.; Meder, S.; Boheim, G. *Angew. Chem., Int. Ed.* **1996**, *35*, 2643–2646. (d) Cross, G. G.; Fyles, T. M.; James, T. D.; Zojaji, M. *Synlett* **1993**, 449–480.

5. (a) Zeng, L.; Oberlies, N. H.; Shi, G.; Gu, Z.-M.; He, K.; McLaughlin, J. *Nat. Prod. Rep.* **1996**, *13*, 275–306. (b) Alali, F. Q.; Liu, X.-X.; McLaughlin, J. *Nat. Prod. Rep.* **1999**, *62*, 504–540.
6. For some selected examples of *Annonaceous* acetogenin syntheses see: (a) Cecil, A. R. L.; Brown, R. C. D. *Org. Lett.* **2002**, *4*, 3715–3718. (b) Koert, U. *Tetrahedron Lett.* **1999**, *40*, 5979–5982. (c) Trost, B. M.; Calkins, T. L.; Bochet, C. G. *Angew. Chem., Int. Ed.* **1997**, *36*, 2632–2635. (d) Marshall, J. A.; Hinkle, K. W. *J. Org. Chem.* **1997**, *62*, 5989–5995. (e) Marshall, J. A.; Chen, M. *J. Org. Chem.* **1997**, *62*, 5996–6000.
7. (a) Bifulco, G.; Caserta, T.; Gomez-Paloma, L.; Piccialli, V. *Tetrahedron Lett.* **2002**, *43*, 9265–9269. (b) Bifulco, G.; Caserta, T.; Gomez-Paloma, L.; Piccialli, V. *Tetrahedron Lett.* **2003**, *44*, 5499–5503.
8. Keinan, E.; Sinha, S. C. *Pure Appl. Chem.* **2002**, *74*, 93–105 and in particular Ref. 21 therein.
9. Towne, T. B.; McDonald, F. E. *J. Am. Chem. Soc.* **1997**, *119*, 6022–6028.
10. (a) Baskaran, S.; Islam, I.; Chandrasekaran, S. *J. Org. Chem.* **1990**, *55*, 891–895. (b) Waddel, T. G.; Carter, A. D.; Miller, T. J. *J. Org. Chem.* **1992**, *57*, 381–383.
11. See Ref. 10 andSinha, S. C.; Sinha, A.; Sinha, S. C.; Keinan, E. *J. Am. Chem. Soc.* **1997**, *119*, 12014–12015.
12. McDonald, F. E.; Schultz, C. C. *Tetrahedron* **1997**, *53*, 16435–16448 and references therein.
13. Donohoe, T. J.; Butterworth, S. *Angew. Chem., Int. Ed.* **2003**, *42*(8), 948–951.
14. Hawskley, D.; Griffin, D. A.; Leeper *J. Chem. Soc., Perkin Trans 1* **2001**, 144–148.
15. Bravo, F.; McDonald, F. E.; Neiwert, W. A.; Do, B.; Hardcastle, K. I. *Org. Lett.* **2003**, *5*, 2123–2126.
16. Morimoto, Y.; Iwai, T.; Kinoshita, T. *Tetrahedron Lett.* **2001**, *44*, 6307–6309.
17. Maerchker, A. In *Organic Reactions*, Wiley: New York, 1965; pp 270–490.
18. (a) Xiong, Z.; Corey, J. *J. Am. Chem. Soc.* **2000**, *122*, 4831–4832. (b) Xiong, Z.; Corey, J. *J. Am. Chem. Soc.* **2000**, *122*, 9328–9329. (c) Morimoto, Y.; Iwai, T.; Kinoshita, T. *J. Am. Chem. Soc.* **2000**, *122*, 7124–7125. (d) Hioki, H.; Kanehara, C.; Ohnishi, Y.; Umemori, Y.; Sakai, H.; Yoshio, S.; Matsushita, M.; Kodama, M. *Angew. Chem., Int. Ed.* **2000**, *39*, 2552–2554.
19. See Ref. 8 and: Koert, U.; Stein, M.; Wagner, H. *Chem. Eur. J.* **1997**, *3*, 1170–1179 and references cited therein.
20. (a) Morimoto, Y.; Iwai, T.; Yoshimura, T.; Takamasa, K. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2005–2010. (b) Wang, X.; Erickson, S. D.; Iimori, T.; Still, W. C. *J. Am. Chem. Soc.* **1992**, *114*, 4128–4137 and references cited therein.
21. (a) Sinha, S. C.; Keinan, E.; Sinha, S. C. *J. Am. Chem. Soc.* **1998**, *120*, 9076–9077. (b) Morimoto, Y.; Iwai, T. *J. Am. Chem. Soc.* **1998**, *120*, 1633–1634. (c) Morimoto, Y.; Iwai, T.; Kinoshita, T. *J. Am. Chem. Soc.* **1999**, *121*, 6792–6797.

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A free radical cyclization approach to indolo-benzodiazocine derivatives

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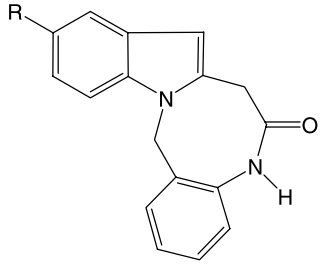
Received 30 July 2004; revised 11 October 2004; accepted 5 November 2004

Available online 8 December 2004

Abstract—A versatile route to indolo[2,1-*d*][1,5]benzodiazocine derivatives has been developed based on a free radical cyclization approach from 1-substituted indole derivatives with appropriately positioned haloacetamide functionalities. Fair yields of the indole- and dihydroindole-fused eight-membered ring derivatives were obtained from *N*-substituted iodo- and bromoacetamide precursors. Synthetic and mechanistic aspects of the regiospecific cyclization are discussed.

1. Introduction

Free radical cyclization is now a useful and well established methodology in heterocyclic synthesis.[1,2] The more successful intramolecular radical cyclizations proceed to form five-membered rings,[3–5] and these are more general than those forming six-membered[6] or seven-membered[7,8] rings. However, cyclization leading to indole fused eight-membered rings is less well investigated.



1 R = H
2 R= OCH_3
3 R= F

As part of a wider program to identify structurally novel antibacterial compounds with, hopefully, new modes of action to combat resistance problems, we became interested in the indolo[2,1-*d*][1,5]benzodiazocinones **1**–**3**, with an indole fused eight-membered ring system. Previous studies in our laboratory had revealed that this system could be accessed in low yields, together with indolo-benzodiazoninones and pyrrolo-indoloquinazolinones, by the photolysis of chloroacetamides.[9]

In an effort to develop a viable regioselective route to indolo-benzodiazocinone derivatives, thermal radical cyclizations via an 8-*exo-trig* process of precursor 1-indolyl haloacetamides, using tributyltin hydride, have been investigated and the results are reported in this paper.

2. Results and discussion

The indolyl substrates required for the radical cyclizations were prepared starting from the 1-substituted indole derivatives **4**–**6**.[9] Then, *N*-alkylation with the appropriate alkyl halides, benzyl halides or reductive amination, followed by acylation of the resulting amines **7**–**13** with the acyl halides, gave the corresponding haloacetamides **14**–**16**. Reaction of the chloroacetamides **14a–h** with sodium iodide in acetonitrile gave the iodoacetamides **15a–h** (Scheme 1). The iodoacetamide **15i** was prepared by direct alkylation of the chloroacetamide **14i** with methyl iodide in DMF after amide anion formation (Scheme 2).

Free radical cyclization reactions of the haloacetamides **14**–**16** were then induced using a 40 mM solution of tributyltin hydride (Bu_3SnH) in the presence of azobisisobutyronitrile (AIBN) in various solvents at reflux.

A slow addition of tributyltin hydride to a boiling toluene solution of the haloacetamides gave the indole fused eight-membered ring compounds **17**–**18** in fair yields, and also the simple reduced compounds **19** (Scheme 1, Table 1). Dehydrogenation of **18a** was achieved in a separate aromatization reaction with Pd/C in boiling toluene to

Keywords: Fused benzodiazocines; Indoles; Radical cyclization; eight-membered ring.
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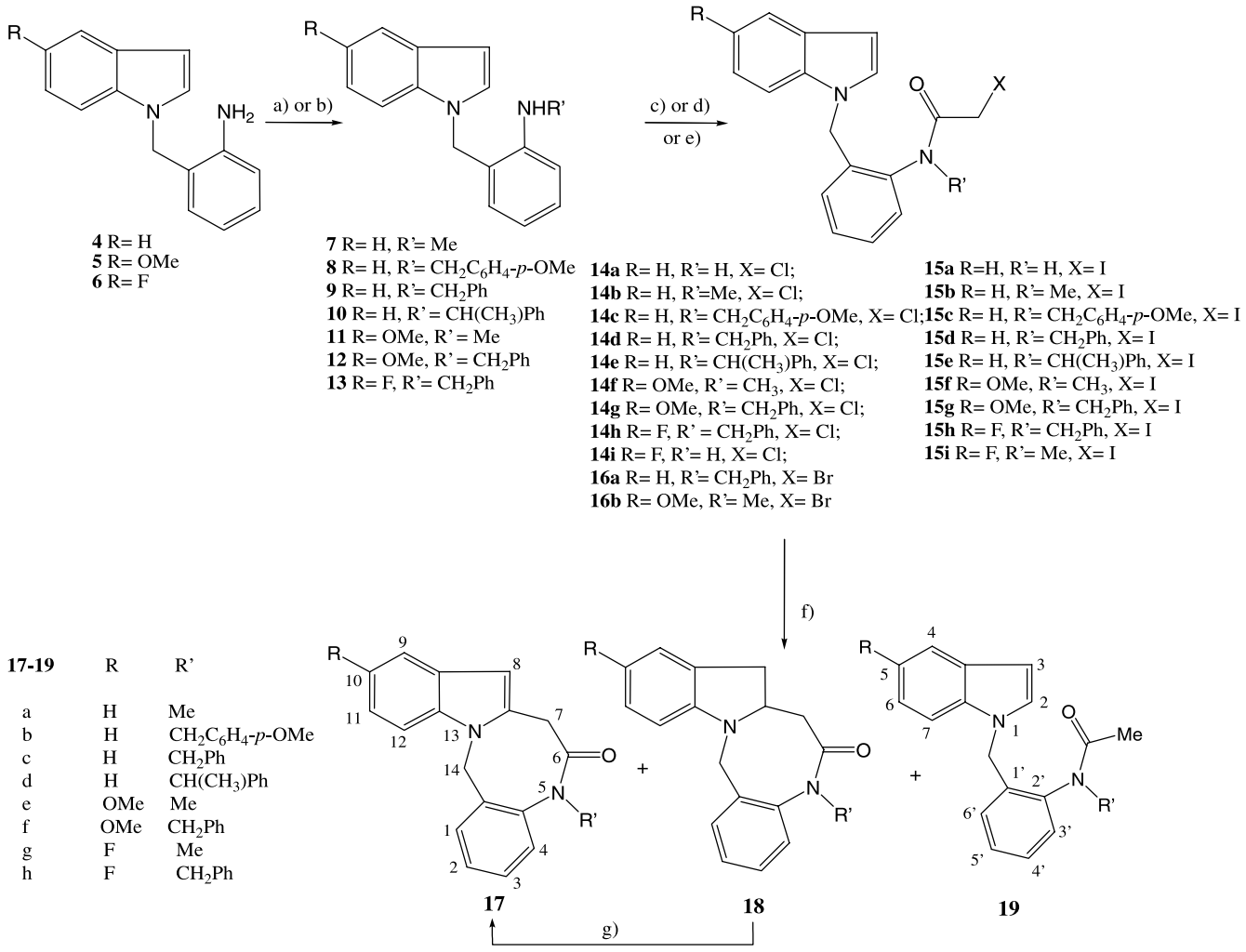
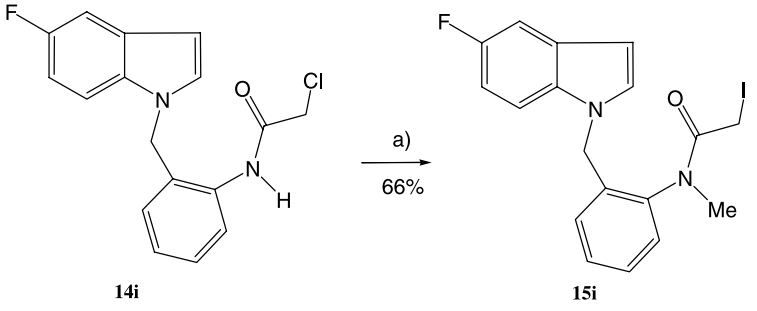
doi:10.1016/j.tet.2004.11.007



Scheme 1. (a) (i) K_2CO_3, (ii) R′X, THF; (b) (i) R′CHO, 5 M HCl-MeOH, (ii) $NaBH_3CN$, MeOH; (c) (i) K_2CO_3, 5 °C, DCM (ii) $ClCH_2COCl$, r.t., 16 h.; (d) (i) NaH (ii) $ClCH_2COCl$, THF, r.t., 16 h.; (e) (i) NaH (ii) $BrCH_2COCl$, THF, r.t., 16 h.; (f) Bu_3SnH, AIBN, boiling solvents; (g) Pd/C, toluene, heat.



Scheme 2. (a) (i) NaH, (ii) MeI, DMF.

give **17a** in 50% yield. In the ^1H NMR spectra of the fused benzodiazocinones **17**, the H-8 proton appeared as a singlet signal at about δ 6.28–6.37, which was clearly separated from other signals. In contrast, the signal attributed to the H-8 protons in compounds of type **18** appeared as a multiplet around δ 2.40–2.50 due to coupling with H-7a. In the ^1H NMR of the acetamides **19**, a diagnostic singlet signal integrating for three protons was apparent at around δ 1.80.

An increase in the cyclization product yields was generally observed as the steric bulk of the substituent group R′ on the haloacetamide nitrogen was increased.[10,11] When the haloacetamides with R′=H (Entries 1 and 3, Table 1) were reacted, the products **19** of simple reduction were obtained.

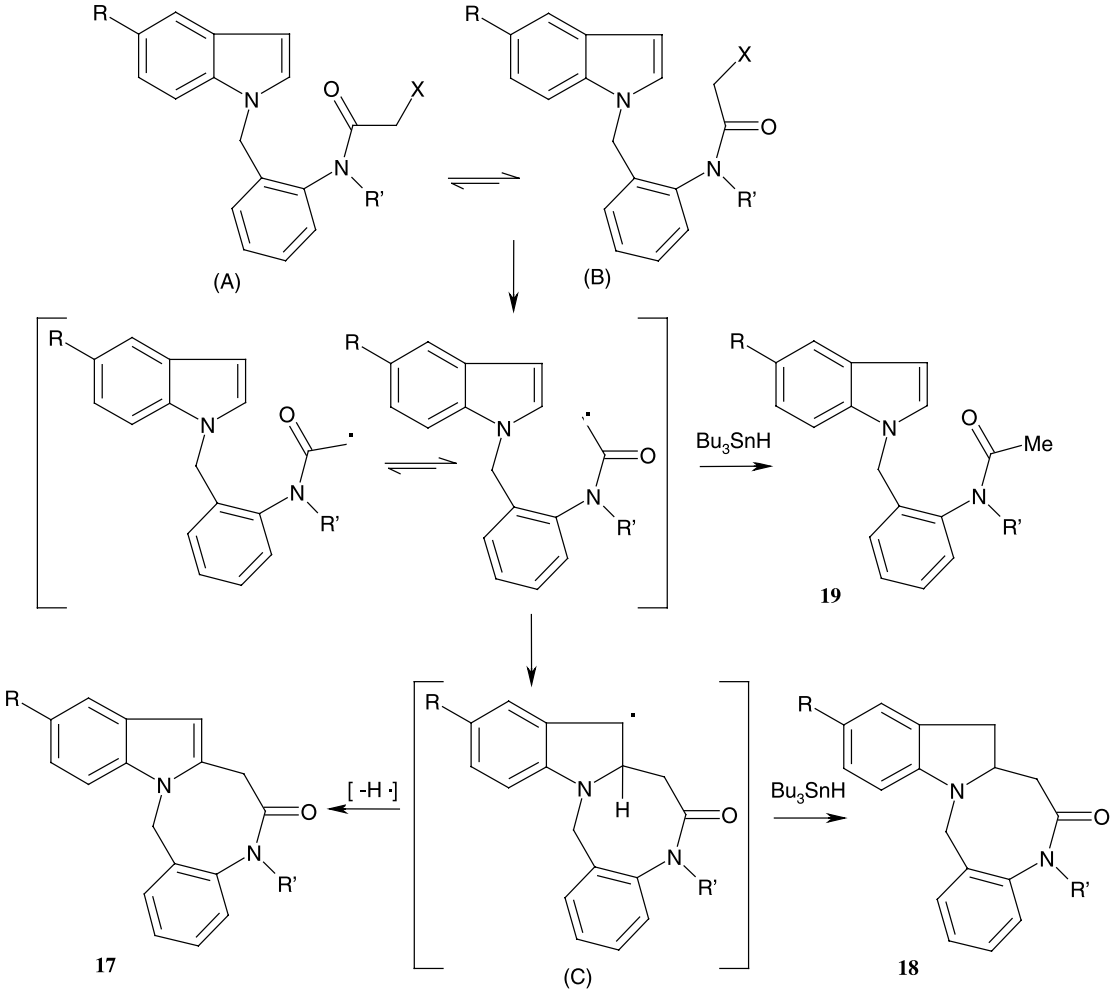
However, when a sufficiently large group (R′) was employed as a substituent on the amide nitrogen, the

Table 1. Radical cyclization of haloacetamides **14–16** using Bu_3SnH/AIBN in boiling toluene

Entry	Substate	R	R′	X	mmol	Yield (%)		
						17	**18**	**19**
1	**14a**	H	H	Cl	0.16	—	—	82
2	**14b**	H	Me	Cl	0.20	—	—	83
3	**15a**	H	H	I	0.21	—	—	85
4	**15b**	H	Me	I	0.13	20	60	20
5	**15c**	H	$CH_2C_6H_4$-*p*-OMe	I	0.33	13	11	7
6	**15d**	H	CH_2Ph	I	0.08	25	43	10
7	**15e**	H	$CH(CH_3)Ph$	I	0.07	22	26[a]	
8	**15f**	OMe	Me	I	0.19	50	—	20
9	**15g**	OMe	CH_2Ph	I	0.12	25	—	<1
10	**15i**	F	Me	I	0.14	35	—	17
11	**16a**	H	CH_2Ph	Br	0.10	54	20[b]	
12	**16b**	OMe	Me	Br	0.12	24	—	33

[a] Mixture of **18d**:**19d** in a ratio of ca. 1:1 (determined by ^1H NMR).
[b] Mixture of **18c**:**19c** in a ratio of ca. 2:1 (determined by ^1H NMR).

cyclization products were formed with iodo or bromoacetamide groups (e.g., entries 8 and 11, Table 1). Ishibashi et al.[5] also observed halogen-dependant differences in radical cyclization versus radical reduction with other *N*-benzylhaloacetamides. In their case, the chloroacetamide afforded cyclization from the *cisoid* amide rotamer, while the iodoacetamide gave predominately the non-cyclized reduction product from the favoured *transoid* rotamer. Clearly amide conformational preferences will also be influenced by the nature of both the *N*-substituents and with the *N*,*N*-disubstituted amides **15b–g**, the very bulky indolylmethyl substituent, together with the methyl or benzylic substituent, would induce a preference for the *cisoid* rotamer of type B (Scheme 3) for the iodoacetamides.



Scheme 3. Proposed cyclization mechanism.

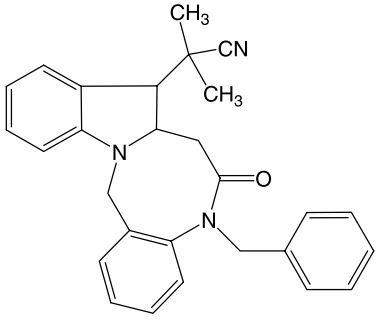
Table 2. Radical cyclization of haloacetamides using Bu_3SnH/AIBN in various solvents

Entry	Substrate	Solvent	Yield (%)		
			17	**18**	**19**
1	**15b**	Mesitylene	44	—	—
2[a]	**15d**	Toluene	25	43	10
3	**15d**	Xylenes	57	—	—
4	**15d**	Mesitylene	70	—	—
5	**15d**	$(CH_3)_3CC_6H_5$	46	—	9
6	**15g**	Mesitylene	29	—	—
7[a]	**15g**	Toluene	25	—	<1
8	**15h**	Mesitylene	23	—	15

[a] From Table 1.

A temperature-dependence study of the cyclization revealed increasing yields with higher boiling point solvents (Table 2).

Cyclizations in high boiling point solvents, for example, xylenes and mesitylene, increased yields of the benzodiazocinones **17** and neither the dihydroindole fused products **18** nor the acetamides **19** were isolated when **15b** or **15d** was the substrate (Table 2). No products from radical attack on the aromatic hydrocarbon solvents were seen.[12] The increased yield of cyclization products and the diminished formation of reduced, uncyclized by-products in high boiling point aromatic solvents suggests that the amide rotamer (B) required for cyclization after radical formation is increased in concentration in the equilibrium at higher temperatures relative to rotamer A.[5] Also, with radical attack on the heteroaromatic ring likely to be slow,[4] the increased temperature would be favourable for this reaction.



20

A 8-*exo-trig* addition of the amidylmethyl radical would give the dihydroindole radical (C) and then an oxidation step to give the products **17** (Scheme 3); the exact details of this latter step in other cyclizations has been the subject of much discussion, but it now seems likely that two possible pathways include hydrogen abstraction by the $(CH_3)_2C^{\cdot}CN$ radical, plus reaction with AIBN.[13] In this connection, it is of interest to note that in one larger scale reaction involving **15d**, some of the radical trapped adduct **20** was isolated.

With a view to ultimately providing access to other *N*-substituted indolo[2,1-*d*][1,5]benzodiazocine-6-ones, a chemoselective means of removal of the *N*-benzyl group in **17c** was investigated. Debenzylation of **17c** was performed using sodium in liquid ammonia[14] to afford **1** in 35% yield (Scheme 4). There was no unreacted **17c**, but some unidentified baseline material was observed in the PTLC of the reaction mixture. Selective addition of a solvated electron was expected at the less-electron rich aromatic ring of the benzyl-protecting group. However, the reaction time was crucial to ensure a reasonable yield of the product. The most suitable time for the cleavage of the benzyl protecting group, and not the eight-membered ring, was 6–8 min.

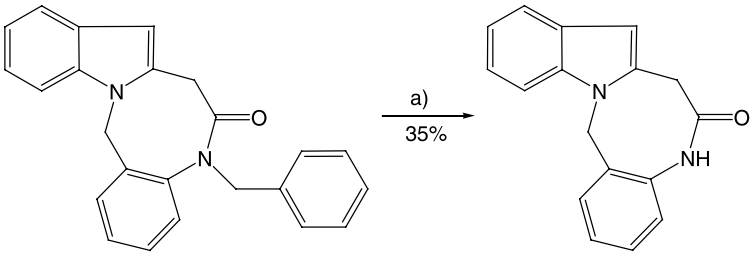
3. Conclusions

It has been shown that, in contrast to photo-initiated reactions, an indole-fused eight-membered ring system can be accessed in fair yield by a thermal free radical cyclization route from 1-substituted indoles. This route has the potential for synthesis of a range of analogues and substituted derivatives.

4. Experimental

4.1. General

Melting points were determined using a Reichert hot-stage



17c **1**

Scheme 4. (a) Na, liq. NH_3, THF.

melting point apparatus and are uncorrected. NMR spectra were recorded on a Varian Unity-300 Fourier transform NMR spectrometer. Unless otherwise stated, the spectra were obtained in $CDCl_3$ and referenced to TMS (proton) and the chloroform mid-line (77 ppm) (carbon). Chemical shifts are reported in parts per million (δ). MS were obtained using a Shimadzu QP-5000 spectrometer. High resolution MS were run using a Fisons/VG Autospec-TOF spectrometer. IR spectra were recorded on a BOMEM MB-100 FTIR. TLC and preparative layer chromatography were performed using Merck silica gel 60 F_{254}. All chromatographic solvent proportions are volume to volume. Column chromatography (silica gel; flash column or normal column) was performed using Merck Kieselgel 60 (0.063–0.200 nm particle size). Solvents were removed under reduced pressure by a Büchi rotary evaporator, and organic solvent extracts were dried with anhydrous Na_2SO_4. Solvent mixtures are v/v ratios. Compounds **4**–**6**, **14a** and **14i** were prepared following the methods reported previously.[9] ^1H and ^{13}C NMR spectra for cyclized and uncyclized derivatives were assigned using the numbering systems illustrated on **17** and **19** (Scheme 1).

4.2. General procedures for the preparation of *N*-substituted amines (7–13)

Procedure A. To a stirred solution of amines (**4**–**6**, 1.0 equiv) in dry DMF under a nitrogen atmosphere was added anhydrous potassium carbonate (K_2CO_3) (1.0 equiv) and the suspension was stirred at 0–5 °C for 5 min. The appropriate alkyl halide (1.0–1.5 equiv) was then added and the reaction mixture stirred at r.t. overnight. Solvent was then removed under reduced pressure. DCM (20 mL) was added to the residue and the mixture was washed with water (4×20 mL). The organic extracts was dried, concentrated, and chromatographed on a column by elution with 1:1 DCM/hexane.

Procedure B. To a solution of amine **4** (1.0 equiv) in absolute methanol (10 mL) with molecular sieves 3 Å was added 5 M HCl–MeOH (0.33 equiv), followed by the appropriate aldehyde (0.25 or 1 equiv) and $NaBH_3CN$ (0.1 or 1.0 equiv). The reaction mixture was stirred at r.t. for 20 h. The mixture was filtered and the filtrate was evaporated to dryness. Water (20 mL) was added and the mixture extracted with DCM (20 mL). The aqueous layer was brought to pH > 10 with solid KOH and extracted with DCM (3×20 mL). The organic extracts were combined and washed with brine (15 mL), dried, concentrated, and chromatographed by elution with 1:1 DCM/hexane.

The following compounds were prepared by one of these general procedures.

4.2.1. 1-[(2-*N*-Methylaminophenyl)methyl]-1*H*-indole (7). Following procedure A, treatment of **4** (0.16 g, 0.71 mmol) in dry DMF (20 mL) with K_2CO_3 (98 mg, 0.71 mmol) and methyl iodide (66 μL, 1.07 mmol) gave **7** (83 mg, 50%) as a colourless solid, mp 52–53 °C; IR (KBr) ν_{max}: 3430 (NH), 2815 (N–CH_3) cm^{-1}: ^1H NMR ($CDCl_3$) δ 2.77 (s, 3H, CH_3), 3.51 (br s, 1H, N*H*), 5.15 (s, 2H, CH_2), 6.53 (d, J=3.6 Hz, 1H, H-3), 6.70 (d, J=8.1 Hz, 1H, H-3′), 6.75 (d, J=7.5 Hz, 1H, Ar), 6.99 (m, J=8.4 Hz, 2H, H-2 and Ar), 7.13–7.33 (m, 3H, H-2′, H-5 and H-6), 7.42 (d, J=8.1 Hz, 1H, H-7), 7.67 (d, J=7.5 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$) δ 31.0 (CH_3), 47.7 (CH_2), 102.2 (C-3), 109.7 (C-7), 110.6 (×2), 117.5, 120.0 (ArC), 121.1 (C-1′), 121.3, 122.0, 127.4, 129.8 (C-3a and ArC), 136.5 (C-7a), 147.5 (C–N); CI-MS m/z 237 ($[M+H]^+$, 97%); HREI-MS m/z calcd for $[M]^+$ $C_{16}H_{16}N_2$: 236.1313, found: 236.1309.

4.2.2. 1-[(2-*N*-(4′-Methoxybenzyl)aminophenyl)methyl]-1*H*-indole (8). Following procedure A, treatment of **4** (0.13 g, 0.59 mmol) in dry DMF (20 mL) with K_2CO_3 (82 mg, 0.59 mmol) and 4-methoxybenzyl bromide (0.12 g, 0.59 mmol) gave **8** (0.14 g, 71%) as a yellow solid; mp 105–106 °C; IR (KBr) ν_{max}: 3425 (NH) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.86 (br s, 1H, N*H*), 3.88 (s, 3H, CH_3), 4.26 (s, 2H, NHCH_2), 5.26 (s, 2H, CH_2), 6.63 (d, J=3.3 Hz, 1H, H-3), 6.77–6.83 (m, 2H, Ar), 6.87 (d, J=8.7 Hz, 2H, Ar), 7.22–7.26 (m, 6H, Ar), 7.32 (t, J=9.0 Hz, 1H, Ar), 7.43 (dd, J=7.5, 1.2 Hz, 1H, H-7), 7.76 (dd, J=7.5, 1.8 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$) δ 47.4 (NHCH_2), 47.7 (CH_2), 55.3 (CH_3), 102.0 (C-3), 109.3, 111.2, 113.8, 117.3, 119.6, 120.6 (C-1′), 121.0, 121.7, 127.2, 128.3, 128.7 (C-3a), 129.2, 129.4, 130.6 (C-7a), 136.2 (C-1″), 146.0 (C-2′), 158.5 (*C*OCH$_3$); CI-MS m/z 343 ($[M+H]^+$, 100%); HREI-MS m/z calcd for $[M]^+$ $C_{23}H_{22}N_2O$: 342.1732, found: 342.1731.

4.2.3. 1-[(2-*N*-Benzylaminophenyl)methyl]-1*H*-indole (9). Following procedure B, treatment of **4** (0.30 g, 1.35 mmol) with 5 M HCl–MeOH (90 μL, 0.45 mmol), benzaldehyde (0.14 mL, 1.35 mmol) and $NaBH_3CN$ (85 mg, 1.35 mmol) gave **9** (0.28 g, 66%) as a yellow solid; mp 89–91 °C; IR (KBr) ν_{max}: 3432 (NH) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.93 (br s, 1H, N*H*), 4.32 (s, 2H, NHCH_2), 5.20 (s, 2H, CH_2), 6.65 (dd, J=3.3, 0.6 Hz, 1H, H-3), 6.76 (d, J=8.1 Hz, 1H, H-3′), 6.83 (td, J=7.5, 1.2 Hz, 1H, Ar), 7.07–7.11 (m, with prominent d, J=3.3 Hz, 2H, H-2 and Ar), 7.18–7.38 (m, 8H, Ar), 7.44 (dd, J=7.2, 1.5 Hz, 1H, H-7), 7.78 (dd, J=7.5, 0.9 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$) δ 48.0 (NHCH_2), 48.3 (CH_2), 102.5 (C-3), 109.8, 111.7, 120.1, 121.0 (C-1′), 121.5, 122.2, 127.4, 127.6, 128.9, 129.2 (C-3a), 129.7, 129.8, 136.6 (C-7a), 139.1 (C-1″), 146.3 (C-2′); CI-MS m/z 313 ($[M+H]^+$, 100%); HREI-MS m/z calcd for $[M]^+$ $C_{22}H_{20}N_2$: 312.1625, found: 312.1621.

4.2.4. 1-{[2-*N*-(1′-Phenylethyl)aminophenyl]methyl}-1*H*-indole (10). Following procedure B, treatment of **4** (0.30 g, 1.42 mmol), with 5 M HCl–MeOH (0.1 mL, 0.50 mmol), acetophenone (40 μL, 0.34 mmol), and $NaBH_3CN$ (10 mg, 0.14 mmol) gave **10** (82 mg, 74%) as an off-white solid; mp 86–88 °C; IR (KBr) ν_{max}: 3425 (NH) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.11 (d, J=6.6 Hz, 3H,CH_3), 3.47 (br s, 1H, N*H*), 4.33 (q, J=6.6 Hz, 1H, CH), 5.20 (s, 2H, CH_2), 6.37 (d, J=8.1 Hz, 1H, Ar), 6.57 (d, J=3.3 Hz, 1H, H-3), 6.64 (t, J=7.5 Hz, 1H, Ar), 6.93–6.95 (m, 2H, Ar), 7.00–7.10 (m, 3H, H-2 and Ar), 7.11–7.23 (m, 5H, Ar), 7.42 (d, J=8.1 Hz, 1H, Ar), 7.66 (d, J=7.5 Hz, 1H, Ar); ^{13}C NMR ($CDCl_3$) δ 25.4 (CH_3), 48.6 (CH_2), 53.2 (CH), 102.7 (C-3), 109.7, 112.6, 117.3, 120.1, 120.8 (C-1′), 121.6, 122.2, 125.8, 127.1, 127.6, 128.8, 129.1 (C-3a), 129.6, 130.1, 136.6 (C-7a), 144.9 (C-1″), 145.8 (C-2′); CI-MS m/z 327 ($[M+H]^+$, 100%); HRCI-MS m/z calcd for $[M+H]^+$ $C_{23}H_{23}N_2$: 317.1861, found: 327.1850.

4.2.5. 1-[(2-*N*-Methylaminophenyl)methyl]-5-methoxy-1*H*-indole (11). Following procedure A, treatment of **5** (0.39 g, 1.54 mmol) with K_2CO_3 (0.21 g, 1.54 mmol) and methyl iodide (0.1 mL, 1.69 mmol) gave **11** (0.29 g, 71%) as a yellow solid; mp 59–60 °C; IR (KBr) ν_{max}: 3429 (NH), 2815 (N–CH_3) cm^{-1}; ^1H NMR δ 2.74 (s, 3H, CH_3), 3.48 (br s, 1H, N*H*), 3.84 (s, 3H, OCH_3), 5.06 (s, 2H, CH_2), 6.42 (d, J=3.6 Hz, 1H, H-3), 6.66–6.73 (m, 2H, Ar), 6.86 (dd, J=8.6, 2.1 Hz, 1H, H-7), 6.92–6.94 (m with prominent d, J=3.3 Hz, 2H, H-2 and Ar), 7.11 (d, J=2.1 Hz, 1H, H-4), 7.22–7.30 (m, 2H, Ar); ^{13}C NMR ($CDCl_3$) δ 31.0 (CH_3), 48.1 (CH_2), 56.2 (OCH_3), 101.8, 102.9 (C-3), 110.5, 110.6, 112.3 (×3), 117.6, 120.7 (C-1′), 128.2 (C-2), 129.5 (C-3a), 129.7, 131.9 (C-7a), 147.5 (C–N), 154.4 (*C*OCH_3); CI-MS *m/z* 267 ($[M+H]^+$, 100%); HRCI-MS *m/z* calcd for $[M+H]^+$ $C_{17}H_{18}N_2O$: 267.1497, found: 267.1490.

4.2.6. 1-[(2-*N*-Benzylaminophenyl)methyl]-5-methoxy-1*H*-indole (12). Following procedure A, treatment of **5** (1.00 g, 4.00 mmol) with K_2CO_3 (0.58 g, 4.17 mmol) and benzyl bromide (0.50 mL, 4.17 mmol) gave **12** (0.80 g, 58%) as a yellow solid; mp 108–110 °C; IR (KBr) ν_{max}: 3428 (NH) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.86 (s, 3H, OCH_3), 4.23 (s, 2H, NHC*H*$_2$), 5.17 (s, 2H, CH_2), 6.48 (d, J=3.3 Hz, 1H, H-3), 6.65 (d, J=8.4 Hz, 1H, H-3′), 6.73 (t, J=7.5 Hz, 1H, Ar), 6.81 (dd, J=8.7, 2.4 Hz, 1H, H-6), 6.98–7.02 (m, 2H, H-2 and Ar), 7.07–7.11 (m, 3H, Ar), 7.18–7.30 (m, 6H, Ar); ^{13}C NMR ($CDCl_3$) δ 48.1 ($NHCH_2$), 48.2 (CH_2), 56.1 (CH_3), 101.9 (C-3), 103.0 (C-4), 110.4, 111.6, 112.2, 117.7, 120.8 (C-1′), 127.4, 128.1, 128.8, 129.6, 129.8, 131.8 (C-7a), 139.0 (C-1″), 146.2 (C-2′), 154.4 (*C*OCH_3); CI-MS *m/z* 343 ($[M+H]^+$, 100%); HREI-MS *m/z* calcd for $[M]^+$ $C_{23}H_{22}N_2O$: 342.1732, found: 342.1731.

4.2.7. 1-[(2-*N*-Benzylaminophenyl)methyl]-5-fluoro-1*H*-indole (13). Following procedure A, treatment of **6** (1.00 g, 4.12 mmol) with K_2CO_3 (0.57 g, 4.12 mmol) and benzyl bromide (0.49 mL, 4.12 mmol) gave **13** (0.65 g, 48%) as colourless needles; mp 189–191 °C; IR (KBr) ν_{max}: 3430 (NH) cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.24 (s, 2H, NHC*H*$_2$), 5.19 (s, 2H, CH_2), 6.50 (d, J=3.3 Hz, 1H, H-3), 6.71 (d, J=8.1 Hz, 1H, Ar), 6.77 (t, J=7.5 Hz, 1H, Ar), 6.90 (td, J=9.0, 2.7 Hz, 1H, H-6), 7.00 (dd, J=7.5, 1.5 Hz, 1H, Ar), 7.05–7.10 (m with prominent d, J=3.3 Hz, 2H, H-2 and Ar), 7.16–7.27 (m, 6H, Ar), 7.30 (dd, J=9.6, 2.4 Hz, 1H, H-7); ^{13}C NMR ($CDCl_3$) δ 48.2 ($NHCH_2$), 48.3 (CH_2), 102.3 (d, J=4.9 Hz, ^{13}C–^{19}F, C3–F), 106.1 (d, J=23.2 Hz, ^{13}C–^{19}F, C6–F), 110.9 (d, J=10.3 Hz, ^{13}C–^{19}F, C7–F), 110.8 (d, J=27.8 Hz, ^{13}C–^{19}F, C4–F), 117.0, 118.1, 120.9 (C-1′), 127.5, 128.8, 129.15, 129.23, 129.5 (d, J=16.3 Hz, ^{13}C–^{19}F, C2a–F), 129.8, 133.1 (C-7a), 138.6 (C-1′), 146.0 (C-2′), 158.2 (d, J=233.1 Hz, ^{13}C–^{19}F, C5–F); CI-MS *m/z* 331 ($[M+H]^+$, 100%); HREI-MS *m/z* calcd for $[M]^+$ $C_{22}H_{19}N_2F$: 330.1532, found: 330.1533.

4.3. General procedures for the preparation of chloroacetamides (14b–d, 14f–h)

Procedure C A mixture of **7** or **11** (1.0 equiv) and anhydrous potassium carbonate (K_2CO_3) (3.7 equiv) in dry DCM was cooled to 0–5 °C and chloroacetyl chloride (2.5 equiv) was added. Stirring was continued with cooling for a further 30 min and the reaction mixture was then allowed to warm to r.t. with stirring overnight. Distilled water (15 mL) was added to the reaction mixture and the aqueous layer was extracted with DCM (3×20 mL). The combined organic extract was dried, then concentrated and the residue chromatographed on a column by elution with DCM.

Procedure D. To a stirred suspension of sodium hydride (NaH) (ca. 50% in mineral oil, 1.1 equiv) in dry THF (5 mL) was added a solution of **8–10** or **12–13** (1.0 equiv) in dry THF (20 mL) under a nitrogen atmosphere and the solution was stirred at r.t. for 30 min. Chloroacetyl chloride (2.5 equiv) in THF (5 mL) was added to the solution and the reaction mixture was stirred at r.t. overnight. Solvent was then removed under reduced pressure. DCM (15 mL) was added to the residue and the mixture was washed with water (3×15 mL). The organic layer was dried, and the solvent then evaporated in vacuo to give a yellow residue. The residue was purified by flash column chromatography with DCM as eluent.

4.3.1. *N*-[(2-(1′-1*H*-Indolyl)methyl]phenyl]-*N*-methyl chloroacetamide (14b). Following procedure C, treatment of **7** (83 mg, 0.35 mmol) with K_2CO_3 (0.18 g, 1.30 mmol) in dry DCM (5 mL), and chloroacetyl chloride (70 μL, 0.88 mmol) gave **14b** (63 mg, 57%) as colourless solid; mp 89–92 °C; IR (KBr) ν_{max}: 2852 (N–CH_3), 1658 (C=O), 796 (C–Cl) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.24 (s, 3H, CH_3), 3.56 (d, J=12.6 Hz, 1H, C*H*HCl), 3.66 (d, J=12.6 Hz, 1H, CH*H*Cl), 5.29 (d, J=5.7 Hz, 2H, CH_2), 6.59 (d, J=3.3 Hz, 1H, H-3), 6.99 (d, J=7.5 Hz, 1H, Ar), 7.06 (d, J=3.0 Hz, 1H, H-2), 7.11–7.39 (m, 4H, Ar), 7.36 (m, 1H, H-6), 7.67 (d, J=7.8 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$) δ 37.5 (CH_3), 41.3 (CH_2Cl), 46.7 (CH_2), 102.7 (C-3), 109.6 (C-7), 119.9, 121.2 (C-1′), 122.0 (C-3′), 125.1 (C-2), 127.0 (C-3a), 127.4, 128.8, 128.9, 129.2, 130.4, 134.1 (C-7a), 136.4 (C-2′), 164.6 (CO); CI-MS *m/z* 313 ($[M+H]^+$, 100%), HRCI-MS *m/z* calcd for $[M+H]^+$ $C_{18}H_{18}N_2O^{35}Cl$: 313.1108, found: 313.1111.

4.3.2. *N*-[(2-(1′-1*H*-Indolyl)methyl]phenyl]-*N*-(4″-methoxylbenzyl)chloroacetamide (14c). Following procedure D, NaH (27 mg ca. 50% in mineral oil, 0.57 mmol) was reacted with a solution of **8** (0.18 g, 0.52 mmol) and chloroacetyl chloride (0.11 mL, 1.42 mmol) to give **14c** (0.20 g, 93%) as a yellow solid; mp 82–84 °C; ^1H NMR ($CDCl_3$) δ 3.51 (d, J=12.6 Hz, 1H, C*H*HCl), 3.62 (d, J=12.6 Hz, 1H, CH*H*Cl), 3.75 (s, 3H, OCH_3), 4.55 (d, J=13.8 Hz, 1H, CONC*H*H), 4.78 (d, J=16.8 Hz, 1H, C*H*H), 4.95 (d, J=13.8 Hz, 1H, CONCH*H*), 5.08 (d, J=16.8 Hz, 1H, CH*H*), 6.51 (d, J=3.3 Hz, 1H, H-3), 6.77–6.80 (m with prominent d, J=8.7 Hz, 3H, H-3′, H-3″, and H-5″), 6.84–6.88 (m with prominent d, J=3.0 Hz, 2H, H-2 and H-6′), 7.00 (d, J=8.1 Hz, 1H, Ar), 7.07 (t, J=7.2 Hz, 1H, H-5), 7.09–7.12 (m with prominent d, J=8.7 Hz, 3H, H-6, H-2″, and H-6″), 7.17–7.24 (m, 2H, Ar), 7.59 (d, J=7.5 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$) δ 41.8 (CH_2Cl), 46.6 (CH_2), 53.8 (CONCH_2), 55.6 (CH_3), 102.7 (C-3), 109.6, 114.3, 120.8, 121.4, 122.3, 128.2, 128.3, 128.8 (C-3a), 129.3, 129.4, 129.9, 130.1, 131.3, 135.9 (C-1′), 136.4 (C-7a), 138.4 (C-2′), 159.7 (*C*OCH_3), 166.8 (CO); CI-MS *m/z* 419 ($[M+H; ^{35}Cl]^+$, 100%), 421 ($[M+H; ^{37}Cl]^+$, 35%); HRCI-MS *m/z* calcd for $[M+H]^+$ $C_{25}H_{24}N_2O_2^{37}Cl$: 421.1497, found: 421.1498.

4.3.3. *N*-[(2-(1′-1*H*-Indolyl)methyl]phenyl]-*N*-benzylchloroacetamide (14d). Following procedure D, NaH (70 mg, 1.76 mmol) was reacted with **9** (0.50 g, 1.60 mmol) and chloroacetyl chloride (0.32 mL, 4.00 mmol) to give **14d** (0.50 g, 81%) as a yellow solid; mp 148–149 °C; ^{1}H NMR ($CDCl_3$) δ 3.56 (d, J=12.9 Hz, 1H, C*H*HCl), 3.66 (d, J=13.2 Hz, 1H, CH*H*Cl), 4.65 (d, J=13.8 Hz, 1H, CONC*H*H), 4.80 (d, J=16.8 Hz, 1H, C*H*H), 5.04 (d, J=13.8 Hz, 1H, CONCH*H*), 5.12 (d, J=16.8 Hz, 1H, CH*H*), 6.54 (d, J=3.0 Hz, 1H, H-3), 6.78 (m, 1H, Ar), 6.85 (d, J=3.0 Hz, 1H, H-2), 6.93 (m, 1H, Ar), 7.04 (d, J=7.8 Hz, 1H, H-7), 7.13 (td, J=7.2, 1.5 Hz, 1H, H-5), 7.22 (td, J=6.9, 0.9 Hz, 1H, H-6), 7.22–7.28 (m, 4H, Ar), 7.30–7.36 (m, 3H, Ar), 7.64 (dd, J=7.5, 0.9 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$) δ 41.3 (CH_2Cl), 46.3 (CH_2), 53.3 (CONCH_2), 102.5 (C-3), 109.3 (C-7), 119.5 (C-5), 121.2 (C-4), 122.1 (C-6), 127.9 (C-2), 128.3, 128.5 (C-3a), 128.8, 129.0, 129.2, 129.7, 129.8, 129.9, 135.7 (C-1′), 136.0 (C-7a), 136.2 (C-1″), 138.3 (C-2′), 166.5 (CO); CI-MS *m/z* 389 ([M+H]$^+$, 100%); HREI-MS *m/z* calcd for [M]$^+$ $C_{24}H_{21}N_2O^{35}Cl$: 388.1342, found 388.1334.

4.3.4. *N*-[(2-(1′-1*H*-Indolyl)methyl]phenyl]-*N*-(1″-phenylethyl)chloroacetamide (14e). A mixture of **10** (87 mg, 0.27 mmol), triethylamine (56 μL, 0.41 mmol), and 4-dimethylaminopyridine (3.3 mg, 0.03 mmol) in dry DCM (10 mL) was stirred at 0 °C. To this solution was added a solution of chloroacetyl chloride (60 μL, 0.54 mmol) in dry DCM (2 mL) and the mixture was stirred at r.t. for 1 h. The reaction mixture was washed with brine and the organic layer was dried and concentrated. The residue was subjected to flash column chromatography (hexane–dichloromethane, 1:1) to give **14e** (36 mg, 33%) as a colourless solid; mp 90–92 °C; ^{1}H NMR ($CDCl_3$, rotamer ratio of *ca* 2:1*, *=minor rotamer) δ 1.58* (d, J=7.2 Hz, 1H, CH_3) 1.64 (d, J=7.2 Hz, 2H, CH_3), 3.53 (d, J=13.2 Hz, 1H, C*H*HCl), 3.63 (d, J=13.2 Hz, 1H, CH*H*Cl), 3.73 (d, J=17.4 Hz, 0.7H, C*H*H), 4.63 (d, J=17.4 Hz, 0.7H, CH*H*), 5.31* (d, J=17.1 Hz, 0.3H, C*H*H), 5.37* (d, J=17.1 Hz, 0.3H, CH*H*), 6.22* (q, J=7.2 Hz, 0.3H, CH), 6.30 (q, J=7.2 Hz, 0.7H, CH), 6.40 (d, J=7.8 Hz, 0.7H, H-3′), 6.50 (d, J=3.3 Hz, 0.7H, H-3), 6.51 (d, J=3.0 Hz, 0.7H, H-2), 6.60* (d, J=3.3 Hz, 0.3H, H-3), 6.78 (d, J=7.8 Hz, 1H, Ar), 7.02–7.04* (m, 0.6H, H-2 and Ar), 7.06–7.16 (m, 3.4H, H-2″, H-6″ and Ar), 7.22 (td, J=7.5, 1.5 Hz, 1H, H-4′ and H-4′*), 7.27–7.36 (m, 5H, H-3″, H-5″ and Ar), 7.39–7.43 (m, 1H, H-7 and H-7*), 7.58 (dd, J=8.1, 2.1 Hz, 0.7H, H-4), 7.67* (d, J=7.8 Hz, 0.3H, H-4); ^{13}C NMR ($CDCl_3$) δ 19.4 (CH_3), 42.3 (CH_2Cl), 46.0 (CH_2), 55.7 (CH), 102.5 (C-3), 109.4, 119.9 (C-5), 121.2 (C-4), 122.1, 128.0 (C-2), 128.4 (C-3′), 128.5 (C-3a), 128.6, 128.9, 129.5, 130.2, 131.4, 134.9 (C-1′), 136.5 (C-7a), 138.3 (C-1″), 139.1 (C–N), 166.3 (CO); CI-MS *m/z* 403 ([M+H]$^+$, 100%); HRCI-MS *m/z* calcd for [M+H]$^+$ $C_{25}H_{24}N_2O^{35}Cl$: 403.1577, found: 403.1559.

4.3.5. *N*-{2-[1′-(5-Methoxy-1*H*-indolyl)methyl]phenyl}-*N*-methylchloroacetamide (14f). Following procedure C, treatment of **11** (0.29 g, 1.09 mmol) with K_2CO_3 (0.56 g, 4.03 mmol) and chloroacetyl chloride (0.22 mL, 2.73 mmol) gave **14f** (0.22 g, 58%) as a yellow solid; mp 71–73 °C; ^{1}H NMR ($CDCl_3$) δ 3.21 (s, 3H, CH_3), 3.52 (d, J=12.6 Hz, 1H, C*H*HCl), 3.62 (d, J=12.6 Hz, 1H, CH*H*Cl), 3.83 (s, 3H, OCH_3), 5.23 (d, J=5.1 Hz, 2H, CH_2), 6.84 (d, J=3.6 Hz, 1H, C-3), 6.83 (dd, J=9.0, 2.4 Hz, 1H, Ar), 6.87 (d, J=7.5 Hz, 1H, Ar), 7.02 (d, J=3.0 Hz, 1H, H-2), 7.05–7.10 (m with prominent d, J=2.4 Hz, 2H, Ar), 7.20 (dd, J=7.5, 1.2 Hz, 1H, Ar), 7.28–7.39 (m, 2H, Ar); ^{13}C NMR ($CDCl_3$) δ 37.5 (CH_3), 41.2 (CH_2Cl), 47.1 (CH_2), 56.1 (OCH_3), 102.3 (C-3), 103.0, 110.4, 112.6, 128.9, 129.0, 129.3 (C-3a), 129.7, 129.9, 131.6 (C-1′), 135.2 (C-7a), 140.6 (C-2′), 154.4 (*C*OCH_3), 166.8 (CO); CI-MS *m/z* 343 ([M+H]$^+$, 100%); HRCI-MS *m/z* calcd for [M+H]$^+$ $C_{19}H_{20}N_2O_2^{35}Cl$: 343.1213, found: 343.1208.

4.3.6. *N*-{2-[1′-(5-Methoxy-1*H*-indolyl)methyl]phenyl}-*N*-benzylchloroacetamide (14g). Following procedure D, compound **12** (0.20 g, 0.58 mmol), NaH (23 mg, 0.64 mmol) and chloroacetyl chloride (0.12 mL, 1.45 mmol) were reacted to give **14g** (0.14 g, 58%) as a yellow solid; mp 166–169 °C; ^{1}H NMR ($CDCl_3$) δ 3.54 (d, J=12.9 Hz, 1H, C*H*HCl), 3.64 (d, J=12.9 Hz, 1H, CH*H*Cl), 3.83 (s, 3H, OCH_3), 4.62 (d, J=13.5 Hz, 1H, CONC*H*H), 4.78 (d, J=16.5 Hz, 1H, C*H*H), 5.04 (d, J=13.8 Hz, 1H, CONCH*H*), 5.08 (d, J=16.5 Hz, 1H, CH*H*), 6.46 (d, J=2.4 Hz, 1H, H-3), 6.79–6.84 (m, 3H, Ar), 6.90–6.93 (m, 2H, Ar), 7.08 (d, J=2.4 Hz, 1H, H-4), 7.21–7.33 (m, 7H, Ar); ^{13}C NMR ($CDCl_3$) δ 41.6 (CH_2Cl), 46.8 (CH_2), 53.5 (CONCH_2), 56.0 (CH_3), 102.2 (C-3), 110.3, 112.6, 128.5, 128.7, 128.9, 129.2 (C-3a), 129.3, 129.5, 129.9, 130.0, 130.1, 131.7 (C-1′), 136.0 (C-7a), 136.3 (C-1″), 138.6 (C-2′), 154.5 (*C*OCH_3), 166.7 (CO); CI-MS *m/z* 419 ([M+H; ^{35}Cl]$^+$, 100%); HRCI-MS *m/z* calcd for [M+H]$^+$ $C_{25}H_{24}N_2O_2^{35}Cl$: 419.1526, found: 419.1506.

4.3.7. *N*-{2-[1′-(5-Fluoro-1*H*-indolyl)methyl]phenyl}-*N*-benzylchloroacetamide (14h). Following procedure D, compound **13** (0.14 g, 0.42 mmol), NaH (18 mg, 0.46 mmol) and chloroacetyl chloride (84 μL, 1.05 mmol) were reacted to give **14h** (0.13 g, 76%) as a yellow solid; mp 135–136 °C; ^{1}H NMR ($CDCl_3$) δ 3.55 (d, J=12.6 Hz, 1H, C*H*HCl), 3.67 (d, J=12.9 Hz, 1H, CH*H*Cl), 4.64 (d, J=13.8 Hz, 1H, CONC*H*H), 4.79 (d, J=16.8 Hz, 1H, C*H*H), 5.02 (d, J=13.8 Hz, 1H, CONCH*H*), 5.11 (d, J=16.5 Hz, 1H, CH*H*), 6.50 (d, J=3.3 Hz, 1H, H-3), 6.79 (ddd, J=9.3, 8.1, 2.7 Hz, 1H, Ar), 6.90 (d, J=3.3 Hz, 1H, H-2), 6.91–6.97 (m, 3H, Ar), 7.22–7.34 (m, 8H, Ar); ^{13}C NMR ($CDCl_3$) δ 41.5 (CH_2Cl), 46.8 (CH_2), 53.6 (CONCH_2), 102.7 (d, J=4.6 Hz, ^{13}C–^{19}F, C-3), 106.2 (d, J=23.0 Hz, ^{13}C–^{19}F, C-6), 110.2 (d, J=9.8 Hz, ^{13}C–^{19}F, C-7), 110.7 (d, J=26 Hz, ^{13}C–^{19}F, C-4), 128.6, 129.0, 129.2, 129.3, 129.5 (C-3a), 129.7, 129.8, 130.0, 130.2, 133.1 (C-1′), 135.7 (C-7a), 136.2 (C-1″), 138.6 (C-2′), 158.2 (d, J=223.6 Hz, ^{13}C–^{19}F, C5–F), 166.9 (CO); CI-MS *m/z* 407 ([M+H]$^+$, 100%); HRCI-MS *m/z* calcd for [M+H]$^+$ $C_{24}H_{21}N_2O^{35}ClF$: 407.1326, found: 407.1319.

4.4. Genernal procedure for the preparation of iodoacetamides (15a–h)

A solution of **14a–h** (1.0 equiv) in acetronitrile containing sodium iodide (NaI) (10.0 equiv) was heated at reflux for 2 h. The solution was cooled and water (10 mL) was added. The solution was then extracted with EtOAc (3×20 mL). The organic extracts were combined, dried, concentrated,

and chromatographed on a column by elution with DCM. The following compounds were prepared by this method.

4.4.1. *N*-[(2-(1′-1*H*-Indoly)methyl]phenyl]-*N*-iodoacetamide (15a). Treatment of **14a** (73 mg, 0.24 mmol) with NaI (0.37 g, 2.48 mmol) in refluxing acetronitrile (5 mL) gave **15a** (83 mg, 87%) as a colourless crystalline solid; mp 101–103 °C; IR (KBr) ν_{max}: 2853 (N–CH_3), 1659 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.53 (s, 2H, CH_2I), 5.27 (s, 2H, CH_2), 6.58 (d, J=3.3 Hz, 1H, H-3), 7.03 (br d, J=3.3 Hz, 2H, H-2 and Ar), 7.12–7.26 (m, 3H, Ar), 7.29–7.32 (m, 2H, Ar and N*H*), 7.53 (br d, J=7.5 Hz, 2H, H-6 and Ar), 7.67 (d, J=7.2 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$) δ −2.8 (CH_2I), 47.5 (CH_2), 102.9 (C-3), 109.8 (C-7), 120.3, 121.5, 122.4, 125.3, 127.1, 127.9, 129.0, 129.2, 131.0, 134.8, 136.5 (C-2′), 166.4 (CO); EI-MS m/z 390 ($[M]^+$, 75%), 264 ($[M-I]^+$, 100%); HREI-MS m/z calcd. for $[M]^+$ $C_{17}H_{15}N_2OI$: 390.0229, found: 390.0233.

4.4.2. *N*-[(2-(1′-1*H*-Indolyl)methyl]phenyl]-*N*-methyliodoacetamide (15b). Treatment of **14b** (0.10 g, 0.32 mmol) with NaI (0.50 g, 3.32 mmol) in refluxing acetonitrile (5 mL) gave **15b** (0.12 g, 93%) as a yellow solid; mp 53–54 °C; ^1H NMR ($CDCl_3$) δ 3.21 (s, 3H, CH_3), 3.30 (d, J=10.2 Hz, 1H, C*H*HI), 3.45 (d, J=9.6 Hz, 1H, CH*H*I), 5.25 (d, J=16.5 Hz, 1H, C*H*H), 5.37 (d, J=16.5 Hz, 1H, CH*H*) 6.58 (d, J=2.7 Hz, 1H, H-3), 6.96 (d, J=8.4 Hz, 1H, Ar), 7.09 (d, J=3.0 Hz, 1H, H-2). 7.11–7.39 (m, 6H, ArH), 7.66 (d, J=7.5 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$) δ −2.9 (CH_2I), 37.7 (CH_3), 47.0 (CH_2), 102.7 (C-3), 109.7 (C-7), 120.1, 121.4, 122.3, 128.3, 128.5, 128.6 (C-3a), 128.9, 129.6, 129.8, 134.9 (C-1′), 136.4 (C-7a), 141.3 (C-2′), 168.3 (CO); CI-MS m/z 405 ($[M+H]^+$, 100%); HREI-MS m/z calcd for $[M]^+$ $C_{18}H_{17}N_2OI$: 404.0386, found: 404.0396.

4.4.3. *N*-[(2-(1′-1*H*-Indolyl)methyl]phenyl]-*N*-(4″-methoxybenzyl)iodoacetamide (15c). Treatment of **14c** (0.20 g, 0.48 mmol) with NaI (0.76 g, 5.04 mmol) in refluxing acetonitrile (10 mL) gave **15c** (0.17 g, 70%) as a yellow solid; mp 64–65 °C; ^1H NMR ($CDCl_3$) δ 3.20 (d, J=9.6 Hz, 1H, C*H*HI), 3.35 (d, J=9.9 Hz, 1H, CH*H*I), 3.72 (s, 3H, OCH_3), 4.51 (d, J=13.8 Hz, 1H, CONC*H*H), 4.77 (d, J=16.8 Hz, 1H, C*H*H), 4.87 (d, J=14.1 Hz, 1H, CONCH*H*), 5.10 (d, J=16.8 Hz, 1H, CH*H*), 6.47 (dd, J=3.3, 0.9 Hz, 1H, H-3), 6.70 (dd, J=8.1, 2.4 Hz, 1H, H-3′), 6.76 (dd, J=9.0, 2.4 Hz, 2H, H-3″ and H-5″), 6.82 (d, J=3.0 Hz, 1H, H-2), 6.95 (dd, J=9.0, 2.4 Hz, 1H, H-6′), 6.98 (d, J=8.4 Hz, 1H, Ar), 7.04 (td, J=6.9, 1.2 Hz, 1H, H-5), 7.05–7.12 (m with prominent d, J=8.7 Hz, 3H, H-6, H-2″, and H-6″), 7.14–7.23 (m, 2H, Ar), 7.56 (dd, J=7.5, 0.9 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$) δ −2.0 (CH_2I), 46.8 (CH_2), 53.1 (CONCH_2), 55.6 (CH_3), 102.7 (C-3), 109.7, 114.3, 120.1, 121.4, 122.2, 128.2, 128.5, 128.7 (C-3a), 129.3, 129.8, 131.2, 135.6 (C-1″), 136.5 (C-7a), 139.2 (C-2′), 159.7 (*C*OCH_3), 168.0 (CO); CI-MS m/z 511 ($[M+H]^+$, 13%); HREI-MS m/z calcd for $[M]^+$ $C_{25}H_{23}N_2O_2I$: 510.0804, found: 510.0810.

4.4.4. *N*-[(2-(1′-1*H*-Indolyl)methyl]phenyl]-*N*-benzyliodoacetamide (15d). Treatment of **14d** (0.50 g, 1.29 mmol) with NaI (1.90 g, 12.9 mmol) in refluxing acetonitrile (25 mL) gave **15d** (0.52 g, 96%) as a yellow solid; mp 101–103 °C; ^1H NMR ($CDCl_3$) δ 3.31 (d, J=9.9 Hz, 1H, C*H*HI), 3.46 (d, J=9.6 Hz, 1H, CH*H*I), 4.62 (d, J=13.8 Hz, 1H, CONC*H*H), 4.85 (d, J=16.8 Hz, 1H, C*H*H), 5.04 (d, J=14.1 Hz, 1H, CONCH*H*), 5.18 (d, J=16.5 Hz, 1H, CH*H*), 6.55 (d, J=3.3 Hz, 1H, H-3), 6.79 (dd, J=7.5, 2.7 Hz, 1H, Ar), 6.88 (d, J=3.3 Hz, 1H, H-2), 7.03 (d, J=6.9 Hz, 1H, Ar), 7.06 (d, J=7.5 Hz, 1H, H-7), 7.11 (td, J=6.6, 0.9 Hz, 1H, H-5), 7.18 (td, J=7.5, 1.2 Hz, 1H, H-6), 7.22–7.28 (m, 4H, Ar), 7.30–7.36 (m, 3H, Ar), 7.64 (dd, J=7.8, 1.2 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$) δ −2.2 (CH_2I), 46.8 (CH_2), 53.7 (CONCH_2), 102.7 (C-3), 109.6, 120.6, 121.4, 122.2, 128.1 (C-2), 128.4, 128.8 (C-3a), 128.9, 129.2, 129.3, 129.7, 129.8, 129.9, 135.6 (C-1′), 136.4 (C-7a), 136.5 (C-1″), 139.3 (C-2′), 168.1 (CO); CI-MS m/z 480 ($[M+H]^+$, 100%); HRCI-MS m/z calcd for $[M+H]^+$ $C_{24}H_{21}N_2OI$: 480.0690, found: 480.0689.

4.4.5. *N*-[(2-(1′-1*H*-Indolyl)methyl]phenyl]-*N*-(1″-phenylethyl)iodoacetamide (15e). Treatment of **14e** (36 mg, 0.09 mmol) with NaI (0.14 g, 0.95 mmol) in refluxing acetonitrile (2 mL) gave **15e** (37 mg, 82%) as a yellow solid; mp 60–61 °C; ^1H NMR ($CDCl_3$, rotamer ratio of ca. 2:1*) δ 1.55* (d, J=7.2 Hz, 1H, CH_3), 1.62 (d, J=6.9 Hz, 2H, CH_3), 3.24 (d, J=9.9 Hz, 1H, C*H*HI), 3.44 (d, J=9.6 Hz, 1H, CH*H*I), 3.80 (d, J=17.1 Hz, 0.7H, C*H*H), 4.69 (d, J=17.1 Hz, 0.7H, CH*H*), 5.30* (d, J=16.8 Hz, 0.3H, C*H*H), 5.39* (d, J=16.8 Hz, 0.3H, CH*H*), 6.24–6.29 (m, 1H, CH and CH*), 6.39 (d, J=7.8 Hz, 0.7H, H-3′), 6.45 (dd, J=3.0, 0.9 Hz, 0.7H, H-3), 6.52–6.54 (m with pominent d, J=3.3 Hz, 1H, H-2 and H-3′*), 6.60 (d, J=3.3 Hz, 0.3H, H-3*), 6.78 (d, J=8.1 Hz, 1H, Ar), 7.03–7.21 (m, 4H, H-5, H-2″, H-6″ and Ar), 7.20–7.21 (m, 1H, H-4′ and Ar), 7.27–7.42 (m, 5H, H-7, H-3″, H-5″ and Ar), 7.57 (dd, J=7.5, 1.5 Hz, 0.7H, H-4), 7.69 (d, J=7.5 Hz, 0.3H, H-4*); ^{13}C NMR ($CDCl_3$) δ −1.3 (CH_2I), −0.8* (CH_2I), 17.4* (CH_3), 19.1 (CH_3), 46.2 (CH_2), 47.1* (CH_2), 55.2* (CH), 55.7 (CH), 102.5 (C-3), 103.0* (C-3), 109.5, 119.9, 120.2*, 121.2, 121.5*, 122.0, 122.4*, 128.1, 128.2, 128.3, 128.5, 128.7, 128.8 (C-3a), 128.87, 128.9, 129.5, 129.8*, 130.1, 130.7*, 131.0, 135.6 (C-1′), 136.4* (ArC), 136.5 (C-7a), 136.66* (ArC), 136.7* (ArC), 138.0 (C-1″), 139.2 (C-2′), 141.3* (C-2), 167.9 (CO), 171.3* (CO); CI-MS m/z 495 ($[M+H]^+$, 100%); HRCI-MS m/z calcd for $[M+H]^+$ $C_{25}H_{24}N_2OI$: 495.0933, found: 495.0926.

4.4.6. *N*-{2-[1′-(5-Methoxy-1*H*-indolyl)methyl]phenyl}-*N*-methyliodoacetamide (15f). Treatment of **14f** (0.21 g, 0.63 mmol) with NaI (0.99 g, 6.61 mmol) in refluxing acetonitrile (10 mL) gave **15f** (0.17 g, 70%) as a yellow solid; mp 75–77 °C; ^1H NMR ($CDCl_3$) δ 3.18 (s, 3H, CH_3), 3.27 (d, J=9.9 Hz, 1H, C*H*HI), 3.42 (d, J=10.2 Hz, 1H, CH*H*I), 3.83 (s, 3H, OCH_3), 5.26 (d, J=16.2 Hz, 1H, C*H*H), 5.37 (d, J=16.5 Hz, 1H, CH*H*), 6.48 (d, J=3.3 Hz, 1H, H-3), 6.83 (dd, J=8.7, 2.7 Hz, 1H, Ar), 6.93 (dd, J=7.2, 2.1 Hz, 1H, Ar), 7.05 (d, J=3.0 Hz, 1H, H-2), 7.08–7.11 (m, 2H, Ar), 7.23 (td, J=8.4, 1.5 Hz, 1H, Ar), 7.35 (d, J=8.7 Hz, 1H, Ar), 7.38 (d, J=9.3 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$) δ −2.7 (CH_2I), 37.7 (CH_3), 47.2 (CH_2), 56.1 (OCH_3), 102.3 (C-3), 103.0, 110.5, 112.6, 128.5, 128.9, 129.6, 129.8, 131.7 (C-1′), 135.0 (C-7a), 141.3 (C-2′), 154.4 (*C*OCH_3), 168.3 (CO); CI-MS m/z 435 ($[M+H]^+$, 100%); HREI-MS m/z calcd for $[M]^+$ $C_{19}H_{19}N_2O_2I$: 434.0491, found: 434.0476.

4.4.7. *N*-{2-[1′-(5-Methoxy-1*H*-indolyl)methyl]phenyl}-*N*-benzyliodoacetamide (15g). Treatment of **14g** (62 mg, 0.15 mmol) with NaI (0.22 g, 1.50 mmol) in refluxing acetonitrile (3 mL) gave **15g** (55 mg, 73%) as a yellow solid; mp 59–61 °C; ^{1}H NMR ($CDCl_3$) δ 3.28 (d, J=9.6 Hz, 1H, C*H*HI), 3.43 (d, J=9.9 Hz, 1H, CH*H*I), 3.84 (s, 3H, OCH_3), 4.60 (d, J=13.8 Hz, 1H, CONC*H*H), 4.81 (d, J=16.8 Hz, 1H, C*H*H), 5.02 (d, J=13.8 Hz, 1H, CONCH*H*), 5.12 (d, J=16.8 Hz, 1H, CH*H*), 6.46 (dd, J=3.3, 0.9 Hz, 1H, H-3), 6.78–6.81 (m, 2H, Ar), 6.83–6.86 (m, 2H, H-2 and Ar), 6.93 (dd, J=9.0, 0.6 Hz, 1H, H-3′), 7.05 (ddd, J=9.0, 6.6, 2.4 Hz, 1H, Ar), 7.09 (d, J=2.4 Hz, 1H, H-4), 7.27–7.29 (m, 4H, Ar), 7.33–7.36 (m, 2H, Ar); ^{13}C NMR ($CDCl_3$) δ −2.5 (CH_2I), 46.9 (CH_2), 53.5 (CON*C*H_2), 56.0 (OCH_3), 102.2 (C-3), 102.9, 110.4, 112.5, 128.4, 128.6 (C-3a), 128.7, 128.9, 129.2, 129.4, 129.7, 129.8, 129.9, 131.8 (C-1′), 135.7 (C-7a), 136.5 (C-1″), 139.4 (C-2′), 154.5 (*C*OCH_3), 168.2 (CO); CI-MS *m/z* 511 ([M+H]$^+$, 100%); HRCI-MS *m/z* calcd for [M+H]$^+$ $C_{25}H_{24}N_2O_2I$: 511.0883, found: 511.0868.

4.4.8. *N*-{2-[1′-(5-Fluoro-1*H*-indolyl)methyl]phenyl}-*N*-benzyliodoacetamide (15h). Treatment of **14h** (74 mg, 0.18 mmol) with NaI (0.27 g, 1.80 mmol) in acetonitrile (4 mL) gave **15h** (77 mg, 89%) as a yellow solid; mp 53–54 °C; ^{1}H NMR ($CDCl_3$) δ 3.28 (d, J=9.9 Hz, 1H, C*H*HI), 3.42 (d, J=9.0 Hz, 1H, CH*H*I), 4.64 (d, J=13.8 Hz, 1H, CONC*H*H), 4.82 (d, J=16.8 Hz, 1H, C*H*H), 4.98 (d, J=13.8 Hz, 1H, CONCH*H*), 5.15 (d, J=16.8 Hz, 1H, CH*H*), 6.49 (d, J=3.3 Hz, 1H, H-3), 6.77 (dd, J=7.5, 2.7 Hz, 1H, Ar), 6.90–6.93 (m with prominent d, J=3.0 Hz, 3H, H-2 and Ar), 7.05 (dd, J=8.9, 2.7 Hz, 1H, Ar), 7.22–7.33 (m, 8H, Ar); ^{13}C NMR ($CDCl_3$) δ −2.6 (CH_2I), 47.0 (CH_2), 53.6 (CON*C*H_2), 102.6 (d, J=5.2 Hz, ^{13}C–^{19}F, C3–F), 106.1 (d, J=22.9 Hz, ^{13}C–^{19}F, C6–F), 110.3 (d, J=9.8 Hz, ^{13}C–^{19}F, C7–F), 110.7 (d, J=26.3 Hz, ^{13}C–^{19}F, C4–F), 128.5, 129.0, 129.2, 129.6, 129.79, 129.83, 129.88, 129.9, 130.5 (C-3a), 133.1 (C-1′), 135.4 (C-7a), 136.5 (C-1″), 139.4 (C-2′), 158.2 (d, J=223.7 Hz, ^{13}C–^{19}F, C5–F), 168.2 (CO); CI-MS *m/z* 499 ([M+H]$^+$, 46%); HREI-MS *m/z* calcd for [M]$^+$ $C_{24}H_{20}N_2OFI$: 498.0604, found: 498.0600.

4.4.9. *N*-{2-[1′-(5-Fluoro-1*H*-indoly)methyl]phenyl}-*N*-methyliodoacetamide (15i). To a suspension of NaH (18.2 mg of ca. 50% dispersion in mineral oil, 0.38 mmol) in dry DMF (5 mL) under nitrogen was cooled to 0–5 °C and a solution of **14i** (0.11 g, 0.35 mmol) in DMF (15 mL) was added dropwise and the mixture was stirred for 30 min at r.t. A solution of methyl iodide (54 μL, 0.87 mmol) in DMF (2 mL) was added and the reaction mixture was stirred at r.t. for a further 16 h. The solvent was evaporated, DCM (20 mL) was added and washed with water (3×15 mL), dried, and evaporated. The crude product was subjected to flash column chromatography (DCM) to give **15i** (76 mg, 66%) as a yellow solid; mp 74–76 °C; ^{1}H NMR ($CDCl_3$) δ 3.17 (s, 3H, CH_3), 3.29 (d, J=9.9 Hz, 1H, C*H*HI), 3.43 (d, J=10.2 Hz, 1H, CH*H*I), 5.29 (d, J=16.5 Hz, 1H, C*H*H), 5.39 (d, J=16.5 Hz, 1H, CH*H*), 6.53 (dd, J=3.9, 0.9 Hz, 1H, H-3), 6.91 (dd, J=9.3, 2.7 Hz, 1H, H-4), 6.95 (dd, J=7.8, 2.1 Hz, 1H, H-3′), 7.12 (d, J=3.3 Hz, 1H, H-2), 7.15 (dd, J=7.2, 2.4 Hz, 1H, H-7), 7.29 (ddd, J=9.6, 7.2, 2.4 Hz, 1H, H-6), 7.32 (dd, J=7.8, 1.5 Hz, 1H, H-6′), 7.36–7.44 (m, 2H, Ar); ^{13}C NMR ($CDCl_3$) δ −3.2 (CH_2I), 37.6 (CH_3), 47.2 (CH_2), 102.7 (d, J=4.6 Hz, C^{13}–F^{19}, C3–F), 106.2 (d, J=23.5 Hz, C^{13}–F^{19}, C6–F), 110.3 (d, J=9.5 Hz, C^{13}–F^{19}, C7–F), 110.8 (d, J=26 Hz, C^{13}–F^{19}, C4–F), 128.7, 129.2 (d, J=10.4 Hz, C^{13}–F^{19}, C3a–F), 129.6, 129.88, 129.93, 130.0, 133.1 (C-7a), 134.6 (C-1″), 141.4 (C-2′), 158.7 (d, J=225.0 Hz, C^{13}–F^{19}, C5–F), 168.4 (CO); CI-MS *m/z* 423 ([M+H]$^+$, 43%); HRCI-MS *m/z* calcd for [M+H]$^+$ $C_{18}H_{17}N_2OFI$: 423.0369, found: 423.0336.

4.4.10. *N*-[(2-(1′-1*H*-Indolyl)methyl]phenyl]-*N*-benzylbromoacetamide (16a). By a procedure similar to that for **15i**, treatment of **9** (0.11 g, 0.34 mmol) with NaH (18 mg, 0.38 mmol) and bromoacetyl chloride (0.10 mL, 0.85 ml) in dry DMF (20 mL) gave **16a**, after purification by column chromatography (1:1 DCM/hexane), (0.10 g, 68%) as a yellow solid; mp 99–101 °C; ^{1}H NMR ($CDCl_3$) δ 3.43 (d, J=1.8 Hz, 2H, CH_2Br), 4.62 (d, J=13.8 Hz, 1H, CONC*H*H), 4.34 (d, J=16.8 Hz, 1H, C*H*H), 5.06 (d, J=13.8 Hz, CONCH*H*), 5.17 (d, J=16.8 Hz, 1H, CH*H*), 6.55 (dd, J=3.3, 0.9 Hz, 1H, H-3), 6.81 (m, 1H, Ar), 6.87 (d, J=3.3 Hz, 1H, H-2), 6.98 (m, 1H, Ar), 7.07 (dd, J=6.9, 0.9 Hz, 1H, H-7), 7.12 (ddd, J=8.4, 7.2, 1.2 Hz, 1H, H-5), 7.18 (td, J=8.5, 1.2 Hz, 1H, H-6), 7.23–7.28 (m, 4H, Ar), 7.30–7.35 (m, 3H, Ar), 7.64 (dd, J=8.0, 1.2 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$) δ 27.0 (CH_2Br), 46.7 (CH_2), 53.6 (CONCH_2), 102.7 (C-3), 109.3 (C-7), 120.1, 121.4, 122.3, 128.1, 128.4, 128.8 (C-3a), 129.0, 129.3, 129.4, 129.9, 130.1, 135.7 (ArC), 136.3 (ArC), 136.4 (C-2′), 138.9 (C-12a), 166.8 (CO); CI-MS *m/z* 433 ([M+H]$^+$, 90%); HREI-MS *m/z* calcd for [M]$^+$ $C_{24}H_{21}N_2O^{79}Br$: 432.0837, found: 432.0851.

4.4.11. *N*-{2-[1′-(5-methoxy-1*H*-indolyl)methyl]phenyl}-*N*-methylbromoacetamide (16b). A mixture of **11** (0.11 g, 0.41 mmol) and anhydrous K_2CO_3 (0.20 g, 1.50 mmol) in dry DCM (5 mL) was cooled to 0–5 °C and bromoacetyl chloride (80 μL, 1.03 mmol) in DCM (3 mL) was added. Stirring was continued at 0–5 °C for a further 30 min and the reaction mixture was then allowed to warm to r.t. with stirring overnight. Distilled water (20 mL) was added to the reaction mixture and the aqueous layer was extracted with DCM (3×20 mL). The combined organic extracts were dried, and concentrated to yield a yellow solid. The crude solid was subjected to flash column chromatography (DCM) to give **16b** (83 mg, 54%) as yellow crystals; mp 58–59 °C; ^{1}H NMR ($CDCl_3$) δ 3.21 (s, 3H, CH_3), 3.53 (d, J=12.6 Hz, 1H, C*H*HBr), 3.63 (d, J=12.6 Hz, 1H, CH*H*Br), 3.84 (s, 3H, OCH_3), 5.25 (d, J=5.4 Hz, 2H, CH_2), 6.49 (dd, J=3.0, 0.6 Hz, 1H, H-3), 6.83 (dd, J=9.0, 2.4 Hz, 1H, Ar), 6.97 (dd, J=7.8, 2.1 Hz, 1H, Ar), 7.02–7.10 (m, 2H, H-2 and Ar), 7.20–7.40 (m, 4H, Ar); ^{13}C NMR ($CDCl_3$) δ 26.4 (CH_2Br), 37.5 (CH_3), 47.1 (CH_2), 56.1 (OCH_3), 102.3 (C-3), 103.2, 110.5, 112.6, 128.9, 129.3 (C-3a), 129.7, 129.8, 129.9, 131.6 (ArC), 135.2 (C-7a), 140.6 (C-2′), 154.4 (*C*OCH_3), 166.8 (CO); CI-MS *m/z* 387 ([M+H]$^+$, 13%); HREI-MS *m/z* calcd for [M]$^+$ $C_{19}H_{19}N_2O_2^{79}Br$: 386.0630, found: 386.0624.

4.5. General procedure for radical cyclization

A solution of tributyltin hydride (Bu_3SnH) (2.0 equiv) and AIBN (1.0 equiv) in an appropriate solvent (40 mM) was added dropwise to the solution of the haloacetamide (**14–16**,

1.0 equiv) in boiling solvent (22 mM) over 4 h, and the mixture was then heated at reflux overnight After removal of the solvent in vacuo, diethyl ether (20 mL) and saturated potassium fluoride solution (20 mL) were added and the mixture was stirred vigorously at r.t. for 2–3 h. The organic layer was separated, dried, concentrated and column chromatographed (DCM eluent).

The following radical cyclizations were performed by this method.

4.5.1. Cyclization of 15b. Compound **15b** (54 mg, 0.13 mmol) in toluene (5 mL) was treated with AIBN (21 mg, 0.13 mmol) and Bu_3SnH (70 μL, 0.26 mmol). The first fraction from the column gave 5,14-dihydro-5-methyl-indolo[2,1-*d*][1,5]benzodiazocin-6-one **17a**, (20 mg, 20%) as a yellow solid; mp 211–212 °C (DCM); IR (KBr) ν_{max}: 2852 (N–CH_3), 1660 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.436 (s, 3H, CH_3), 3.447 (d, $J=13.8$ Hz, 1H, C*H*H-7), 3.67 (d, $J=13.8$ Hz, 1H, CH*H*-7), 4.75 (d, $J=14.1$ Hz, 1H, C*H*H-14), 5.36 (d, $J=13.8$ Hz, 1H, CH*H*-14), 6.37 (s, 1H, H-8), 7.10 (t, $J=7.5$ Hz, 1H, H-10), 7.23 (td, $J=8,1$ Hz, 1H, Ar), 7.38 (d, $J=7.2$ Hz, 1H, Ar), 7.46–7.56 (m, 5H, Ar); ^{13}C NMR ($CDCl_3$) δ 36.2 (CH_2-7), 36.7 (CH_3), 45.2 (CH_2-14), 103.6 (C-8), 108.7, 119.9, 120.6, 121.7, 125.5, 128.0 (C-8a), 129.0, 130.6, 131.8 (C-7a), 132.2, 133.0 (C-14a), 136.8 (C-12a), 144.7 (C-4a), 168.9 (CO); CI-MS *m/z* 277 ($[M+H]^+$, 100%); HRCI-MS *m/z* calcd for $[M+H]^+$ $C_{18}H_{17}N_2O$: 277.1341, found: 277.1341.

The second fraction gave 5,7a,8,14-tetrahydro-5-methyl indolo[2,1-*d*][1,5]benzodiazocin-6-one **18a**, (80 mg, 60%) as a yellow solid; mp 129–130 °C (DCM); ^1H NMR ($CDCl_3$) δ 2.39–2.50 (m, 2H, CH_2-8), 2.60 (dd, $J=15.9$, 9.0 Hz, 1H, C*H*H-7), 3.29 (dd, $J=15.9$, 9.0 Hz, 1H, CH*H*-7), 3.38 (s, 3H, CH_3), 3.84 (d, $J=14.7$ Hz, 1H, C*H*H-14), 4.08 (qd, $J=8.1$, 1.2 Hz, 1H, H-7a), 4.55 (d, $J=15.0$, 1H, CH*H*-14), 6.58 (*t*, $J=7.5$ Hz, 1H, H-10), 6.68 (d, $J=7.8$ Hz, 1H, H-12), 6.99 (d, $J=7.2$ Hz, 1H, Ar), 7.06 (m, 2H, Ar), 7.22–7.39 (m, 3H, Ar), 7.52 (d, $J=7.2$ Hz, 1H, H-9); ^{13}C NMR ($CDCl_3$) δ 36.8 (CH_2-7), 38.0 (CH_3), 42.7 0 (CH_2-8), 45.8 (CH_2-14), 62.4 (CH), 105.1 (C-12), 116.9, 124.8, 126.0, 127.6, 127.7 (C-8a), 128.3, 129.2, 131.6, 135.3 (ArC), 142.4 (ArC), 149.5 (C-4a), 172.1 (CO); CI-MS *m/z* 279 ($[M+H]^+$, 100%); HREI-MS *m/z* calcd for $[M]^+$ $C_{18}H_{18}N_2O$: 278.1419, found: 278.1413.

The third fraction gave *N*-[(2-(1′-1*H*-indolyl)methyl) phenyl]-*N*-methylacetamide **19a**, (20 mg, 20%); mp 58–59 °C; ^1H NMR ($CDCl_3$) δ 1.78 (s, 3H, $COCH_3$), 3.20 (s, 3H, NCH_3), 5.26 (s, 2H, CH_2-8), 6.58 (d, $J=3.0$ Hz, 1H, H-3), 6.90 (d, $J=7.5$ Hz, 1H, Ar), 7.07 (dd, $J=3.0$, 0.6 Hz, 1H, H-2), 7.11–7.20 (m, 4H, Ar), 7.25 (t, $J=7.5$ Hz, 1H, Ar), 7.35 (t, $J=7.5$ Hz, 1H, Ar), 7.66 (dd, $J=7.8$, 1.2 Hz, 1H, Ar); ^{13}C NMR ($CDCl_3$) δ 22.2 (CO*C*H_3), 36.6 (NCH_3), 46.6 (CH_2-8), 102.6, 109.7, 120.1, 121.2, 122.0, 128.2, 128.4, 129.0, 129.4, 135.0 (ArC), 136.3 (ArC), 140.1 (C-2′), 171.7 (CO); CI-MS *m/z* 279 ($[M+H]^+$, 100%); HRCI-MS *m/z* calcd for $[M+H]^+$ $C_{18}H_{19}N_2O$: 279.1497, found: 279.1499.

4.5.2. Cyclization of 15b in mesitylene. Compound **15b** (56 mg, 0.14 mmol) in mesitylene (5 mL) was treated with AIBN (23 mg, 0.14 mmol) and Bu_3SnH (75 μL, 0.28 mmol). The crude material was chromatographed (DCM) to give **18a** (17 mg, 44%).

4.5.3. Cyclization of 15c. Compound **15c** (0.17 g, 0.33 mmol) in toluene (5 mL) was treated with AIBN (37 mg, 0.23 mmol) and Bu_3SnH (0.12 mL, 0.46 mmol). The first fraction from column gave 5,14-dihydro-5-(4′-methoxybenzyl)indolo[2,1-*d*][1,5]benzodiazo cin-6-one **17b** (16 mg, 13%) as a yellow solid; mp 213–215 °C (DCM); ^1H NMR ($CDCl_3$) δ 3.42 (dd, $J=14.1$, 1.2 Hz, 1H, C*H*H-7), 3.68 (d, $J=14.1$ Hz, 1H, CH*H*-7), 3.76 (s, 3H, OCH_3), 4.10 (d, $J=13.8$ Hz, 1H, C*H*H-14), 4.50 (d, $J=13.8$ Hz, 1H, CONC*H*H), 4.93 (d, $J=13.8$ Hz, 1H, CH*H*-14), 5.52 (d, $J=13.8$ Hz, 1H, CONCH*H*), 6.35 (s, 1H, H-8), 6.74 (d, $J=8.7$ Hz, 2H, H-3′ and H-5′), 7.06 (t, $J=7.5$ Hz, 1H, H-10), 7.11 (d, $J=8.7$ Hz, 2H, H-2′ and H-6′), 7.18 (td, $J=7.2$, 0.6 Hz, 1H, H-11), 7.30–7.40 (m, 4H, Ar), 7.46 (dd, $J=7.2$, 1.5 Hz, 1H, Ar), 7.51 (d, $J=7.8$ Hz, 1H, H-9); ^{13}C NMR ($CDCl_3$) δ 36.6 (CH_2-7), 44.8 (CH_2-14), 52.2 (CONCH_2), 55.5 (OCH_3), 103.5 (C-8), 108.7, 114.0, 119.8, 120.5, 121.5, 126.0, 128.0 (ArC), 128.5 (ArC), 129.0, 130.4, 130.9, 132.8 (ArC), 133.1 (ArC), 137.5 (C-12a), 142.8 (C-1′), 159.3 (*C*OCH_3), 168.2 (CO); CI-MS *m/z* 383 ($[M+H]^+$, 100%); HRES-MS *m/z* calcd for $[M+H]^+$ $C_{25}H_{23}N_2O_2$: 383.1763, found: 383.1760.

The second fraction gave 5,7a,8,14-tetrahydro-5-(4′-methoxybenzyl)indolo[2,1-*d*][1,5]benzodiazocin-6-one **18b** (13 mg, 11%) as a yellow solid; mp 107–109 °C (DCM); ^1H NMR ($CDCl_3$) δ 2.39–2.51 (m, 2H, CH_2-8), 2.56 (dd, $J=15.6$, 8.4 Hz, 1H, C*H*H-7), 3.20–3.29 (m with prominent d, $J=14.1$ Hz, 2H, CH*H*-7 and CONC*H*H), 3.76 (d, $J=1.2$ Hz, 3H, OCH_3), 4.01 (q, $J=8.4$ Hz, 1H, H-7a), 4.14 (d, $J=15.0$ Hz, 1H, CONCH*H*), 4.38 (d, $J=14.1$ Hz, 1H, C*H*H-14), 5.51 (d, $J=13.8$ Hz, 1H, CH*H*-14), 6.51–6.56 (m with prominent d, $J=6.9$ Hz, 2H, Ar), 6.75 (d, $J=7.8$ Hz, 2H, H-3′ and H-5′), 6.95 (d, $J=7.5$ Hz, 1H, Ar), 7.04 (t, $J=8.4$ Hz, 1H, Ar), 7.07 (d, $J=7.8$ Hz, 2H, H-2′ and H-6′), 7.22–7.35 (m, 3H, Ar), 7.39 (d, $J=7.5$ Hz, 1H, H-9); ^{13}C NMR ($CDCl_3$) δ 36.7 (CH_2-7), 42.6 (CH_2-8), 45.6 (CH_2-14), 53.1 (CONCH_2), 55.5 (OCH_3), 62.8 (CH), 105.0, 113.9, 116.7, 124.7, 126.7, 127.5, 127.7 (ArC), 128.4, 128.9 (ArC), 129.0, 130.7, 131.3, 136.7 (ArC), 140.4 (ArC), 154.7 (*C*OCH_3), 171.5 (CO); CI-MS *m/z* 385 ($[M+H]^+$, 100%); HRES-MS *m/z* calcd for $[M+H]^+$ $C_{25}H_{25}N_2O_2$: 385.1910, found: 385.1916.

The third fraction gave *N*-[(2-(1′-1*H*-indolyl)methyl)-phenyl]-*N*-(4″-methoxybenzyl)acetamide **19b** (9 mg, 7%) as a yellow solid; mp 58–60 °C (DCM); ^1H NMR ($CDCl_3$) δ 1.81 (s, 3H, CH_3), 3.79 (s, 3H, OCH_3), 4.75 (d, $J=15.9$ Hz, 2H, C*H*H and CONC*H*H), 4.84 (d, $J=13.8$ Hz, 1H, CH*H*), 5.03 (d, $J=17.2$ Hz, 1H, CONCH*H*), 6.53 (dd, $J=3.0$, 1.5 Hz, 1H, H-3), 6.65 (d, $J=7.8$ Hz, 1H, H-3′), 6.83 (m with prominent dd, $J=8.7$, 2.4 Hz, 3H, H-2, H-3′ and H-5′), 6.95 (t, $J=8.7$ Hz, 1H, H-5′), 7.08–7.17 (m, 3H, H-2′, H-6′ and Ar), 7.20–7.23 (m, 2H, Ar), 7.42–7.52 (m, 2H, Ar), 7.63 (d, $J=6.9$ Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$) δ 22.5 (CH_3), 47.4 (CH_2), 52.1 (CONCH_2), 55.5 (OCH_3), 102.5 (C-3), 109.5, 114.2, 111.9, 121.3, 122.1, 128.3, 128.5, 129.2, 129.7, 130.9, 131.3, 132.4 (ArC), 135.9 (ArC), 137.6 (C-1′), 140.3 (C-4a), 159.6 (*C*OCH_3), 168.2 (CO); CI-MS *m/z* 385

([M+H]$^+$, 100%); HRES-MS *m/z* calcd for [M+H]$^+$ $C_{25}H_{25}N_2O_2$: 385.1910, found: 385.1916.

4.5.4. Cyclization of 15d. Compound **15d** (38 mg, 0.08 mmol) in toluene (5 mL) was treated with AIBN (13 mg, 0.08 mmol) and Bu_3SnH (43 μL, 0.16 mmol) The first fraction from the column gave 5-benzyl-5,14-dihydro-indolo[2,1-*d*][1,5]benzodiazocin-6-one **17c** (8 mg, 25%) as a yellow solid; mp 154–155 °C (DCM); IR (KBr) ν_{max}: 1661 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.45 (dd, J=13.8, 1.5 Hz, 1H, C*H*H-7), 3.71 (d, J=13.8 Hz, 1H, CH*H*-7), 4.13 (d, J=14.1 Hz, 1H, CONC*H*H), 4.62 (d, J=13.8 Hz, 1H, C*H*H-14), 4.93 (d, J=13.8 Hz, 1H, CONCH*H*), 5.53 (d, J=14.1 Hz, 1H, CH*H*-14), 6.37 (s, 1H, H-8), 7.07 (t, J=7.8, 1.2 Hz, 1H, H-10), 7.16–7.25 (m, 7H, Ar), 7.34 (d, J=7.8 Hz, 1H, Ar), 7.38–7.42 (m, 2H, Ar), 7.46 (dd, J=8.1, 1.8 Hz, 1H, Ar), 7.52 (d, J=7.8 Hz, 1H, H-9); ^{13}C NMR ($CDCl_3$) δ 36.6 (CH_2-7), 44.8 (CH_2-14), 52.8 (CON*C*H$_2$), 103.6 (C-8), 108.7, 119.8, 120.5, 121.5, 125.9, 128.0 (C-8a), 128.2, 128.8, 129.0, 129.6, 130.5, 132.1, 132.7 (C-7a), 133.0 (C-14a), 136.3 (C-12a), 137.5 (C-1′), 142.8 (C-4a), 168.4 (CO); CI-MS *m/z* 353 ([M+H]$^+$, 100%); HREI-MS *m/z* calcd for [M]$^+$ $C_{24}H_{20}N_2O$: 352.1576, found: 352.1565.

The second fraction gave 5-benzyl-5,7a,8,14-tetrahydro-indolo[2,1-*d*][1,5]benzodiazocin-6-one **18c** (12 mg, 43%) as a yellow solid; mp 141–144 °C (DCM); IR (KBr) ν_{max}: 1647 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.41–2.52 (m, 2H, CH_2-8), 2.57 (dd, J=15.9, 9.3 Hz, 1H, C*H*H-7), 3.22–3.30 (m with prominent d, J=15.3 Hz, 2H, CH*H*-7 and CONC*H*H), 4.02 (qd, J=9.0, 1.2 Hz, 1H, H-7a), 4.14 (d, J=15.0 Hz, 1H, CONCH*H*), 4.50 (d, J=14.1 Hz, 1H, C*H*H-14), 5.51 (d, J=14.1 Hz, 1H, CH*H*-14), 6.51–6.56 (m, 2H, Ar), 6.95 (d, J=6.9 Hz, 1H, Ar), 7.04 (t, J=7.8 Hz, 1H, Ar), 7.14–7.19 (m, 2H, Ar), 7.22–7.27 (m, 5H, Ar), 7.30 (dd, J=9.0, 1.8 Hz, 1H, Ar), 7.40 (dd, J=9.0, 2.4 Hz, 1H, H-9); ^{13}C NMR ($CDCl_3$) δ 36.6 (CH_2-7), 42.5 (CH_2-8), 45.5 (CH_2-14), 53.7 (CON*C*H$_2$), 62.7 (CH), 105.0, 116.8, 124.8, 126.7, 127.6, 127.7 (C-8a), 128.0, 128.5, 128.7, 129.1, 129.4, 131.4 (ArC), 136.7 (ArC), 136.8 (C-1′), 149.6 (C-4a), 171.1 (CO); CI-MS *m/z* 355 ([M+H]$^+$, 100%); HREI-MS *m/z* calcd for [M]$^+$ $C_{24}H_{22}N_2O$: 354.1732, found: 354.1718.

The third fraction gave *N*-[(2-(1′-1*H*-Indolyl)methyl)-phenyl]-*N*-benzylacetamide **19c** (3 mg, 10%) as a yellow solid; mp 158–159 °C (DCM); IR (KBr) ν_{max}: 1676 (C=O), 1358 (CO–CH_3) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.83 (s, 3H, CH_3), 4.74 (d, J=16.8 Hz, 1H, CONC*H*H), 4.80 (d, J=14.1 Hz, 1H, C*H*H), 4.92 (d, J=14.1 Hz, 1H, CH*H*), 5.04 (d, J=16.8 Hz, 1H, CONCH*H*), 6.53 (dd, J=3.3, 0.9 Hz, 1H, H-3), 6.65 (d, J=7.8 Hz, 1H, H-3′), 6.79 (d, J=3.3 Hz, 1H, H-2), 6.94–6.99 (m, 2H, Ar), 7.11 (td, J=7.2, 1.2 Hz, 1H, Ar), 7.15–7.16 (m, 2H, H-5 and H-10), 7.21 (td, J=8.4, 1.8 Hz, 1H, H-11), 7.24–7.29 (m, 3H, Ar), 7.30–7.35 (m, 2H, Ar), 7.64 (ddd, J=7.2, 1.8, 0.6 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$) δ 22.6 (CH_3), 46.4 (CH_2), 52.7 (CON*C*H$_2$), 102.5 (C-3), 109.6, 120.0, 121.3, 122.1, 128.4, 128.5, 128.8 (C-3a), 128.9, 129.2, 129.7, 129.8, 135.9 (C-1′), 136.5 (C-7a), 137.2 (C-1″), 140.3 (C-2′), 170.6 (CO); CI-MS *m/z* 355 ([M+H]$^+$, 100%); HREI-MS *m/z* calcd for [M]$^+$ $C_{24}H_{22}N_2O$: 354.1732, found: 354.1732.

4.5.5. Cyclization of 15d in larger scale. Compound **15d** (0.30 g, 0.68 mmol) in toluene (45 mL) was treated with AIBN (0.12 g, 0.72 mmol) and Bu_3SnH (0.39 mL, 1.44 mmol). The crude material was chromatographed to give **17c** (26 mg, 12%), **18c** (42.8 mg, 19%), **19c** (70 mg, 32%). A further product from the chromatographic separation was compound **20** (10 mg, 4%), obtained as a yellow solid; mp 76–77 °C; IR (KBr) ν_{max}: 1650 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.23 (s, 3H,CH_3), 1.49 (s, 3H, CH_3), 2.21 (d, J=12.3 Hz, 1H, C*H*H-7), 2.58 (dd, J=12.3, 10.5 Hz, 1H, CH*H*-7), 3.01 (d, J=3.3 Hz, 1H, H-8), 3.43 (d, J=15.6 Hz, 1H, C*H*H-14), 3.96 (dd, J=10.5, 3.6 Hz, 1H, H-7a), 4.24 (d, J=15.6 Hz, 1H, CH*H*-14), 4.56 (d, J=13.5 Hz, 1H, CONC*H*H), 5.44 (d, J=13.5 Hz, 1H, CONCH*H*), 6.58 (t, J=7.2 Hz, 1H, H-10), 6.65 (d, J=8.1 Hz, 1H, H-12), 7.12–7.20 (m, 3H, H-2, H-11 and Ar), 7.22–7.29 (m, 5H, Ar), 7.30–7.34 (m, 2H, H-9 and Ar), 7.47 (d, J=7.5 Hz, 1H, H-1); ^{13}C NMR ($CDCl_3$) δ 21.9 (CH_3), 24.5 (CH_3), 36.7 (*C*CN), 44.2 (CH_2-7), 44.3 (CH_2-14), 53.6 (CON*C*H$_2$), 56.1 (C-8), 63.5 (C-7a), 106.8 (C-7), 116.8 (C-5), 124.7 (CN), 125.4 (C-8a), 126.2 (C-4), 126.6, 128.0, 128.1, 128.7 (all ArCH), 129.2 (C-11), 129.37, 129.4 (all ArCH), 132.0 (C-1), 135.3 (C-14a), 136.6 (C-1′), 140.7 (C-4a), 149.1 (C-12a), 171.1 (CO); CI-MS *m/z* 422 ([M+H]$^+$, 100%); HREI-MS *m/z* calcd for [M]$^+$ $C_{28}H_{27}N_3O$: 421.2154, found: 421.2160.

4.5.6. Cyclization of 15d in xylene. Compound **15d** (52 mg, 0.11 mmol) in xylene (5 mL) was treated with AIBN (18 mg, 0.11 mmol) and Bu_3SnH (59 μL, 0.22 mmol). The crude material was chromatographed to give **17c** (22 mg, 57%).

4.5.7. Cyclization of 15d in mesitylene. Compound **15d** (51 mg, 0.11 mmol) in mesitylene (5 mL) was treated with AIBN (18 mg, 0.11 mmol) and Bu_3SnH (59 μL, 0.22 mmol). The crude material was chromatographed to give **17c** (27 mg, 70%).

4.5.8. Cyclization of 15d in *tert*-butylbenzene. Compound **15d** (55 mg, 0.12 mmol) in *tert*-butylbenzene (5 mL) was treated with AIBN (20 mg, 0.11 mmol) and Bu_3SnH (65 μL, 0.22 mmol). The crude material was chromatographed to give **17c** (19 mg, 46%) and **19c** (4 mg, 9%).

4.5.9. Cyclization of 15e in toluene. Compound **15e** (37 mg, 0.07 mmol) in toluene (5 mL) was treated with AIBN (12 mg, 0.07 mmol) and Bu_3SnH (40 μL, 0.15 mmol). The first fraction gave 5,14-dihydro-5-(1′-phenylethyl)indolo[2,1-*d*][1,5]benzodiazocin-6-one **17d** (6 mg, 22%) as a yellow solid; mp 157–159 °C (DCM); ^{1}H NMR ($CDCl_3$, rotamer ratio 52:48*) δ 1.56 (d, J=7.2 Hz, 1.6H, CHCH_3), 1.78* (d, J=7.5 Hz, 1.4 Hz, CHCH_3), 3.33* (d, J=13.8 Hz, 0.5H, C*H*H-7), 3.38 (d, J=12.3 Hz, 0.5H, C*H*H-7), 3.66* (d, J=13.5 Hz, 0.5H, CH*H*-7), 3.72 (d, J=14.1 Hz, 0.5H, CH*H*-7), 3.73* (d, J=13.8 Hz, 0.5H, C*H*H-14), 4.64* (d, J=13.8 Hz, 0.5H, CH*H*-14), 4.88 (d, J=13.8 Hz, 0.5H, C*H*H-14), 5.37 (d, J=13.5 Hz, 0.5H, CH*H*-14), 6.32* (br s, 0.5H, C*H*CH$_3$), 6.32* (s, J=0.5 Hz, H-8), 6.35 (s, J=0.5 Hz, H-8), 6.38 (br s, 0.5H, C*H*CH$_3$), 6.54 (d, J=8.1 Hz, 0.5H, H-4), 7.02–7.56 (m, 12.5H, Ar); ^{13}C NMR ($CDCl_3$) δ 16.4 (CH_3), 19.0* (CH_3), 36.9 (CH_2-7), 44.3* (CH_2-14), 45.2 (CH_2-14), 52.8*

(CH), 53.9 (CH), 103.2* (C-8), 103.3 (C-8), 108.4 (C-15), 119.4*, 119.6, 120.2*, 120.3. 121.1*, 121.3, 127.0* (C-4), 127.7, 128.0, 128.1, 128.2, 128.5, 129.0, 129.4, 130.8 (C-3a), 131.7*, 131.8, 132.8 (C-7a), 133.0* (C-14a), 133.2* (C-7a), 134.2, (C-14a), 137.3* (C-12a), 138.9 (C-12a), 139.5 (C-1′), 140.3* (C-1′), 141.6 (C-4a), 145.5* (C-4a), 164.4 (CO), 168.4* (CO); CI-MS *m/z* 367 ($[M+H]^+$, 100%); HRCI-MS *m/z* calcd for $[M+H]^+$ $C_{25}H_{23}N_2O$: 367.1810, found: 367.1796.

Further elution then gave a mixture of **18d** and **19d** (7 mg, 26%) in a ratio of ca. 1:1 (determined by ^1H NMR spectroscopy).

4.5.10. Cyclization of 15f. Compound **15f** (83 mg, 0.19 mmol) in toluene (5 mL) was treated with AIBN (31 mg, 0.19 mmol) and Bu_3SnH (0.12 mL, 0.38 mmol). The crude material was chromatographed to give, in the first fraction, 5,14-dihydro-10-methoxy-5-methylindolo[2,1-*d*][1,5]benzodiazocin-6-one, **17e** (30 mg, 50%) as a yellow solid; mp 170–173 °C (DCM); ^1H NMR ($CDCl_3$) δ 3.430 (dd, $J=13.8$, 1.2 Hz, 1H, C*H*H-7), 3.434 (s, 3H, CH_3), 3.63 (d, $J=13.5$ Hz, 1H, CH*H*-7), 3.84 (s, 3H, OCH_3), 4.74 (d, $J=13.8$ Hz, 1H, C*H*H-14), 5.27 (d, $J=13.8$ Hz, 1H, CH*H*-14), 6.30 (s, 1H, H-8), 6.90 (dd, $J=9.0$, 2.4 Hz, 1H, H-4), 7.01 (d, $J=2.4$ Hz, 1H, H-9), 7.35–7.39 (m, 3H, Ar), 7.45–7.53 (m, 2H, Ar); ^{13}C NMR ($CDCl_3$) δ 36.1 (CH_2-7), 36.5 (NCH_3), 45.3 (CH_2-14), 56.1 (OCH_3), 102.4 (C-8), 103.2 (C-9), 109.4, 111.7, 125.4, 128.4 (C-8a), 129.0, 130.6, 131.8 (C-7a), 132.2, 133.1 (C-14a), 133.5 (C-12a), 144.8 (C-4a), 154.3 (*C*OCH_3), 168.9 (CO); CI-MS *m/z* 307 ($[M+H]^+$, 100%); HRCI-MS *m/z* calcd for $[M+H]^+$ $C_{19}H_{19}N_2O_2$: 307.1447, found: 307.1444.

Further elution then gave *N*-{2-[1′-(5′-methoxy-1*H*-indolyl)methyl]phenyl}-*N*-methylacetamide **19e** (11 mg, 20%) as a yellow solid; mp 151–153 °C (DCM); ^1H NMR ($CDCl_3$) δ 1.76 (s, 3H, CH_3), 3.19 (s, 3H, NCH_3), 3.84 (s, 3H, OCH_3), 5.20 (s, 2H, CH_2), 6.47 (d, $J=3.0$ Hz, 1H, H-3), 6.80 (dd, $J=8.7$, 2.1 Hz, 1H, Ar), 6.87 (d, $J=7.2$ Hz, 1H, Ar), 6.98–7.07 (m with prominent d, $J=3.6$ Hz, 2H, H-2 and Ar), 7.08 (d, $J=2.1$ Hz, 1H, H-4), 7.10 (d, $J=7.8$ Hz, 1H, Ar), 7.23 (t, $J=8.7$ Hz, 1H, Ar), 7.32 (dd, $J=7.2$, 0.9 Hz, 1H, H-7); ^{13}C NMR ($CDCl_3$) δ 22.2 (CH_3), 36.5 (NCH_3), 46.8 (CH_2), 56.0 (OCH_3), 102.0 (C-3), 103.0, 110.3, 112.5, 128.7, 129.1, 129.16, 129.24, 129.4, 129.7, 131.7 (ArC), 135.1 (ArC), 142.3 (C-2′), 154.5 (*C*OCH_3), 170.9 (CO); CI-MS *m/z* 309 ($[M+H]^+$, 87%); HREI-MS *m/z* calcd for $[M]^+$ $C_{19}H_{20}N_2O_2$: 308.1525, found: 308.1524.

4.5.11. Cyclization of 15g. Compound **15g** (55 mg, 0.11 mmol) in mesitylene (5 mL) was treated with AIBN (18 mg, 0.11 mmol) and Bu_3SnH (59 μL, 0.22 mmol). The crude material was chromatographed to give 5-benzyl-5,14-dihydro-10-methoxyindolo[2,1-*d*][1,5]benzodiazocin-6-one **17f** (12 mg, 29%) as a yellow solid; mp 113–114 °C (DCM); IR (KBr) ν_{max}: 1660 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.42 (dd, $J=15$, 1.2 Hz, 1H, C*H*H-7), 3.68 (d, $J=13.8$ Hz, 1H, CH*H*-7), 3.82 (s, 3H, OCH_3), 4.12 (s, $J=13.8$ Hz, 1H, CONC*H*H), 4.62 (d, $J=13.8$ Hz, 1H, C*H*H-14), 4.85 (d, $J=14.0$ Hz, 1H, CONCH*H*), 5.51 (d, $J=13.8$ Hz, 1H, CH*H*-14), 6.28 (s, 1H, H-8), 6.84 (dd, $J=9.0$, 2.4 Hz, 1H, H-4), 6.99 (d, $J=2.4$ Hz, 1H, H-9), 7.21–7.25 (m, 6H, Ar), 7.31–7.50 (m, 4H, Ar); ^{13}C NMR ($CDCl_3$) δ 36.4 (CH_2-7), 44.9 (CH_2-14), 52.7 (CON*C*H_2), 56.1 (OCH_3), 102.4 (C-8), 109.4, 111.6, 125.9, 128.2, 128.3 (C-8a), 128.8, 129.7, 129.6, 130.0, 130.5, 132.1, 132.7 (C-7a), 132.9 (C-14a), 133.5 (C-12a), 136.4 (C-1′), 142.9 (C-4a), 154.3 (*C*OCH_3), 168.4 (CO); CI-MS *m/z* 383 ($[M+H]^+$, 100%); HRCI-MS *m/z* calcd for $[M+H]^+$ $C_{25}H_{23}N_2O_2$: 383.1760, found: 383.1766.

4.5.12. Cyclization of 15h. Compound **15h** (77 mg, 0.16 mmol) in mesitylene (7 mL) was treated with AIBN (25 mg, 0.16 mmol) and Bu_3SnH (77 μL, 0.31 mmol) The first fraction from the column gave 5-benzyl-10-fluoro-5,14-dihydroindolo[2,1-*d*][1,5] benzodiazocin-6-one **17h** (14 mg, 23%) as a yellow solid; mp 115–116 °C (DCM); IR (KBr) ν_{max}: 1663 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.43 (dd, $J=13.8$, 1.2 Hz, 1H, C*H*H-7), 3.69 (d, $J=13.8$ Hz, 1H, CH*H*-7), 4.13 (d, $J=14.1$ Hz, 1H, C*H*H-14), 4.63 (d, $J=14.1$ Hz, 1H, CONC*H*H), 4.86 (d, $J=13.8$ Hz, 1H, CH*H*-14), 5.52 (d, $J=14.1$ Hz, 1H, CONCH*H*), 6.31 (s, 1H, H-8), 6.90 (dd, $J=9.0$, 2.7 Hz, 1H, Ar), 6.94 (dd, $J=9.0$, 2.4 Hz, 1H, H-9), 7.16 (dd, $J=9.6$, 2.4 Hz, 1H, H-12), 7.19–7.27 (m, 5H, Ar), 7.31–7.40 (m, 3H, Ar), 7.47 (dd, $J=6.6$, 2.1 Hz, 1H, H-1); ^{13}C NMR ($CDCl_3$) δ 36.4 (CH_2-7), 45.0 (CH_2-14), 52.6 (CON*C*H_2), 105.5 (d, $J=4.6$ Hz, C^{13}–F^{19}, C8–F), 105.3 (d, $J=23.5$ Hz, C^{13}–F^{19}, C11–F), 109.3 (d, $J=9.8$ Hz, C^{13}–F^{19}, C12–F), 109.7 (d, $J=26.3$ Hz, C^{13}–F^{19}, C9–F), 126.0, 128.2, 129.2, 129.6, 130.1, 130.7, 132.1, 132.4 (ArC), 134.1 (ArC), 134.6 (ArC), 136.3 (C-1′), 142.8 (C-4a), 158.7 (d, $J=238.6$ Hz, C^{13}–F^{19}, C10–F), 168.2 (CO); CI-MS *m/z* 371 ($[M+H]^+$, 100%); HRCI-MS *m/z* calcd for $[M+H]^+$ $C_{24}H_{20}N_2O_2F$: 371.1560, found: 371.1552.

Further elution then gave *N*-{2-[1′(5′-fluoro-1*H*-indolyl)-methyl)phenyl]-*N*-benzylacetamide **19h** (9 mg, 15%) as a yellow solid; mp 63–64 °C (DCM); ^1H NMR ($CDCl_3$) δ 1.80 (d, 3H, CH_3), 4.70 (d, $J=16.8$ Hz, 1H, CONC*H*H), 4.84 (d, $J=5.1$ Hz, 2H, CH_2), 5.00 (d, $J=16.8$ Hz, 1H, CONCH*H*), 6.48 (dd, $J=2.7$, 0.6 Hz, 1H, H-3), 6.63 (dd, $J=7.5$, 1.2 Hz, 1H, H-3′), 6.79 (m with prominent d, $J=3.3$ Hz, 2H, H-2 and H-4), 6.88 (td, $J=9.0$, 2.4 Hz, 1H, H-6), 6.97 (dd, $J=7.8$, 1.5 Hz, 1H, H-6′), 7.20 (td, $J=7.5$, 1.5 Hz, 1H, Ar), 7.23–7.28 (m, 5H, Ar), 7.29–7.34 (m, 3H, Ar); ^{13}C NMR ($CDCl_3$) δ 22.2 (CH_3), 46.4 (CH_2), 52.5 (CON*C*H_2), 102.1 (d, $J=4.9$ Hz, C^{13}–F^{19}, C3–F), 105.8 (d, $J=11.6$ Hz, C^{13}–F^{19}, C7–F), 109.9 (d, $J=9.8$ Hz, C^{13}–F^{19}, C6–F), 110.3 (d, $J=26.3$ Hz, C^{13}–F^{19}, C4–F), 127.9, 128.2, 128.7, 128.9, 129.0, 129.2, 129.5, 129.67, 129.71, 135.4 (C-7a), 136.9 (C-1″), 140.1 (C-2′), 157.9 (d, $J=233.1$ Hz, C^{13}–F^{19}, C5–F), 170.3 (CO); CI-MS *m/z* 373 $[M+H]^+$, 100%); HRCI-MS *m/z* calcd for $[M+H]^+$ $C_{24}H_{22}N_2O_2F$: 373.1716, found: 373.1704.

4.5.13. Cyclization of 15i. Compound **15i** (60 mg, 0.14 mmol) in toluene (5 mL) was treated with AIBN (23 mg, 0.14 mmol) and Bu_3SnH (75 μL, 0.28 mmol) The first column fraction gave 10-fluoro-5,14-dihydro-5-methyl-indolo[2,1-*d*][1,5]benzodiazocin-6-one **17g** (15 mg, 35%) as a yellow solid; mp 200–202 °C (DCM); ^1H NMR ($CDCl_3$) δ 3.436 (s, 3H, CH_3), 3.439 (d, $J=13.8$ Hz, 1H, C*H*H-7), 3.65 (d, $J=14.1$ Hz, 1H, CH*H*-7), 4.76 (d,

J=13.8 Hz, 1H, C*H*H-14), 5.29 (d, J=13.8 Hz, 1H, CH*H*-14), 6.33 (s, 1H, H-8), 6.97 (td, J=9.0, 2.7 Hz, 1H, H-11), 7.18 (dd, J=9.3, 2.4 Hz, 1H, H-9), 7.35–7.40 (m, 3H, Ar), 7.48 (dd, J=7.5, 1.8 Hz, 1H, Ar), 7.51 (d, J=7.2 Hz, 1H, Ar); ^{13}C NMR ($CDCl_3$) δ 36.2 (CH_2-7), 36.7 (NCH_3), 45.5 (CH_2-14), 103.5 (d, J=4.3 Hz, C^{13}–F^{19}, C8–F), 105.4 (d, J=23.2 Hz, C^{13}–F^{19}, C11–F), 109.3 (d, J=9.5 Hz, C^{13}–F^{19}, C12–F), 109.9 (d, J=49.0 Hz, C^{13}–F^{19}, C9–F), 125.5, 128.2 (d, J=10.1 Hz, C^{13}–F^{19}, C8a–F), 129.0, 130.7, 131.4 (C-7a), 132.1, 134.3 (C-14a), 134.6 (C-12a), 144.6 (C-4a), 158.0 (d, J=232.7 Hz, C^{13}–F^{19}, C10–F), 168.6 (CO); CI-MS m/z 295 ($[M+H]^+$, 100%); HREI-MS m/z calcd for $[M]^+$ $C_{18}H_{15}N_2OF$: 294.1168, found: 294.1159.

Further elution then gave *N*-{2-[1′-(5′-fluoro-1*H*-indolyl)-methyl]phenyl}-*N*-methylacetamide **19g** (7 mg, 17%) as a yellow solid; mp 70–72 °C (DCM); ^{1}H NMR ($CDCl_3$) δ 1.74 (s, 3H, CH_3), 3.17 (s, 3H, NCH_3), 5.23 (s, 2H, CH_2), 6.52 (dd, J=3.3, 0.9 Hz, 1H, H-3), 6.89–6.95 (m, 2H, Ar), 7.06 (dd, J=9.3, 4.5 Hz, 1H, H-4), 7.09 (d, J=3.0 Hz, 1H, H-2), 7.18 (dd, J=7.8, 1.5 Hz, 1H, Ar), 7.25–7.30 (m, 2H, Ar), 7.36 (dd, J=7.5, 1.5 Hz, 1H, Ar); ^{13}C NMR ($CDCl_3$) δ 22.3 (CH_3), 36.7 (NCH_3), 47.1 (CH_2), 102.5 (d, J=4.6 Hz, C^{13}–F^{19}, C3–F), 106.2 (d, J=23.2 Hz, C^{13}–F^{19}, C6–F), 110.2 (d, J=9.7 Hz, C^{13}–F^{19}, C7–F), 110.7 (d, J=26.5 Hz, C^{13}–F^{19}, C4–F), 128.8, 128.9, 129.1, 129.2, 129.5 (d, J=4.5 Hz, C^{13}–F^{19}, C3a–F), 129.9, 130.0, 133.0 (C-1′), 134.6 (C-7a), 142.4 (C-2′), 157.6 (d, J=232.2 Hz, C^{13}–F^{19}, C5–F), 170.7 (CO); CI-MS m/z 297 ($[M+H]^+$, 100%); HREI-MS m/z calcd for $[M]^+$ $C_{18}H_{17}N_2OF$: 296.1325, found: 296.1320.

4.5.14. Cyclization of 16a. Compound, **16a** (44 mg, 0.10 mmol) in toluene (5 mL) was treated with AIBN (16 mg, 0.10 mmol) and Bu_3SnH (53 μL, 0.20 mmol) The crude material was chromatographed to give, in the first fraction, **17c** (19 mg, 54%) and, in the second fraction, a mixture of **18c** and **19c** (7 mg, 20%) in a ratio of ca. 2:1 (determined by ^{1}H NMR spectroscopy).

4.5.15. Cyclization of 16b. Compound **16b** (45 mg, 0.12 mmol) in toluene (5 mL) was treated with AIBN (20 mg, 0.12 mmol) and Bu_3SnH (64 μL, 0.24 mmol) The crude material was chromatographed to give **17e** (9 mg, 24%) (first fraction) and **18e** (12 mg, 33%) (second fraction).

4.5.16. Dehydrogenation of 18a. To a solution of **18a** (98 mg, 0.35 mmol) in dry toluene (5 mL) was added 10% Pd/C (20 mg) The reaction mixture was then heated at reflux for 8 h. The mixture was then cooled, filtered through Celite, and the filtrate evaporated to dryness. The crude yellow solid residue was column chromatographed (DCM) to yield **17a** (48 mg, 50%) and the starting material **18a** (40 mg).

4.5.17. 4.10 5,14-Dihydroindolo[2,1-*d*][1,5]benzodiazocin-6(7*H*)-one (1). Sodium metal (ca. 30 mg) was added to dry THF (1 mL) under nitrogen and then the mixture was cooled using a liquid N_2 bath. Liquid ammonia (condensed at −70 °C, ca. 7 mL) and a solution of **17c** (30 mg, 0.09 mmol) in dry THF (2 mL) was added. The frozen mixture was warmed to −60 °C and the reaction was stirred for 20 min. Solid ammonium chloride was then added until the colour dissipated, at which point the reaction was allowed to warm to r.t. The residue was dissolved in ethyl acetate (30 mL), extracted with water (20 mL), dried and the organic layer concentrated to give a yellow residue. The residue was purified using preparative thin layer chromatography (1% MeOH/DCM) to give **1** (7.8 mg, 35%) as an off white solid; mp 269–271 °C (lit.[9] 268–270 °C); IR (KBr) ν_{max}: 3415 (NH), 1655 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.58 (s, 2H, CH_2-7), 5.18 (s, 2H, CH_2-14), 6.40 (s, 1H, H-8), 7.12 (td, J=7.2, 0.9 Hz, 1H, Ar), 7.24–7.30 (m, 2H, Ar), 7.36–7.41 (m, 1H, Ar), 7.44 (dd, J=7.5, 1.8 Hz, 1H, Ar), 7.48–7.58 (m, 3H, Ar), 7.73 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 35.2 (CH_2-14), 45.5 (CH_2-7), 103.8 (C-8), 108.9, 120.1, 120.7, 121.8, 125.7, 128.1 (C-8a), 128.97, 129.02 (ArC), 130.4, 132.6, 138.3 (ArC), 144.0 (ArC), 170.7 (CO); CI-MS m/z 263 ($[M+H]^+$, 100%); HREI-MS m/z calcd for $[M]^+$ $C_{17}H_{14}N_2O$: 262.1106, found: 262.1104.

Acknowledgements

We gratefully acknowledge support from the Institute for Biomolecular Science, University of Wollongong and a Royal Thai Government Scholarship (to W.S.) for this work.

References and notes

1. Aldabbagh, F.; Bowman, W. R. *Cont. Org. Syn.* **1997**, *4*, 261–280.
2. Bowman, W. R.; Cloonan, M. O.; Krintel, S. L. *J. Chem. Soc., Perkin Trans. 1* **2001**, 2885–2902.
3. el Bialy, S. A. A.; Ohtani, S.; Sato, T.; Ikeda, M. *Heterocycles* **2001**, *54*, 1021–1025.
4. Beckwith, A. L. J.; Storey, J. M. D. *Chem. Commun.* **1995**, 977–978.
5. Tamura, O.; Matsukida, H.; Toyao, A.; Takeda, Y.; Ishibashi, H. *J. Org. Chem.* **2002**, *67*, 5537–5545.
6. Majumdar, K. C.; Basu, P. K. *Heterocycles* **2002**, *57*, 2413–2439.
7. Kaoudi, T.; Quiclet-Sire, B.; Seguin, S.; Zard, S. Z. *Angew. Chem., Int. Ed.* **2000**, *39*, 731–733.
8. Moody, C. J.; Norton, C. L. *J. Chem. Soc., Perkin Trans. 1* **1997**, 2639–2643.
9. Bremner, J. B.; Russell, H. F.; Skelton, B. W.; White, A. H. *Heterocycles* **2000**, *53*, 277–290.
10. Stork, G.; Mah, R. *Heterocycles* **1989**, *28*, 723–727.
11. Bryans, J. S.; Large, J. M.; Parsons, A. F. *Tetrahedron Lett.* **1999**, *40*, 3487–3490.
12. Crich, D.; Hwang, J.-T. *J. Org. Chem.* **1998**, *63*, 2765–2770.
13. Beckwith, A. L. J.; Bowry, V. W.; Bowman, W. R.; Mann, E.; Parr, J.; Storey, J. M. D. *Angew. Chem., Int. Ed.* **2004**, *43*, 95–98.
14. Lizos, D. E.; Murphy, J. A. *Org. Biomol. Chem.* **2003**, *1*, 117–122.

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Tetrahedron
Tetrahedron 61 (2005) 955–963

New polychlorinated pyrrolidinones from the Red Sea marine sponge *Lamellodysidea herbacea*

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Received 27 July 2004; revised 1 October 2004; accepted 5 November 2004

Abstract—Chemical investigation of the dichloromethane extract of the Red Sea marine sponge *Lamellodysidea herbacea* led to the isolation of seven new polychlorinated derivatives **3–6** and **8–10** in addition to the known dysidamide **1**, dysidamide B **2** and dysidamide C **7**. Their structures were established by extensive NMR spectroscopic data. The absolute configuration of compound **9** was determined by X-ray crystallographic diffraction analysis. Dysidamide **1** exhibited neurotoxic effects towards both mesencephalic and cortical murine neurones at 0.8 μg/ml.

1. Introduction

Marine sponges of the genus *Dysidea* have yielded secondary metabolites of a great structural diversity such as sesqui- and sesterterpenes,[1–3] polybrominated derivatives of diphenyl ether[1] and dibenzo-*p*-dioxin types[4,5] and polychlorinated derivatives[6] including amino acids[7] as well as peptides.[8] Some of them exhibited interesting pharmacological activities such as inhibition of iodide transport in thyroid cells,[9] neurotoxicity[10] and inhibition of protein phosphatase.[11]

In our ongoing chemical studies on marine sponges, we have examined a sample of *Lamellodysidea herbacea* (formerly *Dysidea herbacea*) collected in the Red Sea. Chemical investigations of the dichloromethane extract led to the isolation of seven new polychlorinated derivatives **3–6** and **8–10** in addition to the known dysidamide **1**, dysidamide B **2** and dysidamide C **7**, previously reported.[12,13] This paper describes the isolation and structure elucidation of these new polychlorinated derivatives.

2. Results and discussion

The marine sponge *Lamellodysidea herbacea* was collected by hand using SCUBA (−20 m) in the Red Sea and was air-dried. Silica gel flash chromatography (CH_2Cl_2/MeOH) of the dichloromethane extract followed by Sephadex LH-20 and/or reversed-phase HPLC yielded compounds **1–10** in pure form. The known compounds dysidamide **1**, dysidamide B **2** and dysidamide C **7**, originally isolated from the marine sponge *Dysidea herbacea*[12,13] were rapidly identified by comparison with literature values. An X-ray diffraction analysis[14] of dysidamide **1** showed the presence of two conformers in the unit cell, in which all asymmetric carbon centres exhibited (*S*) configuration, as previously reported.[15]

Compounds **3–6** and **8–10** were identified through extensive 1D and 2D NMR spectroscopic data.

Compound **3** was isolated as a colourless glass, $[\alpha]_D = -4.0$ (*c* 0.15, CH_2Cl_2). Electrospray mass spectrum of compound **3** exhibited a cluster ion at *m/z* 440, 442, 444, 446, 448 characteristic of the presence of five chlorine atoms in the molecule. The molecular formula $C_{15}H_{22}NO_3Cl_5$ was deduced from HREIMS and ^{13}C NMR spectrum. In the IR spectrum, absorption bands at 3606, 1741 and 1697 cm^{-1} indicated the presence of an hydroxyl (or NH) and two carbonyl groups, respectively. NMR spectral data were similar to those of dysidamide **1**, pointing out the presence

Keywords: *Lamellodysidea herbacea*; Sponge; Dysidamide; Polychlorinated pyrrolidinones.
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0040–4020/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2004.11.011

Table 1. ^{1}H NMR data of compounds **1**–**4** and **10** recorded in $CDCl_3$ (δ_H, multiplicity, J in Hz)

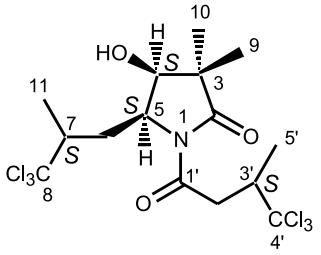
No	1	2	3	4	10
4	4.13 (d, 7.5)	4.10 (br d, 7.3)	4.14 (d, 7.7)	4.12 (d, 7.3)	
5	4.38 (dt, 7.5, 5.3)	4.28 (m)	4.36 (dt, 7.6, 5.2)	4.31 (ddd, 7.3, 4.5, 7.0)	4.52 (t, 6.5)
6	2.17 (m)	2.04 (ddd, 14.1, 6.0, 7.0)	2.16 (m)	2.03 (brdd, 14.0, 6.9)	2.20 (dd, 6.0, 6.8)
		1.85 (m)		1.88 (ddd, 14.3, 5.9, 4.5)	
7	2.97 (m)	2.48 (m)	2.98 (m)	2.48 (m)	2.98 (m)
8		5.93 (d, 2.9)		5.94 (d, 2.8)	
9	1.21 (s)	1.20 (s)	1.19 (s)	1.22 (s)	1.31 (s)
10	1.22 (s)	1.20 (s)	1.21 (s)	1.21 (s)	1.40 (s)
11	1.37 (d, 6.4)	1.17 (d, 6.7)	1.36 (d, 6.5)	1.19 (d, 6.0)	1.47 (d, 6.4)
2′	3.55 (dd, 18.1, 1.6)	3.20 (dd, 18.1, 5.9)	3.19 (dd, 18.1, 5.8)	3.58 (dd, 18.1, 2.6)	3.68 (dd, 18.0, 1.8)
	3.14 (dd, 18.1, 9.4)	2.98 (dd, 18.1, 6.9)	3.03 (dd, 18.1, 7.1)	3.11 (dd, 18.1, 9.3)	3.25 (dd, 18.0, 9.2)
3′	3.29 (m)	2.77 (m)	2.78 (m)	3.30 (ddq, 2.7, 9.2, 6.5)	3.31 (m)
4′		5.95 (d, 3.3)	5.95 (d, 3.3)		
5′	1.34 (d, 6.4)	1.19 (d, 6.6)	1.18 (d, 6.7)	1.35 (d, 6.5)	1.39 (d, 6.6)

of a pyrrolidinone ring system (see Table 1). In comparison with dysidamide **1**, we observed in the ^{1}H NMR spectrum the presence of one additional signal at δ 5.95 (1H, d, J= 3.3 Hz). The ^{13}C NMR spectrum showed the loss of the quaternary carbon signal at δ 104.9 and the presence of one additional methine carbon signal at δ 77.8. Furthermore, we noticed that the methine proton at δ 50.7 was shifted at δ 40.1. This information suggested the replacement of one CCl_3 group by a $CHCl_2$ functionality in **3**. This hypothesis was strengthened by COSY spectrum. Starting from the additional proton at δ 5.95 (H4′), correlations could be observed to the methine proton at δ 2.78 (H3′), which in turn coupled to both methylene protons at δ 3.19–3.03 (H2′) and methyl protons at δ 1.18 (H5′), confirming the presence of a Me–CH($CHCl_2$)–CH_2– side chain in **3**. A second spin system was observed in the COSY spectrum with correlations between the methyl doublet at δ 1.36 (H11) to the methine proton at δ 2.98 (H7), which in turn coupled to the methylene protons at δ 2.16 (H6). These protons further coupled to the methine proton at δ 4.36, which coupled to the methine proton at δ 4.14 (H4), as in dysidamide **1**. Key HMBC correlations between both methylene protons at δ 3.19–3.03 (H2′) and methine proton at δ 4.36 (H5) with the carbonyl C1′ at δ 172.9, unambiguously located each chain on the pyrrolidinone ring as depicted in Figure 2. NOESY correlations between the methyl protons at δ 1.21 in position 10 and both methine protons at δ 4.36 (H5) and 4.14 (H4) and between the methyl protons at δ 1.19 in position 9 with the methylene protons at δ 2.16 indicated that the relative stereochemistry of the new compound **3** was identical to that of dysidamide **1**. We propose the name dysidamide D for this compound **3**.

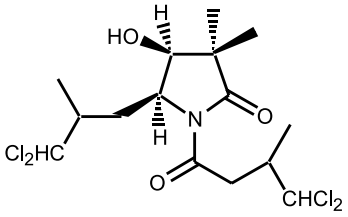
Compound **4** was isolated as a colourless glass, $[\alpha]_D = -3.5$ (c 0.3, CH_2Cl_2). The molecular formula, $C_{15}H_{22}NO_3Cl_5$, deduced from HREIMS indicated that compound **4** was isomer of compound **3**. The ^{1}H NMR spectrum rather matched that of dysidamide B **2** by the presence of the non equivalent methylene protons at δ 2.03–1.88. However, we noticed in the ^{1}H NMR spectrum the loss of one doublet signal at δ 5.95 (see Table 1). The COSY spectrum delineated the presence of two spin systems, whose position was secured by HMBC correlations, as presented in Figure 3. NOESY correlations between the methyl protons Me-10 at δ 1.21 and the methine protons at δ 4.31 (H5) and 4.12 (H4) and between the methyl protons at δ 1.22 and the methylene protons at δ 2.03–1.88 were observed, assigning the stereochemistry of **4** identical to that of dysidamide **1**. Thus, combined 2D NMR data allowed to propose the structure depicted in Figure 1 for compound **4** (see also Tables 1 and 5), we named dysidamide E.

Compound **5** was obtained as white needles, mp 173–174 °C, $[\alpha]_D = -41.8$ (c 1.1, CH_2Cl_2). The molecular formula $C_{16}H_{25}NO_4Cl_6$ was obtained by combined HREIMS and ^{13}C NMR spectral data. The spectral data of **5** were similar to those obtained for dysidamide **1** (see Table 2). The significant difference in ^{1}H NMR spectrum was the presence of two additional signals at δ_H 5.84 and δ 3.70. This last signal gave a carbon resonance in ^{13}C NMR spectrum at δ 52.4, indicating the presence of a methoxyl group in the molecule. HMBC correlations between these methoxyl protons at δ 3.70 to the carbonyl at δ 178.9 suggested the presence of a methyl ester. HMBC correlations between the two singlet methyl protons at δ 1.19 and 1.28 to the carbonyl at δ 178.9, placed the methyl ester function in position 2 on the molecule. We also observed the presence of two resonances at δ 106.1 and 105.0 due to two CCl_3 groups. Also in this case, the COSY spectrum confirmed the presence of the two chains identical to those of dysidamide **1**. In addition, the deshielded proton at δ 5.84, which did not matched any carbon resonance in the HSQC spectrum, showed an HMBC correlation to the carbonyl at δ 169.7, showing the presence of an amide function. At last, key HMBC correlations between the methine proton at δ 2.48–1.45 (H5) to the carbonyl at δ 169.7, suggested the arrangement reported in Figure 1 for compound **5**.

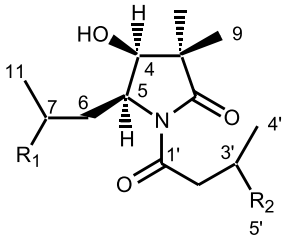
Compound **6** was obtained as white needles, mp 95–96 °C; $[\alpha]_D = -29.6$ (c 0.44, CH_2Cl_2), that analysed for $C_{16}H_{23}NO_4Cl_6$ by HREIMS. The IR spectrum showed absorptions due to the presence of three carbonyl groups (λ 1749, 1723 and 1683 cm^{-1}). The spectral data of **6** were similar to those of compound **5** (see Table 2). The only noticeable difference in the ^{1}H NMR spectrum was the loss of the methine proton at δ 3.59. We observed in the ^{13}C NMR spectrum an additional signal at δ 206.9. These spectral differences were readily accommodated by the replacement of the secondary alcohol group in **5** by a ketone function in position 3 of the molecule. This proposal was



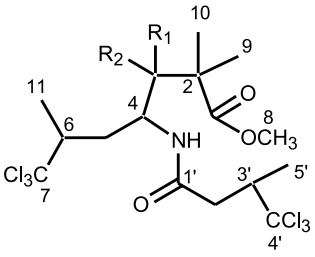
Dysidamide **1**



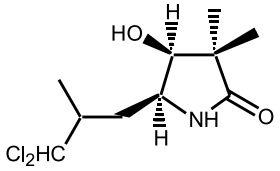
Dysidamide B **2**



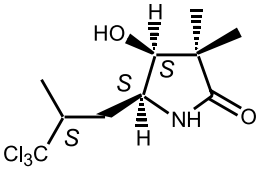
Dysidamide D **3** : R_1= CCl_3, R_2 = $CHCl_2$
Dysidamide E **4** : R_1= $CHCl_2$, R_2 = CCl_3



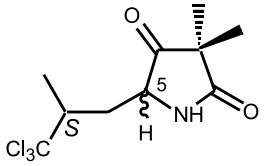
5 : R_1 =H, R_2 = OH
6 : R_1 =R_2 = O



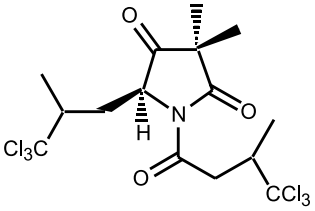
Dysidamide C **7**



Dysidamide F **8**



Dysidamide G **9a** : C_5 (*S*)
5-*Epi*-dysidamide G **9b** : C_5 (*R*)



Dysidamide H **10**

Figure 1. Structures of compounds **1**–**10** isolated from the marine sponge *Lamellodysidea herbacea*.

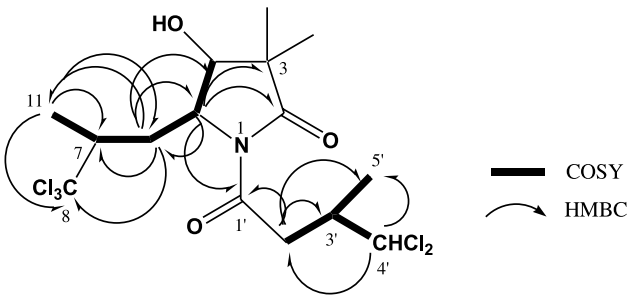


Figure 2. COSY and selected HMBC correlations used to determine the structure of **3**.

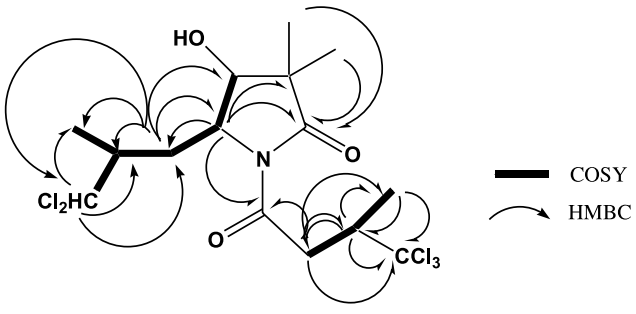


Figure 3. COSY and selected HMBC correlations used to determine the structure of **4**.

Table 2. ^{1}H and ^{13}C NMR data of compounds **5–6** recorded in $CDCl_3$

N°	**5**			**6**		
	δ_C	δ_H (m, *J* in Hz)	HMBC (H→C)	δ_C	δ_H (m, *J* in Hz)	HMBC (H→C)
1	178.9			173.5		
2	45.8			40.5		
3	78.9	3.59 (d, 1.1)	1, 2, 5, 9, 10	206.9		
4	46.7	4.33 (ddd, 12.6, 11.7, 1.1)	5, 6, 11	51.6	5.09 (ddd, 11.8, 9.5, 2.3)	5, 6, 11
5	39.5	2.48 (ddd, 12.8, 12.8, 0.8) 1.45 (ddd, 13.9, 2.5, 10.1)	4, 6, 7, 11, 1′	36.9	2.12 (brdd, 14.2, 0.7) 1.68 (ddd, 14.2, 10.2, 2.3)	4, 6, 7, 11, 1′
6	52.0	2.39 (ddq, 10.1, 1.7, 6.3)	4, 5, 11, 1′	51.7	2.47 (m)	4, 5, 11, 1′
7	106.1			105.3		
8	52.4	3.70 (s)	1	52.9	3.73 (s)	1
9	20.1	1.19 (s)	1, 2, 3,10	21.9	1.42 (s)	1, 2, 3,10
10	24.0	1.28 (s)	1, 2, 3, 9	22.2	1.43 (s)	1, 2, 3, 9
11	16.8	1.41 (d, 6.3)	5, 6, 7	16.3	1.42 (d, 4.2)	5, 6, 7
1′	169.7			170.0		
2′	40.4	2.92 (dd, 14.8, 2.5) 2.13 (dd, 14.8, 10.2)	1′, 3′, 5′	40.3	2.97 (dd, 14.9, 3.1) 2.20 (dd, 14.9, 9.6)	1′, 3′, 5′
3′	51.6	3.17 (ddq, 10.2, 2.5, 6.4)	1′, 2′, 4′, 5′	51.6	3.17 (ddq, 3.1, 9.6, 6.5)	1′, 2′, 4′, 5′
4′	105.0			104.8		
5′	16.8	1.33 (d, 6.4)	2′, 3′, 4′	16.9	1.34 (d, 6.5)	2′, 3′, 4′
NH1′		5.84 (d, 9.8)	3, 4, 1′		6.02 (d, 9.5)	3, 4, 1′

confirmed by interpretation of combined 2D NMR spectra (see Table 2).

Compound **8** was isolated as white needles, mp 171–172 °C, $[\alpha]_D = -36.0$ (*c* 0.84, CH_2Cl_2). Its ESI mass spectrum exhibited a cluster of peaks at *m/z* 288, 290, 292, 294 with a pattern characteristic of a trichlorinated compound. The molecular formula $C_{10}H_{16}NO_2Cl_3$ was determined on the basis of HREIMS and NMR data. Comparison of the NMR data of **8** with those of dysidamide **1** indicated that **8** also contained a pyrrolidinone ring system. The ^{1}H NMR spectrum also showed the presence of a methylene group at δ 2.33–1.73, one methine at δ 2.79 and one methyl doublet at δ 1.40, indicating that **8** possessed the same $MeCH(CCl_3)CH_2$– side chain as dysidamide **1** on C5. NOESY correlations between the methyl protons in position 10 at δ 1.15 and protons H-4 and H-5 and between the methyl protons in position 9 at δ 1.12 with the methylene protons at δ 2.33–1.73 were also observed.

The structure of **8** was secured by hydrolysis of dysidamide **1**. Under mild basic conditions with 0.1% K_2CO_3 in MeOH, dysidamide **1** gave rise to a compound, whose NMR data were in complete accordance with those of the natural product **8**, suggesting (*S*) configuration for all asymmetric centres. We propose the name dysidamide F for compound **8**.

This is the first report of this compound as a natural product. This compound was previously obtained after hydrolysis of dysidamide **1**.[12]

Compound **9** was isolated as colourless needles, mp 109 °C, $[\alpha]_D = -38.5$ (*c* 0.75, CH_2Cl_2). Its ESI mass spectrum exhibited a characteristic cluster of three chlorine atoms for the $[M+H]^+$ peaks at *m/z* 286, 288, 290, 292. From the lower peak at *m/z* 286.0161 (Δ −0.8 mmu) by HREIMS, a molecular formula of $C_{10}H_{14}NO_2Cl_3$ was deduced. Its IR spectrum showed the presence of hydroxyl or NH groups (λ 3419 cm^{-1}), two carbonyl functions (λ 1775, 1711 cm^{-1}), one gem dimethyl (λ 1416, 1389 cm^{-1}). The ^{1}H NMR spectrum recorded in CD_3OD was similar to that of compound **8** but showed that all ^{1}H signals were split into two in a ¾:¼ proportion, suggesting the presence of two diastereoisomers (see Table 3). Compound **9** was crystallized by slow evaporation from a mixture of MeOH/H_2O (7/3) for X-ray studies. The crystals belong to the triclinic system, space group *P1*, chiral. Anomalous dispersion from the chlorine atoms of the molecule permitted us to deduce the relative stereochemistry of the asymmetric carbons, namely C5 and C7.

The crystal is made of two pairs of molecules with occupancy factor = 0.5 in the unit cell. In each pair, one of the two molecules, (Ia), exhibits the *S*-configuration at both quaternary carbons C5(*S*) and C7(*S*) (see Fig. 4). In the crystal packing, molecule (Ia) is connected to either its conformer (Ib) or to its diastereoisomer (II) by two

Table 3. ^{1}H NMR data of compounds **8**, **9a** and **9b** recorded in CD_3OD (δ_H, multiplicity, *J* in Hz)

N°	**8**	**9a**	**9b**
4	3.97 (d, 5.3)		
5	3.86 (ddd, 5.3, 4.5, 9.5)	4.24 (dd, 3.4, 10.9)	4.22 (dd, 5.3, 9.5)
6	2.33 (ddd, 14.1, 10.2, 1.5) 1.73 (ddd, 14.1, 10.2, 4.0)	1.91 (ddd, 13.9, 10.5, 3.4) 2.13 (ddd, 13.9, 10.9, 2.3)	1.80 (ddd, 13.9, 9.5, 5.3) 2.36 (m)
7	2.79 (m)	2.87 (ddq, 10.5, 2.3, 6.5)	3.03 (brdq, 9.5, 6.5)
8	—	—	—
9	1.12 (s)	1.20 (s)	1.21 (s)
10	1.15 (s)	1.23 (s)	1.22 (s)
11	1.40 (d, 6.5)	1.41 (d, 6.5)	1.42 (d, 6.5)

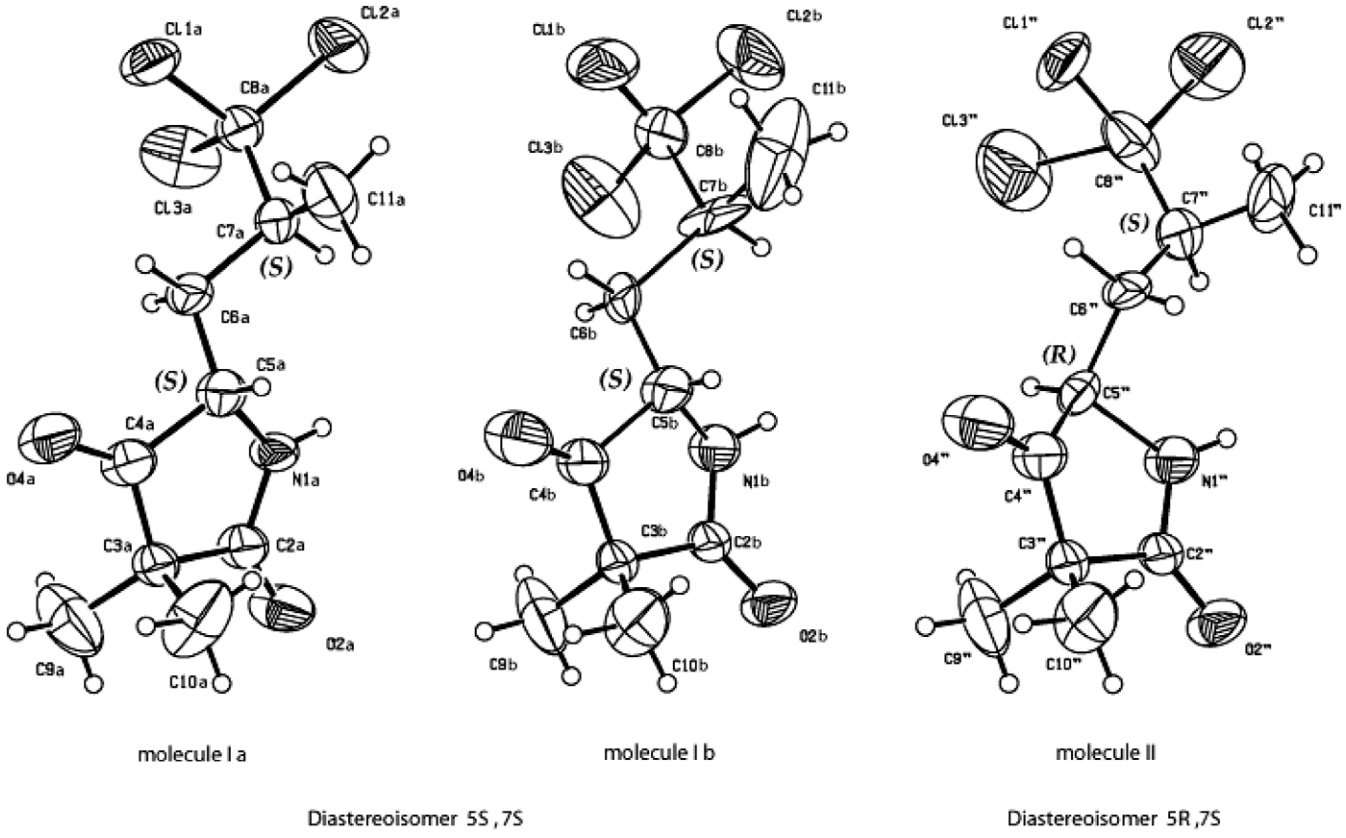


Figure 4. ORTEP-plots showing the molecule Ia: C5(*S*), C7(*S*), Ib: C5(*S*), C7(*S*) and II: C5(*R*), C7(*S*) of compound **9**. Atomic displacement ellipsoids drawn at the 30% probability level, hydrogen atoms drawn as spheres of arbitrary radius.

hydrogen bonds, involving N1a and O2″ or O2b (d= 2.02 Å, angle N–H–O=151.8°) and O2a and N1″ or N1b (d=2.11 Å, angle N–H–O=171.0°), respectively (see Fig. 5).

In the unit cell, molecule (Ia) adopts a N4-envelope conformation as deduced from the torsion angles (see Table 4). The second molecule (Ib) adopts a half-chair conformation and also exhibits *S*-configuration at both quaternary carbons. The third molecule (II) appears to be C5(*R*), C7(*S*) and adopts a half-chair conformation (see Fig. 4).

Hence, the crystal is made of two diastereoisomers in the following proportion ¾:¼ which is somewhat consistent with the ^{1}H NMR results aforementioned. We propose the name dysidamide G for compound **9a** and 5-*epi*-dysidamide G for **9b** (see Fig. 1).

Compound **10** was isolated as a colourless glass; $[\alpha]_D$= −62.8 (c 0.6, CH_2Cl_2). Its ESIMS exhibited a pseudo-molecular ion cluster peak at m/z 474, 476, 478, 480, 482 and 484 indicating the presence of six chlorine atoms in the molecule. The molecular formula was deduced from HREIMS as being $C_{15}H_{19}NO_3Cl_6$, implying the presence of four unsaturations in the molecule. The IR spectrum of **10** showed the presence of three carbonyl groups (1776, 1741, 1708 cm^{-1}) and one gem dimethyl group (1385, 1373 cm^{-1}). The ^{1}H NMR spectrum was similar to that of dysidamide **1** but revealed the disappearance of the methine signal at δ 4.13. The ^{13}C NMR spectrum showed an additional carbonyl signal at δ 209.4. Extensive 1D and 2D spectra were consistent with structure presented in Figure 1. In particular, HMBC correlations between the gem methyl protons at δ 1.40, the methine proton at δ 4.52 and the methylene protons at δ 2.20 with the carbonyl at δ 209.4 supported this structure. NOESY correlation between the methyl at δ 1.31 in position 9 and the methylene protons at δ 2.20 was observed. We propose the name dysidamide H for compound **10**.

Previous intensive studies of the same species, referred to as *Dysidea herbacea* demonstrated that large quantities of the filamentous cyanobacterium *Oscillatoria spongeliae* are responsible for the occurrence of the isolated chlorinated compounds.[16,17] Our work also illustrates and re-enforces the chemical diversity within the genus *Lamellodysidea*.

Dysidamide **1** was tested towards both mesencephalic and cortical murine neurones. In these two neuronal cultures, dysidamide **1** provoked the entire and rapid death of neurones, even at doses of 0.8 μg/ml. No significant effect was observed at 100 ng/ml. Further investigations are in progress to evaluate the neurotoxic effect of the other compounds in the series.

3. Experimental

3.1. General experimental procedures

Silica gel column chromatographies were carried out using

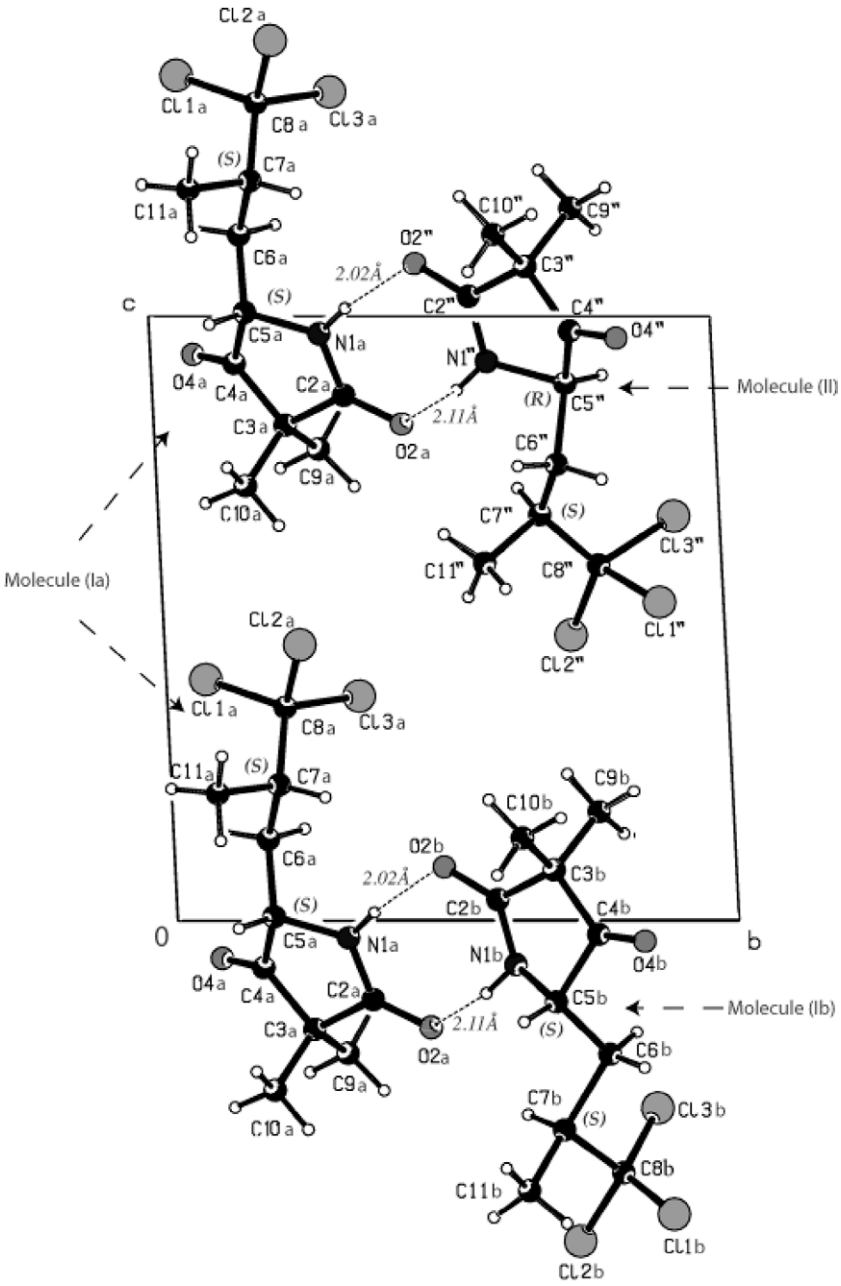


Figure 5. Crystal packing of compound **9**: interpretation of the unit cell content in the **bc** plane. Molecule (Ia) (left column) can be H-bonded either to its conformer (Ib) or its diastereoisomer (II). Note that in the crystallographic refinement, N1b is equivalent to N1″, C2b to C2″, C3b to C3″, C4b to C4″, C9b to C9″, C10 to C10″, O2b to O2″, O4b to O4″, and so are the corresponding attached hydrogens.

Table 4. List of selected torsion angles (°) for compound **9**

Diastereoisomer				C5(*S*) C7(*S*)		C5(*R*) C7(*S*)
				Molecule Ia	Molecule Ib	Molecule II
'Five-member ring'						
N1	C2	C3	C4	12.9	−10.1	−10.1
C2	C3	C4	C5	−21.5	−15.8	27.7
C3	C4	C5	N1	21.8	30.9	−32.2
C4	C5	N1	C2	−14.2	−38.0	27.2
C5	N1	C2	C3	1.5	31.6	−12.9
'Tail'						
C4	C5	C6	C7	−179.7	−172.7	169.1
N1	C5	C6	C7	−68.6	−68.2	70.2
C5	C6	C7	C8	170.2	171.8	95.9
C5	C6	C7	C11	−65.4	−73.0	−127.2

Table 5. ^{13}C NMR data of compounds **1–4** and **10** recorded in $CDCl_3$ and compounds **7–9** recorded in CD_3OD

N°	1	2	3	4	7	8	9a	9b	10
2	178.9	178.8	178.9	178.9	183.9	183.9	180.2	180.2	176.1
3	46.4	46.5	46.3	46.5	46.7	46.7	47.4	47.4	49.1
4	72.9	73.0	73.1	72.9	77.9	77.9	215.1	215.3	209.4
5	57.0	56.7	56.9	56.8	54.8	55.3	60.2	59.9	61.4
6	33.1	31.9	33.2	31.7	33.6	34.6	37.7	37.7	35.1
7	53.5	46.5	53.5	42.8	42.1	53.3	52.7	52.2	50.4
8	105.8	78.4	105.9	78.3	80.5	107.6	106.6	106.6	105.1
9	19.3	19.3	19.3	19.2	18.6	18.8	21.2	21.4	20.8
10	23.9	24.1	24.1	24.0	23.5	23.7	21.3	21.4	22.9
11	16.9	15.1	16.9	15.5	14.9	16.7	16.1	17.0	15.8
1′	172.3	172.7	172.9	172.2					171.0
2′	41.9	40.9	40.8	41.9					42.4
3′	50.7	40.0	40.1	50.7					50.4
4′	104.9	77.8	77.8	105.0					104.7
5′	17.2	15.4	15.2	17.3					17.4

Kieselgel 60 (230–400 mesh, E. Merck). Fractions were monitored by TLC using aluminium-backed sheets (Si gel 60 F254, 0.25 mm thick). Analytical reversed-phase HPLC (Kromasil RP18 column K2185, 4.6×250 mm, MeOH/H_2O) were performed with a L-6200A pump (Merck-Hitachi) equipped with a UV–vis detector (λ= 210 nm) L-4250C (Merck-Hitachi) and a chromato-integrator D-2500 (Merck-Hitachi).

IR spectra were recorded on a Nicolet IMPACT 400D FT-IR spectrophotometer.

Mass spectra were recorded on an API Q-STAR PULSAR I of Applied Biosystem and on a JEOL MS 700BE for low and high-resolution, respectively.

^{13}C NMR spectra were obtained on a Bruker AC300 at 75.47 MHz, ^1H NMR spectra 1D and 2D (COSY, HSQC, HMBC, NOESY) were obtained on a Bruker AVANCE 400.

3.2. Animal material

Specimens of *Lamellodysidea herbacea* (Keller, 1889) (Order Dictyoceratida, Family Dysideidae) were collected by J. Vacelet in the Red Sea at a depth of 20 m in January 1985 during the Ardoukoba expedition. A voucher specimen is available from the Muséum d'Histoire Naturelle in Marseille as collection number MHNM 13660. In the new classification of Hooper and Van Soest, the species *D. herbacea* and *D. chlorea* have been transferred from *Dysidea* to the new genus *Lamellodysidea*.[18] This new genus has been split from *Dysidea* because of the consistent presence in these sponges of an encrusting basal plate and the lack of orientation of the skeleton with respect to the surface. Many sponge specimens from the Indo-Pacific area have been previously identified to *Dysidea herbacea*, which has been originally described from the Red Sea. However, it is likely that this species has been confused with other species such as *D. chlorea*,[18] which could explain the reported chemical diversity.

3.3. Extraction and isolation

The air-dried sponge (288 g) was extracted with 3L methanol at room temperature for 3 days. The concentrated extract (60 g) was partitioned into hexane, CH_2Cl_2, MeOH soluble fractions. The CH_2Cl_2 soluble extract was then fractionated by rapid filtration on a silicagel (Merck silica gel 70–230 mesh) column using dichloromethane with increasing amounts of methanol as eluent. Fraction eluted with 2% MeOH (5.4 g) was subjected to successive reversed-phase HPLC using MeOH/H_2O 7/3 as eluent (flow rate: 1 ml/min, det. wavelength of 210 nm) to yield in order of elution dysidamide B **2** (4.3 mg, at t=12.66 mn), dysidamide D **3** (1.5 mg at t=15.44 mn), dysidamide E **4** (3.0 mg, at t=15.90 mn), dysidamine **1** (450 mg at t= 17.99 mn), compound **5** (11.4 mg at t=19.55 mn), compound **6** (4.4 mg at t=19.56 mn), dysidamide H **10** (6.4 mg at t=20.86 mn). Fraction eluted with 5% MeOH (5.5 g) was first separated on a Sephadex LH20 in 5 subfractions. Subfraction 3 (3.6 g) was successively subjected to reversed-phase column and RP-18 HPLC using MeOH/H_2O 7/3 as eluent to yield dysidamide C **7** (1 mg), dysidamide F **8** (10 mg) and dysidamide G **9a** with 5-*epi*-dysidamide G **9b** (26 mg).

3.3.1. Compound 1. Dysidamide, 4-hydroxy-3,3-dimethyl-1-(4,4,4-trichloro-3-methyl-1-oxobutyl)-5-(3,3,3-trichloro-2-methylpropyl)-2-pyrrolidinone. 0.450 g, 0.15% dry weight white crystals; mp 133 °C; $[\alpha]_D=-16.1$ (c 2.76, CH_2Cl_2), IR ν cm^{-1}: 3447, 1722, 1700, 1385, 1385, 1387, ^1H and ^{13}C NMR data recorded in $CDCl_3$: see Tables 1 and 5; HREIMS $[M+H]^+$ found at m/z 473.9758 (Δ+0.5 mmu) for $C_{15}H_{22}NO_3\ ^{35}Cl_6$.

3.3.2. Compound 2. Dysidamide B, 1-(4,4-dichloro-3-methyl-1-oxobutyl)-5-(3,3-dichloro-2-methylpropyl)-4-hydroxy-3,3-dimethyl-2-pyrrolidinone. 0.0043 g, 0.0015% dry weight colourless glass; $[\alpha]_D=+10.5$ (c 0.43, CH_2Cl_2), IR ν cm^{-1}: 36.7, 1743, 1701, 1388, 1374; ^1H and ^{13}C NMR data recorded in $CDCl_3$: see Tables 1 and 5; HREIMS $[M+H]^+$ found at m/z 406.0500 (Δ−1.0 mmu) for $C_{15}H_{24}NO_3\ ^{35}Cl_4$.

3.3.3. Compound 3. Dysidamide D, 1-(4,4-dichloro-3-methyl-1-oxobutyl)-4-hydroxy-3,3-dimethyl-5-(3,3,3-trichloro-2-methylpropyl)-2-pyrrolidinone. 0.0015 g, 0.05×10^{-2} % dry weight colourless glass; $[\alpha]_D=-4.0$ (c 0.15, CH_2Cl_2); IR ν cm^{-1}: 3606, 1741, 1697, 1386, 1371, ^1H and ^{13}C NMR data recorded in $CDCl_3$: see Tables 1

and 5; HREIMS $[M+H]^+$ found at *m/z* 440.0118 (Δ −0.2 mmu) for $C_{15}H_{23}NO_3{}^{35}Cl_5$.

3.3.4. Compound 4. Dysidamide E, 5-(3,3-dichloro-2-methylpropyl)-4-hydroxy-3,3-dimethyl-1-(4,4,4-trichloro-3-methyl-1-oxobutyl)-2-pyrrolidinone. 0.003 g, 0.10×10^{-2} % dry weight colourless glass; $[\alpha]_D=-3.5$ (*c* 0.3, CH_2Cl_2); IR ν cm^{-1}: 3614, 1745, 1703, 1395, 1381; ^1H and ^{13}C NMR data recorded in $CDCl_3$: see Tables 1 and 5; HREIMS $[M+H]^+$ found at *m/z* 440.0112 (Δ −0.7 mmu) for $C_{15}H_{23}NO_3{}^{35}Cl_5$.

3.3.5. Compound 5. 7,7,7-Trichloro-3-hydroxy-2,2,6-trimethyl-4-(4,4,4-trichloro-3-methyl-1-oxobutyl-amino)-heptanoic acid methyl ester. 0.0114 g, 0.4×10^{-2} % dry weight white needles; mp 173–174 °C, $[\alpha]_D=-41.8$ (*c* 1.1, CH_2Cl_2), IR ν cm^{-1}: 3423, 1698, 1676, 1510, 1393, 1374; ^1H and ^{13}C NMR data recorded in $CDCl_3$: see Table 2; HREIMS $[M+H]^+$ found at *m/z* 505.9988 (Δ −0.5 mmu), for $C_{16}H_{26}NO_4{}^{35}Cl_6$.

3.3.6. Compound 6. 7,7,7-Trichloro-2,2,6-trimethyl-3-oxo-4-(4,4,4-trichloro-3-methyl-1-oxobutylamino)-heptanoic acid methyl ester. 0.004 g, 0.14×10^{-2} % dry weight, white needles; mp 95–96 °C; $[\alpha]_D=-29.6$ (*c* 0.44, CH_2Cl_2), IR ν cm^{-1}: 3414, 1749, 1723, 1683, 1512, 1427; 1389, 1376, ^1H and ^{13}C NMR data recorded in $CDCl_3$: see Table 2; HREIMS $[M+H]^+$ found at *m/z* 503.9821 (Δ −1.5 mmu), calcd 503.9836 for $C_{16}H_{24}NO_4{}^{35}Cl_6$.

3.3.7. Compound 7. Dysidamide C, 5-(3,3-dichloro-2-methylpropyl)-4-hydroxy-3,3-dimethyl-2-pyrrolidinone. 0.001 g, 0.03×10^{-2} % dry weight white needles; ^{13}C NMR data recorded in CD_3OD: see Table 5; ESIMS $[M+H]^+$ found at *m/z* 254.0578 for $C_{10}H_{18}NO_2Cl_2$.

3.3.8. Compound 8. Dysidamide F, 4-hydroxy-3,3-dimethyl-5-(3,3,3-trichloro-2-methylpropyl)-2-pyrrolidinone. 0.010 g, $0.3\ 10^{-2}$ % dry weight white needles; mp 171–172 °C; $[\alpha]_D=-36.0$ (*c* 0.84, CH_2Cl_2); IR ν cm^{-1}: 3610, 3423, 1716, 1392, 1386; ^1H and ^{13}C NMR data recorded in CD_3OD: see Tables 3 and 5; HREIMS $[M+H]^+$ found at *m/z* 288.0319 (Δ −0.6 mmu) for $C_{10}H_{17}NO_2{}^{35}Cl_3$.

3.3.9. Compound 9. Dysidamide G with 5-*epi*-dysidamide G, 3,3-dimethyl-4-oxo-5-(3,3,3-trichloro-2-methylpropyl)-2-pyrrolidinone. 0.026 g, 0.9×10^{-2} % dry weight colourless needles; mp 109 °C, R_f (CH_2Cl_2/acetone 1:1) 0.11; $[\alpha]_D=-38.5$ (*c* 0.75, CH_2Cl_2), IR ν cm^{-1}: 3419, 1775, 1711, 1416, 1389; ^1H and ^{13}C NMR data recorded in CD_3OD: see Tables 3 and 5; HREIMS $[M+H]^+$ found at *m/z* 286.0161 (Δ −0.8 mmu) for $C_{10}H_{15}NO_2{}^{35}Cl_3$.

3.3.10. Compound 10. Dysidamide H, 3,3-dimethyl-4-oxo-1-(4,4,4-trichloro-3-methyl-1-oxobutyl)-5-(3,3,3-trichloro-2-methylpropyl)-2-pyrrolidinone. 0.0064 g, 0.22×10^{-2} % dry weight colourless glass; $[\alpha]_D=-62.8$ (*c* 0.6, CH_2Cl_2), IR ν cm^{-1}: 1776, 1741, 1708, 1385, 1373; ^1H and ^{13}C NMR data recorded in $CDCl_3$: see Tables 1 and 5; HREIMS $[M+H]^+$ found at *m/z* 471.9579 (Δ +0.5 mmu) for $C_{15}H_{20}NO_3{}^{35}Cl_6$.

3.4. X-ray crystallographic analysis

A total of 8577 (4051 unique) reflections were collected up to $2\theta max=50.0°$ in 234 frames of 2° oscillation range using an Enraf-Nonius Kappa-CCD diffractometer with graphite monochromated Mo-*K* radiation ($\lambda=0.71073$ Å) at room temperature. The structure was solved by Patterson method to locate four over six chlorides, the rest of atoms was derived from successive Fourier difference syntheses. Structural parameters were refined based on F^2 using the programs from the SHELX97 package.[19] All non-hydrogen atoms were refined anisotropically, except for C11b and C11″. Hydrogen atoms were assigned idealized locations and given isotropic displacement parameters 1.15× that of the bonded carbon atoms (but 1.2× for the hydrogens in methyl groups).

Crystallographic data for $C_{10}H_{14}Cl_3NO_2$ (FW=286.57), colourless thin needles [the crystal size, $0.1\times0.3\times0.35$ mm^3], triclinic, *P*1 (#1), *a*=6.010 (4) Å, *b*=10.341 (3) Å, *c*=11.103 (4) Å, $\alpha=92.26°$, $\beta=94.12$ (2)°, $\gamma=97.24°$, $V=681.9$ (6) $Å^3$, $Z=2$, $D_{calcd}=1.396$ g/cm^3, μ (Mo-Kα)=0.658 mm^{-1}, observed unique reflections=4047, 358 parameters refined using 11 restraints. For 3210 $F>4\sigma$ (F): *R*1=0.0590 *(based on F)*, *wR*2=0.1614 (based on F^2) and goodness of fit factor=1.051 (based on F^2). $\Delta\rho_{max}=0.29\times10^3$ Å, $\Delta\rho_{min}=-0.21$ e$Å^3$.

Crystallographic data (excluding structure factors) for the structure in this paper have been deposited with the Cambridge Crystallographic Data Centre (CIF file) as supplementary publication number CCDC 244215. Copies of the data can be obtained free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1, EZ, UK [fax: +44(0)1223-336033 or e-mail: http://deposit@ccdc.cam.ac.uk].

Acknowledgements

This work is part of Pierre Sauleau's Thesis, supported by the Leg Prêvost of the MNHN of Paris. We thank Alain Blond, Christelle Caux, Jean-Paul Brouard and Lionel Dubost (MNHN, Paris) for NMR and MS measurements, Nicole Morin (ENS, Paris) for HRMS. We also thank Thomas Debeir of the Hôpital de la Salpêtrière for neurological experiments and Michèle Guyot for helpful discussion.

References and notes

1. Cameron, G. M.; Stapleton, B. L.; Simonsen, S. M.; Brecknell, D. J.; Garson, M. J. *Tetrahedron* **2000**, *56*, 5247–5252.
2. Dumrongchai, N.; Ponglimanont, C.; Stapleton, B. L.; Garson, M. J. *ACGC Chem. Commun.* **2001**, *13*, 17–22.
3. Rudi, A.; Yosief, T.; Schleyer, M.; Kashman, Y. *Org. Lett.* **1999**, *1*, 471–473.
4. Utkina, N. K.; Denisenko, V. A.; Scholokova, O. V.; Virovaya, M. V.; Gerasimenko, A. V.; Popov, D. Y.; Krasokhin, V. B.; Popov, A. M. *J. Nat. Prod.* **2001**, *64*, 151–153.

5. Utkina, N. K.; Denisenko, V. A.; Virovaya, M. V.; Scholokova, O. V.; Prokof'eva, N. G. *J. Nat. Prod.* **2002**, *65*, 1213–1215.
6. Harrigan, G. G.; Goetz, G. H.; Luesch, H.; Yang, S.; Likos, J. *J. Nat. Prod.* **2001**, *64*, 1133–1138.
7. Unson, M. D.; Rose, C. B.; Faulkner, D. J.; Brinen, L. S.; Steiner, J. R.; Clardy, J. *J. Org. Chem.* **1993**, *58*, 6336–6343.
8. Stapleton, B. L.; Cameron, G. M.; Garson, M. J. *Tetrahedron* **2001**, *57*, 4603–4607.
9. Van Sande, J.; Deneubourg, J.; Beauwens, F.; Braekman, J. C.; Daloze, D.; Dumont, J. E. *Mol. Pharmacol.* **1990**, *37*, 583–589.
10. Sakai, R.; Kamiya, H.; Murata, M.; Shimamoto, K. *J. Am. Chem. Soc.* **1997**, *119*, 4112–4116.
11. Gunasekera, S. P.; McCarthy, P. J.; Kelly-Borges, M.; Lobkovsky, E.; Clardy, J. *J. Am. Chem. Soc.* **1996**, *118*, 8759–8760.
12. Gebreyesus, T.; Yosief, T.; Carmely, S.; Kashman, Y. *Tetrahedron Lett.* **1988**, *31*, 3863–3864.
13. Isaacs, S.; Berman, R.; Kashman, Y.; Gebreyesus, T.; Yosief, T. *J. Nat. Prod.* **1991**, *54*, 83–91.
14. Chiaroni A. Personal communication, 2004.
15. Carmely, S.; Gebreyesus, T.; Kashman, Y.; Skelton, B. W.; White, A. H.; Yosief, T. *Aust. J. Chem.* **1990**, *43*, 1881–1888.
16. Unson, M. D.; Faulkner, D. J. *Experientia* **1993**, *49*, 349–353.
17. Faulkner, D. J.; Unson, M. D.; Bewley, C. A. *Pure Appl. Chem.* **1994**, *66*, 1983–1990.
18. Cook, S. de C.; Bergquist, P. R. In Hooper, J. N. A., Van Soest, R. W. M., Eds.; Systema Porifera: A Guide to the Classification of Sponges; Kluwer Academic/Plenum: New York, 2002; Vol. 1, pp 1061–1066.
19. Sheldrick, G. M. *SHELX97. Program for the Refinement of Crystal Structures from Diffraction Data*; University of Göttingen: Germany, 1997.

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Tetrahedron 61 (2005) 965–970

Bis[3-(triethoxysilyl)propyl]tetrasulfide: the first liquid sulfur-transferring agent useful for conversion of nucleoside phosphites to the phosphorothioates

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Received 22 September 2004; revised 2 November 2004; accepted 4 November 2004

Available online 10 December 2004

Abstract—This paper describes bis[3-(triethoxysilyl)propyl]tetrasulfide, which is the first liquid sulfur-transferring agent useful for the conversion of nucleoside phosphite intermediates in the synthesis of the phosphorothioates using a phosphoramidite strategy. This liquid reagent is preferable to existing solid sulfur-transferring agents, because it enables solid-phase synthesis with an automated DNA/RNA synthesizer, while avoiding the risk of accumulation of reagent deposits in the delivery tubes and valves of the synthesizer during sulfurization.

1. Introduction

Oligodeoxyribonucleotide phosphorothioates have attracted a great deal of attention as promising antisense molecules;[1] thus, the development of an efficient method for synthesis of these substances has become important.[1] Synthesis is now most conventionally carried out in a solid phase using the phosphoramidite method.[2] For the phosphoramidite approach, the invention of a sulfurizing agent useful for the conversion of nucleoside phosphites to phosphorothioates remains important. Thus far, a number of sulfurizing agents have been suggested, including elemental sulfur,[3] 3*H*-1,2-benzodithiol-3-one 1,1-dioxide (Beaucage reagent),[4] tetraethylthiuram disulfide,[5] dibenzoyl tetrasulfide,[6] bis(ethoxythiocarbonyl) tetrasulfide,[7] benzyl(triethyl)ammonium tetrathiomolybdate,[8] bis(arenesulfonyl) disulfides [e.g. bis(benzenesulfonyl) disulfide, bis(*p*-toluenesulfonyl) disulfide, bis(*p*-methoxybenzenesulfonyl) disulfide, and bis(*p*-chlorobenzenesulfonyl) disulfide[9]], diphenylacetyl disulfide,[10] bis(ethoxythiocarbonyl) disulfide,[11] propylene sulfide in the presence of $ReOCl_3[P(C_6H_5)_3]_2$ as a catalyst,[12] bis(*O*,*O*-diisopropoxyphosphinothioyl) disulfide,[13] 3-methyl-1,2,4-dithiazolin-5-one,[14] 3-ethoxy-1,2,4-dithiazolidin-5-one,[15–17] 1,2,4-dithiazolidine-3,5-dione,[16,17] 3-amino-1,2,4-dithiazol-5-thione,[18] and 2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulfide (Lawesson's reagent).[19] However, these reagents are solids with insufficient solubility in solvents suitable for this type of synthesis, such as acetonitrile and toluene. Accordingly, use of these reagents for solid-phase synthesis is often difficult because the reagent deposits in delivery tubes and valves, thereby causing serious mechanical problem. In contrast, there would be no such risk if a liquid reagent could be used that is freely/highly soluble in various organic solvents. Furthermore, high solubility of the reagent would enable the use of a high-concentration solution, and thus avoids the need for undesired large volume of solvent. This issue is particularly relevant in the case of large-scale syntheses, for example, in the synthesis of substances practically used for antisense therapy. Thus, although liquid sulfurizing agents useful for solid-phase synthesis are critical, no such reagents have been found to date. The liquid reagent, di-*tert*-butyl disulfide, was previously suggested,[20] but this reagent requires UV irradiation in order to become active,[21] and thus it is only useful for liquid-phase synthesis. Consequently, there is still no useful liquid sulfurizing agent known for application in solid-phase synthesis. This paper describes bis[3-(triethoxysilyl)propyl] tetrasulfide (TEST) $\{[(C_2H_5O)_3SiCH_2CH_2CH_2SS]_2\}$[22] as the first example of a liquid sulfurizing agent found to be useful not only for the liquid-phase synthesis, but also the solid-phase synthesis of nucleoside phosphorothioates through a phosphoramidite approach.

Keywords: Nucleotides; Sulfur compounds; Thiophosphates.
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doi:10.1016/j.tet.2004.11.003

2. Results and discussion

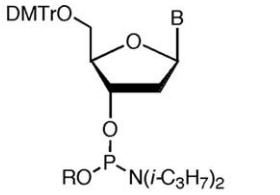
First, the utility of TEST was examined for the liquid-phase preparation of the dinucleoside phosphorothioate **9**. To this end, the nucleoside phosphoramidite **4** (0.20 mmol) and the nucleoside **5** (0.20 mmol) were condensed using benzimidazolium triflate (BIT)[23] (0.20 mmol) as a promoter in the presence of powdery molecular sieves (MS) 3A (ca. 50 mg)[24] in acetonitrile (1.0 mL) (25 °C, 1–10 min). Subsequently, the resulting product (the dinucleoside phosphite), without isolation, was sulfurized in the same flask by adding neat TEST (0.22 mmol) only or a mixture of TEST (0.22 mmol) and an additive reagent (in the amount shown in Table 1).[25] Yield of the target phosphorothioate **9** was estimated by the ^{31}P NMR analysis of an aliquot of the reaction mixture collected at suitable intervals. Table 1 summarizes the results, which demonstrated that it is crucial to use DMAP or *N*-methylimidazole (*N*-MeIm) as an additive in more than 3 equiv with respect of the amount of TEST in order to smoothly accomplish sulfurization and to obtain a high yield of the target product. For example, sulfurization in the presence of DMAP in three to 5 equiv to TEST was completed within 1 min to provide **9** in an almost quantitative yield. In contrast, the sulfurization in the absence of the additive (a reaction promoter), i.e. the reaction in neat TEST was not completed within 1 min, and the yield of **9** after 1 min was ca. 75%. This reaction required 10 min for completion. The amount of the promoter was also important. Less than 3 equiv of the reaction promoter with respect to TEST did not enable a sufficiently rapid reaction. For instance, when 1 equiv of DMAP was used, the reaction required 5 min for completion. The crude product always included a small amount of phosphate, but the ratio of the phosphorothioate **9** versus the undesired phosphate (S/O ratio) was generally high. For example, the S/O ration in the crude product obtained by synthesis using 3 equiv of DMAP with respect to TEST was >99.5:0.5. In general, this strategy could be applied for the preparation of other dinucleoside phosphorothioates such as **6**, **7**, and **8**, using **1**, **2**, and **3**, respectively, as the phosphoramidite. The isolated yields of **6**, **7**, and **8**, prepared via 1-min sulfurization using a mixture of TEST (1.1 equiv to the phosphoramidite) and DMAP (3.3 equiv to the phosphoramidite) were as follows: **6**, 95%; **7**, 92%; and **8**, 96%. The S/O ratios in these products were all >99.5:0.5. In all cases, no damage of the nucleoside bases, carbohydrate moieties, or protecting groups was observed.

Table 1. Synthesis of the dinucleotide phosphorothioate **9**: effect of an additive on sulfurization using TEST

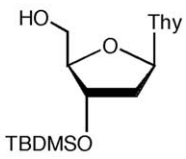
Additive (equiv[a])	Reaction time/min	Product yield/%
None	1	75
None	5	95
None	10	100
DMAP (1.1)	1	98
DMAP (1.1)	5	100
DMAP (3.3)	1	100
N-Melm (1.1)	1	81
N-Melm (1.1)	5	98
N-Melm (3.3)	1	94
N-Melm (3.3)	5	100
Benzimidazole (3.3)	1	100
HMPA (3.3)	1	96

[a] Molar equivalents with respect to the amount of TEST.

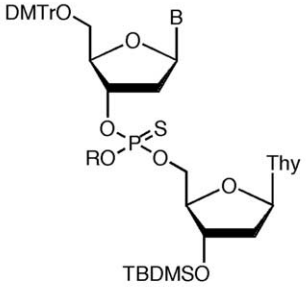
Furthermore, we investigated the utility of TEST in the synthesis of the methyl phosphorothioate **10** and the 2-cyanoethyl phosphorothioate **11**, in which internucleotide-bond formation was achieved using **12** or **13**, respectively, as the phosphoramidite under similar conditions to those described above. However, in these two cases, the undesired and extensive elimination of the methyl and cyanoethyl groups took place. Thus, the present method was not deemed useful for the synthesis of methyl and cyanoethyl phosphorothioates.



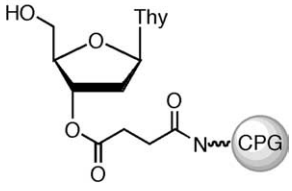
1, B = Ade^{AOC}; R = CH_2=$CHCH_2$
2, B = Cyt^{AOC}; R = CH_2=$CHCH_2$
3, B = Gua^{All,AOC}; R = CH_2=$CHCH_2$
4, B = Thy; R = CH_2=$CHCH_2$
12, B = Thy; R = CH_3
13, B = Thy; R = $NCCH_2CH_2$



5



6, B = Ade^{AOC}; R = CH_2=$CHCH_2$
7, B = Cyt^{AOC}; R = CH_2=$CHCH_2$
8, B = Gua^{All,AOC}; R = CH_2=$CHCH_2$
9, B = Thy; R = CH_2=$CHCH_2$
10, B = Thy; R = CH_3
11, B = Thy; R = $NCCH_2CH_2$



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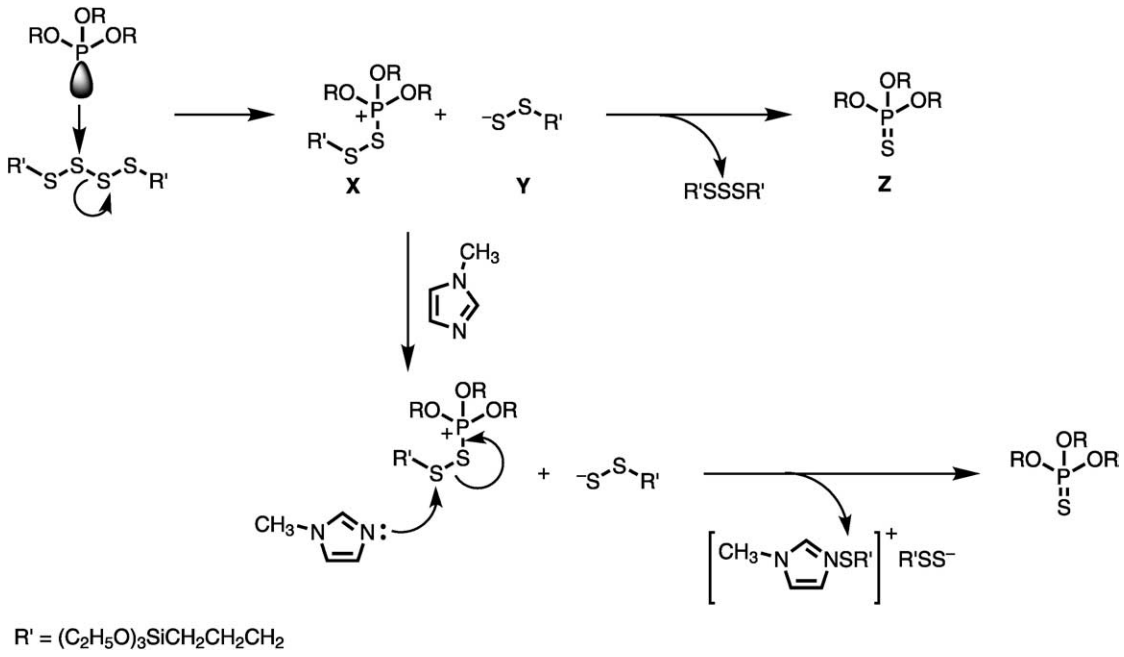
In the present sulfurization reaction, the promotion of *N*-MeIm could be explained as follows. The mechanism of the reaction in the absence of a promoter can be conceived as the equation shown in the first line of Scheme 1. Thus, first the phosphite reacts with TEST to form the phosphonium disulfide **X** and the disulfide anion **Y**. Then, the anion **Y** nucleophilically attacks the sulfur atom of **X**, giving the phosphorothioate **Z** and R′SSSR′ [R′=$(C_2H_5O)_3SiCH_2$-CH_2CH_2].[26] In this process, the reaction of **X** and **Y** may be the rate-determining step, but **Y** is not sufficiently nucleophilic. Therefore, the rate of the reaction is rather slow. However, when *N*-MeIm, the nucleophilicity of which is higher than that of the disulfide ion **Y**, is present, then *N*-MeIm will react with **X** in place of **Y**, as shown in the equation in the second line of Scheme 1.

Consequently, the replacement is more smoothly accomplished to produce the phosphorothioate **Z**. Here, the above-mentioned demethylation in the synthesis of methyl phosphorothioates may be caused by the nucleophilic



Scheme 1. Proposed mechanism of sulfurization of a phosphite to a phosphorothioate.

attack of *N*-MeIm or by the disulfide anion **Y**. However, in the synthesis of cyanoethyl phosphorothioates, *N*-MeIm may act as a base deblocking the cyanoethyl group via a β-elimination process.

We next examined the utility of TEST for the solid-phase synthesis of nucleotide phosphorothioates via the phosphoramidite method on an automated Applied Biosystems Model 392 DNA/RNA synthesizer. As shown in the solution-phase synthesis, it also appears to be crucial for solid-phase synthesis that one uses DMAP or *N*-MeIm as a promoter in order to obtain good results. However, because DMAP is a solid reagent that is not highly soluble in organic solvents, this material has the potential of depositing in the synthesizer, causing mechanical problems. On the other hand, *N*-MeIm is a freely or highly soluble liquid in various organic solvents, and thus there is no risk of depositing in a machine when such a solvent is used for dissolving the reagent. Thus, we chose *N*-MeIm as the promoter. Furthermore, in contrast to the liquid-phase synthesis carried out in a flask, it is impossible to add a neat form of TEST to the reaction mixture in the case of solid-phase synthesis achieved using an automated machine; thus, in the solid-phase synthesis, we had to use TEST as a solution in a suitable solvent. Therefore, before starting our synthesis, we tested the stability of TEST in the presence of *N*-MeIm in an organic solvent. The test was carried out using 0.25 M TEST/1.25 M *N*-MeIm solution in various media by keeping the samples at 25 °C for 7 days. The solvents tested included benzene, dichloromethane, ethyl acetate, acetone, anisole, acetonitrile, a 1:1 mixture of dichloromethane and acetonitrile, a 1:4 mixture of dichloromethane, methanol, *tert*-butyl alcohol, DMSO, *N*-methyl-2-pyrrolidone, DMF, and HMPA. Here, it was revealed that TEST is stable in *tert*-butyl alcohol, *N*-methyl-2-pyrrolidone, DMF, and HMPA, but it underwent decomposition in other solvents, generating a colorless precipitate of sulfur. Among the four solvents that did not decompose TEST, we chose DMF for use in the following examinations, because this solvent is very inexpensive, non-toxic, and appears to be safer for use in reactions on this type of machine.[26] We next attempted to determine which conditions would be suitable for solid-phase synthesis by the preparation of TpsT (ps = phosphorothioate linkage), using **4** as the phosphoramite unit. The synthesis was examined on a 1.0-μmol scale, starting from **14**, i.e. thymidine bound at its 3′-terminal oxygen via a long chain alkylamine chain to controlled pore glass; the chain elongation was performed according to the protocol shown in Table 2. In this synthesis, the sulfurization of the phosphite intermediate was examined by the use of (i) a 0.1 M TEST, 0.5 M *N*-MeIm/DMF solution (method A), (ii) a 0.1 M TEST, 2.5 M *N*-MeIm/DMF solution (method B), (iii) a 0.25 M TEST, 1.25 M *N*-MeIm/DMF solution (method C), (iv) a 0.25 M TEST, 1.25 M *N*-MeIm/*N*-methyl-2-pyrrolidone solution (method D), (v) a 0.5 M TEST, 2.5 M *N*-MeIm/DMF solution (method E), or (vi) a 1.0 M solution of TEST in *N*-MeIm (method F). After elongation of the nucleotide linkage was complete, TpsT was isolated by treatment with $Pd_2[(C_6H_5CH{=}CH)_2CO]_3\cdot CHCl_3$ in the presence of $(C_6H_5)_3P$ and butylammonium formate in THF (50 °C, 1 h) for deblocking the allyl protector, and then with conc. ammonia

Table 2. Reaction sequence of the solid-phase synthesis

Step	Operation	Reagent(s)	Time (min)
1	Washing	CH_3CN	0.4
2	Detritylation	3% Cl_3CCOOH	1.3
3	Washing	CH_3CN	0.8
4	Coupling	0.1 M Amidite/CH_3CN	1.0
5	Washing	CH_3CN	0.2
6	Capping	Ac_2O/2,6-lutidine/THF (1:1:8) + *N*-methylimidazole/THF	0.3
7	Washing	CH_3CN	0.2
8	Sulfurization	0.1 M TEST/0.5 M *N*-methylimidazole/DMF	10.0
9	Washing	CH_3CN	0.6

(30 min) for detaching the nucleotide from the solid support. The purity and yield of the product were determined by the ^{31}P NMR analysis of the crude material. Among the sulfurization methods, method A was found to be the best, and the reaction was complete in 10 min. In this case, TpsT was obtained in a 98% isolated yield and the S/O ratio of the product was >99.5:0.5. However, in the case of the syntheses employing method B, C, D, E, or F for 10 min, the phosphorothioate was obtained in only an 89 (method B), 49 (method C), 37 (method D), 25 (method E) or 37% (method F) yield, respectively. In these cases, completion of the sulfurization required longer than 10 min. It should be noted that one of these methods caused technical problems with regard to the delivery tubes and machine valves; this was the case not only during but also after the sulfurization process, as the substances used for sulfurization and those generated by sulfurization were highly soluble in the reaction solvent. Subsequently, we carried out the preparation of a longer oligomer, $(Tps)_{19}T$, via the procedure shown in Table 2, in which the sulfurization was performed in 10 min by method A.[27] The chain elongation was achieved at a 97.2% average coupling yield to give the desired product in a 78% overall yield, which had high purity in the crude form, as shown in Figure 1. The S/O ratio of this product was 99:1.

3. Conclusion

Here, we disclosed the first example of a liquid sulfurizing agent, TEST, which was found to be useful for the conversion of nucleoside phosphites to the phosphorothioates. We compared the utility of TEST with that of the Beaucage reagent, a representative sulfurizing agent that is currently the most widely used reagent for such purpose. With regard to its reactivity in solid-phase synthesis, TEST is unfortunately inferior to the Beaucage reagent. For example, in the solid-phase synthesis of TpsT, sulfurization using a 0.1 M solution of the Beaucage reagent in acetonitrile was completed in 0.25 min to give the target product in a 98% yield (the S/O ratio in the product =99.0:1.0). On the other hand, sulfurization using a 0.1 M TEST and 0.5 M *N*-methylimidazole solution in DMF was rather slow, i.e. required 10 min for completion in order to obtain the desired product in a comparable yield (98%) to that shown in the synthesis using the Beaucage reagent. However, TEST was found to be superior in some respects to the Beaucage reagent. The clearest advantage of TEST was that this reagent is a liquid. This feature is quite beneficial in the context of solid-phase synthesis, because there is no risk that the reagent will deposit in the delivery tubes and the synthesizer valves. Another advantage of TEST is that it is highly soluble in various organic solvents such as acetonitrile and THF. This characteristic is also an important advantage for solution-phase synthesis on a large scale, because it circumvents the need for use of an undesirably large volume of solvent. Another advantage of TEST is that this compound is commercially available at a reasonable price, i.e. it costs less than the Beaucage reagent.[28] The low-cost availability of this novel reagent will prove to be an important feature for large-scale synthesis. Thus, TEST is more useful than the Beaucage reagent in such cases. Furthermore, TEST is quite stable in a moist environment and does not undergo decomposition with storage under ordinary conditions, nor does it decompose during synthetic procedures, including workup. In contrast, the Beaucage reagent is very sensitive to moisture. In fact, the Beaucage reagent is decomposed by moisture present in the atmosphere during storage and is also decomposed by water in a reaction solvent or in that used during a workup to generate certain acidic species, causing the cleavage of the 5′-*O*-dimethoxytrityl protector and depurination of deoxyadenosines and deoxyguanosines. In conclusion, these merits of TEST more than compensate for the low reactivity of this reagent, which was found to be a sufficiently useful sulfurizing agent in the synthesis of nucleoside phosphorothioates via the phosphoramidite approach.

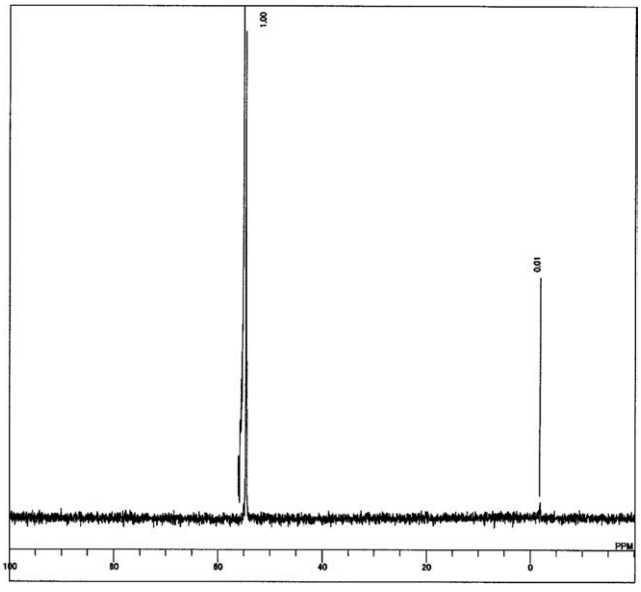


Figure 1. The ^{31}P NMR spectrum of $(Tps)_{19}T$ in crude form.

4. Experimental

4.1. General

A UV spectrum was measured on a JASCO V-500 spectrometer. NMR spectra were taken on a JEOL JNM-α400 or ECA-500 instrument. The ^{1}H, ^{13}C, and ^{31}P NMR chemical shifts are described as δ values in ppm relative to $(CH_3)_4Si$ (for ^{1}H and ^{13}C) and 85% H_3PO_4, respectively. ESI-TOF high-resolution mass (HRMS) spectra were obtained on Applied Biosystems Voyager MDE and Mariner spectrometers, respectively. HPLC analysis was carried out using a COSMOSIL $5C_{18}$-MS column (Nacalai Tesque, ODS-5 mm, 4.6–250 mm) on a Waters 2695 Separations Module chromatograph with a Waters 2996 Photodiode Array detector. Column chromatography was performed using Nacalai Tesque silica gel 60 (neutrality, 75 mm). Unless otherwise noted, synthetic reactions were carried out at ambient temperature. The reactions requiring anhydrous conditions were achieved under an argon atmosphere in flasks dried by heating at 400 °C under 1.0–3.0 mmHg, or by washing with a 5% solution of

dichlorodimethylsilane in dichloromethane followed by anhydrous dichloromethane, and then heating at 100 °C.

4.2. Materials and solvents

TEST (Gelest), DMAP (Tokyo Kasei), *N*-methylimidazole (Nakarai tesque), benzimidazole (Tokyo Kasei), triphenylphosphine oxide (Tokyo Kasei), 5′-*O*-(*p*,*p*′-dimethoxytrityl) thymidine 3′-(methyl *N*,*N*′-diisopropylphosphoramidite) (**12**) (Glen Research), 5′-*O*-(*p*,*p*′-dimethoxytrityl)thymidine 3′-(2-cyanoethyl *N*,*N*′-diisopropylphosphoramidite) (Glen Research) (**13**) and the thymidine derivative anchored on CPG (**14**) (Applied Biosystems) were commercially supplied. Nucleoside phosphoramidite **1**,[29,30] **2**,[29,30] **3**[29,30] and **4**,[29] 5′-*O*-free nucleoside **5**,[31] benzimidazolium triflate,[23a] and $Pd_2[C_6H_5CH{=}CH]_2CO]_3{\cdot}CHCl_3$[28] were prepared by the reported methods. Diethyl ether, THF, and toluene were used after drying by reflux over sodium-benzophenone ketyl. Acetonitrile, DMF, and dichloromethane were distilled from calcium hydride. Other organic reagents were used as commercially supplied without any purification. Solid and amorphous organic substances were used after drying over P_2O_5 at 50–60 °C for 8–12 h under 1.0–3.0 mmHg. Powdery molecular sieves (MS) 3A was employed after drying the commercially supplied one (Nacalai tesque) at 200 °C for 12 h under 1.0–3.0 mmHg.

4.3. General procedure for the liquid-phase preparation of dinucleoside phosphorothioates 6–9

A mixture of the nucleoside phosphoramidite **1**–**4** (0.20 mmol), the nucleoside **5** (0.20 mmol), and powdery molecular sieves (MS) 3A (ca. 50 mg) in acetonitrile (1.0 mL) was stirred at 25 °C for 30 min. To the resulting mixture was added benzimidazolium triflate (0.20 mmol) and stirring was continued for 5 min. TEST (0.22 mmol) and a promoter (0.66 mmol) were added to the reaction mixture, which was stirred at 25 °C. An aliquot of the reaction mixture was sampled at intervals, dissolved in $CDCl_3$, and subjected to ^{31}P NMR analysis using triphenylphosphine oxide as a standard to estimate the yield of the target phosphorothioates. After confirming the completion of the reaction, the reaction mixture was filtered to remove MS 3A and the filtrate was concentrated to give a viscous oil. This crude product was chromatographed on silica gel (50 g) to give a pure sample of **6**–**9**.

4.3.1. Allyl N^6-(allyloxycarbonyl)-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyadenyn-3′-yl 3′-*O*-(*tert*-butyldimethylsilyl)-thymidin-5′-yl phosphorothioate (6). ^1H NMR ($CDCl_3$) 0.09 (s, 6H), 0.90 (s, 9H), 1.93, 1.94 (2 s, 3H), 2.06–2.19 (m, 1H), 2.24–2.31 (m, 1H), 2.73–2.78 (m, 1H), 3.00–3.11 (m, 1H), 3.39–3.46 (m, 2H), 3.77 (s, 6H), 4.01–4.06 (m, 1H), 4.17–4.31 (m, 2H), 4.40–4.43 (m, 2H), 4.48–4.64 (m, 2H), 4.76, 4.77 (2 s, 2H), 5.22–5.46 (m, 5H), 5.82–6.02 (m, 2H), 6.26, 6.32 (2 t, J=6.0 Hz, 1H), 6.47 (t, J=6 Hz, 1H), 6.79, 6.81 (2 s, 4H), 7.22–7.39 (m, 9H), 8.17, 8.18 (2 s, 1H), 8.68 (s, 1H), 8.81 (s, 1H), 9.78, 9.88 (2 br s, 1H). ^{31}P NMR ($CDCl_3$) 67.6, 67.2; HRMS (ESI^+) calcd for $C_{54}H_{67}N_7O_{13}PSSi^+$ ($M+H^+$) 1112.4019, found 1112.4447.

4.3.2. Allyl N^4-(allyloxycarbonyl)-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxycytosyn-3′-yl 3′-*O*-(*tert*-butyldimethylsilyl)-thymidin-5′-yl phosphorothioate (7). ^1H NMR ($CDCl_3$) 0.06, 0.08 (2 s, 6H), 0.87, 0.88 (2 s, 9H), 1.88, 1.99 (2 s, 3H), 2.02–2.31 (m, 3H), 2.79–2.94 (m, 1H), 3.41–3.44 (m, 2H), 3.74–3.80 (m, 6H), 3.92–4.78 (m, 9H), 5.16–5.37 (m, 5H), 5.77–6.03 (m, 2H), 6.24–6.32 (m, 2H), 6.82–6.84 (m, 4H), 6.96 (d, J=6.0 Hz, 1H), 7.21–7.36 (m, 9H), 8.05–8.15 (m, 2H), 9.34, 9.37 (2 s, 1H). ^{31}P NMR ($CDCl_3$) 68.8, 68.7; HRMS (ESI^+) calcd for $C_{53}H_{66}N_5O_{14}PSSiNa^+$ ($M+Na^+$) 1110.3738, found 1110.3726.

4.3.3. Allyl N^2-(allyloxycarbonyl)-O^6-(allyl)-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyguanyn-3′-yl 3′-*O*-(*tert*-butyldimethylsilyl)-thymidin-5′-yl phosphorothioate (8). ^1H NMR ($CDCl_3$) 0.09 (s, 6H), 0.89, 0.90 (2 s, 9H), 1.94, 1.95 (2 s, 3H), 2.08–2.31 (m, 2H), 2.69–2.77 (m, 1H), 2.91–3.01 (m, 1H), 3.32–3.49 (m, 2H), 3.77 (s, 6H), 3.98–4.70 (m, 8H), 5.01–5.10 (m, 2H) 5.21–5.50 (m, 7H) 5.80–6.02 (m, 2H), 6.10–6.41 (m, 3H), 6.77–6.80 (m, 4H), 7.17–7.40 (m, 9H), 7.67–7.95 (2 s, 2H), 8.80, 9.02 (2 s, 1H), 8.81 (s, 1H), 9.78, 9.88 (2 br, 1H). ^{31}P NMR ($CDCl_3$) 67.7, 67.3; HRMS (ESI^+) calcd for $C_{57}H_{71}N_7O_{14}PSSi^+$ ($M+H^+$) 1168.4281, found 1168.4284.

4.3.4. Allyl 5′-*O*-(4,4′-dimethoxytrityl)-thymidin-3′-yl 3′-*O*-(*tert*-butyldimethylsilyl)-thymidin-5′-yl phosphorothioate (9). ^1H NMR ($CDCl_3$) 0.08, 0.10 (2 s, 6H), 0.89, 0.90 (2 s, 9H), 1.46 (s, 3H), 1.92, 1.93 (2 s, 3H), 2.05–2.62 (m, 4H), 3.40–3.49 (m, 2H), 3.80 (s, 6H), 3.94–4.62 (m, 7H), 5.20–5.41 (m, 4H), 5.78–5.96 (m, 1H), 6.12–6.28 (m, 1H), 6.39–6.44 (m, 1H), 6.85 (d, J=4.0 Hz, 4H), 7.25–7.41 (m, 9H), 7.57–7.58 (m, 1H), 8.54–8.67 (m, 2H). ^{31}P NMR ($CDCl_3$) 67.5, 67.3; HRMS (ESI^+) calcd for $C_{50}H_{63}N_4O_{13}PSSiNa^+$ ($M+Na^+$) 1041.3642, found 1041.3511.

4.4. Solid-phase synthesis of $(Tps)_{19}T$

The synthesis of oligodeoxyribonucleotide phosphorothioates were carried out according to the reaction cycle shown in Table 2, respectively, using suitable phosphoramidites as monomer units. In the synthesis via the allyl/AOC-protected phosphoramidite approach, the solid-anchored product after chain elongation was treated with a mixture of $Pd_2[(C_6H_5CH{=}CH)_2CO]_3{\cdot}CHCl_3$ and $(C_6H_5)_3P$ in the presence of *n*-butylammonium formate in THF at 50 °C for 60 min and then with conc. ammonia at 25 °C for 60 min to give the target oligomer.

Acknowledgements

This study was funded in part by contributions from Mitsui Chemicals, and from Grants-in-Aid for Scientific Research (Nos. 16011223 and 16350086) from the Ministry of Education, Science, Sports and Culture of Japan.

References and notes

1. Representative reviews: (a) Ulmann, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 543–584. (b) Milligan, J. F.; Matteucci, M. D.; Martin, J. C. *J. Med. Chem.* **1993**, *36*, 1923–1937. (c) Varma,

R. S. *Synlett* **1993**, 621–637. (d) Stec, W. J.; Wilk, A. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 709–722. (e) Ling, R.; Yoshida, M.; Mariano, P. S. *J. Org. Chem.* **1996**, *61*, 4439–4449.

2. (a) Beaucage, S. L.; Iyer, R. P. *Tetrahedron* **1993**, *49*, 6123–6194 and references cited therein. (b) Hayakawa, Y. *Bull. Chem. Soc. Jpn.* **2001**, *74*, 1547–1565.
3. Burgers, P. M. J.; Eckstein, F. *Tetrahedron Lett.* **1978**, *40*, 3835–3838.
4. (a) Iyer, R. P.; Phillips, L. R.; Egan, W.; Regan, J. B.; Beaucage, S. L. *J. Org. Chem.* **1990**, *55*, 4693–4699. (b) Iyer, R. P.; Egan, W.; Regan, J. B.; Beaucage, S. L. *J. Am. Chem. Soc.* **1990**, *112*, 1253–1254.
5. Vu, H.; Hirscbein, B. L. *Tetrahedron Lett.* **1991**, *32*, 3005–3008.
6. Rao, M. V.; Reese, C. B.; Zhengyun, Z. *Tetrahedron Lett.* **1992**, *33*, 4839–4842.
7. Zhang, Z.; Nichols, A.; Alsbeti, M.; Tang, J. X.; Tang, J. Y. *Tetrahedron Lett.* **1998**, *39*, 2467–2470.
8. Rao, M. V.; Macfarlane, K. *Tetrahedron Lett.* **1994**, *35*, 6741–6744.
9. Efimov, V. A.; Kalinkina, A. L.; Chakhmakhcheva, O. G.; Hill, T. S.; Jayaraman, K. *Nucleic Acids Res.* **1995**, *23*, 4029–4033.
10. (a) Roelen, H. C. P. F.; Kamer, P. C. J.; van den Elst, H.; van der Marel, G. A.; van Boom, J. H. *Recl. Trav. Chim. Pays-Bas* **1991**, *110*, 325–331. (b) Cheruvallath, Z. S.; Wheeler, P. D.; Cole, D. L.; Ravikumar, V. T. *Nucleosides Nucleotides* **1999**, *18*, 485–492. (c) Cheruvallath, Z. S.; Carty, R. L.; Moore, M. N.; Capaldi, D. C.; Krotz, A. H.; Wheeler, P. D.; Turney, B. J.; Craig, S. R.; Gaus, H. J.; Scozzari, A. N.; Cole, P. L.; Ravikumar, V. T. *Org. Proc. Res. Dev.* **2000**, *4*, 199–204.
11. (a) Eleuteri, A.; Cheruvallath, Z. S.; Capaldi, D. C. *Nucleosides Nucleotides* **1999**, *18*, 1803–1807. (b) Cheruvallath, Z. S.; Kumar, R. K.; Rentel, C.; Cole, D. L.; Ravikumar, V. T. *Nucleosides, Nucleotides, Nucleic Acids* **2003**, *22*, 461–468.
12. Arterburn, J. B.; Perry, M. C. *Tetrahedron Lett.* **1997**, *38*, 7701–7704.
13. Stec, W. J.; Uznanski, B.; Wilk, A. *Tetrahedron Lett.* **1993**, *34*, 5317–5720.
14. Xu, Q.; Musier-Forsyth, K.; Hammer, R. P.; Barany, G. *Nucleic Acids Res.* **1996**, *24*, 1602–1607.
15. Xu, Q.; Barany, G.; Hamner, R. P.; Nusier-Forsyth, K. *Nucleic Acids Res.* **1996**, *24*, 3643–3644.
16. Zhang, Z.; Nichols, A.; Tang, J. X.; Han, Y.; Tang, J. Y. *Tetrahedron Lett.* **1999**, *40*, 2095–2098.
17. Tang, J. Y.; Han, Y.; Tang, J. X.; Zhang, Z. *Org. Proc. Res. Dev.* **2000**, *4*, 194–198.
18. Zhang, Z.; Han, Y.; Tang, J. X.; Tang, J.-Y. *Tetrahedron Lett.* **2002**, *43*, 4347–4349.
19. Ju, J.; McKenna, C. E. *Bioorg. Med. Chem. Lett.* **2003**, *12*, 1643–1645.
20. Sopchik, A. E.; Bentrude, W. G. *Tetrahedron Lett.* **1981**, *22*, 307–310.
21. According to an examination carried out by our group, di-*tert*-butyl disulfide does not serve as an effective sulfurizing agent without UV irradiation.
22. TEST is commercially available from Gelest and Shin-etsu, Tokyo, Japan. This liquid reagent is freely soluble in various organic solvents including toluene, tetrahydrofuran, acetonitrile, and *N*-methylimidazole at ambient temperature.
23. (a) Hayakawa, Y.; Kataoka, M.; Noyori, R. *J. Org. Chem.* **1996**, *61*, 7996–7997. (b) Hayakawa, Y.; Kawai, R.; Hirata, A.; Sugimoto, J.; Kataoka, M.; Sakakura, A.; Hirose, M.; Noyori, R. *J. Am. Chem. Soc.* **2001**, *123*, 8165–8176.
24. Hayakawa, Y.; Hirata, A.; Sugimoto, J.; Kawai, R.; Sakakura, A.; Kataoka, M. *Tetrahedron* **2001**, *57*, 8823–8826.
25. *N*-Methyl-2-pyrrolidone and HMPA are rather expensive, and HMPA is highly toxic. *tert*-Butyl alcohol, the melting point of which is ca. 26 °C, runs the risk of freezing in the delivery tubes of the synthesizer.
26. Production of $(C_2H_5O)_3Si(CH_2)_3SSS(CH_2)_3Si(OC_2H_5)_3$ was confirmed by HRMS (ESI^+): calcd for $C_{18}H_{42}O_6S_3Si_2Na^+$ 529.1574, found 529.1893.
27. In the solid-phase synthesis of a dinucleoside phosphorothioate, 5 min of sulfurization suffices to obtain a high yield of the product. In contrast, for the synthesis of this size of oligomers, i.e. dimers, more time (at least 10 min) is required for sulfurization in order to obtain a satisfactory yield of the target product.
28. According to recent catalogs, the respective prices of TEST and the Beaucage reagent are as follows: TEST costs \$280/mol (Gelest), \$400/mol (Shin-etsu), \$2,500/mol (Fulka), and the Beaucage reagent costs \$26,800/mol (Aldrich).
29. Hayakawa, Y.; Wakabayashi, S.; Kato, H.; Noyori, R. *J. Am. Chem. Soc.* **1990**, *112*, 1691–1696.
30. Sophie, B. H.; Hayakawa, Y. *Nucleosides Nucleotides* **1999**, *18*, 1771–1787.
31. Ogilvie, K. K. *Can J. Chem.* **1973**, *51*, 3799–3807.

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Optimized polymer–enzyme electrostatic interactions significantly improve penicillin G amidase efficiency in charged PEGA polymers

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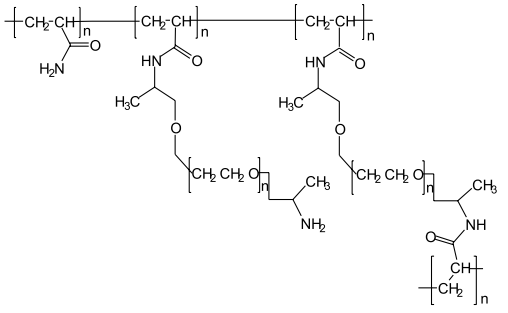
Received 5 August 2004; revised 12 October 2004; accepted 4 November 2004

Available online 30 November 2004

Abstract—Hydrolytic yields as high as 80% were obtained by using penicillin G amidase (PGA) on substrates anchored on optimized positively charged PEGA polymers. By increasing the amount of permanent charges inside the polymer, electrostatic interactions between the positively charged $PEGA^+$ and the negatively charged PGA (pI = 5.2–5.4) were strengthened, thus favouring the accessibility of the bulky enzyme (MW = 88 kDa) inside the pores. The effect of different amounts of charges on polymer swelling and protein retention inside the polymer was investigated and correlated to the enzyme efficiency demonstrating that electrostatic interactions predominate over swelling properties in determining enzyme accessibility.

1. Introduction

Evaluation of libraries of compounds generated by combinatorial chemistry has been appreciated during the last decade as an efficient and rapid approach to synthesise and screen arrays of compounds. Combinatorial biocatalysis represents a complementary approach to combinatorial chemistry and exploits the amazing selectivity and activity of enzymes at mild experimental conditions and with a low environmental impact. This approach requires polymer materials that are compatible with enzyme activity. Meldal et al. successfully developed a range of PEGA polymers (cross-linked acrylamide and poly(ethylene glycol) (Scheme 1) that have been used successfully in both enzymatic acylation and hydrolysis.[1]



Scheme 1. Structure of $PEGA_{1900}$, co-polymer of poly(ethylene glycol) and acrylamide.

$PEGA_{1900}$ was found to be completely permeable to proteins with molecular masses of up to 35 kDa,[2,3] whereas incomplete enzyme access was observed when larger enzymes, such as penicillin G acylase (PGA, 88 kDa) were used.[4]

Penicillin G amidase, due to its high specificity for phenylacetic group, is the enzyme of first choice for the protection/deprotection of functional groups in solution and it is expected to open up alternative opportunities for releasing compounds from polymeric supports via enzymatic selective cleavage of ad hoc designed linkers.[5]

Recently, we demonstrated that the introduction of permanent charges into PEGA polymer (by replacing 20% (w/w) of the amide monomers with monomers containing quaternary amine derivatives) increased swelling is observed due to electrostatic repulsions between equal

Keywords: Penicillin G amidase; PEGA polymers; Solid phase biocatalysis; Hydrolysis; Electrostatic interaction.
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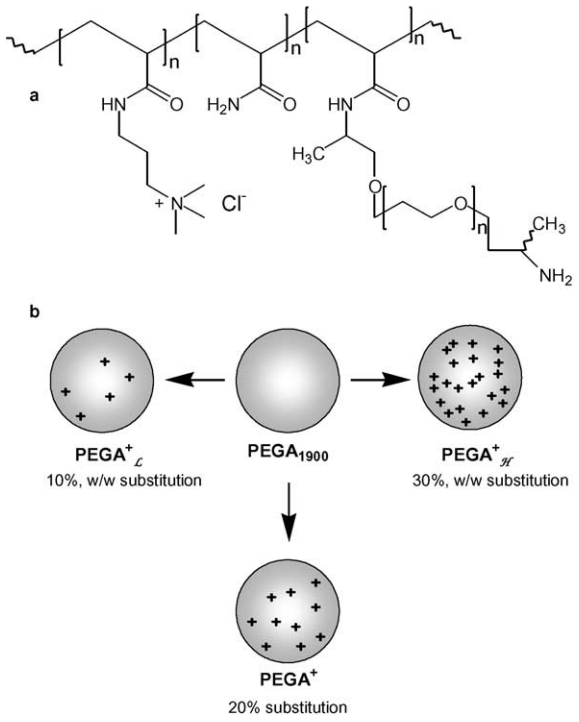
0040–4020/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2004.11.015

charges which cause the enlargement of pores.[6] In addition, oppositely charged enzymes are drawn inside due to favourable electrostatic attraction between polymer and protein. For example, we showed that these novel resins, PEGA$^+$, could be used effectively for the hydrolysis of phenylacetamides by PGA, achieving conversions of up to 48%.[6]

Following the promising results obtained with PEGA$^+$, and being aware that practical applications of enzymes in solid phase synthesis necessitate complete enzyme accessibility to the polymer, we report here on the further improvement of the positively charged PEGA resins. By studying the effect of charge density on enzyme–polymer electrostatic interactions, optimal diffusion of PGA into the resin can be achieved, thus assuring efficient hydrolytic activity.



Scheme 2. Structure of novel charged PEGA polymers. Partial substitution of acrylamide monomers on $PEGA_{1900}$ (a) with positively charged monomers leads to (b) $PEGA_L^+$ (10%, w/w substitution) or PEGA$^+$ (20%, w/w substitution) or $PEGA_H^+$ (30%, w/w substitution).

Table 1. Characteristics of novel charged PEGA polymers

Resin	Solid[a] (%)	Monomer substitution[b] (%, w/w)
$PEGA_L^+$	4.78	11.40
PEGA$^+$	6.40	22.50
$PEGA_H^+$	5.09	30.40

[a] Resins as supplied by Polymerlab, wet in methanol.
[b] Weight of charged monomer on the total polymer.

2. Results and discussion

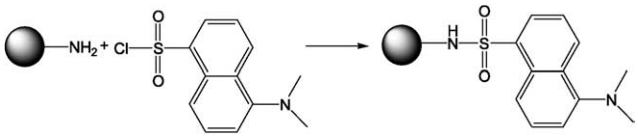
2.1. Characterisation of novel charged PEGA polymers

PEGA$^+$ has a polyacrylamide backbone in which acrylamide units are partially substituted (20%, w/w) with units carrying 3-(trimethylammonium)propyl chloride side chains (Scheme 2) which confer permanent positive charges to the polymer.[6]

During the present study novel charged PEGA polymers have been developed by varying the percentage of substitution of the acrylamide monomers with charged monomers from 10%, w/w (low density, $PEGA_L^+$) to 30%, w/w (high density, $PEGA_H^+$) (Table 1).

The three polymers have a declared loading of about 0.2 mmol/g_{dry} (Table 2). All the different PEGA$^+$ polymers were designed so to maintain constant the amount of primary amino groups and the presence of different percentages of charged monomers does not affect the chemical reactivity of the amino functions, as demonstrated experimentally by their chemical acylation with Fmoc-Phe (Table 2). Experimental results are in agreement with the loading declared by the manufacturer.

The amino group distribution was studied in all the polymers by exploiting two photon microscopy.[7] The amino groups were coupled with the fluorescent probe dansyl chloride as reported in Scheme 3.



Scheme 3. The amino group distribution in charged PEGA polymers was determined by coupling the fluorophore dansyl chloride.

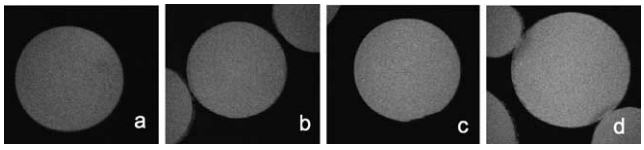


Figure 1. Fluorescence images of PEGA polymers. (a) $PEGA_{1900}$, (b) $PEGA_L^+$, (c) PEGA$^+$, (d) $PEGA_H^+$. The beads have an average diameter size of 300 μm.

Table 2. Loading and chemical reactivity of charged PEGA polymers

Resin	Chemical accessibility (mmol/g_{dry})				
	Number of acylation step				Average[a]
	1	2	3	4	
$PEGA_L^+$	0.21	0.26	0.31	0.26	0.27
PEGA$^+$	0.01	0.23	0.24	0.22	0.23
$PEGA_H^+$	0.16	0.20	0.22	0.22	0.22

[a] Calculated on saturation data: second, third and fourth acylation step. Complete acylation was confirmed by ninhydrin test.

After the dansylation the beads were analysed with two photon microscopy, TPM. The visual analysis of the beads showed a uniform distribution of the amino groups inside the polymers (Fig. 1).

Despite the similar loading determined for all the PEGA polymers (~0.2 mmol/g_{dry}), when the beads were suspended in Kpi buffer, an increase in fluorescence intensity was observed moving from neutral $PEGA_{1900}$ to $PEGA^{+}_{H}$ (Fig. 2). This is most probably ascribable to the presence of quaternary ammonium groups in a microenvironment that favours a shift in the equilibrium towards the highly fluorescent form of the dansylated residues.

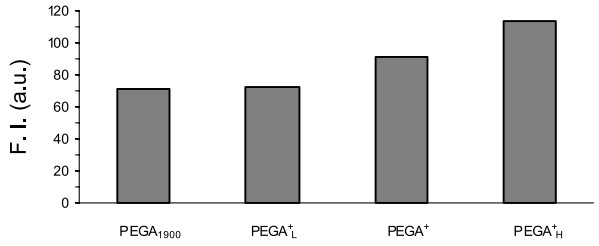


Figure 2. Fluorescence intensity (expressed in terms of adsorbance units) of charged PEGA beads after dansylation.

2.2. Effect of permanent charge density on swelling properties

It has been demonstrated that improved polymer swelling can promote more efficient enzyme diffusion into the polymer pores. Our previous studies on charged $PEGA^{+}$ showed that electrostatic repulsion between the positive charges inside the polymer causes pore enlargement and improved swelling. Of course, the extent of the phenomenon strongly depends both on buffer pH and ionic strength.[8]

Figure 3 reports the swelling behaviour of the PEGA resins

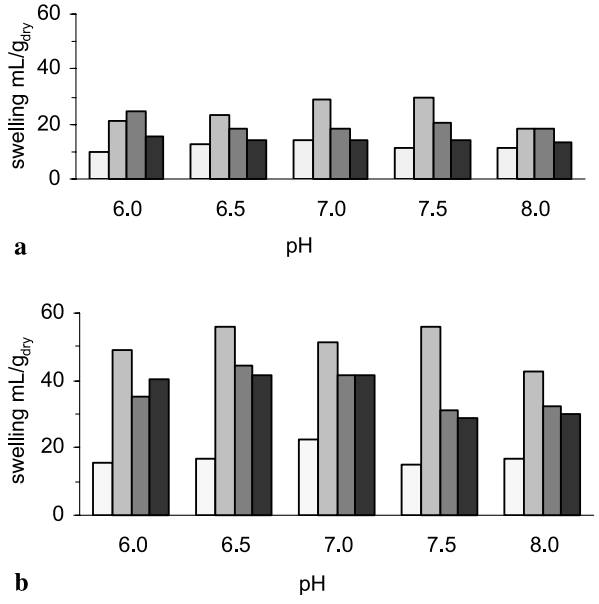


Figure 3. Swelling behaviour (mL/g_{dry}) of neutral polymer $PEGA_{1900}$ (white), $PEGA^{+}_{L}$ (grey), $PEGA^{+}$ (dark grey) and $PEGA^{+}_{H}$ (black) depending on pH. Data were collected in 0.1 M (a) and 0.001 M (b) buffer Kpi.

considered in this study as a function of ionic strength variations and in a range of pH values from 6.0 to 8.0.

The largest extent of swelling was observed for the $PEGA^{+}_{L}$ polymer. It is quite remarkable that the substitution of only 10%, w/w of the acrylamide in the original neutral ($PEGA_{1900}$) polymer monomers translates into a threefold improvement, so that the swelling capacity increases from 15 mL/g_{dry} (observed for $PEGA_{1900}$) to more than 50 mL/g_{dry}.

The data confirm the behaviour previously observed for $PEGA^{+}$: the swelling of all charged PEGA resins is considerably favoured by low ionic strength.[8] This behaviour is mainly due to the interference coming from the buffer ionic species which weaken the interactions between the permanent charges of the polymer. Data obtained using ultra pure water support this hypothesis, demonstrating how swelling reaches a maximum as the concentration of ionic species in solution is minimized (Fig. 4).

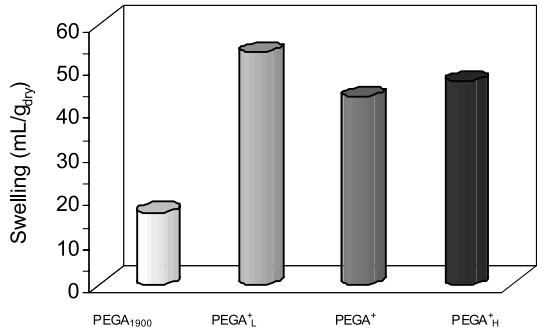


Figure 4. Swelling properties of charged PEGA and neutral $PEGA_{1900}$ in ultra pure water.

In principle, a larger number of positive charges inside the polymer should favour chain repulsion and pore enlargement, thus improving swelling capacity. The unexpected lower swelling capacity of $PEGA^{+}_{H}$ could be ascribed to an exceeding concentration of counter-ions inside the polymer which could cause a sort of 'rigidity' of the resin.

Therefore, the $PEGA^{+}_{L}$ seems to be characterized by the right balance between positive charges and polymer structure so as to maximize the resin solvation and pore enlargement.

2.3. Enzyme–resin interaction

We have previously reported that PGA can be attracted by $PEGA^{+}$ thanks to electrostatic interactions occurring between the positive charges of the polymer and the enzyme which has an overall negative charge at pH 8.0 (five negative charges).[8] In principle this phenomenon could favour the accessibility of the protein inside the resin.

The effect of the electrostatic interactions between PGA and the different positively charged PEGA was evaluated by determining the amount of protein retained by each resin in a Kpi buffer at pH 8.0 (0.001 M).

The protein retention was determined by the Pierce method.[9] As expected, $PEGA_H^+$ exerts the largest capacity for enzyme retention (70% of protein in solution, pH 8.0) due to the highest density of positive charges (Fig. 5). It is noteworthy that despite the fact that $PEGA_L^+$ shows highest swelling capacity (Fig. 4), PGA permeates $PEGA_H^+$ more efficiently, thus indicating the overwhelming role of electrostatic interactions in determining enzyme accessibility (Fig. 5).

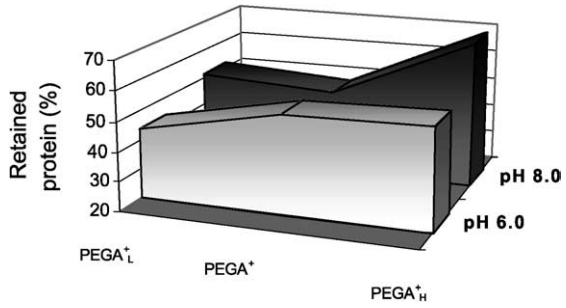


Figure 5. Comparison of retained PGA by PEGA polymers (%) when suspended in Kpi buffer 0.001 M at pH 6.0 and pH 8.0.

The strong effect of electrostatic interactions on protein accessibility inside the polymer was evidenced by comparing the protein retention at pH 6.0 (Fig. 5). At pH 6.0 the negative charge density on PGA decreases, thus causing the weakening of the electrostatic interactions with the positive resin and $PEGA_H^+$ in particular. This is reflected by a considerable decrease in protein retention by $PEGA_H^+$ from 70 to 50%.

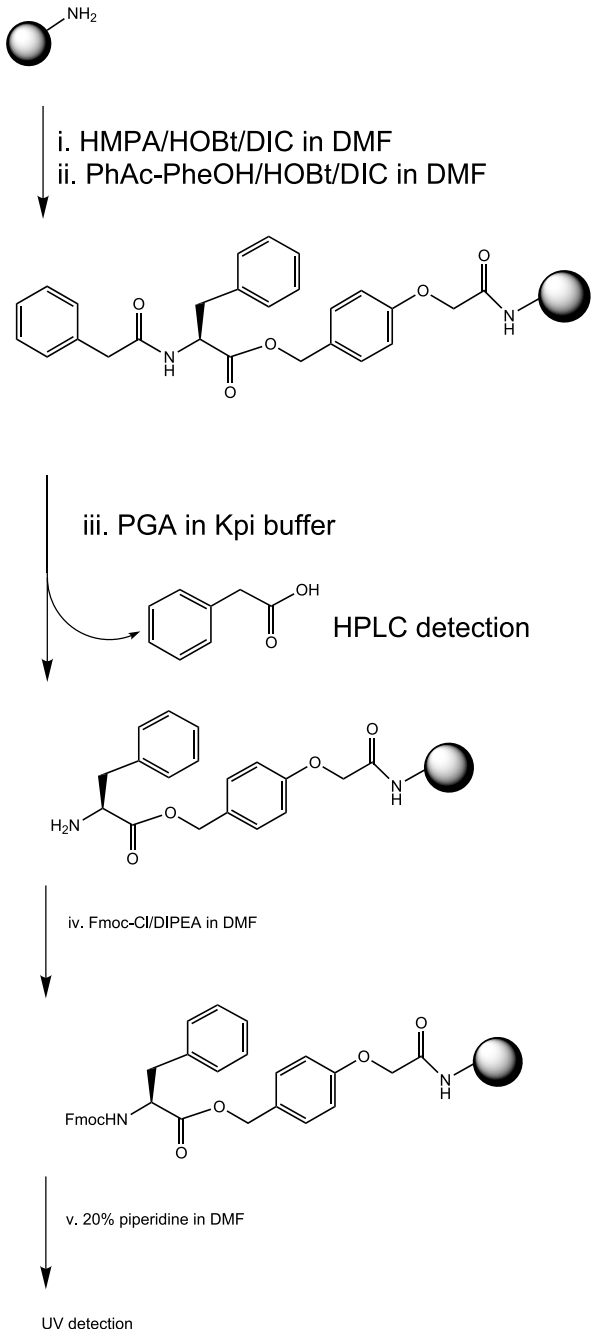
Although both swelling and electrostatic interactions positively affect the enzyme accessibility and diffusion, the present data clearly demonstrate the major relevance of electrostatic interactions. Then it appears that, by using the appropriate solid support in controlled experimental conditions, optimal enzyme diffusion into the resin can be achieved together with favourable interactions with the polymer backbone. In the case of PGA, maximum protein retention was achieved working with $PEGA_H^+$ at pH 8.0 in dilute buffer (0.001 M) (14.4 $\mu g/mg_{dry}$ of retained PGA).

2.4. PGA efficiency in the hydrolysis of PEGA-bound substrates

In order to evaluate the enzymatic efficiency on charged PEGA polymers, the substrate PhAc-Phe-Wang was linked to the polymers as described in Section 4 (Scheme 4). The release of phenylacetic acid was quantified by HPLC. After enzymatic hydrolysis, the obtained results were confirmed by acylating the free amino groups of Phe-Wang with Fmoc-Cl and then by quantifying (UV) the Fmoc released upon chemical cleavage.

The hydrolysis study was performed at pH 8.0, which guarantees optimal PGA hydrolytic activity. The results of enzymatic hydrolysis (Fig. 6) are in perfect agreement with the data of protein retention (Fig. 5).

$PEGA_H^+$ in dilute buffer allows 80% conversion to be achieved, which is a quite impressive improvement



Scheme 4. Chemical synthesis of PhAc-Phe-Wang-PEGA and enzymatic hydrolysis by PGA. Yields are determined by HPLC determination of released PhAcOH and by UV. Results were confirmed by TFA cleavage of the Wang linker (see Section 4).

compared to about 10% obtained with $PEGA_L^+$ and 50% with $PEGA^+$.

Figure 6 clearly indicates that, by choosing the appropriate pH and ionic strength, it was possible to take full advantage of the innovative properties of the $PEGA_H^+$ polymers. Experimental conditions strongly promote and reinforce PGA–polymer interactions thus maximising protein diffusion.

On the other hand, the results clearly indicate that $PEGA_L^+$, although endowed with the highest swelling capacity, is

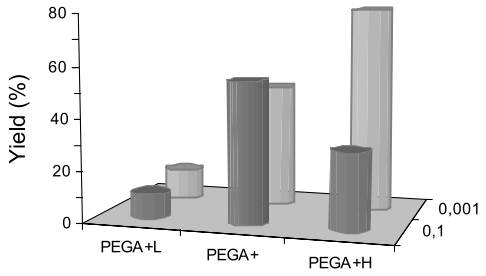


Figure 6. Enzymatic hydrolysis catalysed by PGA on $PEGA_L^+$, $PEGA^+$ and $PEGA_H^+$ in Kpi buffer 0.1 and 0.001 M (pH 8.0) at 24 h reaction and 30 °C.

less efficient for PGA applications due to its lower protein retention caused by the fewer positive charges.

3. Conclusions

This study demonstrates that large enzymes can be efficiently applied in solid phase chemistry. In particular, the high hydrolytic conversions obtained by using PGA on $PEGA_H^+$ attest that the selectivity of this enzyme can be fully exploited, thus opening new perspectives for the enzymatic cleavage of ad hoc designed 'safety catch' linkers.[5]

4. Experimental

4.1. General

$PEGA_{1900}$ and charged PEGA ($PEGA_L^+$, $PEGA^+$ and $PEGA_H^+$ were supplied by Polymer Laboratories (UK). The polymers were supplied with a declared loading of 0.1–0.2 mmol/g_{dry}. 4-Hydroxymethylphenoxyacetic acid (HMPA), *N*,*N*′-diisopropylcarbodiimide (DIC), 1-hydroxybenzotriazole (HOBT), *N*-Fmoc-L-phenylalanine (Fmoc-Phe), 4-dimethylaminopyridine (DMAP), *N*-ethyldiisopropylamine (DIPEA), phenylacetyl-L-phenylalanine (PhAc-L-Phe), 9-fluorenylmethyl chloroformiate (Fmoc-chloride) and phenylacetic acid (PhAcOH) were purchased from Sigma-Aldrich. All the solvents were of HPLC grade and obtained from Labscan. *N*,*N*-Dimethylformamide (DMF) was Biotech grade, >99% (Aldrich).

Penicillin G amidase (PGA) (solution: 16–32 U/mg) was obtained from Fluka and lyophilized. The specific activity after lyophilisation was 17 U/mg (benzylpenicillin units). One unit corresponds to the amount of preparation that hydrolyses 1 μmol of benzylpenicillin (solution of 10% in Na_2HPO_4/NaH_2PO_4 buffer, 0.02 M, pH 8.0) per minute at 37 °C.

4.2. Chemical synthesis

Before and after any chemical step, resins were washed three times with MeOH/DMF (1:1), MeOH, DCM (dichloromethane) and DMF and filtered. Swelling of polymers was determined by suspending them in the appropriate solvent. After 90 min this suspension was filtered by gravity force and weighed. All the reactions (loading determination, chemical syntheses) were performed at room temperature, under constant stirring on a blood rotator (40 rpm) and repeated at least three times.

4.2.1. Ninhydrin test. Three solutions were prepared: solution A: 5 g ninhydrin in 100 mL of ethanol; solution B: 80 g phenol in 20 mL of ethanol; solution C: 2 mL of a KCN solution (0.001 mequiv in pyridine) diluted to 100 mL. One drop of each solution was added on a small sample of resin. The mixture was then heated to 200 °C. A blue colour was evidence of the presence of unreacted amines. Yellow indicated all amines had reacted.

4.2.2. Determination of loading. The chemical accessibility was determined by reacting them with Fmoc-Phe (3 equiv) in the presence of DIC (4 equiv) and DMAP (0.1 equiv) in dry DMF. The process was repeated until the ninhydrin test gave a negative result. The Fmoc group was removed and quantified by suspending the resins in a 20% solution of piperidine in DMF and filtering the mixture after 2 h. The loading of the resin was calculated from the filtrate using Eq. 1

$$\text{Loading (mequiv/g}_{dry}) = \frac{\text{Abs(290 nm)} \cdot V\text{(ml)}}{4950 \cdot g_{dry}} \quad (1)$$

where Abs (290 nm) represents the absorbance at 290 nm, *V* (mL) the volume of piperidine (20% in DMF) used, 4950 the ε and g_{dry} the amount of dry resin.

4.2.3. Synthesis of PhAc-Phe-Wang-PEGA. Before chemical synthesis the resins were washed three times with MeOH/DMF, MeOH, CH_2Cl_2 and DMF. The syntheses were performed on a gram scale (wet weight polymer) (Scheme 4). The solvent content after each chemical synthesis was calculated by drying samples at 110 °C.

The resins were weighed in reactor syringes and suspended in DMF. The Wang linker was attached using HMPA (3 equiv) in the presence of DIC (4 equiv) and HOBT (6 equiv). The mixtures were allowed to mix on a blood rotator overnight. The resins were then filtered (Vacuum-System, Stepbio) and washed with MeOH/DMF, MeOH, DCM and DMF. The synthesis step was repeated until the ninhydrin test gave a negative result indicating all amines had reacted.

Next, L-Phe-PhAc (3 equiv) was added in the presence of DIC (4 equiv) and DMAP (0.1 equiv) in DMF. The reaction was performed in two cycles, the first of 2 h and the second overnight. After each cycle the resin was filtered and washed with MeOH/DMF, MeOH, DCM and DMF. The non-reacted OH groups were capped with acetic anhydride (10 equiv) in DMF overnight.

4.2.4. Dansylation. *Experimental conditions*. N-terminal dansylation was performed by treating the resin with 2 equiv of dansyl chloride and 2 equiv of triethylamine in DMF for 30 min.[10]

4.2.5. TPM experiments. A standard commercial multiphoton system (Biorad MRC1024, Coherent Mira 900) coupled to a Nikon TE300 microscope was used. Images

were acquired using Nikon lens. Optical sections were obtained by focusing the laser beam at different depths, within the sample ($\lambda = 770$ nm). Dansylated PEGA beads were suspended in Kpi buffer (0.1 M) and analysed with TPM.

4.3. Enzymatic reactions

Prior to the enzymatic reactions, the peptide carrying polymers were washed three times with the buffer used for the enzymatic hydrolysis.

The resin was suspended in 6 mL of the appropriate buffer in the presence of 5 mg of lyophilized PGA (85U). The reactions were incubated at 30 °C for 24 h on a blood rotator. Afterwards, the reaction mixtures were filtered and washed using 36 mL (12×3 mL) of MeCN/H_2O (1:1). The filtrate was recovered in a flask, dried under vacuum, re-dissolved in 1 mL of MeCN/H_2O (1:1), centrifuged, and filtered through 0.45 μm membrane filters. The samples were then analysed with a RP-HPLC system as detailed below.

4.3.1. Cleavage of Wang-linker. After the enzymatic reactions, the remaining peptide on the solid support was cleaved with a solution of TFA/H_2O (95:5) for 2 h. The filtrate was collected, the resins were then washed with a 1:1 mixture of MeCN/H_2O, filtered, collected, and dried under vacuum. The residues were re-dissolved in 1 mL of MeCN/H_2O (1:1) and analysed by RP-HPLC as detailed below.

4.3.2. HPLC analysis. Reverse phase HPLC analysis was carried out on Gilson 321 system equipped with a Gilson 255 UV at 260 nm detector using a C_{18} column. A flow of 1 mL/min (50% MeCN and H_2O with 0.1% TFA in both phases) was used and enzymatic conversions were then calculated by comparison with standard solutions.

4.3.3. Coupling with Fmoc chloride. After the enzymatic hydrolysis, free amino groups were reacted with Fmoc chloride (3 equiv) in the presence of DIPEA (3 equiv) in dry DMF for 2 h. The Fmoc group was then removed and quantified as reported previously.

4.3.4. Determination of retained protein. The amount of protein that had accessed the PEGA polymers (Fig. 5) was determined by the Pierce method, using bicinchoninic acid kit (Sigma). Standard PGA solutions with known enzyme concentrations were used as standards. Analyses were performed as reported in literature.[9]

Acknowledgements

The authors would like to acknowledge the EC (Combiocat project), Wellcome Trust and MIUR (Rome, Italy) for funding. Thanks are due to Dr. Gail McConnel for TPM experiments.

References and notes

1. (a) Meldal, M. *Tetrahedron Lett.* **1992**, *33*, 3077–3080. (b) St. Hilaire, P. M.; Willert, M.; Juliano, M. A.; Juliano, L.; Meldal, M. *J. Comb. Chem.* **1999**, *1*, 509–523. (c) Meldal, M. *Biopolymers* **2002**, *66*, 93–100. (d) Meldal, M.; Svendsen, I.; Juliano, L.; Juliano, M. A.; Nery, E. D.; Scharfstein, J. *J. Pept. Sci.* **1998**, *4*, 83–91.
2. (a) Ulijn, R. V.; Baragaña, B.; Halling, P. J.; Flitsch, S. L. *J. Am. Chem. Soc.* **2002**, *124*, 10988–10989. (b) Ulijn, R. V.; Bisek, N.; Flitsch, S. L. *Org. Biomol. Chem.* **2003**, *1*, 621–622.
3. Humphrey, C. E.; Turner, N. J.; Easson, M. A. M.; Flitsch, S. L.; Ulijn, R. V. *J. Am. Chem. Soc.* **2003**, *125*, 13952–13953.
4. Kress, J.; Zanaletti, R.; Amour, A.; Ladlow, M.; Frey, J. G.; Bradley, M. *Chem. Eur. J.* **2002**, *8*, 3769–3772.
5. (a) Reents, R.; Jeyaraj, D. A.; Waldmann, H. *Adv. Synth. Catal.* **2001**, *343*, 501–513. (b) Waldmann, H.; Reidel, A. *Angew. Chem., Int. Ed.* **1997**, *36*, 647–649.
6. Basso, A.; De Martin, L.; Gardossi, L.; Margetts, G.; Brazendale, I.; Bosma, A. Y.; Ulijin, R. V.; Flitsch, S. L. *Chem. Commun.* **2003**, 1296–1297.
7. (a) Bosma, A. Y.; Ulijn, R. V.; McConnell, G.; Girkin, J.; Halling, P. J.; Flitsch, S. L. *Chem. Commun.* **2003**, 2790–2791. (b) Ulijn, R. V.; Brazendale, I.; Margetts, G.; Flitsch, S. L.; McConnel, G.; Girkin, J.; Halling, P. J. *J. Comb. Chem.* **2003**, *5*, 215–217.
8. Basso, A.; Ulijn, R. V.; Flitsch, S. L.; Margetts, G.; Brazendale, I.; Ebert, C.; De Martin, L.; Linda, P.; Verdelli, S.; Gardossi, L. *Tetrahedron* **2004**, *60*, 589–594.
9. Giacomini, C.; Villarino, A.; Franco-Fraguas, L.; Batista-Vieira, F. *J. Mol. Catal. B: Enzym.* **1998**, *4*, 313–327.
10. Zheng, Y.; Gattás-Asfura, K. M.; Konka, V.; Leblanc, R. M. *Chem. Commun.* **2002**, 2350–2351.

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Synthesis of tri- and tetracyclic diterpenes. Cyclisations promoted by SmI_2

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Received 14 July 2004; revised 3 September 2004; accepted 29 September 2004

Available online 10 December 2004

Abstract—Several tri- and tetracyclic diterpenes have been synthesised from zamoranic acid. The key step is the cyclisation of a dicarbonyl 13,14-secoderivative by SmI_2.

1. Introduction

Tri- and tetracyclic diterpenes are very common in nature, showing a variety of skeletons, and many of them are very important not only due to their structures but also from the biological point of view.[1] Abietanes, pimaranes, isopimaranes, cassanes, cleistanthanes, totaranes and the trinor-derivatives, podocarpanes, are tricyclic diterpenes. Among the tetracyclic systems are kauranes, beyeranes or hibaenes and isohibaenes (Fig. 1). They show biological profiles as antifungal,[2] antiinflamatory,[3] antioxidant,[4] antibiotic,[5] antitumoural,[6] antimalarial,[7] cardioactive,[8] and antiviral[9] compounds and are inhibitors of nitric oxide.[10] Additionally some of this class of species are active as phytoalexines,[11] making these compounds particularly interesting. Due to their wide distribution and interesting bioactivities many synthetic studies have been reported.[12]

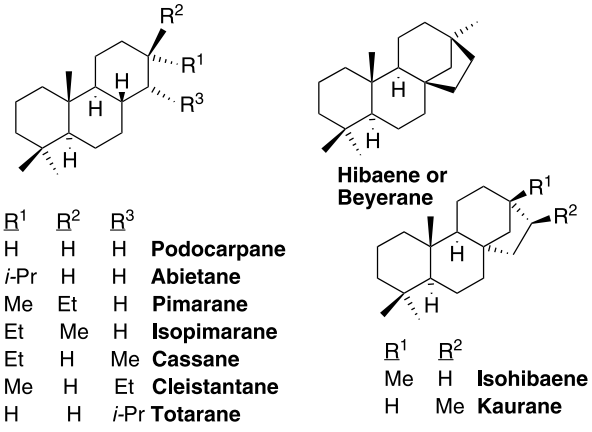

Figure 1. Diterpene tri- and tetracyclic skeletons.

As part of our continuing efforts to synthesise this kind of biologically active compound, a series of tri-and tetracyclic diterpenes were synthesised from zamoranic acid. This compound is a labdane diterpene,[13] which has already been used to prepare biologically active natural products, such as sesquiterpene drimanes,[14] labdanolides, such as limonidilactone,[15] diterpenes with the isofregenedane skeleton, such as chrysolic acid and isofregenedol.[16] Recently we have reported the synthesis of tri- and tetracyclic diterpenes[17] and (+)-totarol.[18]

In this paper we show that when the trinorderivative of zamoranic acid, the dialdehyde (14,15,16-trinorlabdan-13,17-dial), **23**, is treated with SmI_2, it gives rise to the podocarpanic diol **36** as the major product, an advanced intermediate for the synthesis of tricyclic compounds with the isopimarane, cassane, cleistanthane and rearranged abietane skeletons.

2. Results and discussion

Our general approach to the tricyclic diterpenes from zamoranic acid is outlined in Scheme 1. To allow access to tricyclic diterpenes of diverse skeletons such as **II**, our plan

Keywords: Samarium iodide; Stereoselective cyclisation; Tri- and tetracyclic diterpenes; Zamoranic acid.
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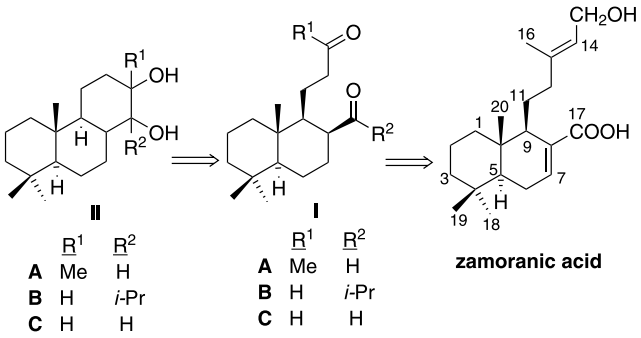
doi:10.1016/j.tet.2004.09.116



Scheme 1.

leads to the corresponding dicarbonyl compounds **I** as intermediates for subsequent cyclisation by SmI_2. The synthesis of the dicarbonyl compounds **I** was planned as resulting from the degradation of the side chain of zamoranic acid and then adjustment of the functionalisation or substitution at C-17. The synthesis of tricyclic diterpenes, such as pimaranes, or tetracyclic species, such as hibaenes **IIA**, was planned via an intermediate obtained by cyclisation of **IA**. The synthesis of totaranes, and particularly (+)-totarol, can be envisaged from diol **IIB** as intermediate, this being obtained by cyclisation of **IB** with SmI_2. As highlighted above in order to obtain tricyclic diterpenes with a range of different skeletons, the synthesis of the podocarpanic diol **IIC** was planned from the dialdehyde **IC**.

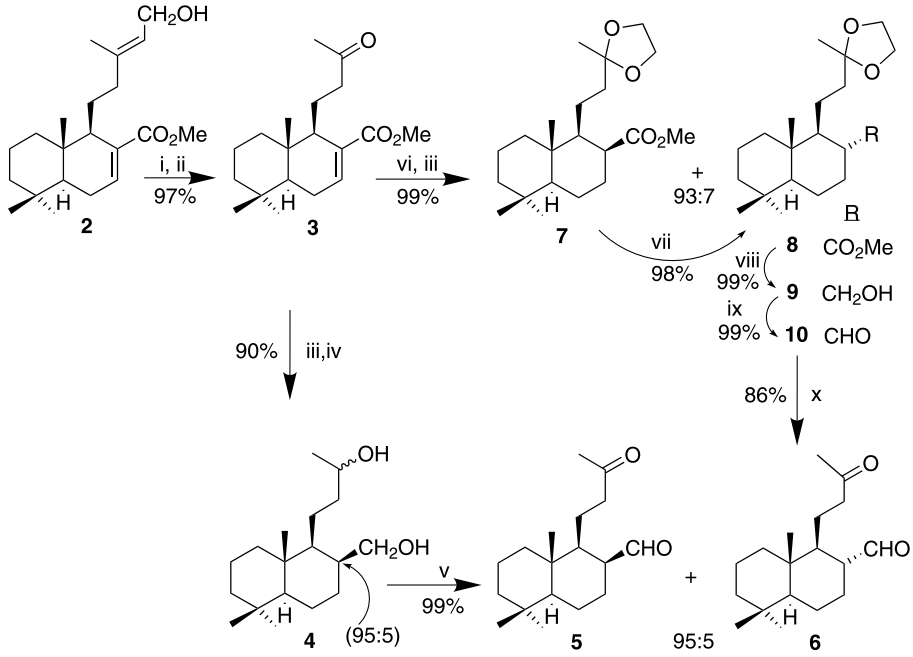
In the paper we describe the synthesis of compounds with pimarane and hibaene skeletons and of (+)-totarol, and demonstrate the potential of the podocarpanic diol **36** as an intermediate for the synthesis of different tricyclic diterpene skeletons. Initially, we describe the synthesis of the dicarbonyl compounds, then the cyclisation with SmI_2 of each of these, and finally the synthesis of the diterpenes.

2.1. Degradation of the side chain and synthesis of dicarbonyl compounds

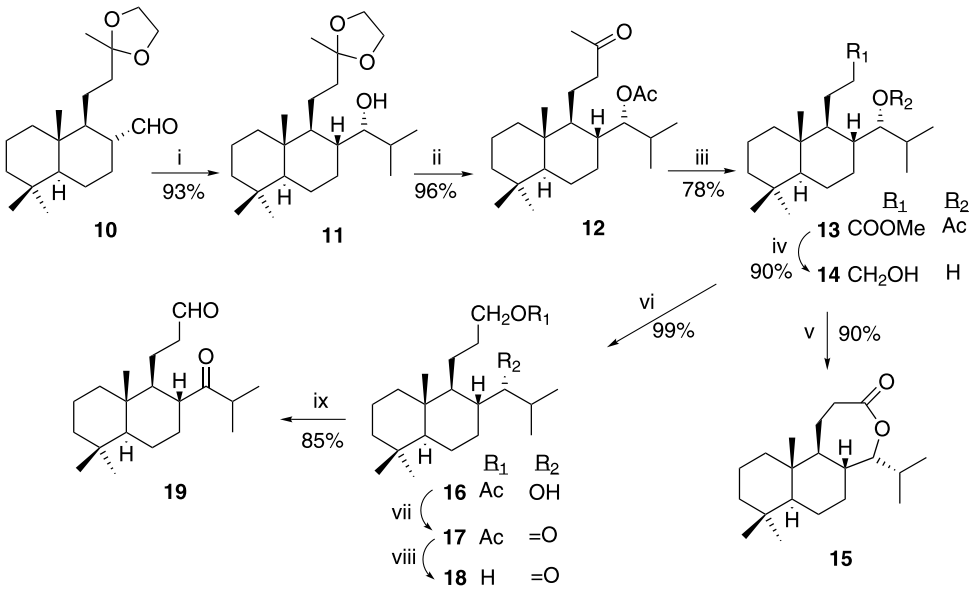
2.1.1. Synthesis of dicarbonyl compounds 5 and 6. The synthesis of the intermediates **5** and **6** is shown in Scheme 2. Chemoselective oxidation of the allyl alcohol olefin unit of zamoranic acid **2** with OsO_4[19] afforded the corresponding diol, which was subsequently cleaved with LTA (lead tetracetate)[20] to yield the methyl ketone **3**. Hydrogenation of **3** in the presence of PtO_2,[21] followed by reduction with DIBAL-H[22] led to compound **4** with very good diastereoselectivity, we realised that LAH could also be used for the latter part of this transformation. Oxidation of **4** using Swern technique[23] gave **5** and **6**; Collins's reagent[24] could also facilitate this transformation.

However, aldehyde **6** was alternatively synthesised from **7** in an excellent yield (Scheme 2). Hydrogenation of the dioxolane derivative of **3** gives **7** and **8** stereoselectively (93:7, 98%). The coupling constants of H-8 in the ^{1}H NMR spectra for **7** (J=1.6, 4.8 Hz) and **8** (J=4.3, 11.8 Hz) confirm the stereochemical assignment for C-8. Treatment of **7** with NaOMe[25] gave diastereomer **8**, which was easily converted to **6** by a reduction–oxidation sequence through the intermediates **9** and **10**, these transformations confirm the C-8 stereochemistry of **5** and **6**.

2.1.2. Synthesis of dicarbonylic compound 19. Addition of isopropylmagnesium chloride[26] to aldehyde **10** (Scheme 3) proceeded with complete stereoselectivity to give exclusively the 14*R*-hydroxy derivative **11**, in excellent yield. The structure of **11** was confirmed by X-ray analysis of one of its derivatives (compound **15**, Scheme 3), showing that the reaction of the nucleophile has taken place from the less hindered *Si* face of the carbonyl group.



Scheme 2. (i) OsO_4, NMO, ^{t}BuOH/THF/H_2O, rt, 18 h; (ii) LTA, benzene, rt, 2 h; (iii) H_2/PtO_2/Et_2O, rt, 36 h for **3** to **4** and 10 h for **3** to **7** and **8**; (iv) DIBAL-H, toluene, −78 °C, 30 min; (v) $(COCl)_2$, DMSO, Et_3N, DCM, −78 °C, 30 min; (vi) $(CH_2OH)_2$, *p*-TsOH, benzene, reflux, 21 h; (vii) (a) NaOMe/MeOH, reflux 12 h; (b) CH_2N_2; (viii) LAH, Et_2O, rt, 1 h; (ix) CrO_3/Py, DCM, rt, 30 min; (x) *p*-TsOH/acetone, rt, 14 h.



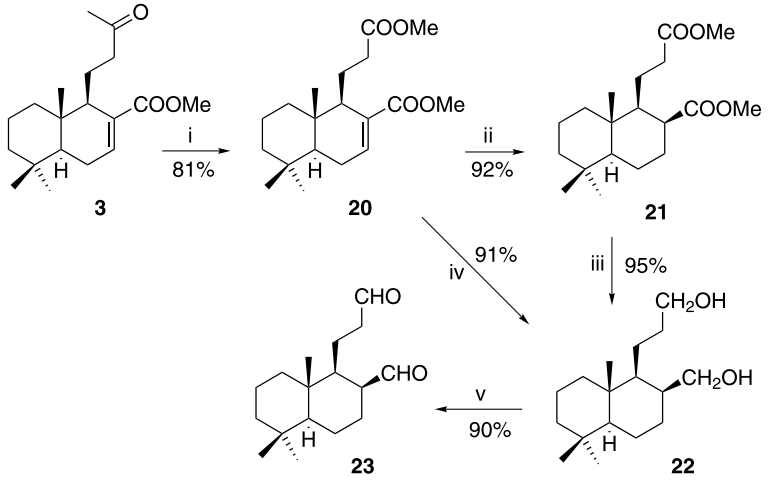
Scheme 3. (i) *i*-PrMgCl, THF, 0 °C, 1 h; (ii) (a) Ac_2O, Py, 70 °C, 2 h; (b) *p*-TsOH, Me_2CO, rt, 14 h; (iii) (a) HMDSNa, TMSCl, −78 °C, 2 h; (b) OsO_4, NMO, rt, 48 h; (c) H_5IO_6, THF, HOH, rt, 1 h; (d) CH_2N_2, Et_2O, rt, 3 h; (iv) LAH, Et_2O, rt, 1 h; (v) TPAP, NMO, rt, 30 min; (vi) Ac_2O, Py, rt, 30 min; (vii) TPAP, NMO, rt, 2 h; (viii) K_2CO_3, MeOH, rt, 4 h; (ix) CrO_3, Py, rt, 1 h.

The transformation of **11** into secotararane **13** through the methyl ketone **12** required acetylation at 70 °C, due to the hindrance of the hydroxyl group, followed by deprotection.

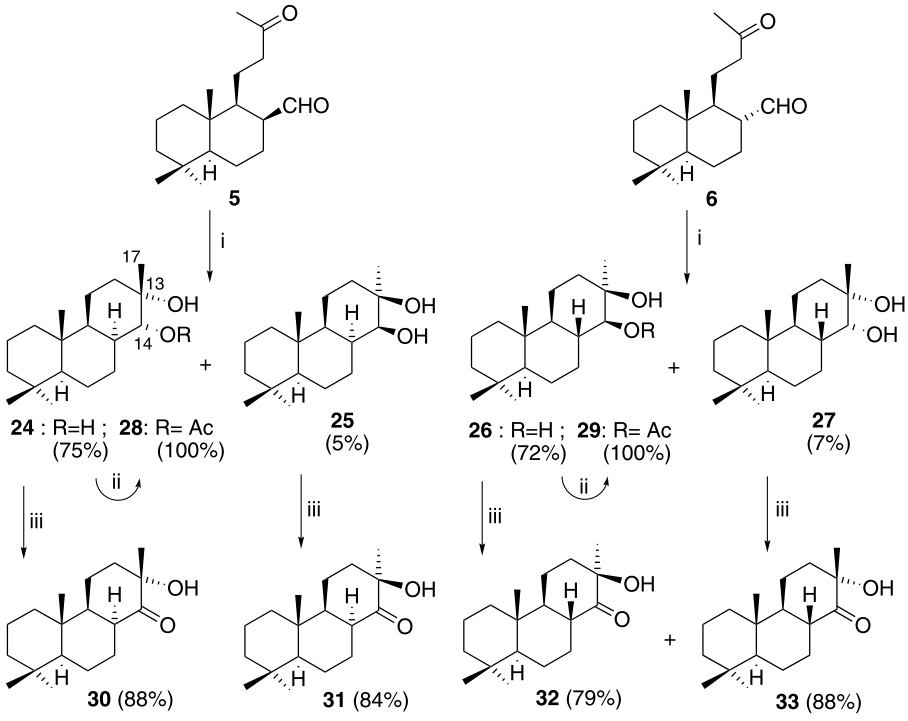
Degradation of **12** by the haloform reaction[27] did not work and it was therefore necessary to employ a sequence of reactions. Thus, capture of the kinetic enolate of **12** as a trimethylsilyl enol ether[28] and *cis*-hydroxylation, followed by cleavage with H_5IO_6[29] and esterification[30] of the resulting acid give **13**. Reduction of **13** with LAH gave the diol **14** in excellent overall yield from **10**. TPAP/NMO[31] oxidation of **14** gave the lactone **15**, whose structure was confirmed by single crystal X-ray[32] structure determination corroborating the 14*R* configuration in **11**. Due to this unwanted lactonisation, the oxidation of **14** to the dicarbonyl system **19** needed for the cyclisation was carried out in an indirect way: chemoselective acetylation of the primary hydroxyl group of **14** with Ac_2O in pyridine over 30 min gave **16**, oxidation of the secondary alcohol of the resulting monoacetyl derivative gave **17**, hydrolysis of the primary acetoxy group, and subsequent oxidation gave **19** through the intermediates **16**, **17** and **18** in a 85% global yield from **16**. (Scheme 3).

2.1.3. Synthesis of dialdehyde 23. Dialdehyde **23** can be obtained from the methylketone **3** by degradation of the side chain by one carbon, hydrogenation of Δ^7 and adjustment of the functionality (Scheme 4). The first two steps can be interchanged, but a better yield is obtained using the degradation–hydrogenation sequence.

Degradation of the side chain of **3** by the same sequence as before—enol ether formation, oxidation with OsO_4, cleavage with H_5IO_6 and final esterification with $TMSCHN_2$—gives diester **20** in an excellent global yield of 81%. For the hydrogenation of **20** several conditions were tested, the best yield of **21** (92%) and excellent diastereoselection (8*S*) being obtained using PtO_2 in EtOH. Reduction of **21** with



Scheme 4. (i) (a) LDA, TMSCl, −78 °C, 3 h; (b) OsO_4, NMO, rt, 24 h; (c) H_5IO_6, THF, HOH, rt, 3 h; (d) $TMSCHN_2$, MeOH, C_6H_6, rt, 12 h; (ii) H_2/PtO_2, EtOH, rt, 12 h; (iii) LAH, Et_2O, rt, 1 h; (iv) Li/NH_3 −78 °C, 2 h; (v) CrO_3, Py, rt, 1 h.



Scheme 5. (i) SmI_2, THF/MeOH, rt, 4 h; (ii) Ac_2O/Py, rt, 12 h; (iii) DMSO, $(COCl)_2$, Et_3N, CH_2Cl_2, −78 °C, 30 min.

LAH gives **22** in a 95% yield. The transformation of **20** to **22** can be done directly by reduction of **20** with Li in NH_3[33] (91%). Oxidation of **22** with CrO_3 in pyridine gives **23** in a 90% yield.

2.2. Cyclisation with SmI_2

2.2.1. Cyclisation of 5 and 6 with SmI_2. As shown in Scheme 5, treatment of **5** with SmI_2[34] led to a diastereomeric mixture of **24** (75%) and **25** (5%). Under the same conditions, **6** afforded **26** (72%) and **27** (7%).

The stereochemical assignment of H-14 (Fig. 1) in the acetyl derivatives of major diols **28** and **29** was established as β and α, respectively, by measurement of the coupling constants between H-14 and H-8, which indicate that these hydrogens are *trans*, dihedral angle 180°. The stereochemistry of Me-17 was determined by NOE experiments (Fig. 2).

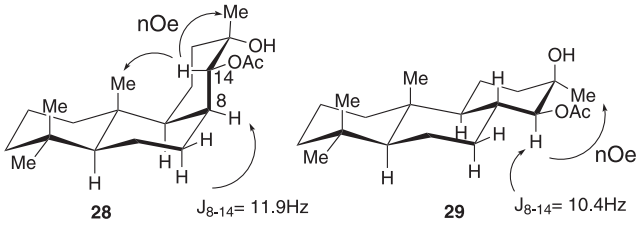


Figure 2.

The stereochemistry of H-14 in the minor components, **25** and **27**, was determined to be *cis* based on the coupling constants between H-8 and H-14 (J_{8-14}=4.3 Hz in **25** and J_{8-14}<1.0 Hz in **27**). We were not able to establish directly the stereochemistry of Me-17 in the minor components **25** and **27** so we oxidised C-14 in the four diols to give **30–33** (Scheme 5). H-8 coupling constants in compounds **30–33** corroborate that the stereochemistry of C-8 is 8*S* for **30** and **31** (J_{H-8}=2.0, 6.5, 6.5 Hz) and 8*R* for **32** and **33** (J_{H-8}=4.0,

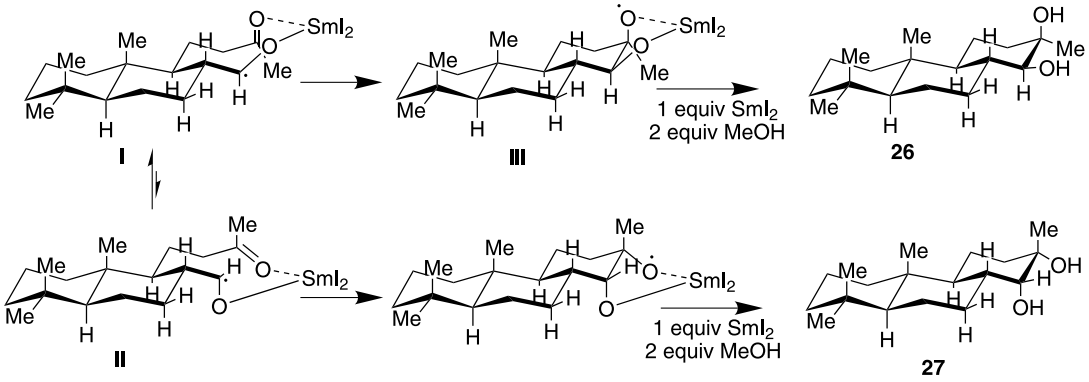


Figure 3.

11.8, 11.8 Hz) in agreement with that obtained above for **24**–**27** in the pinacol cyclisation.

Formation of **32** from **26** (with the stereochemistry already fixed at C-13) and **33** from **27**, allowed assignment of the stereochemistry at C-13 of **27** as opposite to that in **26** at C-13. The same reasoning led to assignment of the stereochemistry at C-13 for compound **25**, due to its relationship with compound **24**.

The SmI_2 promoted cyclisation results in a *cis* disposition of the hydroxyl groups. As proposed in Figure 3, this is probably due to chelation of the oxygen to samarium in the coupling reaction.[34] The higher proportion of **26** is probably due to the greater stability of intermediate **III**, which allows the metal centre to be in a pseudoequatorial position.

Following the same rationalisation, the formation of compound **24** as the major isomer can be explained. The structure of **24** was corroborated by X-ray[35] (Fig. 4).

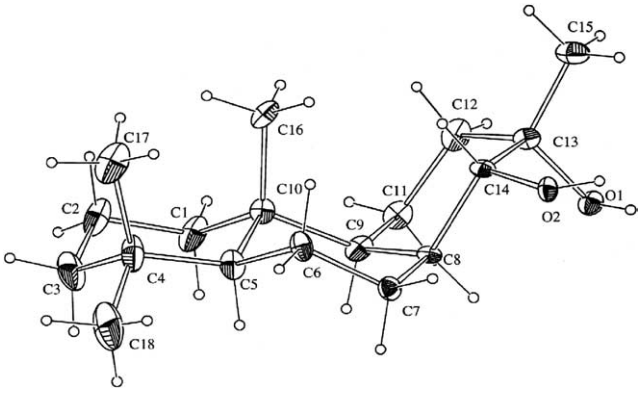
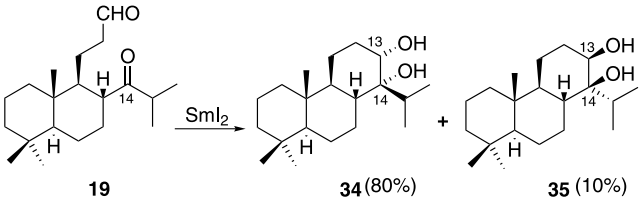


Figure 4. ORTEP view of compound **24**.

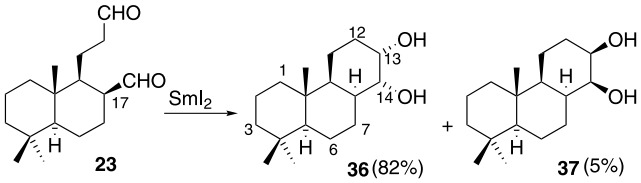
Thus, efficient generation of *dinor*-diterpenes with a tricyclic skeleton such as **24** permits access to complex tri- and tetracyclic compounds, as will be discussed later.

2.2.2. Cyclisation of 19 with SmI_2. Treatment of **19** with SmI_2[34] (0.1 M, in THF prepared in situ) gave a mixture of the totarane diastereomers **34** (80%) and **35** (10%), which were separated by column chromatography (Scheme 6). NMR, including HMQC, HMBC and NOE experiments established the structures of **34** and **35**. The signal for H-13 of **34** corresponded to an axial hydrogen (δ 3.55, dd, J= 11.1, 4.1 Hz) and so the hydroxyl groups are both α. The signal for H-13 in **35** corresponds to an equatorial hydrogen (δ 3.83 ppm, t, J=2.0 Hz). The structure for the major diol **34** is that expected from a pinacol coupling with SmI_2.[34]



Scheme 6.

2.2.3. Cyclisation of 23 with SmI_2. Reaction of **23** (Scheme 7) with a solution of SmI_2[34] (0.1 M in THF) generated in situ gives the mixture of **36** (80%) and **37** (5%), which were separated by column chromatography.



Scheme 7.

The structures of **36** and **37** as stereoisomeric podocarpane diols were established by extensive NMR studies, including $^{1}H/^{13}C$ HMQC and HMBC. C-13 and C-14 stereochemistry for the tricyclic compounds **36** and **37**, considering that C-8 retains the stereochemistry of the starting material, was deduced from the multiplicity and the coupling constants of the geminal hydrogens to the hydroxyl groups. Signals for H-13 (4.00 ppm, 1H, q, J=3.2 Hz) and for H-14 (3.80 ppm, 1H, dd, J=11.5, 3.2 Hz) in the ^{1}H NMR spectrum of **36**, indicate that H-13 is equatorial and H-14 axial, and thus the union of rings B and C has to be *cis*.

In the ^{1}H NMR spectrum of **37**, the signal due to H-14 appears as a triplet of coupling constant 9.2 Hz and that due to H-13 as a multiplet. The configuration of C-13 and C-14 (13*R*,14*S*) in **37** was suggested by the NOE between H-14 and H-8 that indicates a probable *cis* relationship between these hydrogens. The multiplicity of H-14 as a triplet and its coupling constant of 9.2 Hz may be interpreted by three possible hypotheses, all of which were studied using the molecular modelling programs Maestro and MacroModel[36] to generate conformations, with all possible ring conformations permitted. Firstly, it is conceivable that epimerisation has taken place at position 8. Conformational analysis of either possible *cis* diol with position 8 epimerised shows that the dihedral angles between H-14 and H-13 and H-8 would be either diequatorial/diequatorial or diaxial/diequatorial for all energetically accessible conformations, which is not consistent with the observed coupling constants of 9.2 Hz, confirming the assumption stated earlier that position 8 retains its stereochemistry. Alternatively, it could be that this minor product was due to an alternate cyclisation mechanism in which the diol unit adopted the *trans* configuration, permitting a diaxial/diaxial arrangement of these protons. Though the established mechanism gives rise to the *cis* product, the *trans*-diol is a known minor side-product of SmI_2 mediated pinacol cyclisation,[14n] presumably via alternate cyclisation mechanisms. Molecular modelling studies show that such a *trans*-diol arrangement would be expected to produce H-8, H-14 and H-13, H-14 dihedral angles of 173–174 and 168–169°, respectively for energetically-accessible conformations, consistent with the observed coupling constant. Finally, we may propose that in a *trans-anti-cis* tricyclic system as in **37**, the three rings adopt a conformation in which H-14 has similar dihedral angles, fairly close to 0°, with H-8 and H-13, explaining the identical 9.2 Hz coupling constant. Molecular models with ring B in a chair conformation (Fig. 5, I) show that H-14 has dihedral angles with H-8 and H-13α of

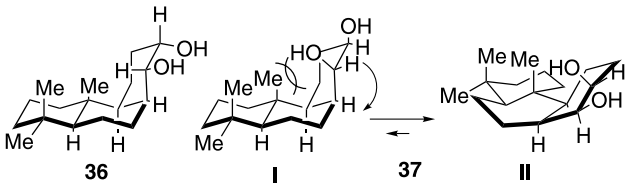


Figure 5.

−54° and 53, while the energetically-accessible ring B boat form (Fig. 5, II) shows corresponding dihedral angles of 42 and −54°, respectively. It is possible that an intermediate conformation between these extremes, which would show dihedral angles close to 0°, is sufficiently low-energy to be significantly populated, giving rise to the observed coupling constant. When the three rings adopt a chair conformation, as in **37**(**I**) the very significant steric hindrance between Me-20 and the hydroxyl of C-14 can immediately be seen. Conformation **37**(**II**) avoids this hindrance, and results in the two conformations being equally accessible (energy difference using MMFF94S <1.0 kJ/mol whether modelled in vacuo or with the $CHCl_3$ GB/SA solvent model) despite the boat conformation for ring B (Fig. 6). Since cyclisation with SmI_2 of the similar compounds **5** and **6** (Scheme 5) has been shown to give only the *cis* diols, we propose the *cis* stereochemistry for the hydroxy groups of **37**.

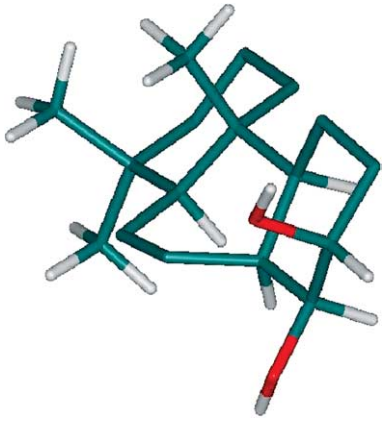


Figure 6.

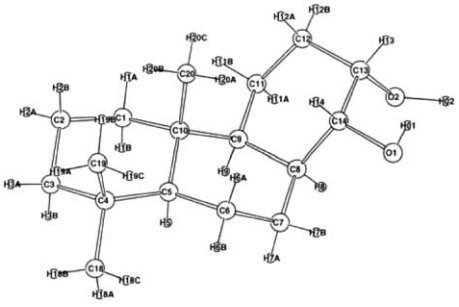


Figure 7. ORTEP view of compound **36**.

The structures of **36** and **37** correspond to those of the 8-*epi*-podocarpanes.

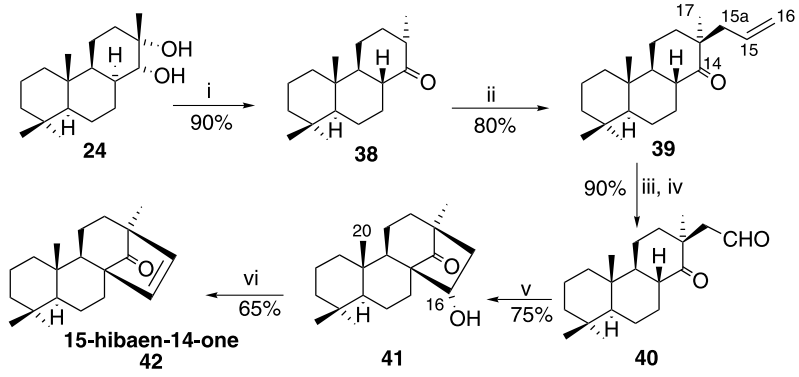
Structure and configuration for C-8, C-13 and C-14 (8*S*,13*S*,14*R*) of the major diol **36** were confirmed by X-ray [37] (Fig. 7).

2.3. Preparation of natural products

Having thus established the synthesis of the tricyclic compounds, we describe some applications of this methodology for the synthesis of natural products with different skeletons such as the tetracyclic diterpene, 15-hibaene-14-one,[38] (+)-totarol and others.

2.3.1. Synthesis of pimaranes and hibaenes: 40 and 42. Compound **38** (Scheme 8) was obtained by reaction of **24** with *p*-TsOH/THF (90% yield). In this reaction an epimerisation has taken place from the *trans-anti-cis* system to the more stable *trans-anti-trans* system, in which Me-17 has adopted the more stable equatorial position. Treatment of **38** with LDA/allyl bromide gave **39**, with the stereochemistry predicted by House[39] as outlined below.

Oxidation with OsO_4 and subsequent cleavage with $NaIO_4$ achieved transformation of **39** into **40**, in an excellent yield. (Scheme 8). Compound **40** is a diterpene with a pimarane skeleton that can be considered as an advanced intermediate for the synthesis of other natural pimaranes. Tetracyclic compound **41** was formed on reaction of **40** with KOH/MeOH. The stereochemistry of C-8, C-13 and C-16 in **41** was determined by differential NOE experiments. Of the four possible stereoisomers only the one shown in Figure 8 would show a NOE (6%) between H-16 (δ 4.68 ppm) and Me-20. In addition, a NOE (6%) between H-16 and the *pro-R* hydrogen on C-15 (δ 2.43 ppm) was observed.



Scheme 8. (i) *p*-TsOH/THF, 80 °C, 30 min; (ii) KHMDS, allyl-MgBr, THF, −78 °C, 90 min; (iii) OsO_4, NMO, $^tBuOH/THF/H_2O$, rt, 2 h; (iv) $NaIO_4$, THF/H_2O, rt, 25 min; (v) KOH/MeOH, reflux, 36 h; (vi) Py, TsCl, 90 °C, 36 h.

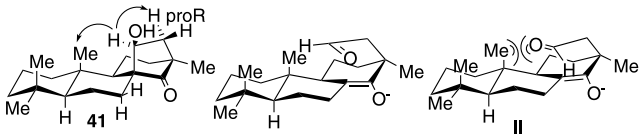


Figure 8.

It should be noted that this cyclisation reaction has taken place with high diastereoselectivity. The aldehyde only reacts on the *Re*-face (Fig. 8) due to the more favourable orientation of the carbonyl group in the intermediate when Me-20 is further away from the C=O.

Finally, elimination of the hydroxyl group was achieved by treatment of **41** with TsCl/Py affording **42**, in 65% yield [mp 95–98 °C; $[\alpha]_D$ −33° (*c* 0.5, $CHCl_3$)], and whose spectroscopic properties are coincident with those of 15-hibaen-14-one; (lit.[38]) mp 98–99 °C, $[\alpha]_D$ −29° (*c* 0.8, $CHCl_3$).

2.3.2. Synthesis of totaranes: (+)-totarol, 49. Transformation of **34** into (+)-totarol **49** requires the dehydration of the tertiary hydroxyl group and ring C aromatisation (Scheme 9). Oxidation of **34** with TPAP/NMO gave **43** in good yield, but all attempts to dehydrate and aromatise **43** using different conditions and reagents (DDQ,[40] TsCl/Py, $POCl_3$/Py, $SOCl_2$/Py, $HClO_4$, CF_3COOH, and HCOOH, HI) failed to give the desired results.

The aromatisation of ring C was therefore attempted from ketone **46**, obtained by pinacol rearrangement from **34**. Treatment of **34** with *p*-TsOH, and subsequent column chromatography, gave **46** (61%) and the minor compounds **45** and **44**. The formation of the cyclohexanone **46** is explained by the migration of hydride from C-13 to C-14, although in the acidic conditions of the reaction an epimerization at C-14 takes place to provide the more stable β-isopropyl group. The aldehyde **45** is formed by ring C contraction in the pinacol reaction and compound **44** is a dimer (M^+ *m/z*: 580) formed by reaction of the starting material with the aldehyde **45**.

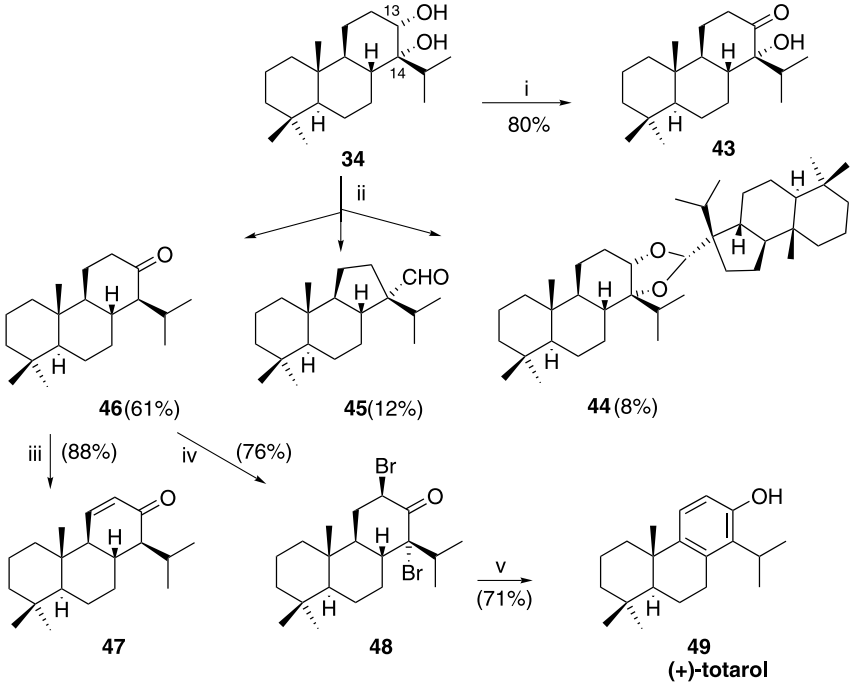
The aromatisation of **46** was attempted via enone **47**, obtained by reaction of **46** with PhSeCl/LDA[41] followed by oxidation of the selenide with *m*-CPBA at 0 °C. All attempts at aromatisation of **47** with DDQ, SeO_2 at 0 °C or even Pd/C[42] in refluxing *m*-xylene failed to give totarol.

Aromatisation of ring C was finally achieved by a halogenation–dehydrohalogenation sequence via intermediate **48**. Treatment of **46** with $CuBr_2$/MeCN[43] gave the dibromide **48** in moderate yield, and subsequent elimination with Li_2CO_3/LiBr[44] gave a good yield of **49**[45] [mp 129–130 °C; $[\alpha]_D$ +41° (*c* 0.4, $CHCl_3$)], whose spectroscopic properties are coincident with those of (+)-totarol; (lit.[45]) mp 131–132 °C, $[\alpha]_D$ +42° (*c* 0.8, $CHCl_3$).

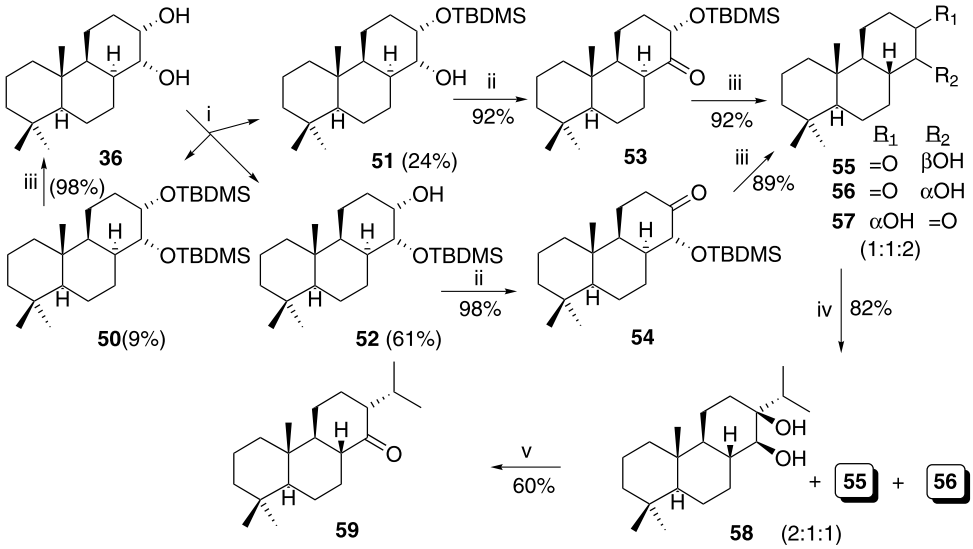
With the synthesis of (+)-totarol from zamoranic acid, we have opened a new synthetic route to tricyclic diterpenes with alkyl groups at C-14 (totaranes, cassanes or cleistanthanes) using the cyclisation of their 13,14-secoderivatives.

2.3.3. Synthesis of abietanes, totaranes, cleistanthanes, cassanes, isopimaranes and abeoabietanes. The podocarpanediols **36** and **37** obtained in the cyclisation have been tested as advanced intermediates for the synthesis of tricyclic diterpenes with different skeletons. First of all we did a study of the chemoselective protection of the hydroxy groups at C-13 and C-14.

Acetylation is not useful because a mixture of both



Scheme 9. (i) TPAP, NMO, rt, 30 min; (ii) *p*-TsOH, benzene, 60 °C, 48 h; (iii) (a) LDA, PhSeCl, THF, HMPA, −78 to 0 °C, 1 h; (b) *m*-CPBA, DCM, rt, 5 min; (iv) $CuBr_2$, MeCN, 50 °C, 72 h; (v) Li_2CO_3, LiBr, DMF, 140 °C, 16 h.



Scheme 10. (i) TBDMSCl, imidazole, rt, 22 h; (ii) TPAP, NMO, rt, 30 min; (iii) TBAF, THF, rt, 2 h; (iv) *i*-PrMgCl, THF, 0 °C, 3 h; (v) HI, benzene, 90 °C, 1 h.

monoacetyl derivatives is always obtained, due to acetyl migration.

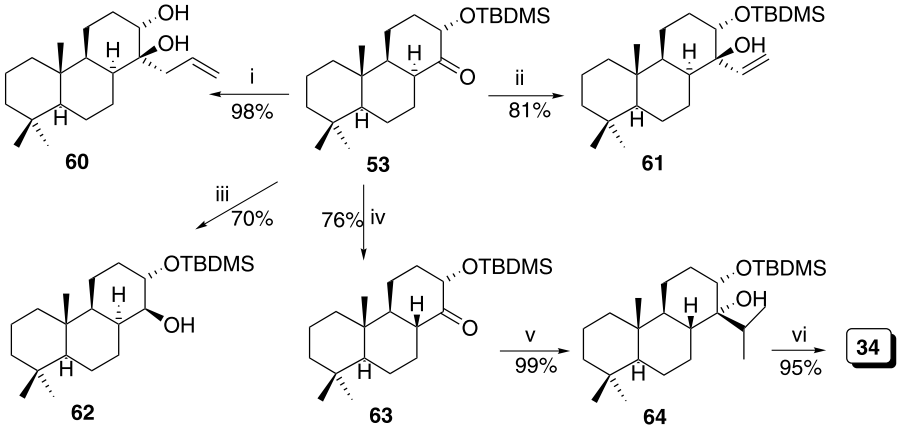
Reaction of **36** with TBDMSCl and imidazole[46] (Scheme 10) gives good chemoselectivity yielding **52**(61%), **51**(24%) and **50** (9%). The latter compound can easily be transformed into **36** by treatment with TBAF.[47]

TPAP oxidation of **51** and **52** gives the carbonyl compounds **53** and **54**, respectively in quantitative yield, and from these we were able to obtain access to the diterpenes with different skeletons. TBAF deprotection of **54** and **53** gives a mixture of acyloins **55**, **56** and **57** (1:1:2) in which not only has the deprotection taken place, but also an inversion at C-8 has been produced. When the reaction is carried out on each compound separately with *i*PrMgCl, the same of reaction mixture was obtained and **55**, **56** and the diol **58** were produced in the same 1:1:2 ratio as before. Treatment of **58** with HI[48] leads to the pinacolinic transposition to the ketone **59**, with the abietane skeleton.

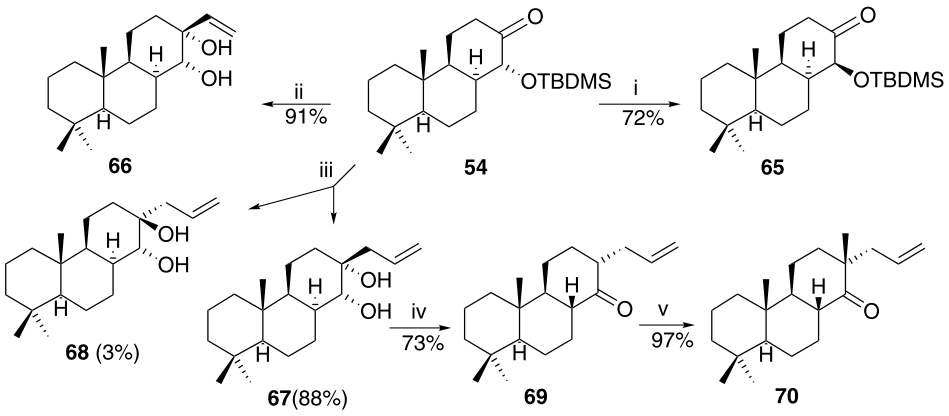
Since acyloins could not be used separately, the organometalic addition was done directly on the carbonyl compounds **53** and **54**.

The organometalic addition was tested with the minor carbonyl compound **53** (Scheme 11) and gave rise directly to various diterpenes with the totarane skeleton, or indirectly to a diterpene with a cleistanthane skeleton.

Addition of allyl magnesium bromide to **53** followed by treatment with TBAF gives **60** while treatment of **53** with vinyl magnesium bromide gives intermediate **61**. These compounds are advanced intermediates for the synthesis of diterpenes with a cleistanthane skeleton. Reaction of **53** with isopropylmagnesium chloride does not give the addition compound but the reduction product **62** instead. Addition of isopropylmagnesium chloride to **63**, the epimer of **53** at C-8 (which was obtained by treatment of **53** with NaMeO in methanol), gives the addition product **64** that by deprotection gives diol **34** with a totarane skeleton. Nevertheless, the best procedure found for the preparation of totaranes is by the secototarane route already described.



Scheme 11. (i) (a) allyl-MgBr, THF, 0 °C, 1 h; (b) TBAF, THF, rt, 90 min; (ii) vinyl-MgBr, THF, 0 °C, 15 min; (iii) *i*-PrMgCl, THF, 0 °C, 92 h; (iv) NaOMe, MeOH, rt, 12 h; (v) *i*-PrMgCl, THF, 0 °C, 90 min; (vi) TBAF, THF, 3 h.



Scheme 12. (i) NaOMe, MeOH, rt, 4 h; (ii) (a) vinyl-MgBr, THF, 0 °C, 15 min; (b) TBAF, THF, rt, 1 h; (iii) (a) allyl-MgBr, THF, 0 °C, 1 h; (b) TBAF, THF, rt, 90 min; (iv) *p*-TsOH, benzene, 60 °C, 24 h; (v) MeI, KHMDS, THF, −78 °C, 24 h.

All attempts to add isopropylmagnesium chloride to **54** and its epimer at C-14, **65** (obtained by treatment of **54** with NaMeO in methanol) gave no satisfactory results. Addition of allylmagnesium bromide and vinylmagnesium bromide to **54** gave excellent yields, the derivatives **67** (88%) and **68** (3%) being isolated from the reaction with the first organometallic and **66** (91%) from reaction of the second. (Scheme 12).

Compound **67** could be considered a precursor of the cytotoxic agent incanone,[49] a diterpene with an abeoabietane skeleton. Compounds **67** and **66** could also be considered as advanced intermediates for the synthesis of diterpenes with cassane and isopimarane skeletons, as has been established in the synthesis of the homoisopimarane **70** (epimer at C-13 of **39**, a homopimarane used in the synthesis of hibaenone **42**). Treatment of **67** with *p*-toluensulfonic acid gives ketone **69**, which leads to **70** upon treatment with MeI in the presence of KHMDS.[50] As in the synthesis of **39**, the alkyl groups, that have been introduced, occupy the axial positions predicted by House.

3. Conclusions

Several dicarbonyl compounds were obtained from zamoranic acid. These advanced intermediates undergo an efficient stereoselective cyclisation promoted by SmI_2, leading to tri- and tetracyclic diterpenes with different carbon skeletons. The synthesis of biologically active natural products with abietane, cassane, abeoabietane, cleistanthane and isopimarane skeletons using **58**, **60** or **61**, **66**, **67**, **69** and **70** as advanced intermediates is under development.

4. Experimental

Unless otherwise stated, all chemicals were purchased as the highest purity commercially available and were used without further purification. Melting points were determined with a Kofler hot stage melting point apparatus and are uncorrected. IR spectra were recorded on a BOMEM 100 FT IR spectrophotometer. ^1H and ^{13}C NMR spectra were performed in deuterochloroform and referenced to the residual peak of $CHCl_3$ at δ 7.26 ppm and δ 77.0 ppm for ^1H and ^{13}C, respectively, in a Bruker WP-200 SY and a BRUKER DRX 400 MHz. Chemical shifts are reported in δ ppm and coupling constants (J) are given in Hz. MS were performed in a VG-TS 250 spectrometer at 70 eV ionising voltage. Mass spectra are presented as *m/z* (% rel. int.). HRMS were recorded in a VG Platform (Fisons) spectrometer. Optical rotations were determined in a Perkin–Elmer 241 polarimeter in 1 dm cells. Diethyl ether, THF and benzene were distilled from sodium, and pyridine and dichloromethane were distilled from calcium hydride under an argon atmosphere.

4.1. Preparation of 3

To a solution of **2** (460 mg, 1.42 mmol) in *t*-BuOH/THF/H_2O (10:3:1), was added 4-methylmorpholine *N*-oxide (NMO) (196 mg, 1.67 mmol) and OsO_4 (2.5% in *t*-BuOH, 20 μL). The solution was stirred and checked by TLC during 18 h then Na_2SO_3 aqueous saturated was added and the solution stirred for 30 min, diluted with Et_2O, washed with HCl 7%, water and brine, dried over Na_2SO_4 and the solvent evaporated at low vacuum to give, after chromatography (Hex/AcOEt 8:2), the dihydroxylated compound (520 mg, 99%).

To a solution of the latter compound (348 mg, 0.94 mmol) in benzene (13 mL), LTA (1.25 g, 2.81 mmol) was added at room temperature and the solution stirred for 2 h. After this time, the mixture was diluted with Et_2O and washed with water, $NaHCO_3$ 10%, water and brine, dried (Na_2SO_4) and the solvent evaporated at low vacuum to give the crude material that was chromatographed on silica gel (Hex/AcOEt 9:1) to give compound **3** (280 mg, 98%).

4.1.1. Methyl 13-oxo-14,15-dinor-7-labden-17-oate: 3. λ_{max}^{EtOH}: 220 (ε=4500); $[\alpha]_D^{22}$ −52.0° (c=0.9, $CHCl_3$). IR (film): 2949, 2866, 1717, 1647, 1458, 1435, 1389, 1364, 1316, 1267, 1246, 1215, 1163, 1109, 1063, 1049, 974, 785, 758, 702, 665 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ: 6.71 (1H, m, H-7), 3.69 (3H, s, OMe), 2.85 (1H, ddd, J=4.6, 11.1, 17.1 Hz, H-12), 2.40 (1H, ddd, J=4.6, 11.1, 17.1 Hz, H-12), 2.11 (3H, s, COMe), 2.10–0.91 (12H, m), 0.89, 0.86 and 0.81 (3Me, s each, Me-18, Me-19 and Me-20). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 39.2 (C-1); 18.4 (C-2); 41.9 (C-3); 32.6 (C-4); 49.3 (C-5); 23.8 (C-6); 137.5 (C-7); 134.6

(C-8); 50.3 (C-99); 36.8 (C-10); 21.7 (C-11); 45.1 (C-12); 207.9 (C-13); 29.4 (C-16); 168.7 (C-17); 32.9 (C-18); 21.7 (C-19); 13.9 (C-20); 51.0 (–CO_2*Me*). EIMS *m/z* (%): 306 (M^+, 4), 275 (98), 219 (19), 190 (16), 150 (43), 133 (17), 119 (36), 109 (100), 73 (84), 61 (61). Anal. Calcd for $C_{19}H_{30}O_3$: C, 74.47; H, 9.87; found: C, 74.49; H, 9.84.

4.2. Preparation of 4

Compound **3** (10.3 g, 33.7 mmol), dissolved in Et_2O (90 mL), was hydrogenated in the presence of PtO_2 (400 mg). After 36 h the catalyst was filtered off, and the solvent removed, to give the crude mixture (10.3 g).

Diisobutylaluminium hydride (78.4 mL of a 1.5 M solution in toluene, 117 mmol) was added dropwise to a stirred solution of the crude mixture (10.3 g) in DCM (340 mL), under argon at −78 °C. The solution was stirred for 30 min, quenched with water (5 mL) and allowed to warm to room temperature; upon gelling the slurry was stirred with solid ($NaHCO_3$/Na_2SO_4), and diluted with AcOEt. The mixture was stirred vigorously until, upon standing, the solution cleared. The organic phase was decanted and filtered through a pad of celite. The filtrate was evaporated in vacuo and purified by column chromatography on silica gel to give a mixture (95:5) of diol **4** and its epimer on C-8 (8.54 g, 90%).

4.2.1. 14,15-Dinor-labda-13,17-diol: 4. IR (film): 3341, 2934, 2868, 1464, 1387, 1132, 1086, 1028, 955 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ: 3.85–3.55 (3H, m, H-13 and CH_2OH), 2.10–0.92 (19H, m), 1.20 (3H, m, Me-16), 0.86, 0.80 and 0.70 (3Me, s each, Me-18, Me-19 and Me-20). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 39.2 (C-1), 18.6 (C-2), 42.0 (C-3), 33.2 (C-4), 56.6 (C-5), 17.8 (C-6), 29.6/29.4 (C-7), 39.6/39.7 (C-8), 53.4 (C-9), 38.0 (C-10), 20.7/21.6 (C-11), 37.5/38.0 (C-12), 67.7/68.5 (C-13), 22.7/23.8 (C-16), 61.4 (C-17), 33.4 (C-18), 21.5 (C-19), 15.7 (C-20). Anal. Calcd for $C_{18}H_{34}O_2$: C, 76.54; H, 12.13; found: C, 76.52; H, 12.10.

4.3. Preparation of 5 and 6

Dimethylsulfoxide (220 μL, 3.10 mmol) was added dropwise to a solution of oxalyl chloride (133 μL, 1.55 mmol) in dry DCM (4 mL) at −78 °C. The mixture was stirred for 5 min, then diol **4** (104 mg, 0.37 mmol) in dichloromethane (7 mL) was added. The solution was stirred for a further 30 min at −78 °C, then quenched with triethylamine (1.3 mL, 9.34 mmol) and allowed to warm to room temperature. The mixture was then poured into water and extracted with AcOEt. The extract was washed with water and brine, dried over Na_2SO_4, and evaporated in vacuo, giving the aldehydes **5** and **6** (95:5, 103 mg, 99%).

4.3.1. (8*S*)-13-Oxo-14,15-dinor-17-labdanal: 5. IR (film): 2940, 2870, 1721, 1452, 1389, 1366, 1263, 1167, 1109, 1028, 1003 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 9.95 (1H, br s, –CHO), 2.75 (1H, ddd, J=4.6, 11.1, 17.1 Hz, H-12), 2.38 (1H, ddd, J=4.6, 11.1, 17.1 Hz, H-12), 2.34–0.89 (15H, m), 2.16 (3H, s, MeCO), 0.85, 0.78 and 0.74 (3Me, s each, Me-18, Me-19 and Me-20); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 38.7 (C-1), 18.7 (C-2), 42.0 (C-3), 33.3 (C-4), 53.5 (C-5), 18.9 (C-6), 26.6 (C-7), 47.5 (C-8), 55.8 (C-9), 38.7 (C-10), 19.6 (C-11), 42.6 (C-12), 208.3 (C-13), 29.8 (C-16), 204.7 (C-17), 33.5 (C-18), 21.5 (C-19), 15.3 (C-20). Anal. Calcd for $C_{18}H_{30}O_2$: C, 77.65; H, 10.86; found: C, 77.44; H, 10.70.

4.3.2. (8*R*)-13-Oxo-14,15-dinor-17-labdanal: 6. IR (film) 2944, 2851, 1719, 1458, 1389, 1364, 1186, 1171, 972, 937, 719 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 9.53 (1H, d, J= 4.2 Hz, –CHO), 2.65–2.35 (3H, m), 2.07 (3H, s, MeCO), 1.90–0.86 (14H, m), 0.86, 0.85 and 0.82 (3Me, s each, Me-18, Me-19 and Me-20); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 38.6 (C-1); 18.4 (C-2), 41.9 (C-3), 33.2 (C-4), 54.5 (C-5), 20.1 (C-6), 26.8 (C-7), 49.9 (C-8), 53.8 (C-9), 37.8 (C-10), 22.8 (C-11), 44.6 (C-12), 208.2 (C-13), 29.7 (C-16), 205.1 (C-17), 33.3 (C-18), 21.7 (C-19), 14.0 (C-20). Anal. Calcd for $C_{18}H_{30}O_2$: C, 77.65; H, 10.86; found: C, 77.78; H, 11.05.

4.4. Preparation of compounds 7 and 8

To a solution of compound **3** (360 mg, 1.18 mmol) in benzene (20 mL) was added ethylene glycol (254 μL) and a catalytic amount of *p*-TsOH (12 mg) and the solution was refluxed in a Dean–Stark apparatus. After 21 h the mixture was cooled and diluted with Et_2O, washed with 10% $NaHCO_3$, water and brine, dried over Na_2SO_4 and the solvent removed to give the corresponding protected compound (412 mg, 100%). Then, this product (320 mg, 0.91 mmol), was dissolved in ether (3 mL) and hydrogenated in the presence of PtO_2 (22 mg). After 10 h the catalyst was filtered off, the solvent removed subsequent chromatography of the crude mixture (Hex/AcOEt 95:5) afforded compounds **7** (290 mg, 92%) and **8** (25 mg, 7%).

4.4.1. Methyl (8*S*)-13-ethylendioxy-14,15-dinor-17-labdanoate: 7. $[\alpha]_D^{22}$ +26.8° (*c*=0.41, $CHCl_3$); IR (film): 2947, 1728, 1464, 1445, 1387, 1373, 1206, 1171, 1140, 1098, 1063, 947, 858 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 3.97–3.89 (4H, m, CH_2O), 3.63 (3H, s, OMe), 2.62 (1H, dt, J=1.6, 4.8 Hz, H-8), 2.32–0.80 (16H, m), 1.33 (3H, s, Me-16), 0.83, 0.79 and 0.74 (3Me, s each, Me-18, Me-19 and Me-20); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 39.2 (C-1), 18.7 (C-2), 42.1 (C-3), 33.3 (C-4), 56.5 (C-5), 19.2 (C-6), 29.5 (C-7), 38.6 (C-8), 54.0 (C-9), 39.2 (C-10), 20.7 (C-11), 37.6 (C-12), 110.5 (C-13), 23.5 (C-16), 175.8 (C-17), 33.5 (C-18), 21.5 (C-19), 14.0 (C-20), 50.8 (–CO_2*Me*), 64.4/64.5 (O(CH_2)$_2$O); EIMS *m/z* (%): 352 (M^+, 1), 337 (5), 123 (5), 87 (100); EIHRMS: calcd for $C_{21}H_{36}O_4$ (M^+) 352.2614, found (M^+) 352.2618.

4.4.2. Methyl (8*R*)-13-ethylendioxy-14,15-dinor-17-labdanoate: 8. $[\alpha]_D^{22}$ −3.6° (*c*=1.62, $CHCl_3$); IR (film): 2945, 1736, 1462, 1433, 1375, 1327, 1296, 1258, 1157, 1109, 1063, 972, 947, 855 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 3.94–3.83 (4H, m, CH_2O), 3.65 (3H, s, –OMe), 2.36 (1H, dt, J=4.3, 11.8 Hz, H-8), 1.25 (3H, s, Me-16), 0.85, 0.82 and 0.80 (3Me, s each, Me-18, Me-19 and Me-20); ^{13}C NMR (100 MHz, $CDCl_3$) 40.3 (C-1), 18.5 (C-2), 42.0 (C-3), 33.1 (C-4), 52.6 (C-5), 23.4 (C-6), 30.7 (C-7), 47.0 (C-8), 54.5 (C-9), 38.1 (C-10), 20.7 (C-11), 38.4 (C-12), 109.8 (C-13), 23.5 (C-16), 176.9 (C-17), 33.3 (C-18), 21.7 (C-19), 13.9 (C-20), 51.3 (CO_2*Me*), 64.4/64.5 (O(CH_2)$_2$O); EIMS *m/z* (%): 352 (M^+, 1), 337 (3), 87 (100); EIHRMS: calcd for $C_{21}H_{36}O_4$ (M^+) 352.2614, found (M^+) 352.2619.

4.5. Reaction of 7 with NaOMe/MeOH: 8

Compound **8** was also obtained from **7** as follows. To a solution of NaOMe/MeOH (2 N), prepared carefully from Na metal (92 mg) over MeOH (2 mL), was added compound **7** (150 mg, 0.43 mmol) dissolved in MeOH (1.5 mL) at room temperature. The solution was heated under reflux for 12 h. After that, the mixture was diluted with Et_2O, washed with saturated aqueous NH_4Cl, H_2O, brine, dried over Na_2SO_4 and the solvent removed at low vacuum to give 144 mg of the crude product. Subsequent esterification with CH_2N_2 afforded compound **8** (148 mg, 98%).

4.6. Reduction of 8: 9

To a solution of **8** (2.54 g, 7.22 mmol) in dry Et_2O (40 mL), LAH (300 mg, 7.89 mmol) was added. The reaction was stirred at room temperature for 1 h. The reaction was cooled to 0 °C and wet EtOAc was added and the mixture filtered. The organic phase was dried over Na_2SO_4, filtered and evaporated affording **9** (2.34 g, 99%).

4.6.1. 13-Ethylendioxy-14,15-dinor-8-*epi*-labdan-17-ol: 9. $[\alpha]_D^{22}$ −5.6° (c=0.95, $CHCl_3$); IR (film): 3461, 2886, 1464, 1387, 1277, 1252, 1210, 1204, 1107, 1063, 972, 947, 858, 785 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 4.00–3.80 (4H, m, –OC_2H_4O–), 3.67 (1H, dd, J=3.2, 10.8 Hz, H_A-17), 3.47 (1H, dd, J=5.9, 10.8 Hz, H_B-17), 1.95–1.00 (15H, m), 1.00–0.96 (2H, m), 1.29 (3H, s, H-16), 0.84, 0.80 and 0.80 (3Me, s each, Me-18, Me-19 and Me-20); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 42.2 (C-1), 18.7 (C-2), 38.8 (C-3), 33.3 (C-4), 55.0 (C-5), 21.3 (C-6), 29.6 (C-7), 41.9 (C-8), 52.7 (C-9), 38.4 (C-10), 22.9 (C-11), 41.0 (C-12), 110.1 (C-13), 23.5 (C-16), 66.4 (C-17), 33.4 (C-18), 21.8 (C-19), 14.1 (C-20), 64.6 (–OC_2H_4O–); EIMS m/z (%): 309 (M^+ −15, 2), 120 (8), 87 (100), 69 (12).

4.7. Oxidation of 9: 10

To a solution of pyridine (0.44 mL, 5.38 mmol) in dry DCM (6 mL) was added CrO_3 (43 mg, 0.43 mmol). The mixture was stirred for 30 min at room temperature. **9** (88 mg, 0.27 mmol) in dry DCM (8 mL) was added. After this time ether was added and the mixture filtered. The organic layer was washed with 2 N aqueous HCl, a 6% aqueous solution of $NaHCO_3$, water and brine. The organic layer was dried over Na_2SO_4 and evaporated to give the expected compound **10** (86 mg 99%).

4.7.1. 13-Ethylendioxy-14,15-dinor-8-*epi*-labdan-17-al: 10. IR (film): 2900, 1723, 1462, 1385, 1206, 1063, 855 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 9.50 (1H, d, J=4.2 Hz, H-17), 4.00–3.80 (4H, m, –OC_2H_4O–), 2.40–2.20 (1H, m, H-8), 1.82–0–98 (14H, m), 0.98–0.70 (2H, m), 1.25 (3H, s, H-16), 0.85, 0.83 and 0.81 (3Me, s each, Me-18, Me-19 and Me-20); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 40.2 (C-1), 18.7 (C-2), 42.2 (C-3), 33.4 (C-4), 54.8 (C-5), 20.3 (C-6), 27.0 (C-7), 51.1 (C-8), 54.2 (C-9), 38.7 (C-10), 23.4 (C-11), 38.7 (C-12), 110.0 (C-13), 23.7 (C-16), 205.5 (C-17), 33.6 (C-18), 22.0 (C-19), 14.3 (C-20), 64.7 (–OC_2H_4O–); EIHRMS: calcd for $C_{20}H_{34}O_3$ (M^+) 322.2508, found (M^+) 322.2512.

4.8. Deprotection of 10: 6

To a solution of **10** (87 mg, 0.27 mmol) in acetone (which had been distilled over $KMnO_4$ (10 mL)) was added *p*-toluensulfonic acid (2 mg, 0.01 mmol). The reaction mixture was stirred for 14 h and extracted with EtOAc followed by successive washing of the organic layer with 5% aqueous $NaHCO_3$ solution, water and brine. The organic layer was dried and evaporated to give a crude mixture, which was chromatographed on silica gel to yield the expected compound **6** (65 mg, 86%).

4.9. Reaction of 10 with isopropyl magnesium chloride: 11

To a solution of **10** (2.0 g, 6.20 mmol) in dry THF (20 mL) was added isopropylmagnesium chloride 2 M in THF (30 mL, 60 mmol) at 0 °C, under an argon atmosphere. The reaction mixture was stirred for 1 h at room temperature and then treated with a saturated NH_4Cl aqueous solution, extracted with EtOAc and washed with water and brine. The organic layer was dried over Na_2SO_4 and evaporated yielded desired compound **11** (2.11 g, 93%).

4.9.1. 13-Methyl-13-ethylendioxy-13,14-*seco*-totaran-14-ol: 11. $[\alpha]_D^{22}$ −6.6° (c=1.43, $CHCl_3$); IR (film): 3516, 2932, 1723, 1468, 1385, 1053, 851 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 4.00–3.86 (4H, m, –OC_2H_4O–), 3.22 (1H, d, J= 9.6 Hz, H-14), 1.82–1.00 (15H, m), 1.31 (3H, s, H-13a), 1.02 (3H, d, J=6.3 Hz, H-16), 1.00–0.78 (2H, m), 0.82 (3H, d, J=6.8 Hz, H-17), 0.84, 0.80 and 0.80 (3Me, s each, Me-18, Me-19 and Me-20); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 40.9 (C-1), 19.0 (C-2), 42.4 (C-3), 33.6 (C-4), 54.9 (C-5), 21.4 (C-6), 24.4 (C-7), 40.5 (C-8), 51.9 (C-9), 38.8 (C-10), 22.7 (C-11), 39.4 (C-12), 110.5 (C-13), 23.8 (C-13a), 77.0 (C-14), 31.4 (C-15), 22.1 and 21.0 (C-16 and C-17), 33.6 (C-18), 19.0 (C-19), 14.3 (C-20), 64.8 (–OC_2H_4O–); EIMS m/z (%): 351 (M^+ −15, 2), 115 (8), 87 (100).

4.10. Preparation of 12

A solution of compound **11** (1.23 g, 3.36 mmol) in pyridine (5 mL) and Ac_2O (10 mL) was stirred at 70 °C for 2 h. Then ice was added, the solution stirred for 30 min, and diluted with EtOAc, the organic layer was washed with 10% HCl and water, dried (Na_2SO_4) and evaporated. Purification by chromatography on silica gel yielded quantitatively the corresponding acetate.

To a solution of the latter compound (1.56 g, 3.82 mmol) in distilled acetone over $KMnO_4$ (10 mL), was added *p*-toluensulfonic acid (70 mg, 0.42 mmol). The reaction mixture was stirred for 14 h and extracted with EtOAc followed by successive washing of the organic layer with 5% aqueous $NaHCO_3$ solution, water and brine. The organic layer was dried over Na_2SO_4 and evaporated to give a crude mixture, which was chromatographed on silica gel to yield the expected compound **12** (1.33 g, 96%).

4.10.1. 13-Methyl-14*R*-acetoxy-13,14-*seco*-totaran-13-one: 12. $[\alpha]_D^{22}$ +18.0° (c=1.99, $CHCl_3$); IR (film): 2928, 1728, 1462, 1370, 1244, 1115 and 1020 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 4.68 (1H, d, J=9.6 Hz, H-14), 2.61

(1H, dddd, J=17.2, 12.2, 7.4, 4.8 Hz, H-8), 2.11 (3H, s, H-13), 2.09 (3H, s, MeCOO–), 2.58–1.00 (14H, m), 1.00–0.50 (3H, m), 0.88 (3H, d, J=6.6 Hz, H-16), 0.83 (3H, d, J=6.6 Hz, H-17), 0.85, 0.82 and 0.81 (3Me, s each, Me-18, Me-19 and Me-20); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 39.9 (C-1), 18.8 (C-2), 42.2 (C-3), 33.3 (C-4), 54.8 (C-5), 21.3 (C-6), 25.9 (C-7), 39.1 (C-8), 51.8 (C-9), 39.1 (C-10), 22.1 (C-11), 45.9 (C-12), 209.7 (C-13), 30.0 (C-13a), 78.9 (C-14), 30.0 (C-15), 19.8 and 18.8 (C-16 and C-17), 33.6 (C-18), 22.1 (C-19), 14.5 (C-20), 21.4 (*Me*COO–), 171.3 (Me*C*OO–); EIMS *m*/*z* (%): 364 (M^+, 8), 304 (38), 247 (62), 217 (8), 177 (20), 123 (70), 69 (100); EIHRMS: calcd for $C_{23}H_{40}O_3$ (M^+) 364.2977, found (M^+) 364.2970.

4.11. Preparation of 13

To a solution of **12** (40 mg, 0.110 mmol) in dry THF (1 mL) was added NaHMDS 1.0 M (0.60 mL, 0.600 mmol), at −78 °C under an argon atmosphere. The solution was stirred for 10 min. Then distilled TMSCl (0.20 mL, 1.60 mmol) was added. The mixture was stirred for 2 h and NEt_3 (1 mL) was added. After 30 min, the resulting mixture was diluted with EtOAc and washed with 5% aqueous $NaHCO_3$ solution, water and brine. The organic layer was dried over Na_2SO_4 and the solvent evaporated to give a crude mixture (58 mg).

To a solution of the crude mixture (58 mg) in ^{t}BuOH/THF/H_2O (3/2/1 mL) was added *N*-methylmorpholine *N*-oxide (NMO) (45 mg, 0.33 mmol) and a solution of OsO_4 2.5% (0.01 mL) in ^{t}BuOH. The reaction mixture was stirred at room temperature for 48 h and a saturated aqueous solution of $Na_2S_2O_3$ (5 mL) was added. Extraction with AcOEt was followed by successive washing of the organic layer with a 10% aqueous $Na_2S_2O_3$ solution, 2 N aqueous HCl solution, water and brine. The organic layer was dried over Na_2SO_4 and the solvent evaporated to yield the expected crude mixture (53 mg).

To a solution of the crude mixture (53 mg) in THF/H_2O (3/1 mL) was added H_5IO_6 (170 mg, 0.75 mmol). The reaction mixture was stirred at room temperature for 1 h. After this time the mixture was diluted with water and a 10% aqueous NaOH solution until the pH was >7.0, and extracted with AcOEt. The aqueous layer was acidified with concd HCl, extracted with AcOEt, and washed with water and brine. The organic layer was dried and evaporated. A saturated solution of CH_2N_2 in Et_2O was added. After 3 h the solution was evaporated giving a crude mixture (61 mg), which was chromatographed on silica gel (*n*-hexane/AcOEt, 95/5) to give the expected compound **13** (32 mg, 78%).

4.11.1. Methyl 14*R*-acetoxy-13,14-*seco*-totaran-13-oate: 13. $[\alpha]_D^{22}$ +20.8° (c=0.64, $CHCl_3$); IR (film): 1732, 1458, 1370, 1244, 1171, 1032 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 4.73 (1H, d, J=9.6 Hz, H-14), 3.64 (3H, s, –COOMe), 2.60–2.00 (2H, m, H-12), 2.07 (3H, s, MeCOO–), 2.00–1.00 (14H, m), 0.89 (3H, d, J=6.6 Hz, H-16), 0.84 (3H, d, J=6.6 Hz, H-17), 1.00–0.60 (2H, m), 0.85, 0.83 and 0.81 (3Me, s each, Me-18, Me-19 and Me-20); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 39.9 (C-1), 18.8 (C-2), 42.2 (C-3), 33.4 (C-4), 54.8 (C-5), 21.4 (C-6), 25.9 (C-7), 39.5 (C-8), 51.6 (C-9), 39.1 (C-10), 23.4 (C-11), 35.6 (C-12), 174.5 (C-13), 78.8 (C-14), 30.7 (C-15), 19.8 and 19.6 (C-16 and C-17), 33.6 (C-18), 22.2 (C-19), 14.5 (C-20), 51.6 (–COO*Me*), 21.4 (*Me*COO–), 171.3 (Me*C*OO–); EIMS *m*/*z* (%): 380 (M^+, 4), 320 (48), 277 (30), 233 (27), 182 (30), 136 (83), 69 (100); EIHRMS: calcd for $C_{23}H_{40}O_4$ (M^+) 380.2927, found (M^+) 380.2919.

4.12. Reduction of 13: 14

To a solution of **13** (15 mg, 0.039 mmol) in dry Et_2O (2 mL) was added LAH (5 mg, 0.130 mmol). The mixture was stirred at room temperature for 1 h. The reaction mixture was cooled at 0 °C and wet EtOAc was added and the mixture filtered, the organic phase was dried over Na_2SO_4, filtered and the solvent evaporated affording **14** (11 mg, 90%).

4.12.1. 13,14-*seco*-Totaran-13,14*R*-diol: 14. $[\alpha]_D^{22}$ −10.6° (c=0.72, $CHCl_3$); IR (film): 3372, 2926, 1464, 1387, 1262, 1057, 1009, 970, 909, 735 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.65 (2H, dd, J=9.2, 5.6 Hz, H-13), 3.25 (1H, d, J=9.6 Hz, H-14), 2.30 (2H, br s, OH), 1.80–1.00 (16H, m), 1.02 (3H, d, J=7.0 Hz, H-16), 0.81 (3H, d, J=7.0 Hz, H-17), 1.00–0.70 (2H, m), 0.84, 0.80 and 0.80 (3Me, s each, Me-18, Me-19 and Me-20); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 39.6 (C-1), 19.0 (C-2), 42.4 (C-3), 33.5 (C-4), 54.9 (C-5), 21.4 (C-6), 24.6 (C-7), 40.4 (C-8), 51.3 (C-9), 38.7 (C-10), 24.4 (C-11), 34.8 (C-12), 63.5 (C-13), 77.3 (C-14), 31.4 (C-15), 21.4 and 19.2 (C-16 and C-17), 33.6 (C-18), 22.1 (C-19), 14.2 (C-20); EIMS *m*/*z* (%): 267 (M^+ −43, 3), 238 (100), 123 (46), 95 (25), 69 (32).

4.13. Oxidation of 14 with TPAP: 15

To a mixture of **14** (41 mg, 0.130 mmol), *N*-methylmorpholine *N*-oxide (20 mg, 0.15 mmol) and molecular sieves (60 mg), in anhydrous DCM (2 mL), was added TPAP (6 mg, 0.017 mmol) under argon, at room temperature. The reaction mixture was stirred for 30 min and then filtered on silica gel (EtOAc), the organic layer was evaporated to give a crude mixture which was chromatographed on silica gel (*n*-hexane/AcOEt, 9/1) to yield **15** (35 mg, 90%).

4.13.1. 13,14-*seco*-Totaran-13,14*R*-olide: 15. $[\alpha]_D^{22}$ +20.0° (c=0.42, $CHCl_3$); IR (film): 1710, 1520, 1430 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.78 (1H, dd, J=10.3, 3.7 Hz, H-14), 2.90–2.80 and 2.40–2.30 (2H, m, H-12), 1.95–1.92 (1H, m, H-15), 1.90–1.86 (2H, m, H-11), 1.80–1.76 (2H, m, H-7), 1.74–1.66 (4H, m, H-1 and H-6), 1.63–1.58 (1H, m, H-8), 1.45–1.37 and 1.20–1.10 (2H, m, H-3), 1.01 (3H, d, J=6.6 Hz, H-16), 1.00–0.92 (1H, m, H-9), 0.90 (3H, d, J=6.6 Hz, H-17), 0.94–0.90 (3H, m, H-2 and H-5), 0.84 (3H, s, Me-18), 0.79 (6H, s, Me-19 and Me-20); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 38.8 (C-1), 19.1 (C-2), 42.2 (C-3), 33.1 (C-4), 55.3 (C-5), 22.0 (C-6), 25.8 (C-7), 40.1 (C-8), 53.0 (C-9), 37.8 (C-10), 17.9 (C-11), 33.1 (C-12), 174.8 (C-13), 85.6 (C-14), 28.7 (C-15), 20.7 and 18.6 (C-16 and C-17), 33.5 (C-18), 21.6 (C-19), 14.8 (C-20); EIMS *m*/*z* (%): 306 (M^+, 1), 234 (100), 201 (14), 178 (12), 123 (60), 81 (58).

4.14. Acetylation of 14: 16

A solution of compound **14** (43 mg, 0.140 mmol) in pyridine (1.5 mL) and Ac_2O (3 mL) was stirred at room temperature for 30 min. Then ice was added, the solution stirred for 30 min and diluted with EtOAc, the organic layer was washed with 10% HCl and water, dried over Na_2SO_4 and evaporated the solvent. Purification by chromatography on silica gel yielded desired compound **16** (48 mg, 99%).

4.14.1. 13-Acetoxy-13,14-*seco*-totaran-14*R*-ol: 16. $[\alpha]_D^{22}$ −6.1° ($c=1.46$, $CHCl_3$); IR (film): 3536, 2959, 1738, 1464, 1387, 1366, 1258, 1040 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 4.08–3.84 (2H, m, H-13), 3.12 (1H, d, $J=9.6$ Hz, H-14), 2.00 (3H, s, MeCOO–), 1.80–1.08 (14H, m), 0.98 (3H, d, $J=6.6$ Hz, H-16), 1.00–0.80 (4H, m), 0.78 (3H, d, $J=6.6$ Hz, H-17), 0.81, 0.75 and 0.75 (3Me, s each, Me-18, Me-19 and Me-20); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 39.4 (C-1), 18.9 (C-2), 42.3 (C-3), 33.4 (C-4), 54.8 (C-5), 21.3 (C-6), 24.9 (C-7), 40.6 (C-8), 51.3 (C-9), 38.5 (C-10), 24.3 (C-11), 30.5 (C-12), 65.1 (C-13), 77.1 (C-14), 31.4 (C-15), 20.9 and 19.3 (C-16 and C-17), 33.6 (C-18), 22.0 (C-19), 14.2 (C-20), 171.4 (Me*C*OO–), 21.2 (*Me*COO–); EIMS *m*/*z* (%): 352 (M^+, 2), 309 (10), 280 (82), 249 (30), 205 (40), 177 (15), 123 (100), 69 (88); EIHRMS: calcd for $C_{22}H_{40}O_3$ (M^+) 352.2977, found (M^+) 352.2975.

4.15. Oxidation of 16 with TPAP: 17

To a mixture of **16** (48 mg, 0.140 mmol), *N*-methylmorpholine *N*-oxide (26 mg, 0.190 mmol) and molecular sieves (80 mg) in anhydrous DCM (4 mL), under argon, at room temperature was added TPAP (7 mg, 0.020 mmol). The reaction mixture was stirred for 2 h and then filtered on silica gel (EtOAc), the organic layer was evaporated to give a crude product which was chromatographed on silica gel (*n*-hexane/AcOEt, 9/1) to yield **17** (48 mg, 98%).

4.15.1. 13-Acetoxy-13,14-*seco*-totaran-14-one: 17. $[\alpha]_D^{22}$ −11.6° ($c=0.83$, $CHCl_3$); IR (film): 2940, 1738, 1705, 1244 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 3.90 (2H, t, $J=5.8$ Hz, H-13), 2.80–2.58 (2H, m, H-8 and H-15), 2.00 (3H, s, MeCOO–), 1.88–1.10 (12H, m), 1.09 (3H, d, $J=6.8$ Hz, H-16), 1.06 (3H, d, $J=6.8$ Hz, H-17), 1.10–0.60 (4H, m), 0.83, 0.83 and 0.81 (3Me, s each, Me-18, Me-19 and Me-20); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 38.7 (C-1), 18.7 (C-2), 42.3 (C-3), 33.4 (C-4), 54.6 (C-5), 21.4 (C-6), 30.6 (C-7), 41.8 (C-8), 52.3 (C-9), 38.2 (C-10), 27.0 (C-11), 31.7 (C-12), 65.1 (C-13), 218.5 (C-14), 51.6 (C-15), 18.4 and 18.3 (C-16 and C-17), 33.6 (C-18), 22.0 (C-19), 14.4 (C-20), 171.4 (Me*C*OO–), 21.4 (*Me*COO–); EIMS *m*/*z* (%): 350 (M^+, 14), 290 (8), 265 (82), 219 (16), 123 (45), 69 (100); EIHRMS: calcd for $C_{22}H_{38}O_3$ (M^+) 350.2821, found (M^+) 350.2823.

4.16. Hydrolysis of 17: 18

To **17** (21 mg, 0.060 mmol) a solution of 3% K_2CO_3 in methanol (4 mL) was added with stirring at room temperature. After 4 h the solvent was evaporated and diluted with AcOEt. The organic layer was washed with a 2 M aqueous solution of HCl, water and brine. The solution was dried and evaporated to yield the expected compound **18** (18 mg, 97%).

4.16.1. 13-Hydroxy-13,14-*seco*-totaran-14-one: 18. $[\alpha]_D^{22}$ −20.4° ($c=0.74$, $CHCl_3$); IR (film): 3441, 2934, 1705, 1458, 1387, 1053 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 3.66–3.38 (2H, m, H-13), 2.82–2.58 (2H, m, H-8 and H-15), 2.09–1.15 (12H, m), 1.08 (3H, d, $J=7.0$ Hz, H-16), 1.06 (3H, d, $J=7.0$ Hz, H-17), 1.02–0.83 (4H, m), 0.84, 0.84 and 0.81 (3Me, s each, Me-18, Me-19 and Me-20); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 38.9 (C-1), 18.7 (C-2), 42.2 (C-3), 33.3 (C-4), 54.6 (C-5), 21.4 (C-6), 31.7 (C-7), 41.7 (C-8), 52.3 (C-9), 38.2 (C-10), 26.6 (C-11), 34.6 (C-12), 63.1 (C-13), 219.5 (C-14), 51.2 (C-15), 18.3 and 18.3 (C-16 and C-17), 33.6 (C-18), 22.0 (C-19), 14.3 (C-20); EIMS *m*/*z* (%): 308 (M^+, 8), 265 (7), 237 (16), 139 (38), 109 (30), 95 (46), 69 (100); EIHRMS: calcd for $C_{20}H_{36}O_2$ (M^+) 308.2715, found (M^+) 308.2712.

4.17. Oxidation of 18: 19

To a solution of pyridine (0.4 mL, 4.90 mmol) in dry DCM (5 mL) was added CrO_3 (200 mg, 2.00 mmol). The mixture was stirred for 30 min at room temperature. Then **18** (90 mg, 0.290 mmol) in dry DCM (5 mL) was added. After 30 min ether was added and the mixture filtered. The organic layer was washed with 2 M aqueous HCl, a 6% aqueous solution of $NaHCO_3$, water and brine. The organic layer was dried and evaporated to give the expected compound **19** (80 mg, 90%).

4.17.1. 14-Oxo-13,14-*seco*-totaran-13-al: 19. IR (film): 1709, 1464, 1387, 1368 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 9.62 (1H, s, H-13), 2.81–2.00 (4H, m, H-8, H-12 and H-15), 1.93–1.12 (12H, m), 1.07 (6H, d, $J=6.8$ Hz, H-16 and H-17), 1.12–0.63 (2H, m), 0.84, 0.84 and 0.81 (3Me, s each, Me-18, Me-19 and Me-20); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 38.8 (C-1), 18.7 (C-2), 41.5 (C-3), 33.4 (C-4), 54.6 (C-5), 21.4 (C-6), 31.6 (C-7), 41.4 (C-8), 52.4 (C-9), 38.2 (C-10), 25.6 (C-11), 35.7 (C-12), 202.8 (C-13), 218.8 (C-14), 51.0 (C-15), 18.4 and 18.2 (C-16 and C-17), 33.6 (C-18), 22.0 (C-19), 14.3 (C-20).

4.18. Preparation of 20

To a solution of diisopropylamine (0.80 mL, 5.62 mmol) in THF (10 mL), cooled at −78 °C and under an argon atmosphere was added *n*-BuLi 1.6 M in hexane (3.52 mL, 5.62 mmol). The solution was stirred for 10 min. Then distilled TMSCl (3.52 mL, 28.1 mmol) was added and compound **3** (862 mg, 2.81 mmol) in THF (12 mL) was added via cannula. The mixture was stirred for 3 h and NEt_3 (4 mL) was added. After 30 min, the resulting mixture was diluted with AcOEt and washed with 5% aqueous $NaHCO_3$ solution (2 mL), water and brine. The organic layer was dried over Na_2SO_4 and evaporated to give the corresponding silyl enol ether (1.06 g).

To a solution of later compound (1.00 g, 2.65 mmol) in ^{t}BuOH/THF/H_2O (20/7/3 mL) was added *N*-methylmorpholine *N*-oxide (NMO) (450 mg, 3.18 mmol) and a solution of OsO_4 2.5% (60 μL) in ^{t}BuOH. The reaction mixture was stirred at room temperature for 24 h and a saturated aqueous

solution of $Na_2S_2O_3$ (10 mL) was added. Extraction with AcOEt was followed by successive washing of the organic layer with a 10% aqueous $Na_2S_2O_3$ solution, 2 N aqueous HCl solution, water and brine. The organic layer was dried and evaporated to yield the expected hydroxyketone (820 mg). To a solution of this material (820 mg, 2.55 mmol) in THF/H_2O (15/5 mL) was added H_5IO_6 (1.40 g, 6.32 mmol). The reaction mixture was stirred at room temperature for 3 h. After this time the mixture was diluted with water and a 10% aqueous NaOH solution until the pH was >7.0, and extracted with AcOEt. The aqueous layer was acidified with concd HCl, extracted with AcOEt, and washed with water and brine. The organic layer was dried over Na_2SO_4 and evaporated to give the acid. To a solution of this acid (800 mg, 2.72 mmol) in MeOH/benzene (4/4 mL) was added $TMSCHN_2$ 2 M in hexane (1.4 mL, 2.8 mmol). After 15 h the solvent was evaporated to give **20** (732 mg, 81% from compound **3**).

4.18.1. Dimethyl 14,15,16-trinor-7-labden-13,17-dioate: 20. IR (film): 3100, 2928, 2855, 1717, 1645, 1435, 1248, 1040, 914, 733 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 10.60 (1H, br s, –COOH), 6.80–6.60 (1H, m, H-7), 3.67 (3H, s, –COOMe), 2.75 (1H,,ddd, $J=16.1$, 11.3, 4.8 Hz, H_A-12), 2.30 (1H, ddd, $J=16.1$, 11.3, 5.4 Hz, H_B-12), 2.10–0.90 (12H, m), 0.87, 0.83 and 0.79 (3Me, s each, Me-18, Me-19 and Me-20); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 39.2 (C-1), 18.4 (C-2), 42.0 (C-3), 32.7 (C-4), 50.3 (C-5), 23.2 (C-6), 138.2 (C-7), 134.4 (C-8), 49.4 (C-9), 36.9 (C-10), 24.0 (C-11), 35.4 (C-12), 179.1 (C-13), 169.3 (C-17), 33.0 (C-18), 21.8 (C-19), 14.1 (C-20), 51.3 (–COO*Me*).

4.19. Hydrogenation of 20: 21

To a solution of **20** (39 mg, 0.120 mmol) in EtOH (3 mL), PtO_2 (10 mg, 0.044 mmol) was added. The reaction mixture was stirred for 12 h under hydrogen atmosphere and then filtered on silica gel (AcOEt), the organic layer was evaporated to yield the expected compound **21** (36 mg, 92%).

4.19.1. Dimethyl 14,15,16-trinor-labdan-13,17-dioate: 21. IR (film): 2949, 2880, 1734, 1458, 1437, 1389, 1366, 1258, 1210, 1163 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 3.59 and 3.57 (3H, s each, –COOMe), 2.70–2.10 (3H, m, H-8 and H-12), 2.00–1.00 (13H, m), 1.00–0.80 (1H, m), 0.77, 0.73 and 0.67 (3Me, s each, Me-18, Me-19 and Me-20); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 42.2 (C-1), 18.8 (C-2), 39.2 (C-3), 33.5 (C-4), 56.5 (C-5), 19.3 (C-6), 29.4 (C-7), 38.9 (C-8), 53.0 (C-9), 38.7 (C-10), 21.9 (C-11), 32.9 (C-12), 174.8 (C-13), 175.9 (C-17), 33.7 (C-18), 21.7 (C-19), 14.1 (C-20), 51.7 and 51.4 (–COO*Me*); EIMS *m/z* (%): 324 (M^+, 20), 292 (59), 264 (25), 221 (39), 201 (15), 177 (43), 149 (70), 123 (95), 91 (100), 77 (90).

4.20. Reduction of 21: 22

To a solution of **21** (32 mg, 0.098 mmol) in dry Et_2O (4 mL) was added LAH (12 mg, 0.320 mol). The reaction was stirred at room temperature for 1 h. The reaction was cooled at 0 °C, then wet AcOEt was added and the mixture filtered, the organic phase was dried over Na_2SO_4, filtered and evaporated affording **22** (27 mg, 95%).

4.20.1. 14,15,16-Trinor-labdan-13,17-diol: 22. Mp 141–144 °C (Et_2O); $[\alpha]_D^{22}$ +44.6° ($c=0.84$, $CHCl_3$); IR (film): 3364, 2924, 2851, 1464, 1387, 1262, 1026, 801, 735 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 3.75–3.55 (4H, m, H-13 and H-17), 2.05–1.10 (16H, m), 0.90–0.80 (1H, m), 0.86, 0.81 and 0.71 (3Me, s each, Me-18, Me-19 and Me-20); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 39.2 (C-1), 18.7 (C-2), 42.0 (C-3), 33.3 (C-4), 56.6 (C-5), 18.0 (C-6), 29.6 (C-7), 39.9 (C-8), 53.0 (C-9), 38.2 (C-10), 21.7 (C-11), 31.6 (C-12), 63.3 (C-13), 61.6 (C-17), 33.5 (C-18), 21.5 (C-19), 15.7 (C-20); EIMS *m/z* (%): 268 (M^+, 2), 177 (8), 123 (33), 81 (61), 69 (96), 53 (100).

4.21. Reaction of 20 with Li/NH_3: 22

Lithium (175 mg, 22 mmol), ^{t}BuOH (5 mL) and a solution of compound **20** (290 mg, 0.900 mmol) in THF (5 mL) were added to liquid NH_3 at −78 °C (50 mL). The reaction mixture was stirred at −78 °C for 2 h. The solution was brought up to room temperature and then treated with a saturated NH_4Cl aqueous solution. Extraction with AcOEt, washing with water and brine followed by drying (Na_2SO_4) and evaporation of the solvent left a crude product (232 mg), which was chromatographed on silica gel (*n*-hexane/AcOEt 9/1) to give **22** (220 mg, 91%).

4.22. Oxidation of 22: 23

To a solution of pyridine (0.44 mL, 5.38 mmol) in dry DCM (6 mL) was added CrO_3 (266 mg, 2.68 mmol). The mixture was stirred for 30 min at room temperature. Then **22** (60 mg, 0.22 mmol) in dry DCM (8 mL) was added. After 30 min ether was added and filtered. The organic layer was washed with 2 N aqueous HCl, a 6% aqueous solution of $NaHCO_3$, water and brine. The organic layer was dried over Na_2SO_4 and evaporated to give the expected compound **23** (52 mg, 90%).

4.22.1. 14,15,16-Trinor-labdan-13,17-dial: 23. IR (film): 2950, 1709, 1462, 1389 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 9.83 and 9.63 (1H, s each, –CHO), 2.80–2.20 (3H, m, H-8 and H-12), 2.10–0.80 (14H, m), 0.85, 0.80 and 0.77 (3Me, s each, Me-18, Me-19 and Me-20); EIMS *m/z* (%): 264 (M^+, 2), 221 (3), 123 (8), 84 (100), 69 (12).

4.23. Preparation of SmI_2

In a standard procedure 1.07 g (7.1 mmol) of Samarium powder was placed in a schlenk, which was flamed under vacuum. Then, 1,2-diiodoethane (1.26, 4.4 mmol) in THF (47 mL) was added via cannula. The mixture was stirred under an argon atmosphere for 1.30 h. After that, a deep blue solution of SmI_2 in THF was obtained.

4.24. Preparation of compounds 24 and 25

To a solution of compound **5** (590 mg, 2.1 mmol) in dry THF (21 mL) and MeOH (430 μL), was added at room temperature a solution of SmI_2 (0.1 M in THF, 52 mL, 5.3 mmol), under an argon atmosphere. After 4 h of stirring, the solution was diluted with Et_2O, the organic layers washed with aqueous saturated NH_4Cl, brine, dried (Na_2SO_4) and evaporated in vacuo to afford, after column

chromatography on silica gel, the products **24** (550 mg, 75%) and **25** (44 mg, 5%).

4.24.1. (8*S*)-15,16-Dinor-pimaran-13α,14α-diol: 24. Mp 140–144 °C (*n*-hexane/AcOEt); $[\alpha]_D^{22}$ +23.0° (*c*=1.24, $CHCl_3$); IR (film): 3437, 2984, 1383, 1371, 1049 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 3.63 (1H, d, *J*=11.8 Hz, H-14), 2.20–1.30 (13H, m), 1.26 (3H, s, Me-17), 1.20–1.00 (4H, m), 0.86, 0.84 and 0.83 (3Me, s each, Me-18, Me-19 and Me-20); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 40.7 (C-1), 18.7 (C-2), 42.0 (C-3), 33.0 (C-4), 56.0 (C-5), 18.4 (C-6), 27.2 (C-7), 38.0 (C-8), 49.4 (C-9), 39.0 (C-10), 20.1 (C-11), 27.2 (C-12), 71.3 (C-13), 73.6 (C-14), 28.2 (C-17), 34.0 (C-18), 22.0 (C-19), 15.6 (C-20); EIMS *m/z* (%): 280 (M^+, 5), 262 (18), 244 (9), 177 (12), 123 (41), 107 (26), 95 (51), 81 (62), 67 (69), 54 (100). Anal. Calcd for $C_{18}H_{32}O_2$: C, 77.09; H, 11.50; found: C, 77.12; H, 11.13.

4.24.2. (8*S*)-15,16-Dinor-pimaran-13β,14β-diol: 25. IR (film): 3374, 2924, 1462, 1385, 1368, 1146, 1074, 945 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 3.38 (1H, d, *J*=4.3 Hz, H-14), 1.23 (3H, s, Me-17), 1.01 (3H, s, Me-20), 0.87 and 0.85 (2Me, s each, Me-18 and Me-19). Anal. Calcd for $C_{18}H_{32}O_2$: C, 77.09; H, 11.50; found: C, 77.05; H, 11.21.

4.25. Acetylation of 24: 28

A solution of compound **24** (81 mg, 0.2 mmol) in pyridine (1 mL) and Ac_2O (1 mL) was stirred at room temperature for 12 h. Then, ice was added, the solution was stirred for 30 min and diluted with AcOEt, the organic layer was washed with HCl 10% and water, dried over Na_2SO_4 and evaporated. Purification by chromatography on silica gel gave the monoacetyl derivative **28** in a quantitative yield.

4.25.1. (8*S*)-14α-Acetoxy-15,16-dinor-pimaran-13α-ol: 28. Mp 127–131 °C (*n*-hexane/AcOEt); $[\alpha]_D^{22}$ +18.8° (*c*=0.24, $CHCl_3$); IR: 3400, 1721, 1281, 1236, 1144, 1127, 1109, 1024, 1001, 978, 943 cm^{-1}; ^1H (400 MHz, $CDCl_3$) δ: 5.24 (1H, d, *J*=11.9 Hz, H-14), 2.30–2.20 (1H, m, H-8), 2.14 (3H, s, OAc), 1.87 and 1.82 (2H, m, H-1), 1.58–1.53 (1H, m, H-9), 1.51–1.20 (10H, m), 1.40–1.15 (2H, m, H-3), 0.85–0.82 (1H, m, H-5), 1.12 (3H, s, Me-17), 1.09 (3H, s, Me-20), 0.85 and 0.84 (2Me, s each, Me-18 and Me-19); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 40.5 (C-1), 18.6 (C-2), 41.9 (C-3), 33.1 (C-4), 56.1 (C-5), 18.5 (C-6), 27.7 (C-7), 35.1 (C-8), 49.3 (C-9), 39.0 (C-10), 19.9 (C-11), 35.9 (C-12), 71.0 (C-13), 76.9 (C-14), 28.2 (C-17), 33.9 (C-18), 21.9 (C-19), 19.0 (C-20), 20.8 (–OCO*Me*), 170.7 (–O*C*OMe); EIMS *m/z* (%): 322 (M^+, 5), 262 (14), 205 (41), 177 (12), 136 (39), 107 (60), 77 (100); HRMS: calcd for $C_{20}H_{34}O_3$ (M^+) 322.2508, found (M^+) 322.2511.

4.26. Preparation of compounds 26 and 27

To a solution of compound **6** (230 mg, 0.83 mmol) in dry THF (10 mL) and MeOH (230 μL), was added at room temperature a solution of SmI_2 (0.1 M in THF, 25 mL, 2.6 mmol), under an argon atmosphere. After 4 h of stirring, the solution was diluted with Et_2O, the organic layers washed with saturated aqueous NH_4Cl, brine, dried (Na_2SO_4) and evaporated in vacuo to afford, after column chromatography on silica gel, the products **26** (167 mg, 72%) and **27** (16 mg, 7%).

4.26.1. (8*R*)-15,16-Dinor-pimaran-13β,14β-diol: 26. IR (film): 3356, 2940, 2855, 1458, 1373, 1069, 1018 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 2.90 (1H, d, *J*=10.2 Hz, H-14), 2.20–1.00 (17H, m), 1.23 (3H, s, Me-17), 0.85, 0.82 and 0.82 (3Me, s each, Me-18, Me-19 and Me-20); EIMS *m/z* (%): 280 (M^+, 1), 262 (14), 244 (12), 177 (6), 123 (10), 107 (32), 54 (100). Anal. Calcd for $C_{18}H_{32}O_2$: C, 77.09; H, 11.50; found: C, 77.32; H, 11.07.

4.26.2. (8*R*)-15,16-Dinor-pimaran-13α,14α-diol: 27. IR (film): 3434, 1452, 1385, 1371, 1125, 1040, 1005 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 3.29 (1H, br s, H-14), 2.18–1.02 (17H, m), 1.21 (3H, s, Me-17), 0.85, 0.82 and 0.82 (3Me, s each, Me-18, Me-19 and Me-20); EIMS *m/z* (%): 280 (M^+, 2), 262 (18), 244 (14), 177 (28), 54 (100).

4.27. Acetylation of 26: 29

A solution of compound **26** (120 mg, 0.3 mmol) in pyridine (1 mL) and Ac_2O (1 mL) was stirred at room temperature for 12 h. Then, ice was added, the solution stirred for 30 min and diluted with AcOEt, the organic layer was washed with HCl 10% and water, dried over Na_2SO_4 and evaporated. Purification by chromatography on silica gel gave the monoacetyl derivative **29** in a quantitative yield.

4.27.1. (8*R*)-14β-Acetoxy-15,16-dinor-pimaran-13β-ol: 29. IR (film): 3451, 2918, 2849, 1721, 1452, 1377, 1238, 1127, 1022, 912 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 4.52 (1H, d, *J*=10.4 Hz, H-14), 2.12 (3H, s, OAc), 2.10–1.10 (17H, m), 1.09 (3H, s, Me-17), 0.86, 0.84 and 0.82 (3Me, s each, Me-18, Me-19 and Me-20); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 39.2 (C-1), 18.8 (C-2), 42.0 (C-3), 33.1 (C-4), 54.8 (C-5), 19.1 (C-6), 30.4 (C-7), 36.3 (C-8), 53.7 (C-9), 36.7 (C-10), 20.7 (C-11), 36.8 (C-12), 70.6 (C-13), 82.7 (C-14), 27.5 (C-17), 33.8 (C-18), 21.8 (C-19), 14.2 (C-20), 20.8 (–OCO*Me*), 170.6 (–O*C*OMe); HRMS: calcd for $C_{20}H_{34}O_3$ (M^+) 322.2508, found (M^+) 322.2505.

4.28. Oxidation of 24: 30

Swern oxidation of **24** (395 mg, 1.41 mmol) (as described above for compound **5**), afforded after column chromatography over silica gel (Hex/AcOEt 8:2) compound **30** (350 mg, 88%).

4.28.1. (8*S*)-13α-Hydroxy-15,16-dinor-pimaran-14-one: 30. Mp 122–125 °C (*n*-hexane/AcOEt); IR (film): 3450, 2950, 1713, 1462, 1389, 1370, 1146, 1071, 997, 912, 739 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 3.24 (1H, dt, *J*=2.7, 6.0 Hz, H-8), 2.30–2.20 (2H, m), 2.00–1.30 (11H, m), 1.30 (3H, s, Me-17), 0.85, 0.81 and 0.75 (3Me, s each, Me-18, Me-19 and Me-20); EIMS *m/z* (%): 279 (M^++1, 25), 235 (30), 217 (20), 177 (22), 153 (23), 107 (58), 95 (71), 81 (75),69 (83), 53 (100). Anal. Calcd for $C_{18}H_{30}O_2$: C, 77.65; H, 10.86; found: C, 77.59; H, 10.94.

4.28.2. (8*R*)-13β-Hydroxy-15,16-dinor-pimaran-14-one: 32. Swern oxidation of compound **26** (52 mg, 0.18 mmol) (described above for compound **5**), gave compound **32**

(41 mg, 79%). IR (film): 3482, 2947, 2866, 1703, 1447, 1385, 1256, 1144 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 2.91 (1H, dt, $J=3.8$, 11.8 Hz, H-8), 2.20–1.10 (13H, m), 1.27 (3H, s, Me-17), 0.96, 0.85 and 0.83 (3Me, s each, Me-18, Me-19 and Me-20); EIMS *m/z* (%): 278 (M^+, 3), 235 (15), 217 (10), 153 (30), 81 (70), 53 (100). Anal. Calcd for $C_{18}H_{32}O_2$: C, 77.65; H, 10.86; found: C, 77.72; H, 10.53.

4.28.3. (8*S*)-13β-Hydroxy-15,16-dinor-pimaran-14-one: 31. Swern oxidation of compound **25** (40 mg, 0.14 mmol) (described above for compound **5**), gave compound **31** (33 mg, 84%). IR (film): 3470, 2930, 1697, 1458, 1379, 1366, 1200, 1169, 1142, 1111, 1053, 990, 955 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 4.00 (1H, s, –OH), 2.86 (1H, t, $J=7.0$ Hz, H-8), 2.18–1.12 (12H, m), 1.39 (3H, s, Me-17), 0.86, 0.81 and 0.79 (3Me, s each, Me-18, Me-19 and Me-20); EIMS *m/z* (%): 278 (M^+, 2), 235 (12), 217 (22), 177 (26), 153 (18), 107 (43), 95 (64), 53 (100).

4.28.4. (8*R*)-13α-Hydroxy-15,16-dinor-pimaran-14-one: 33. Swern oxidation of compound **27** (24 mg, 0.08 mmol), gave compound **33** (20 mg, 88%). IR (film): 3451, 2945, 1705, 1462, 1370, 1179, 1163, 1034, 993, 963 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 4.08 (1H, s, –OH), 2.52 (1H, dt, $J=4.3$, 11.8 Hz, H-8), 2.19–1.08 (12H, m), 1.36 (3H, s, Me-17), 0.95, 0.85 and 0.83 (3Me, s each, Me-18, Me-19 and Me-20); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 39.3 (C-1), 18.9 (C-2), 41.9 (C-3), 33.2 (C-4), 58.1 (C-5), 20.5 (C-6), 25.0 (C-7), 45.0 (C-8), 54.5 (C-9), 37.7 (C-10), 21.5 (C-11), 40.9 (C-12), 216.0 (C-14), 25.0 (C-17), 33.5 (C-18), 21.8 (C-19), 13.9 (C-20); EIMS *m/z* (%): 322 ($M^+ + 1$, 75), 236 (100), 218 (45), 177 (22), 123 (61), 95 (26), 81 (22), 67 (19); HRMS: calcd for $C_{18}H_{30}O_2$ (M^+) 278.2246, found (M^+) 278.2240.

4.29. Reaction of 19 with SmI_2: 34 and 35

To a solution of **19** (220 mg, 0.720 mmol) in THF (10 mL) was added, under an argon atmosphere, dry methanol (1 mL) and a solution of SmI_2 prepared in situ 0.1 M (20 mL). The mixture was stirred for 2 h. The reaction mixture was diluted with AcOEt and a saturated NH_4Cl aqueous solution was added, extracted with AcOEt and washed with a 10% aqueous $Na_2S_2O_3$ solution, water and brine. The organic layer was dried over Na_2SO_4 and evaporated to give a crude product, which was chromatographed on silica gel (*n*-hexane/AcOEt, 9/1) to give **34** (173 mg, 80%) and **35** (21 mg, 10%).

4.29.1. Totaran-13α,14α-diol: 34. Mp 140 °C (*n*-hexane/AcOEt); $[\alpha]_D^{22}$ −7.6° ($c=0.82$, $CHCl_3$); IR: 3468, 2928, 1458, 1387, 1055, 1032 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 3.55 (1H, dd, $J=11.1$, 4.1 Hz, H-13), 2.27–2.17 (1H, m, H-15), 1.89–1.79 (2H, m, H-7), 1.79–1.69 and 1.57–1.47 (2H, m, H-12), 1.73–1.60 (2H, m, H-11), 1.73–1.63 and 0.95–0.85 (2H, m, H-1), 1.59–1.47 and 1.45–1.35 (2H, m, H-2), 1.49–1.37 (1H, m, H-8), 1.43–1.33 and 1.17–1.07 (2H, m, H-3), 1.33–1.23 (2H, m, H-6), 1.11–1.01 (1H, m, H-9), 1.07 (3H, d, $J=7.1$ Hz, H-16), 0.99 (3H, d, $J=7.1$ Hz, H-17), 0.88–0.78 (1H, m, H-5), 0.83 (3H, s, Me-18), 0.81 (3H, s, Me-19) and 0.79 (3H, s, Me-20); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 39.5 (C-1), 18.9 (C-2), 42.0 (C-3), 33.0 (C-4), 54.0 (C-5), 21.4 (C-6), 26.2 (C-7), 39.8 (C-8), 49.1 (C-9), 36.8 (C-10), 22.7 (C-11), 31.2 (C-12), 71.9 (C-13), 76.7 (C-14), 32.6 (C-15), 18.6 and 18.1 (C-16 and C-17), 33.4 (C-18), 21.8 (C-19), 14.1 (C-20); EIMS *m/z* (%): 308 (M^+, 3), 265 (88), 229 (52), 177 (8), 109 (30), 95 (38), 69 (100); EIHRMS: calcd for $C_{20}H_{36}O_2$ (M^+) 308.2715, found (M^+) 308.2712.

4.29.2. Totaran-13β,14β-diol: 35. $[\alpha]_D^{22}$ −19.8° ($c=0.63$, $CHCl_3$); IR (film): 3432, 2938, 1460, 1387, 1368, 1263, 1028, 1001, 970 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 3.83 (1H, t, $J=2.0$ Hz, H-13), 2.07–1.97 (1H, m, H-15), 1.93–1.83 (2H, m, H-12), 1.79–1.69 (1H, m, H-8), 1.73–1.63 and 0.94–0.84 (2H, m, H-1), 1.70-1.60 (2H, m, H-11), 1.52–1.42 (2H, m, H-2), 1.50–1.40 (2H, m, H-6), 1.45–1.35 and 1.15–1.05 (2H, m, H-3), 1.35–1.20 and 1.00–0.90 (2H, m, H-7), 1.13–1.03 (1H, m, H-9), 1.02 (3H, d, $J=7.0$ Hz, H-16), 1.00 (3H, d, $J=7.0$ Hz, H-17), 0.86–0.76 (1H, m, H-5), 0.86 (6H, s, Me-18 and Me-20) and 0.82 (3H, s, Me-19); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 39.7 (C-1), 16.9 (C-2), 42.0 (C-3), 33.1 (C-4), 55.3 (C-5), 19.0 (C-6), 27.4 (C-7), 42.2 (C-8), 51.0 (C-9), 37.6 (C-10), 22.0 (C-11), 28.6 (C-12), 68.9 (C-13), 76.4 (C-14), 31.5 (C-15), 20.1 and 19.5 (C-16 and C-17), 33.6 (C-18), 22.0 (C-19), 14.0 (C-20); EIMS *m/z* (%): 308 (M^+, 60), 265 (90), 229 (35), 153 (42), 123 (55), 69 (100); EIHRMS: calcd for $C_{20}H_{36}O_2$ (M^+) 308.2715, found (M^+) 308.2708.

4.30. Reaction of 23 with SmI_2: 36 and 37

To a solution of **23** (700 mg, 2.65 mmol) in THF (20 mL) was added, under an argon atmosphere, dry methanol (6 mL) and a solution of SmI_2 prepared in situ 0.1 M (70 mL). The mixture was stirred for 2 h. After that, the reaction mixture was diluted with AcOEt and a saturated NH_4Cl aqueous solution was added, extracted with AcOEt and washed with a 10% aqueous $Na_2S_2O_3$ solution, water and brine. The organic layer was dried over Na_2SO_4 and evaporated to give a crude product, which was chromatographed on silica gel (*n*-hexane/AcOEt, 9/1) to give **36** (568 mg, 82%) and **37** (40 mg, 5%).

4.30.1. 8-*epi*-Podocarpa-13α,14α-diol: 36. Mp 113–116 °C (*n*-hexane/AcOEt); $[\alpha]_D^{22}$ +33.9° ($c=1.09$, $CHCl_3$); IR (film): 3390, 2940, 2845, 1265, 1100 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 4.00 (1H, q, $J=3.2$ Hz, H-13), 3.85 (1H, dd, $J=11.5$, 3.2 Hz, H-14), 2.20–2.00 (3H, m, H-7 and H-8), 1.89–1.79 and 0.94–0.84 (2H, m, H-1), 1.73–1.63 (4H, m, H-2 and H-11), 1.69–1.59 (2H, m, H-12), 1.56–1.46 (3H, m, H-6 and H-9), 1.45–1.35 and 1.17–1.07 (2H, m, H-3), 0.99 (3H, s, Me-20), 0.92–0.82 (1H, m, H-5), 0.86 (3H, s, Me-18), 0.83 (3H, s, Me-19); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 40.8 (C-1), 18.6 (C-2), 41.9 (C-3), 33.1 (C-4), 55.3 (C-5), 18.3 (C-6), 26.6 (C-7), 36.0 (C-8), 48.7 (C-9), 38.8 (C-10), 18.7 (C-11), 29.3 (C-12), 69.5 (C-13), 70.2 (C-14), 34.0 (C-18), 22.0 (C-19), 18.9 (C-20); EIMS *m/z* (%): 266 (M^+, 2), 153 (11), 123 (14), 107 (45), 89 (37), 77 (100), 67 (34).

4.30.2. 8-*epi*-Podocarpa-13β,14β-diol: 37. Mp 130–133 °C (*n*-hexane/AcOEt); $[\alpha]_D^{22}$ +3.4° ($c=1.02$, $CHCl_3$); IR (film): 3390, 2940, 2845, 1265 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 3.64 (1H, t, $J=9.2$ Hz, H-14), 3.42–3.32 (1H, m, H-13), 2.30–2.20 and 1.53–1.43 (2H, m, H-7),

2.00–1.90 (2H, m, H-11), 1.95–1.85 and 1.73–1.63 (2H, m, H-12), 1.91–1.81 and 0.92–0.82 (2H, m, H-1), 1.70–1.60 (1H, m, H-8), 1.63–1.53 and 1.35–1.25 (2H, m, H-2), 1.53–1.43 (3H, m, H-6 and H-9), 1.45–1.35 and 1.20–1.10 (2H, m, H-3), 1.02 (3H, s, Me-20), 0.89–0.79 (1H, m, H-5), 0.87 (3H, s, Me-18), 0.82 (3H, s, Me-19); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 40.6 (C-1), 18.6 (C-2), 41.9 (C-3), 33.1 (C-4), 56.0 (C-5), 18.2 (C-6), 26.7 (C-7), 41.9 (C-8), 49.1 (C-9), 38.8 (C-10), 23.0 (C-11), 31.1 (C-12), 76.6 (C-13), 74.2 (C-14), 33.9 (C-18), 21.9 (C-19), 19.3 (C-20); EIMS *m/z* (%): 266 (M^+, 70), 233 (15), 215 (15), 123 (95), 107 (48).

4.31. Reaction of 24 with *p*-TsOH: 38

A solution of compound **24** (160 mg, 0.57 mmol) in benzene (5 mL) was added at room temperature *p*-TsOH (5 mg) and the resulting mixture heated at 80 °C for 30 min. The solution was cooled and diluted with Et_2O, and the organic layer washed with $NaHCO_3$ 10%, water and brine, dried over Na_2SO_4 and the solvent was removed at low vacuum to give, after column chromatography on silica gel, compound **38** (130 mg, 90%).

4.31.1. (8*R*,13*S*)-15,16-Dinor-pimaran-14-one: 38. IR (film): 2928, 2866, 1709, 1447, 1371, 1260, 1121, 1090, 1063, 1036, 984 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.30 (1H, dq, J=12.4, 6.2 Hz, H-13), 2.21 (1H, ddt, J=1.2, 4.0, 12.0 Hz, H-8), 2.07 (1H, ddt, J=12.8, 6.2, 3.2 Hz, H-12), 1.95–0.78 (15H, m), 0.98 (3H, d, J=6.5 Hz, Me-17), 0.93, 0.85 and 0.82 (3Me, s each, Me-18, Me-19 and Me-20). Anal. Calcd for $C_{18}H_{30}O$: C, 82.38; H, 11.52; found: C, 82.12; H, 11.73.

4.32. Reaction of 38 with allyl bromide: 39

To a solution of **38** (114 mg, 0.435 mmol) in THF (2.1 mL) was added a solution of KHMDS (957 μL, 0.5 M in toluene) at −78 °C, under argon. After stirring the mixture for 5 min, allyl bromide (51 μL, 1.4 mmol) was added and stirred for 90 min. Then $NaHCO_3$ (1 mL) was added, diluted with ether, washed with water, brine, dried (Na_2SO_4) and the solvent removed to give, after chromatography, compound **39** (105 mg, 80%).

4.32.1. 15a-Homo-15-pimaren-14-one: 39. IR (film): 3077, 2944, 2866, 1705, 1640, 1456, 1375, 1256, 1121, 1094, 1032, 991, 914, 864, 833 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.62–5.58 (1H, m, H-15), 5.05–5.02 (2H, m, =CH_2), 2.45 (1H, dt, J=4.3, 11.9 Hz, H-8), 2.45 (1H, dd, J_{AB}=14.0 Hz, J_{AX}=7.2 Hz, H_A-15a), 2.15 (1H, dd, J_{BA}=14.0 Hz, J_{BX}=7.6 Hz, H_B-15a), 1.90–0.80 (16H, m), 0.97 (3H, s, Me-17), 0.94, 0.85 and 0.83 (3Me, s each, Me-18, Me-19 and Me-20); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 38.1 (C-1), 18.9 (C-2), 41.5 (C-3), 33.1 (C-4), 57.2 (C-5), 19.5 (C-6), 26.7 (C-7), 45.3 (C-8), 54.4 (C-9), 37.6 (C-10), 20.7 (C-11), 39.1 (C-12), 47.7 (C-13), 216.6 (C-14), 41.9 (C-15a), 117.9 (C-15), 133.1 (C-16), 21.8 (C-17), 33.5 (C-18), 22.3 (C-19), 13.9 (C-20); HRMS: calcd for $C_{21}H_{34}O$ (M^+) 302.2610, found (M^+) 302.2614.

4.33. Preparation of 40

To a solution of **39** (30 mg, 0.1 mmol) in *t*-BuOH/THF/H_2O (10:3:1), was added NMO (16 mg, 0.12 mmol) and OsO_4 (2.5% in *t*-BuOH, 10 μL). The solution was stirred and checked by TLC during 2 h, then saturated aqueous Na_2SO_3 was added and the solution stirred for 30 min, diluted with Et_2O, washed with HCl 7%, water and brine, dried over Na_2SO_4 and the solvent evaporated. The residue was dissolved in THF/H_2O (2:1, 1 mL) and $NaIO_4$ (82 mg, 0.39 mmol) was added at room temperature. The mixture was stirred for 25 min, then diluted with Et_2O and washed with $NaHCO_3$ 10%, $NaHSO_3$ 10%, water and brine, dried (Na_2SO_4) and the solvent evaporated at low vacuum to give the crude material, which was chromatographed on silica gel (Hex/AcOEt 95:5) to give compound **40** (27 mg, 90%).

4.33.1. 14-Oxo-pimaran-16-al: 40. IR (film): 2940, 2866, 2733, 1705, 1456, 1381, 1321, 1256, 1121, 1067, 1034, 1005, 972, 916, 854, 833, 733 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 9.65 (1H, t, J=2.7 Hz, –CHO), 2.77 (1H, d, J=2.7 Hz, H_A-15), 2.50 (1H, d, J=2.7 Hz, H_B-15), 2.46 (1H, dt, J=3.8, 11.3 Hz, H-8), 1.14 (3H, s, Me-17), 198–0.78 (16H, m), 0.95, 0.84 and 0.82 (3Me, s each, Me-18, Me-19 and Me-20); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 38.4 (C-1), 18.9 (C-2), 41.9 (C-3), 33.1 (C-4), 56.8 (C-5), 19.7 (C-6), 26.7 (C-7), 45.4 (C-8), 54.5 (C-9), 37.7 (C-10), 20.6 (C-11), 39.1 (C-12), 46.2 (C-13), 214.7 (C-14), 50.3 (C-15), 200.6 (C-16), 22.9 (C-17), 33.5 (C-18), 21.8 (C-19), 13.9 (C-20). Anal. Calcd for $C_{20}H_{32}O_2$: C, 78.90; H, 10.59; found: C, 79.00; H, 10.23.

4.34. Reaction of 40 with KOH/MeOH: 41

To a solution of **40** (20 mg, 0.07 mmol) in MeOH was added KOH (20 mg). The solution was heated under reflux for 36 h. After that, the mixture was diluted with Et_2O, washed with water, brine, dried (Na_2SO_4) and the solvent evaporated at low vacuum to give the crude material which was chromatographed on silica gel (Hex/AcOEt 9:1) to give compound **41** (16 mg, 75%).

4.34.1. 16*S*-Hydroxy-14-hibaone: 41. $[\alpha]_D^{22}$ +10.2° (c=0.25, $CHCl_3$); IR (film): 3482, 1717, 1314, 1256, 1155, 1042, 721 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.68 (1H, m, H-16), 2.43 (1H, dd, J=8.1, 14.0 Hz, H-15), 1.90–0.80 (14H, m), 1.42 (1H, dd, J=3.0, 14.0 Hz, H-15), 1.23 (1H, m, H-9), 1.04 (2Me, s, Me-17 and Me-20), 0.90 (1H, m, H-5), 0.87 and 0.82 (2Me, s each, Me-18 and Me-19); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 39.8 (C-1), 18.4 (C-2), 41.5 (C-3), 33.0 (C-4), 55.4 (C-5), 18.4 (C-6), 23.9 (C-7), 56.2 (C-8), 60.5 (C-9), 39.1 (C-10), 18.3 (C-11), 41.5 (C-12), 47.1 (C-13), 221.5 (C-14), 43.5 (C-15), 67.6 (C-16), 19.5 (C-17), 33.5 (C-18), 21.7 (C-19), 15.5 (C-20); HRMS: calcd for $C_{20}H_{32}O_2$ (M^+) 304.2402, found (M^+) 304.2406.

4.35. Treatment of 41 with TsCl/Py: 42

To a solution of compound **41** (13 mg, 0.04 mmol) in pyridine was added tosyl chloride (25 mg), under argon. The solution was heated at 90 °C and checked by TLC. After 36 h the mixture was cooled and diluted with AcOEt, washed with HCl 7%, $NaHCO_3$ 10%, water and brine, dried (Na_2SO_4) and the solvent removed to give, after CC (Hex/AcOEt 9:1) compound **42** (8 mg, 65%).

4.35.1. 15-Hibaen-14-one: 42. Mp 95–98 °C; $[\alpha]_D^{22}$ −33.2° (c=0.5, $CHCl_3$); IR: 2953, 1759, 1177, 721 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 6.12 and 5.89 (1H, d each, J=7.0 Hz, H-15 and H-16), 1.94 (1H, dt, J=4.7, 13.3 Hz, H-7), 1.70–0.84 (15H, m), 1.05 (3H, s, Me-17), 0.87, 0.87 and 0.83 (3Me, s each, Me-18, Me-19 and Me-20); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 39.2 (C-1), 18.5 (C-2), 41.7 (C-3), 33.1 (C-4), 54.6 (C-5), 19.0 (C-6), 27.2 (C-7), 55.4 (C-8), 55.9 (C-9), 38.2 (C-10), 18.4 (C-11), 35.3 (C-12), 50.9 (C-13), 220.0 (C-14), 133.0 (C-15), 132.3 (C-16), 16.5 (C-17), 33.5 (C-18), 21.9 (C-19), 15.5 (C-20). Anal. Calcd for $C_{20}H_{30}O$: C, 83.86; H, 10.56; found: C, 83.76; H, 10.66.

4.36. Oxidation of 34 with TPAP: 43

To a mixture of **34** (31 mg, 0.10 mmol), *N*-methylmorpholine *N*-oxide (20 mg, 0.15 mmol) and molecular sieves (50 mg) in anhydrous DCM (3 mL), TPAP (10 mg, 0.028 mmol) was added under argon, at room temperature. The reaction mixture was stirred for 30 min and then filtered on silica gel (EtOAc), the organic layer was evaporated to give a crude mixture, which was chromatographed on silica gel (DCM) to yield **43** (24 mg, 80%).

4.36.1. 14α-Hydroxy-totaran-13-one: 43. $[\alpha]_D^{22}$ +9.5° (c=0.48, $CHCl_3$); IR: 3470, 2932, 1709, 1464, 1387, 1273, 1123 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 3.28 (1H, br s, –OH), 2.52–2.39 (2H, m, H-12), 2.16–2.00 (1H, m, H-15), 1.98–1.08 (14H, m), 1.03 (3H, d, J=7.0 Hz, H-16), 1.00–0.60 (1H, m), 0.86, 0.83 and 0.81 (3Me, s each, Me-18, Me-19 and Me-20), 0.72 (3H, d, J=7.0 Hz, H-17); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 39.1 (C-1), 19.0 (C-2), 42.2 (C-3), 33.3 (C-4), 54.8 (C-5), 21.9 (C-6), 28.9 (C-7), 45.8 (C-8), 52.2 (C-9), 37.6 (C-10), 22.2 (C-11), 35.9 (C-12), 217.2 (C-13), 81.2 (C-14), 35.9 (C-15), 17.5 and 15.9 (C-16 and C-17), 33.6 (C-18), 21.9 (C-19), 14.1 (C-20); EIMS m/z (%): 306 (M^+, 26), 264 (22), 235 (93), 123 (46), 95 (50), 69 (100); HRMS: calcd for $C_{20}H_{34}O_2$ (M^+) 306.2559, found (M^+) 306.2554.

4.37. Reaction of 34 with *p*-TsOH: 44, 45 and 46

Diol **34** (136 mg, 0.440 mmol) dissolved in benzene (10 mL) was stirred at 60 °C in the presence of *p*-toluensulfonic acid (30 mg, 0.150 mmol) for 48 h under an argon atmosphere. The mixture reaction was diluted with AcOEt and washed successively with a 5% aqueous solution of $NaHCO_3$, water and brine. The organic layer was dried and evaporated to give a crude (126 mg), which was chromatographed on silica gel (*n*-hexane/benzene, 9/1) to yield **44** (19 mg, 8%), **45** (15 mg, 12%) and **46** (74 mg, 61%).

Compound **44**. $[\alpha]_D^{22}$ +20.3° (c=1.20, $CHCl_3$); IR (film): 2942, 1262, 1200 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 4.78 (1H, s, –OCHO–), 3.94 (1H, d, J=7.0 Hz, H-13), 2.32–2.23 (2H, m, H-15), 2.23–1.00 (32H, m), 0.98 (3H, d, J=6.9 Hz, H-16′), 0.95 (3H, d, J=6.9 Hz, H-17′), 0.88 (3H, s), 0.87 (3H, d, J=6.9 Hz, H-16), 0.86 (3H, s), 0.85 (3H, s), 0.84 (3H, s), 0.83 (3H, d, J=6.9 Hz, H-17), 0.81 (3H, s), 0.79 (3H, s), 1.00–0.80 (2H, m); EIMS m/z (%): 579 (M^+−1, 2), 537 (3), 319 (98), 273 (100), 217 (16).

4.37.1. 14*R*-(13→14)-abeo-Totaran-13-al: 45. $[\alpha]_D^{22}$ +16.6° (c=1.25, $CHCl_3$); IR (film): 2944, 1724, 1458, 1389, 1370, 1198 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 9.43 (1H, s, –CHO), 2.29–2.09 (1H, m, H-15), 2.09–1.00 (16H, m), 1.00 (3H, d, J=6.6 Hz, H-16), 1.00–0.80 (1H, m), 0.85, 0.81 and 0.78 (3Me, s each, Me-18, Me-19 and Me-20), 0.75 (3H, d, J=6.6 Hz, H-17); ^{13}C NMR (50 MHz, $CDCl_3$, δ ppm): 40.5 (C-1), 19.0 (C-2), 42.7 (C-3), 33.7 (C-4), 57.9 (C-5), 22.5 (C-6), 24.2 (C-7), 45.0 (C-8), 55.6 (C-9), 37.3 (C-10), 24.2 (C-11), 27.4 (C-12), 205.2 (C-13), 60.6 (C-14), 28.3 (C-15), 19.9 and 19.8 (C-16 and C-17), 33.9 (C-18), 21.9 (C-19), 13.2 (C-20); EIMS m/z (%): 290 (M^+, 50), 261 (80), 177 (49), 123 (100), 95 (64), 69 (92). EIHRMS: calcd for $C_{20}H_{34}O$ (M^+) 290.2610, found (M^+) 290.2519.

4.37.2. 14-*epi*-Totaran-13-one: 46. Mp 132 °C (*n*-hexane/AcOEt); $[\alpha]_D^{22}$ +19.9° (c=1.19, $CHCl_3$); IR: 2944, 1724, 1458, 1389, 1370, 1198 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 2.30 (1H, ddd, J=13.7, 6.1, 3.5 Hz, H_A-12), 2.25 (1H, dd, J=13.7, 6.1 Hz, H_B-12), 2.05–1.90 (4H, m), 1.75–1.07 (12H, m), 1.04 (3H, d, J=6.8 Hz, H-16), 0.97 (3H, d, J=6.8 Hz, H-17), 0.95–0.88 (1H, m), 0.86, 0.84 and 0.83 (3Me, s each, Me-18, Me-19 and Me-20); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 42.2 (C-1), 19.0 (C-2), 42.4 (C-3), 33.4 (C-4), 55.1 (C-5), 22.0 (C-6), 34.0 (C-7), 41.0 (C-8), 54.8 (C-9), 37.6 (C-10), 25.7 (C-11), 39.6 (C-12), 213.9 (C-13), 61.0 (C-14), 26.8 (C-15), 21.1 and 17.5 (C-16 and C-17), 33.7 (C-18), 22.0 (C-19), 14.4 (C-20); EIMS m/z (%): 290 (M^+, 100), 248 (98), 177 (18), 137 (21), 98 (48), 77 (29); EIHRMS: calcd for $C_{20}H_{34}O$ (M^+) 290.2610, found (M^+) 290.2611.

4.38. Preparation of 47

To a solution of diisopropylamine (60 μL, 0.420 mmol) in THF (0.5 mL), cooled at −78 °C and under an argon atmosphere, was added *n*-BuLi 1.6 M in hexane (0.20 mL, 0.32 mmol). After 10 min, a solution of **46** (20 mg, 0.069 mmol) in THF (0.1 mL) was added via cannula with stirring. The resulting mixture was stirred for 30 min at −78 °C, then the solution was brought up to 0 °C, and then recooled to −78 °C. A solution of phenylselenium chloride (65 mg, 0.34 mmol) in THF (2 mL) and HMPA (0.05 mL) was added via cannula. The reaction mixture was stirred at room temperature for 1 h, and washed with a saturated NH_4Cl aqueous solution, extracted with AcOEt and washed with water and brine. Evaporation of the solvent followed by chromatography on silica gel (*n*-hexane/AcOEt, 98/2) yielded the phenylselenide (30 mg).

To a solution of the latter compound (58 mg, 0.130 mmol) in dry DCM (5 mL) at 0 °C, was added *m*-CPBA (33 mg, 0.191 mmol) dissolved in dry DCM (0.1 mL). The reaction mixture was stirred for 5 min at room temperature and then treated with a 6% aqueous solution of $Na_2S_2O_3$. Extraction with AcOEt, followed by successive washing of the organic layer with a 10% aqueous $Na_2S_2O_3$ solution, 5% aqueous $NaHCO_3$ solution, water and brine. The organic layer was dried over Na_2SO_4 and evaporated. Purification by chromatography on silica gel (*n*-hexane/benzene, 8/2) yielded desired compound **47** (33 mg, 88%).

4.38.1. 14-*epi*-11-Totaren-13-one: 47. $[\alpha]_D^{22}$ +15.1° (c=

0.49, $CHCl_3$); IR (film): 2932, 2872, 1680, 1464, 1458, 1387, 1366, 1271, 1121, 1084 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 6.86 (1H, d, $J=10.2$ Hz, H-11), 5.93 (1H, dd, $J=10.2$, 2.2 Hz, H-12), 2.34–2.10 (2H, m, H-9 and H-14), 2.10–1.00 (12H, m), 1.16 (3H, d, $J=7.4$ Hz, H-16), 0.95 (3H, d, $J=7.4$ Hz, H-17), 1.00–0.80 (1H, m), 0.88, 0.84 and 0.82 (3Me, s each, Me-18, Me-19 and Me-20); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 38.4 (C-1), 18.8 (C-2), 42.1 (C-3), 33.4 (C-4), 57.9 (C-5), 21.7 (C-6), 31.8 (C-7), 39.1 (C-8), 56.3 (C-9), 37.2 (C-10), 131.5 (C-11), 149.4 (C-12), 187.1 (C-13), 54.6 (C-14), 25.9 (C-15), 21.2 and 17.4 (C-16 and C-17), 33.3 (C-18), 21.6 (C-19), 15.8 (C-20); EIMS, *m/z*: 288 (M^+, 43), 246 (29), 177 (12), 137 (38), 108 (100), 69 (68); EIHRMS: calcd for $C_{20}H_{32}O$ (M^+) 288.2453, found (M^+) 288.2462.

4.39. Reaction of 46 with $CuBr_2$: 48

To a solution of **46** (14 mg, 0.048 mmol) in MeCN (0.5 mL) was added $CuBr_2$ (14 mg, 0.063 mmol). The reaction mixture was stirred at 50 °C, under an argon atmosphere for 72 h, then the mixture was diluted with AcOEt and water. Extraction with AcOEt was followed by successive washing of the organic layer with 5% aqueous solution of $NaHCO_3$, water and brine. The organic layer was dried over Na_2SO_4 and evaporated. Purification by chromatography on silica gel (*n*-hexane/benzene, 99/1) yielded compound **48** (16 mg, 76%).

4.39.1. 12β,14α-Dibromo-totaran-13-one: 48. $[\alpha]_D^{22}$ −19.2° ($c=0.48$, $CHCl_3$); IR (film): 3553, 2932, 1732, 1653, 1458, 1387, 1082 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 5.75 (1H, dd, $J=13.2$, 6.2 Hz, H-12), 2.62–2.44 (1H, m), 2.42–2.22 (1H, m, H-15), 2.12–1.00 (13H, m), 1.32 (3H, d, $J=7.0$ Hz, H-16), 1.14 (3H, d, $J=7.0$ Hz, H-17), 1.00–0.80 (1H, m), 0.90, 0.86 and 0.84 (3Me, s each, Me-18, Me-19 and Me-20); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 39.6 (C-1), 18.9 (C-2), 41.9 (C-3), 33.4 (C-4), 54.1 (C-5), 21.0 (C-6), 27.8 (C-7), 45.1 (C-8), 51.6 (C-9), 37.8 (C-10), 39.3 (C-11), 54.9 (C-12), 224.7 (C-13), 83.8 (C-14), 36.7 (C-15), 20.2 and 18.0 (C-16 and C-17), 33.7 (C-18), 22.1 (C-19), 14.6 (C-20); EIMS *m/z* (%): 473, 471 and 469 (M^+, 51, 100 and 57), 446 (9), 445 (28), 423 (10), 413 (19), 392 (11), 391 (41), 365 (9), 343 (17), 329 (9), 309 (23), 287 (11), 269 (5), 259 (5), 187 (6), 177 (9).

4.40. Reaction of 48 with Li_2CO_3/LiBr: 49, (+)-totarol

To a solution of **45** (10 mg, 0.022 mmol) in DMF (1 mL) Li_2CO_3 (5 mg, 0.068 mmol) and LiBr (6 mg, 0.068 mmol) were added. The reaction mixture was heated at 140 °C for 17 h. Then ice was added, the solution stirred for 30 min and diluted with AcOEt, the organic layer was washed with 2 M aqueous HCl solution, water and brine. The organic layer was dried over Na_2SO_4 and evaporated to give a crude mixture which was chromatographed on silica gel (*n*-hexane/AcOEt, 98/2) to give compound **49**, (+)-totarol (4.5 mg, 71%).

4.40.1. (+)-Totarol: 49. Mp 129–130 °C (*n*-hexane); $[\alpha]_D^{22}$ +41.2° ($c=0.4$, $CHCl_3$); IR: 3482, 2932, 1458, 1387, 1263, 1103, 665 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 7.00 (1H, d, $J=8.4$ Hz, H-12), 6.51 (1H, d, $J=8.4$ Hz, H-11), 3.41 (1H, m, H-15), 3.00–2.80 (2H, m, H-7), 2.30–1.40 (9H, m), 1.33 (3H, d, $J=7.0$ Hz, H-16), 1.32 (3H, d, $J=7.0$ Hz, H-17), 1.17 (3H, s, Me-20), 0.94 (3H, s, Me-18) and 0.90 (3H, s, Me-19); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 39.8 (C-1), 19.8 (C-2), 42.0 (C-3), 33.5 (C-4), 49.7 (C-5), 19.6 (C-6), 28.9 (C-7), 133.9 (C-8), 143.3 (C-9), 38.0 (C-10), 123.1 (C-11), 114.5 (C-12), 152.1 (C-13), 131.1 (C-14), 27.3 (C-15), 20.6 (C-16 and C-17), 33.4 (C-18), 21.8 (C-19), 25.4 (C-20); EIHRMS: calcd for $C_{20}H_{30}O$ (M^+) 286.2567, found (M^+) 286.2572.

4.41. Reaction of 36 with TBDMSCl: 50, 51 and 52

To a solution of diol **51** (48 mg, 0.180 mmol) in DMF (1.6 mL), TBDMSCl (36 mg, 0.234 mmol), imidazole (37 mg, 0.540 mmol) and DMAP (6 mg, 0.049 mmol) were added. The reaction mixture was stirred for 22 h and then treated with a 5% aqueous $NaHCO_3$ solution. The solution was extracted with AcOEt and washed with water and brine. The organic layer was dried over Na_2SO_4 and evaporated to give a crude mixture (64 mg) which was chromatographed on silica gel (DCM) yielding **50** (8 mg, 9%), **51** (16 mg. 24%) and **52** (42 mg, 61%).

4.41.1. 13α,14α-*t*-Butyldimethylsilyloxy-8-*epi*-podocarpane: 50. Mp 138 °C (*n*-hexane/AcOEt); $[\alpha]_D^{22}$ +16.8° ($c=0.57$, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ: 3.85–3.78 (1H, m, H-13), 3.72 (1H, dd, $J=13.2$, 2.9 Hz, H-14), 2.28–1.00 (16H, m), 0.98–0.88 (1H, m), 0.87 and 0.85 (9H s each, $^tBu$$Me_2$Si–), 0.93, 0.81 and 0.79 (3Me, s each, Me-18, Me-19 and Me-20), 0.03, 0.01, 0.00 and −0.02 (3H, s, each, ^{t}BuMe_2Si); EIMS *m/z* (%): 495 ($M^+ + 1$, 2), 437 (60), 231 (100), 147 (20).

4.41.2. 13α-*t*-Butyldimethylsilyloxy-8-*epi*-podocarpan-14α-ol: 51. Mp 104 °C (*n*-hexane/AcOEt); $[\alpha]_D^{22}$ +21.3° ($c=0.79$, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ: 3.99 (1H, dd, $J=5.9$, 2.9 Hz, H-14), 3.70 (1H, dt, $J=10.3$, 2.9 Hz, H-13), 2.25–2.18 (1H, m), 2.00–1.92 (1H, m), 1.83–1.08 (14H, m), 0.98, 0.87 and 0.83 (3Me, s each, Me-18, Me-19 and Me-20), 0.91 (9H, s, $^tBu$$Me_2$Si–), 0.90–0.80 (1H, m), 0.08 (6H, s, ^{t}BuMe_2Si–); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 41.0 (C-1), 18.7 (C-2), 42.0 (C-3), 33.1 (C-4), 55.1 (C-5), 18.5 (C-6), 26.4 (C-7), 37.2 (C-8), 48.6 (C-9), 38.7 (C-10), 18.9 (C-11), 20.3 (C-12), 71.1 (C-13), 70.5 (C-14), 34.0 (C-18), 22.0 (C-19), 18.7 (C-20), 25.8 (Me_3*C*Si–), 25.8 (*Me*$_3$CSi–), −4.8 and −4.5 (*Me*$_2$Si–); EIMS *m/z* (%): 362 ($M^+ - 18$, 2), 323 (8), 231 (100), 175 (18), 149 (48), 75 (80); EIHRMS: calcd for $C_{23}H_{45}O_2Si$ ($M^+ + 1$) 381.3189, found ($M^+ + 1$) 381.3184.

4.41.3. 14α-*t*-Butyldimethylsilyloxy-8-*epi*-podocarpan-13α-ol: 52. Mp 85 °C (*n*-hexane/AcOEt); $[\alpha]_D^{22}$ +17.8° ($c=1.16$, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ: 3.92 (1H, dd, $J=13.2$, 2.9 Hz, H-14), 3.90–3.80 (1H, m, H-13), 2.20–1.00 (16H, m), 0.90–0.80 (1H, m), 0.99, 0.86 and 0.84 (3Me, s each, Me-18, Me-19 and Me-20), 0.92 (9H, s, $^tBu$$Me_2$Si–), 0.12 and 0.10 (6H, s, ^{t}BuMe_2Si–); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 41.0 (C-1), 18.5 (C-2), 42.2 (C-3), 33.3 (C-4), 56.0 (C-5), 18.8 (C-6), 28.8 (C-7), 36.0 (C-8), 49.6 (C-9), 39.2 (C-10), 18.9 (C-11), 27.1 (C-12), 71.9 (C-13), 69.9 (C-14), 34.2 (C-18), 22.2 (C-19), 19.4 (C-20), 26.1 (Me_3*C*Si–), 26.1 (*Me*$_3$CSi–), −4.5 and −4.0 (*Me*$_2$Si–);

EIMS *m/z* (%): 380 (M^+, 2), 323 (30), 231 (95), 149 (48), 149 (48), 75 (100). EIHRMS: calcd for $C_{23}H_{44}O_2Si$ (M^+) 380.3111, found (M^+) 380.3117.

4.42. Reaction of 50 with TBAF: 36

To compound **50** (60 mg, 0.160 mmol) TBAF 1.0 M in THF (1 mL) was added. The mixture was stirred under an argon atmosphere for 2 h then diluted with water. The solution was extracted with AcOEt, and washed with water and brine. The organic layer was dried over Na_2SO_4 and evaporated to give a crude product which was chromatographed on silica gel (*n*-hexane/AcOEt, 95/5) to yield **36** (41 mg, 98%).

4.42.1. Oxidation of 51 with TPAP: 53. To a mixture of **51** (36 mg, 0.095 mmol), *N*-methylmorpholine *N*-oxide (30 mg, 0.220 mmol) and molecular sieves (25 mg) in anhydrous DCM (4 mL) under argon, TPAP (10 mg, 0.028 mmol) was added, at room temperature. The reaction mixture was stirred for 30 min and then filtered on silica gel (EtOAc), the organic layer was evaporated to give a crude which was chromatographed on silica gel (DCM) to yield **53** (33 mg, 92%).

4.42.2. 13α-*t*-Butyldimethylsilyloxy-8-*epi*-podocarpan-14-one: 53. Mp 123 °C (*n*-hexane/AcOEt); $[\alpha]_D^{22}$ +15.4° ($c=0.76$, $CHCl_3$); IR: 2920, 1711, 1254, 1094, 1065, 833 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 3.91 (1H, dd, $J=5.8$, 3.2 Hz, H-13), 3.14 (1H, t, $J=6.5$ Hz, H-8), 2.37–2.27 (2H, m, H-7), 2.15–2.10 (2H, m, H-12), 2.03–1.93 (1H, m, H-9), 1.79–1.69 and 0.94–0.84 (2H, m, H-1), 1.50–1.40 (2H, m, H-2), 1.44–1.33 and 1.18–1.08 (4H, m, H-3 and H-11), 0.95–0.74 (3H, m, H-5 and H-6), 0.91 (9H, s, ^tBuMe$_2$Si–), 0.88 (3H, s, Me-18), 0.83 (3H, s, Me-19), 0.79 (3H, s, Me-20), 0.09 and 0.06 (3H, s each, ^{t}Bu*Me*$_2$Si–); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 40.0 (C-1), 19.0 (C-2), 41.9 (C-3), 33.0 (C-4), 55.2 (C-5), 18.4 (C-6), 24.5 (C-7), 42.7 (C-8), 51.3 (C-9), 39.1 (C-10), 18.8 (C-11), 32.4 (C-12), 74.8 (C-13), 214.6 (C-14), 33.8 (C-18), 21.9 (C-19), 16.2 (C-20), 25.7 (Me$_3$*C*Si–), 25.7 (*Me*$_3$CSi–), −5.2 and −4.9 (*Me*$_2$Si–); EIMS *m/z* (%): 379 (M^++1, 2), 353 (8), 321 (100), 229 (8), 75 (18).

4.43. Oxidation of 52 with TPAP: 54

To a mixture of **52** (32 mg, 0.084 mmol), *N*-methylmorpholine *N*-oxide (28 mg, 0.204 mmol) and molecular sieves (25 mg) in anhydrous DCM (4 mL), under argon, at room temperature was added TPAP (10 mg, 0.028 mmol). The reaction mixture was stirred for 30 min and then filtered on silica gel, the organic layer was evaporated to give a crude which was chromatographed on silica gel (DCM) to yield **54** (31 mg, 98%).

4.43.1. 14α-*t*-Butyldimethylsilyloxy-8-*epi*-podocarpan-13-one: 54. $[\alpha]_D^{22}$ −2.5° ($c=1.43$, $CHCl_3$); IR: 2940, 2920, 2820, 1732, 1458, 1254, 1117, 857, 779 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 4.40 (1H, d, $J=11.5$ Hz, H-14), 2.56 (1H, ddd, $J=14.5$, 8.7, 5.1 Hz, H_A-12), 2.36 (1H, dt, $J=14.5$, 5.1 Hz, H_B-12), 2.15–2.00 (3H, m, H_A-7, H-8 and H_A-11), 1.89–1.79 and 1.00–0.90 (2H, m, H-1), 1.70–1.65 (1H, m, H_B-11), 1.64–1.54 (2H, m, H_B-7 and H-9), 1.55–1.40 (2H, H-6), 1.48–1.37 and 1.21–1.11 (4H, m, H-2 and H-3), 1.14 (3H, s, Me-20), 1.03–0.93 (1H, m, H-5), 0.94 (9H, s, ^tBuMe$_2$Si–), 0.90 (6H, s, Me-18 and Me-19), 0.17 and 0.14 (3H, s c/u, ^{t}Bu*Me*$_2$Si–). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 41.0 (C-1), 18.6 (C-2), 42.0 (C-3), 33.2 (C-4), 53.7 (C-5), 18.2 (C-6), 26.5 (C-7), 44.2 (C-8), 48.8 (C-9), 38.3 (C-10), 23.6 (C-11), 39.0 (C-12), 211.2 (C-13), 77.0 (C-14), 33.5 (C-18), 21.8 (C-19), 18.3 (C-20), 25.9 (Me$_3$*C*Si–), 25.8 (*Me*$_3$CSi–), −5.6 and −4.2 (*Me*$_2$Si–); EIMS *m/z* (%): 379 (M^++1, 2), 353 (5), 321 (100), 229 (23), 197 (10), 149 (28), 105 (25), 77 (53).

4.44. Reaction of 53 or 54 with TBAF: 55, 56 and 57

To compound **53** (60 mg, 0.160 mmol) TBAF 1.0 M in THF (1 mL) was added. The mixture was stirred under an argon atmosphere for 2 h and diluted with water. The solution was extracted with AcOEt, and washed with water and brine. The organic layer was dried over Na_2SO_4 and evaporated to give a crude which was chromatographed on silica gel (*n*-hexane/AcOEt, 95/5) to yield **55** (10 mg, 23%), **56** (10 mg, 23%) and **57** (20 mg, 46%).

When the reaction was carried out under the same condition with **54** (146 mg, 0.39 mmol), TBAF 1.0 M in THF (2 mL), after the work up **55** (22 mg, 22%), **56** (22 mg, 22%) and **57** (44 mg, 44%) were obtained.

4.44.1. 14β-Hydroxy-podocarpan-13-one: 55. Mp 95 °C (*n*-hexane/AcOEt); $[\alpha]_D^{22}$ −8.4° ($c=0.75$, $CHCl_3$); IR: 2926, 2843, 1724, 1458, 1387, 1088 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 3.72 (1H, d, $J=10.2$ Hz, H-14), 2.53 (1H, ddd, $J=14.2$, 6.8, 2.8 Hz, H_A-12), 2.39–2.29 (1H, m, H-8), 2.32 (1H, ddd, $J=14.2$, 6.4, 3.2 Hz, H_B-12), 2.10–2.03 (1H, m, H_A-7), 1.80–1.05 (13H, m), 1.05–0.90 (1H, m), 0.89, 0.84 and 0.83 (3Me, s each, Me-18, Me-19 and Me-20); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 39.7 (C-1), 19.0 (C-2), 42.2 (C-3), 33.4 (C-4), 55.1 (C-5), 21.3 (C-6), 32.6 (C-7), 47.1 (C-8), 52.3 (C-9), 37.9 (C-10), 26.9 (C-11), 38.5 (C-12), 211.4 (C-13), 80.2 (C-14), 33.8 (C-18), 22.1 (C-19), 14.3 (C-20); EIMS *m/z* (%): 264 (M^+, 90), 235 (15), 163 (38), 123 (57), 95 (62), 69 (100); EIHRMS: calcd for $C_{17}H_{28}O_2$ (M^+) 264.2089, found (M^+) 264.2097.

4.44.2. 14α-Hydroxy-podocarpan-13-one: 56. Mp 85 °C (*n*-hexane/AcOEt); $[\alpha]_D^{22}$ −14.2° ($c=0.48$, $CHCl_3$); IR: 3669, 2936, 1705 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 4.05 (1H, ddd, $J=12.0$, 6.8, 1.6 Hz, H-14), 2.43 (1H, ddd, $J=9.2$, 6.8, 3.6 Hz, H_A-12), 2.32 (1H, ddt, $J=12.0$, 4.0, 1.6 Hz, H-8), 1.97 (1H, dq, $J=13.6$, 4.0 Hz, H_A-7), 1.83 (1H, dq, $J=12.8$, 2.8 Hz, H_A-11), 1.76–1.66 and 1.00–0.90 (2H, m, H-1), 1.76–1.66 and 1.35–1.25 (2H, m, H-6), 1.65–1.55 and 1.45–1.35 (2H, m, H-2), 1.55–1.45 (1H, m, H_B-7), 1.50–1.40 (1H, m, H_B-11), 1.47–1.37 and 1.25–1.15 (2H, m, H-3), 1.42–1.32 (1H, m, H_B-12), 1.25–1.15 (1H, m, H-9), 0.94 (3H, s, Me-20), 0.86 (3H, s, Me-18), 0.84 (3H, s, Me-19) and 0.84–0.74 (1H, m, H-5); ^{13}C NMR (100 MHz, $CDCl_3$, δppm): 39.2 (C-1), 18.8 (C-2), 41.8 (C-3), 33.1 (C-4), 54.4 (C-5), 20.3 (C-6), 25.8 (C-7), 47.4 (C-8), 57.7 (C-9), 37.6 (C-10), 21.8 (C-11), 35.6 (C-12), 213.1 (C-13), 74.9 (C-14), 33.4 (C-18), 21.8 (C-19), 13.8 (C-20); EIMS *m/z* (%): 264 (M^+, 100), 231 (10), 163 (18), 123 (43), 95

(42), 69 (57); EIHRMS: calcd for $C_{17}H_{28}O_2$ (M^+) 264.2089, found (M^+) 264.2095.

4.44.3. 13α-Hydroxy-podocarpan-14-one: 57. Mp 90 °C (*n*-hexane/AcOEt); $[\alpha]_D^{22}$ −72.5° (*c*=1.57, $CHCl_3$); IR: 3445, 2926, 1717, 1458 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 4.10 (1H, dd, *J*=6.4, 5.2 Hz, H-13), 2.59 (1H, dt, *J*=11.9, 4.2 Hz, H-8), 2.05–1.95 and 1.30–1.20 (2H, m, H-7), 1.90–1.80 (2H, m, H-12), 1.75–1.65 and 1.25–1.15 (2H, m, H-6), 1.75–1.65 and 1.03–0.93 (2H, m, H-1), 1.60–1.50 (2H, m, H-11), 1.50–1.40 (2H, m, H-2), 1.47–1.37 and 1.20–1.10 (2H, m, H-3), 1.30–1.20 (1H, m, H-9), 0.90 (3H, s, Me-20), 0.88 (3H, s, Me-18), 0.83 (3H, s, Me-19) and 0.88–0.78 (1H, m, H-5); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 39.5 (C-1), 18.7 (C-2), 41.9 (C-3), 33.4 (C-4), 54.9 (C-5), 20.8 (C-6), 27.2 (C-7), 45.2 (C-8), 53.2 (C-9), 37.4 (C-10), 18.2 (C-11), 30.6 (C-12), 73.0 (C-13), 215.3 (C-14), 33.6 (C-18), 21.9 (C-19), 13.6 (C-20); EIMS *m/z* (%): 264 (M^+, 91), 235 (16), 205 (15), 163 (32), 123 (50), 95 (52), 69 (100); EIHRMS: calcd for $C_{17}H_{28}O_2$ (M^+) 264.2089, found (M^+) 264.2081.

4.45. Addition of isopropylmagnesium chloride to 55, 56 and 57: 58

To a solution of **55**, **56** and **57** (25 mg, 0.095 mmol) in dry THF (1 mL) under an argon atmosphere, isopropylmagnesium chloride 2 M in Et_2O (3 mL, 6 mmol) was added at 0 °C. The reaction mixture was stirred for 3 h at room temperature and then treated with a saturated NH_4Cl aqueous solution, extracted with EtOAc and washed with water and brine. The organic layer was dried over Na_2SO_4 and evaporated to give a crude mixture, which was chromatographed on silica gel (*n*-hexane/AcOEt, 9/1) to yield **55** (5 mg, 20%), **56** (5 mg, 20%) and **58** (10.5 mg, 42%).

These results were the same when the reaction was carried out with any of these compounds separately.

4.45.1. Abietan-13β,14β-diol: 58. Mp 65 °C (*n*-hexane/AcOEt); $[\alpha]_D^{22}$ −19.1° (*c*=0.54, $CHCl_3$); IR: 3445, 2926, 2868, 1464, 1387, 1368, 1263, 1026 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 3.16 (1H, d, *J*=9.5 Hz, H-14), 2.25–2.15 and 1.00–0.90 (2H, m, H-7), 2.10–2.00 (1H, m, H-15), 1.95–1.85 (2H, m, H-2), 1.78–1.68 and 1.73–1.63 (2H, m, H-1), 1.75–1.65 and 1.23–1.13 (2H, m, H-12), 1.75–1.65 (1H, m, H-8), 1.70–1.60 and 1.35–1.25 (2H, m, H-6), 1.51–1.41 (2H, m, H-11), 1.45–1.35 and 1.20–1.10 (2H, m, H-3), 0.92 (3H, d, *J*=6.9 Hz, H-16), 0.87 (3H. d, *J*=6.9 Hz, H-17), 0.85 (3H, s, Me-19), 0.85 (3H, s, Me-18), 0.83 (3H, s, Me-20), 0.88–0.78 (1H, m, H-5) and 0.75–0.63 (1H, m, H-9). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 39.2 (C-1), 18.8 (C-2), 42.1 (C-3), 33.1 (C-4), 55.0 (C-5), 21.2 (C-6), 31.4 (C-7), 38.8 (C-8), 53.3 (C-9), 36.6 (C-10), 18.7 (C-11), 27.1 (C-12), 71.9 (C-13), 77.2 (C-14), 33.4 (C-15), 17.6 and 16.2 (C-16 and C-17), 33.5 (C-18), 21.8 (C-19), 14.2 (C-20); EIMS *m/z* (%): 308 (M^+, 1), 265 (100), 229 (27), 69 (78); EIHRMS: calcd for $C_{20}H_{36}O_2$ (M^+) 308.2715, found (M^+) 308.2721.

4.46. Reaction of 58 with HI: 59

Diol **58** (13 mg, 0.041 mmol) dissolved in benzene (2 mL) was refluxed in the presence of an aqueous solution of HI 57% (0.13 mL) at 90 °C for 1 h. The solution was diluted with AcOEt and washed with a 6% aqueous solution of $NaHSO_3$, a 10% aqueous solution of $NaHCO_3$, water and brine. The organic layer was dried over Na_2SO_4 and evaporated to give a crude mixture which was chromatographed on silica gel (*n*-hexane/AcOEt, 95/5) to yield **59** (7 mg, 60%).

4.46.1. Abietan-14-one: 59. $[\alpha]_D^{22}$ −4.0° (*c*=0.30, $CHCl_3$); IR (film): 2942, 2868, 1707, 1458, 1387, 1368, 1260 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 2.20 (1H, dt, *J*=11.6, 4.0 Hz, H-8), 2.15–0.95 (17H, m), 0.93, 0.85 and 0.83 (3Me, s each, Me-18, Me-19 and Me-20), 0.89 (3H, d, *J*=6.7 Hz, H-16), 0.84 (3H, d, *J*=7.2 Hz, H-17), 0.85–0.75 (1H, m); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 39.5 (C-1), 19.2 (C-2), 42.2 (C-3), 33.4 (C-4), 56.8 (C-5), 20.7 (C-6), 26.6 (C-7), 50.3 (C-8), 54.7 (C-9), 37.8 (C-10), 24.7 (C-11), 29.1 (C-12), 58.4 (C-13), 214.4 (C-14), 26.2 (C-15), 21.7 and 19.2 (C-16 and C-17), 33.8 (C-18), 22.1 (C-19), 14.1 (C-20); EIMS *m/z* (%): 290 (M^+, 100), 248 (15), 177 (35), 153 (23), 123 (48), 98 (99), 69 (72); EIHRMS: calcd for $C_{20}H_{34}O$ (M^+) 290.2610, found (M^+) 290.2604.

4.47. Preparation of 60

To a solution of **53** (18 mg, 0.048 mmol) in dry THF (2 mL) was added allylmagnesium bromide 1 M in Et_2O (1 mL, 1 mmol) at 0 °C, under an argon atmosphere. The reaction mixture was stirred for 1 h at room temperature and then treated with a saturated NH_4Cl aqueous solution, extracted with EtOAc and washed with water and brine. The organic layer was dried and evaporated to give a crude mixture, which was chromatographed on silica gel (*n*-hexane/AcOEt, 7/3) to give the allyl derivative (17 mg). To a solution of TBAF 1.0 M in THF (1 mL), the latter compound (17 mg, 0.040 mmol) was added. The mixture was stirred under an argon atmosphere for 90 h and diluted with water. The solution was extracted with AcOEt, washed with water and brine. The organic layer was dried over Na_2SO_4 and evaporated to give a crude which was chromatographed on silica gel (*n*-hexane/AcOEt, 7/3) to yield **60** (12.5 mg, 98%).

4.47.1. 8-*epi*-15(17→16)-abeo-Totaran-13α,14β-diol: 60. $[\alpha]_D^{22}$ −37.3° (*c*=0.37, $CHCl_3$); IR (film): 3449, 2926 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 5.96 (1H, dddd, *J*=17.3, 10.1, 7.7, 7.5 Hz, H-15), 5.23 (1H, dd, *J*=10.1, 2.1 Hz, H_A-16), 5.18 (1H, dd, *J*=17.3, 2.1 Hz, H_B-16), 3.62 (1H, dd, *J*=11.1, 4.6 Hz, H-13), 2.59 (1H, dd, *J*=14.1, 7.7 Hz, H_A-15a), 2.36 (1H, dd, *J*=14.1, 7.5 Hz, H_B-15a), 2.12 (1H, ddd, *J*=13.4, 6.1, 3.1 Hz, H-8), 1.85–1.75 and 1.34–1.24 (2H, m, H-12), 1.70–1.60 and 1.10–1.00 (2H, m, H-3), 1.56–1.45 (3H, m, H-7 and H-9), 1.45–1.35 (2H, m, H-11), 1.44–1.34 and 1.19–1.09 (2H, m, H-1), 1.40–1.30 (2H, m, H-2), 1.35–1.23 (3H, m, H-5 and H-6), 0.90 (3H, s, Me-19), 0.87 (3H, s, Me-20), 0.86 (3H, s, Me-18); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 42.4 (C-1), 16.4 (C-2), 44.2 (C-3), 33.3 (C-4), 46.1 (C-5), 19.2 (C-6), 20.0 (C-7), 39.4 (C-8), 45.7 (C-9), 36.7 (C-10), 22.1 (C-11), 29.3 (C-12), 73.0 (C-13), 75.7 (C-14), 36.8 (C-15a), 134.1 (C-15), 119.5 (C-16), 33.9 (C-18), 22.5 (C-19), 17.7 (C-20); EIMS *m/z* (%): 306 (M^+, 5), 265 (100), 229 (70), 173 (11), 123 (40), 95 (42), 69 (65);

EIHRMS: calcd for $C_{20}H_{34}O_2$ (M^+) 306.2559, found (M^+) 306.2566.

4.48. Reaction of 53 with vinylmagnesium bromide: 61

To a solution of **53** (40 mg, 0.106 mmol) in dry THF (1 mL) was added vinylmagnesium bromide 1 M in Et_2O (2 mL, 2 mmol), at 0 °C, under an argon atmosphere. The reaction mixture was stirred for 15 min at room temperature and then treated with a saturated NH_4Cl aqueous solution, extracted with EtOAc and washed with water and brine. The organic layer was dried over Na_2SO_4 and evaporated to give a crude mixture, which was chromatographed on silica gel (DCM) to yield **61** (35 mg, 81%).

4.48.1. 13α-*t*-Butyldimethylsilyloxy-17-nor-8-*epi*-15-cleistanten-14β-ol: 61. $[\alpha]_D^{22}$ −2.4° (c=0.74, $CHCl_3$); IR (film): 3450, 2920 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 6.23 (1H, dd, J=17.4, 10.9 Hz, H-15), 5.30 (1H, dd, J=17.4, 1.5 Hz, H_A-16), 5.12 (1H, dd, J=10.9, 1.5 Hz, H_B-16), 3.52 (1H, dd, J=8.4, 4.1 Hz, H-13), 2.13 (1H, ddd, J=10.4, 6.2, 2.8 Hz, H-8), 1.88–1.78 and 1.47–1.37 (2H, m, H-12), 1.75–1.65 and 1.02–0.92 (2H, m, H-3), 1.69–1.59 and 1.58–1.48 (2H, m, H-7), 1.61–1.51 (1H, m, H-9), 1.44–1.34 and 1.19–1.09 (2H, m, H-1), 1.40–1.30 (4H, m, H-2 and H-6), 1.21–1.11 (3H, m, H-5 and H-11), 0.93 (3H, s, Me-20), 0.87 (9H, s, ^tBuMe$_2$Si–), 0.87 (3H, s, Me-19), 0.83 (3H, s, Me-18), 0.03 (6H, s, ^{t}BuMe_2Si–); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 42.6 (C-1), 19.5 (C-2), 43.6 (C-3), 33.5 (C-4), 48.8 (C-5), 19.4 (C-6), 21.1 (C-7), 39.5 (C-8), 46.2 (C-9), 37.2 (C-10), 20.6 (C-11), 30.3 (C-12), 75.2 (C-13), 76.9 (C-14), 143.8 (C-15), 112.9 (C-16), 33.2 (C-18), 22.6 (C-19), 17.6 (C-20), 26.1 (Me$_3$*C*Si–), 26.1 (*Me*$_3$CSi–), −4.6 and −3.9 (*Me*$_2$Si–); EIMS *m*/*z* (%): 406 (M^+, 10), 349 (28), 257 (40), 217 (7), 171 (42), 131 (52), 75 (100); EIHRMS: calcd for $C_{25}H_{46}O_2Si$ (M^+) 406.3267, found (M^+) 406.3262.

4.49. Reaction of 53 with isopropylmagnesium chloride: 62

To a solution of **53** (195 mg, 0.520 mmol) in dry THF (1 mL) was added isopropylmagnesium chloride 2 M in Et_2O (4 mL, 8 mmol), at 0 °C, under an argon atmosphere. The reaction mixture was stirred for 2 h at room temperature and then treated with a saturated NH_4Cl aqueous solution, extracted with EtOAc and washed with water and brine. The organic layer was dried over Na_2SO_4 and evaporated to yield compound **62** (137 mg, 70%).

4.49.1. 13α-*t*-Butyldimethylsilyloxy-8-*epi*-podocarpan-14β-ol: 62. $[\alpha]_D^{22}$ −20.0° (c=0.36, $CHCl_3$); IR (film): 3584, 2928, 1464, 1389, 1258, 1103, 1080, 837, 775 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 3.42–3.33 (2H, m, H-13 and H-14), 1.98–1.00 (16H, m), 0.89 (9H, s, ^tBuMe$_2$Si–), 0.86, 0.84 and 0.84 (3Me, s each, Me-18, Me-19 and Me-20), 1.00–0.85 (1H, m), 0.06 (6H, s, ^{t}BuMe_2Si–); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 42.7 (C-1), 19.5 (C-2), 44.2 (C-3), 33.5 (C-4), 47.8 (C-5), 16.3 (C-6), 22.4 (C-7), 36.7 (C-8), 46.3 (C-9), 36.7 (C-10), 20.1 (C-11), 32.6 (C-12), 73.2 (C-13), 77.4 (C-14), 34.1 (C-18), 22.7 (C-19), 18.1 (C-20), 26.1 (Me$_3$*C*Si–), 26.1 (*Me*$_3$CSi–), −3.8 and −4.4 (*Me*$_2$Si–).

4.50. Reaction of 53 with NaMeO/MeOH: 63

To a solution of NaMeO/MeOH (2 M) (prepared by adding sodium (50 mg, 2.17 mmol) to MeOH (1.1 mL)), was added at room temperature a solution of **53** (50 mg, 0.132 mmol) in MeOH (3 mL). After 12 h the reaction mixture was diluted with AcOEt and then treated with a saturated aqueous solution of NH_4Cl (5 mL), extracted with AcOEt and washed with water and brine. The organic layer was dried over Na_2SO_4 and evaporated to give a crude (43 mg) which was chromatographed on silica gel (DCM) to give **62** (38 mg, 76%).

4.50.1. 13α-*t*-Butyldimethylsilyloxy-podocarpan-14-one: 63. $[\alpha]_D^{22}$ −33.9° (c=0.43, $CHCl_3$); IR (film): 2942, 2857, 1728, 1254, 1159 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 4.10 (1H, dd, J=12.2, 6.4 Hz, H-13), 2.27–2.20 (1H, m, H_A-12), 2.20 (1H, dt, J=13.4, 4.0 Hz, H-8), 1.92 (1H, dq, J=13.4, 3.6 Hz, H-7β), 1.83 (1H, dq, J=12.8, 2.7 Hz, H-11α), 1.76–1.66 and 0.97–0.87 (2H, m, H-1), 1.71–1.61 (1H, m, H_B-12), 1.67–1.57 (2H, m, H-2), 1.50–1.40 (1H, m, H-7α), 1.47–1.37 (1H, m, H-11β), 1.48–1.40 (2H, m, H-6), 1.43–1.33 and 1.15–1.08 (2H, m, H-3), 1.16–1.06 (1H, m, H-9), 0.92 (3H, s, Me-20), 0.89 (9H, s, ^tBuMe$_2$Si–), 0.85 (3H, s, Me-18), 0.82 (3H, s, Me-19), 0.82–0.72 (1H, m, H-5), 0.13 and 0.01 (3H, s each, ^{t}BuMe_2Si–); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 39.2 (C-1), 20.4 (C-2), 41.8 (C-3), 33.1 (C-4), 54.8 (C-5), 18.8 (C-6), 26.1 (C-7), 47.8 (C-8), 57.0 (C-9), 37.5 (C-10), 22.6 (C-11), 35.9 (C-12), 77.0 (C-13), 210.8 (C-14), 33.5 (C-18), 21.8 (C-19), 13.8 (C-20), 25.8 (Me$_3$*C*Si–), 25.8 (*Me*$_3$CSi–), −5.4 and −4.6 (*Me*$_2$Si–); EIMS *m*/*z* (%): 363 (M^+ −15, 5), 321 (100), 229 (10), 183 (12), 129 (11), 75 (27).

4.51. Reaction of 63 with isopropylmagnesium chloride: 64

To a solution of **63** (28 mg, 0.074 mmol) in dry THF (1 mL) was added isopropylmagnesium chloride 2 M in Et_2O (10 mL, 20 mmol), at 0 °C, under an argon atmosphere. The reaction mixture was stirred for 90 min at room temperature and then treated with a saturated NH_4Cl aqueous solution, extracted with EtOAc and washed with water and brine. The organic layer was dried over Na_2SO_4 and evaporated to yield compound **64** (31 mg, 99%).

4.51.1. 13α-*t*-Butyldimethylsilyloxy-totaran-14α-ol: 64. $[\alpha]_D^{22}$ −2.1° (c=0.77, $CHCl_3$); IR (film): 3567, 2949, 1472, 1387, 1362, 1256, 1071, 837, 775 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 3.52 (1H, dd, J=10.3, 4.4 Hz, H-13), 2.25–2.15 (1H, m, H-15), 1.88–1.78 (2H, m, H-7), 1.73–1.63 and 0.95–0.85 (2H, m, H-1), 1.60–1.58 (8H, m, H-2, H-6, H-11 and H-12), 1.47–1.37 (1H, m, H-8), 1.40–1.30 and 1.17–1.07 (2H, m, H-3), 1.25–1.15 (1H, m, H-9), 1.01 (3H, d, J=7.3 Hz, H-16), 0.95 (3H, d, J=7.2 Hz, H-17), 0.90 (9H, s, ^tBuMe$_2$Si–), 0.88–0.78 (1H, m, H-5), 0.83 (3H, s, Me-18), 0.80 (3H, s, Me-19), 0.79 (3H, s, Me-20), 0.06 and 0.05 (3H, s each, ^{t}BuMe_2Si–); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 39.5 (C-1), 18.9 (C-2), 42.0 (C-3), 33.0 (C-4), 53.8 (C-5), 21.5 (C-6), 27.1 (C-7), 39.5 (C-8), 48.8 (C-9), 36.7 (C-10), 22.6 (C-11), 31.0 (C-12), 73.5 (C-13), 77.0 (C-14), 32.0 (C-15), 18.5 and 18.0 (C-16 and C-17), 33.4 (C-18), 21.8 (C-19), 14.0 (C-20), 25.9

(Me_3CSi–), 25.8 (Me_3CSi–), −3.6 and −4.6 (Me_2Si–); EIMS, *m/z* (%): 422 (M^+, 2), 379 (52), 321 (11), 273 (22), 231 (36), 147 (30), 75 (100); EIHRMS: calcd for $C_{26}H_{50}O_2Si$ (M^+) 422.3580, found (M^+) 422.3586.

4.52. Reaction of 64 with TBAF: 34

To compound **64** (50 mg, 0.120 mmol) TBAF 1.0 M in THF (1 mL) was added. The mixture was stirred under an argon atmosphere for 3 h and diluted with water. The solution was extracted with AcOEt, and washed with water and brine. The organic layer was dried over Na_2SO_4 and evaporated to give a crude product which was chromatographed on silica gel (*n*-hexane/AcOEt, 95/5) to yield the diol **34** (35 mg, 95%).

4.53. Reaction of 54 with NaMeO/MeOH: 65

To a solution of NaMeO/MeOH (2 M), prepared by addition of sodium (75 mg, 3.26 mmol) to MeOH (1.6 mL), a solution of **54** (90 mg, 0.240 mmol) in MeOH (10 mL) was added at room temperature. After 4 h the reaction mixture was diluted with AcOEt and then treated with a saturated NH_4Cl aqueous solution (5 mL), extracted with AcOEt and washed with water and brine. The organic layer was dried over Na_2SO_4 and evaporated to give a crude (85 mg) which was chromatographed on silica gel (DCM) to give **65** (65 mg, 72%).

4.53.1. 14β-*t*-Butyldimethylsilyloxy-*8-epi*-podocarpan-13-one: 65. $[\alpha]_D^{22}$ −29.7° (*c*=0.81, $CHCl_3$); IR (film): 2944, 2053, 1732, 1472, 1389, 1362, 1254, 1136, 974, 868, 837, 779 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 3.81 (1H, d, *J*=10.4 Hz, H-14), 2.40–1.00 (16H, m), 1.00–0.80 (1H, m), 0.91 (9H, s, $^tBuMe_2Si$–), 0.88, 0.83 and 0.81 (3Me, s each, Me-18, Me-19 and Me-20), 0.13 and 0.03 (3H, s each, $^tBuMe_2Si$–); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 39.5 (C-1), 18.8 (C-2), 42.0 (C-3), 32.8 (C-4), 54.9 (C-5), 21.2 (C-6), 32.8 (C-7), 46.4 (C-8), 53.1 (C-9), 37.1 (C-10), 26.1 (C-11), 39.3 (C-12), 209.5 (C-13), 82.1 (C-14), 33.6 (C-18), 21.8 (C-19), 14.1 (C-20), 25.8 (Me_3CSi–), 25.8 (Me_3CSi–), −4.3 and −5.7 (Me_2Si–); EIMS *m/z* (%): 378 (M^+, 2), 363 (6), 321 (100), 197 (7), 129 (20), 75 (18); EIHRMS: calcd for $C_{23}H_{42}O_2Si$ (M^+) 378.2954, found (M^+) 378.2946.

4.54. Reaction of 54 with vinylmagnesium chloride: 66

To a solution of **65** (117 mg, 0.308 mmol) in dry THF (2 mL) was added vinylmagnesium bromide 1 M in Et_2O (10 mL, 3 mmol), at 0 °C, under an argon atmosphere. The reaction mixture was stirred for 15 min at room temperature and then treated with a saturated NH_4Cl aqueous solution, extracted with EtOAc and washed with water and brine. The organic layer was dried and evaporated to give a crude mixture, which was chromatographed on silica gel (DCM) to yield the vinyl derivative (93 mg). To a solution of TBAF 1.0 M in THF (2 mL), the latter compound (93 mg) was added. The mixture was stirred under an argon atmosphere for 1 h and diluted with water. The solution was extracted with AcOEt, washed with water and brine. The organic layer was dried over Na_2SO_4 and evaporated to give a crude which was chromatographed on silica gel (*n*-hexane/AcOEt, 7/3) to yield **66** (82 mg, 91%).

4.54.1. 17-nor-8-*epi*-15-Pimaren-13α,14α-diol 66. $[\alpha]_D^{22}$ +25.1° (*c*=0.71, $CHCl_3$); IR (film): 3387, 2932, 1458, 1387, 1368, 1042 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 5.87 (1H, dd, *J*=17.3, 10.7 Hz, H-15), 5.38 (1H, dd, *J*=17.3, 1.4 Hz, H_A-16), 5.18 (1H, dd, *J*=10.7, 1.4 Hz, H_B-16), 3.80 (1H, d, *J*=11.8 Hz, H-14), 2.26 (1H, s, –OH), 2.25–2.15 (1H, m, H_A-7), 2.06 (1H, dddd, *J*=11.7, 10.4, 6.0, 3.0 Hz, H-8), 1.90–1.80 and 0.92–0.82 (2H, m, H-1), 1.83-1.68 (2H, m, H-11), 1.78–1.68 and 1.62–1.52 (2H, m, H-12), 1.60–1.50 (1H, m, H-9), 1.58–1.47 and 1.32–1.22 (2H, m, H-6), 1.48–1.45 (1H, m, H_B-7), 1.45–1.35 and 1.20–1.10 (2H, m, H-3), 1.43–1.33 (2H, m, H-2), 1.03 (3H, s, Me-20), 0.88–0.78 (1H, m, H-5), 0.87 (3H, s, Me-18), 0.83 (3H, s, Me-19); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 40.6 (C-1), 18.6 (C-2), 41.9 (C-3), 33.1 (C-4), 56.0 (C-5), 18.3 (C-6), 27.2 (C-7), 36.5 (C-8), 49.0 (C-9), 39.0 (C-10), 19.7 (C-11), 33.9 (C-12), 74.2 (C-13), 71.6 (C-14), 144.6 (C-15), 113.9 (C-16), 34.0 (C-18), 21.9 (C-19), 19.2 (C-20); EIMS *m/z* (%): 292 (M^+, 8), 264 (26), 220 (38), 191 (18), 137 (60), 109 (53), 95 (70), 69 (78), 55 (100); EIHRMS: calcd for $C_{19}H_{32}O_2$ (M^+) 292.2402, found (M^+) 292.2398.

4.55. Reaction of 54 with allylmagnesium chloride: 67 and 68

To a solution of **54** (108 mg, 0.240 mmol) in dry THF (16 mL) was added allylmagnesium bromide 1 M in Et_2O (12 mL, 12 mmol), at 0 °C, under an argon atmosphere. The reaction mixture was stirred for 1 h at room temperature and then treated with a saturated NH_4Cl aqueous solution, extracted with EtOAc and washed with water and brine. The organic layer was dried (Na_2SO_4) and evaporated to give a crude mixture, which was chromatographed on silica gel (*n*-hexane/AcOEt, 9/1) to yield the corresponding allyl derivatives (82 mg). To a solution of TBAF 1.0 M in THF (2 mL), the latter compounds (82 mg) were added. The mixture was stirred under an argon atmosphere for 90 min and diluted with water. The solution was extracted with AcOEt, and washed with water and brine. The organic layer was dried over Na_2SO_4 and evaporated to give a crude product which was chromatographed on silica gel (*n*-hexane/AcOEt, 7/3) to yield **67** (65 mg, 88%) and **68** (3 mg, 3%).

4.55.1. 8-*epi*-15(17→16)-abeo-Abiet-16-en-13α,14α-diol: 67. $[\alpha]_D^{22}$ +23.3° (*c*=0.36, $CHCl_3$); IR (film): 3435, 2924, 2866, 2361, 1456, 1038 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 5.96–5.78 (1H, m, H-16), 5.16–5.11 (2H, m, H-17), 3.69 (1H, d, *J*=9.4 Hz, H-14), 2.42 (1H, dd, *J*=14.9, 7.8 Hz, H_A-15), 2.30 (1H, dd, *J*=14.9, 7.7 Hz, H_B-15), 2.25–2.15 (2H, m, H-7), 1.92 (1H, dddd, *J*=9.4, 9.0, 6.0, 3.0 Hz, H-8), 1.87–1.77 and 0.92–0.82 (2H, m, H-1), 1.80–1.70 and 1.60–1.50 (6H, m, H-2, H-11 and H-12), 1.50–1.40 (3H, m, H-6 and H-9), 1.45–1.35 and 1.15–1.05 (2H, m, H-3), 0.98 (3H, s, Me-20), 0.89–0.79 (1H, m, H-5), 0.87 (3H, s, Me-18), 0.84 (3H, s, Me-19); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 40.6 (C-1), 18.6 (C-2), 41.9 (C-3), 33.0 (C-4), 56.0 (C-5), 18.3 (C-6), 27.2 (C-7), 38.0 (C-8), 49.0 (C-9), 38.9 (C-10), 19.9 (C-11), 33.2 (C-12), 72.8 (C-13), 71.8 (C-14), 45.2 (C-15), 134.0 (C-16), 118.6 (C-17), 34.0 (C-18), 21.9 (C-19), 19.0 (C-20); EIMS *m/z* (%): 265 (M^+ −41, 100), 247 (36), 229 (64), 205 (22), 177

(12), 153 (44), 123 (42), 77 (80); EIHRMS: calcd for $C_{20}H_{34}O_2Si$ (M^+) 306.2559, found (M^+) 306.2552.

4.55.2. 8-*epi*-15(17→16)-abeo-Abiet-16-en-13β,14α-diol: 68. $[\alpha]_D^{22}$ +9.3° (c=1.28, $CHCl_3$); IR (film): 3397, 2936, 2868, 1638, 1456, 1389, 1368, 1263, 1053, 1028, 995, 972, 909, 741 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 5.88 (1H, dddd, J=17.3, 10.1, 7.6, 7.2 Hz, H-16), 5.15 (1H, dd, J=10.1, 1.8 Hz, H_A-17), 5.12 (1H, dd, J=17.3, 1.8 Hz, H_B-17), 3.85 (1H, d, J=12.3 Hz, H-14), 2.43 (1H, dd, J=14.0, 7.2 Hz, H_A-15), 2.35 (1H, dd, J=14.0, 7.6 Hz, H_B-15), 2.25–2.15 (1H, m, H-7β), 1.92–1.80 (3H, m, H_A-1, H-8 and H_A-11), 1.73–1.41 (4H, m, H_A-2 and H_B-11 and H-12), 1.51–1.48 (1H, m, H-9), 1.50–1.25 (5H, m, H_B-2, H_A-3, H-6 and H-7α), 1.24–1.08 (1H, m, H_B-3), 1.05 (3H, s, Me-20), 0.88–0.78 (2H, m, H_B-1 and H-5), 0.85 (3H, s, Me-18), 0.83 (3H, s, Me-19); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 40.8 (C-1), 18.7 (C-2), 41.9 (C-3), 33.1 (C-4), 55.6 (C-5), 18.1 (C-6), 26.8 (C-7), 38.7 (C-8), 48.5 (C-9), 39.0 (C-10), 21.5 (C-11), 33.4 (C-12), 75.3 (C-13), 75.5 (C-14), 34.6 (C-15), 133.9 (C-16), 118.8 (C-17), 33.9 (C-18), 22.0 (C-19), 19.1 (C-20); EIMS m/z (%): 306 (M^+, 3), 288 (4), 265 (62), 247 (21), 229 (40), 177 (8), 123 (34), 95 (40), 69 (100); EIHRMS: calcd for $C_{20}H_{34}O_2Si$ (M^+) 306.2559, found 306.2550.

4.56. Reaction of 67 with *p*-TsOH: 69

A solution of *p*-toluensulfonic acid in benzene 0.12 M (2 mL) was added to compound **67** (16 mg, 0.055 mmol) and stirred under an argon atmosphere for 24 h. at 60 °C. After that the mixture was diluted with AcOEt and water, extracted with AcOEt, and washed with 5% $NaHCO_3$, H_2O and brine. It was then dried over Na_2SO_4, filtered and the solvent evaporated. The crude product (15 mg) was chromatographed on silica gel, eluting with *n*-hexane/benzene (9/1) giving **69** (12 mg, 73%).

4.56.1. 13*R*-15(17→16)-abeo-Abiet-16-en-14-one: 69. $[\alpha]_D^{22}$ −6.8° (c=0.77, $CHCl_3$); IR (film): 1709, 1462, 1445, 1389, 1260, 1036, 910, 802 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 5.76 (1H, dddd, J=19.4, 14.5, 9.1, 2.3 Hz, H-16), 5.02 (1H, dd, J=14.5, 1.5 Hz, H_A-17), 4.99 (1H, dd, J=2.3, 1.5 Hz, H_B-17), 2.65–2.55 (2H, m, H-15), 2.24 (1H, dt, J=12.2, 4.6 Hz, H-8), 2.29–2.19 (1H, m, H-13), 2.19–2.09 and 1.23–1.13 (2H, m, H-12), 1.95–1.85 and 1.45–1.35 (2H, m, H-7), 1.85–1.75 and 1.38–1.12 (2H, m, H-3), 1.85–1.75 and 1.18–1.08 (2H, m, H-11), 1.75–1.65 and 0.95–0.85 (2H, m, H-1), 1.74–1.54 (4H, m, H-2 and H-6), 1.18–1.08 (1H, m, H-9), 0.93 (3H, s, Me-20), 0.87 (3H, s, Me-18), 0.83 (3H, s, Me-19), 0.83–0.73 (1H, m, H-5); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 39.2 (C-1), 18.9 (C-2), 41.9 (C-3), 33.1 (C-4), 58.1 (C-5), 20.5 (C-6), 26.3 (C-7), 49.6 (C-8), 54.4 (C-9), 37.6 (C-10), 24.3 (C-11), 32.8 (C-12), 49.9 (C-13), 213.8 (C-14), 33.6 (C-15), 136.8 (C-16), 115.9 (C-17), 33.5 (C-18), 21.8 (C-19), 13.8 (C-20); EIMS m/z (%): 288 (M^+, 100), 267 (8), 203 (10), 177 (25), 109 (82), 95 (58), 69 (85); EIHRMS: calcd for $C_{20}H_{32}O$ (M^+) 288.2453, found 288.2458.

4.57. Preparation of 70

To a solution of **69** (58 mg, 0.201 mmol) in THF (0.5 mL), KHMDS 0.5 M in toluene (2 mL, 1 mmol) was added at −78 °C, under an argon atmosphere. After 5 min distilled MeI (5 mL, 3.21 mmol) was added. The mixture was stirred for 24 h After that, 5% $NaHCO_3$ was added, the mixture extracted with AcOEt, washed with 5% $NaHCO_3$, H_2O and brine, dried over Na_2SO_4, filtered and the solvent evaporated. The crude mixture (65 mg) was chromatographed on silica gel (13 g), eluting with *n*-hexane/AcOEt (100/0.5 mL) to give **70** (57 mg, 97%).

4.57.1. 15a-Homo-15-isopimaren-14-one: 70. $[\alpha]_D^{22}$ −29.0° (c=0.31, $CHCl_3$); IR (film): 2938, 2868, 1703, 1638, 1458, 1387, 1370, 1260, 1098, 912 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 5.77 (1H, dddd, J=19.4, 14.5, 9.1, 2.3 Hz, H-15), 5.02 (1H, dd, J=14.5, 1.5 Hz, H_A-16), 4.99 (1H, dd, J=2.3, 1.5 Hz, H_B-16), 2.45 (1H, dt, J=11.9, 4.3 Hz, H-8), 2.47–2.43 (1H, m, H_A-15a), 2.18–2.05 (1H, m, H_B-15a), 2.00–1.00 (15H, m), 1.10 (3H, s, Me-17), 1.00–0.80 (1H, m), 0.93, 0.83 and 0.81 (3Me, s each, Me-18, Me-19 and Me-20); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 42.2 (C-1), 19.1 (C-2), 43.1 (C-3), 33.4 (C-4), 57.3 (C-5), 20.1 (C-6), 27.1 (C-7), 45.3 (C-8), 54.6 (C-9), 37.7 (C-10), 20.9 (C-11), 37.2 (C-12), 47.1 (C-13), 217.2 (C-14), 39.4 (C-15a), 135.5 (C-15), 117.6 (C-16), 22.1 (C-17), 33.7 (C-18), 23.2 (C-19), 14.1 (C-20). EIMS m/z (%): 302 (M^+, 100), 287 (33), 244 (12), 177 (30), 123 (93), 95 (56), 69 (83); EIHRMS: calcd for $C_{21}H_{34}O$ (M^+) 302.2610, found 302.2602.

Acknowledgements

The authors thank the Spanish DGI for financial support (BQU2002-02049), and the Spanish Ministerio de Educación y Cultura for a doctoral fellowship to S. C. M. and to M. A. C and Junta de Castilla y León for financial support (SA072/03).

References and notes

1. Hanson, J. R. *Nat. Prod. Rep.* **2003**, *20*, 70 and references cited therein.
2. (a) Schaller, F.; Wolfender, J.-L.; Hostettmann, K. *Helv. Chim. Acta* **2001**, *84*, 222. (b) Scaller, F.; Rahalison, L.; Islam, N.; Potterat, O.; Hostettmann, K.; Stoeckli-Evans, H.; Mavi, S. *Helv. Chim. Acta* **2000**, *83*, 407. (c) Hostettmann, K.; Terreaux, C. *Chimia* **2000**, *54*, 652. (d) Hostettmann, K.; Schaller, F. *Antimicrobial Diterpenes*, United States Patent No. 5,929,124, 2000. (e) Rasoamiaranjanahary, L.; Guilet, D.; Marston, A.; Randimbivololona, F.; Hostettmann, K. *Phytochemistry* **2003**, *64*, 543.
3. (a) Kim, Y.-H.; Chung, B. S.; Sankara, U. *J. Nat. Prod.* **1988**, *51*, 1080. (b) Kang, H.-S.; Kim, Y.-H.; Lee, C.-S.; Lee, J.-J.; Choi, I.; Pyun, K.-H. *Cellular Immunol.* **1996**, *170*, 212. (c) Kang, H.-S.; Song, H. K.; Lee, J.-J.; Pyun, K.-H.; Choi, I. *Mediators Inflamm.* **1998**, *7*, 257.
4. (a) Chien, S.-C.; Kuo, Y.-H. *Helv. Chim. Acta* **2004**, *87*, 554. (b) Kuo, Y.-H.; Chien, S.-C.; Kuo, C.-C. *Planta Medica* **2001**, *68*, 1020. (c) Haraguchi, H.; Ishikawa, H.; Kubo, I. *Planta Medica* **1997**, *63*, 213.

5. (a) Woldemichael, G. M.; Wáchter, G.; Singh, M. P.; Maise, W. M.; Timmermann, B. N. *J. Nat. Prod.* **2003**, *66*, 242. (b) Ulubelen, A.; Öksüz, S.; Kolak, U.; Bosk-Johansson, C.; Çelik, C.; Voelter, W. *Planta Medica* **2000**, *66*, 458.
6. (a) Iwamoto, M.; Ohtsu, H.; Tokuda, H.; Nishino, H.; Matsunaga, S.; Tanaka, R. *Bioorg. Med. Chem.* **2001**, *9*, 1911. (b) Awale, S.; Tezuda, Y.; Banskota, A. H.; Shimoji, S.; Taira, K.; Kadota, S. *Tetrahedron* **2002**, *58*, 5503. (c) Sairafianpour, M.; Christensen, J.; Stærk, D.; Budnik, B. A.; Kharazmi, A.; Bagherzadeh, K.; Jaroszewski, J. W. *J. Nat. Prod.* **2001**, *64*, 1398. (d) Mei, S.-X.; Jiang, B.; Niu, X.-M.; Li, M.-L.; Yang, H.; Na, Z.; Lin, Z.-W.; Li, C.-M.; Sun, H.-D. *J. Nat. Prod.* **2002**, *65*, 633. (e) Minami, T.; Wada, S.; Tokuda, H.; Tanaabe, G.; Muraoka, O.; Tanaka, R. *J. Nat. Prod.* **2002**, *65*, 1921. (f) Chen, X.; Ding, J.; Ye, Y.-M.; Zang, J.-S. *J. Nat. Prod.* **2002**, *65*, 1016.
7. Ziegler, H. L.; Jensen, T. H.; Christensen, J.; Stærk, D.; Hägerstrand, H.; Sittie, A. A.; Olsen, C. E.; Staalsø, T.; Ekpe, P.; Jaroszewski, J. W. *Planta Medica* **2002**, *68*, 547.
8. (a) Kolak, U.; Ari, S.; Birman, H.; Hasançebi, S.; Ulubelen, A. *Planta Medica* **2001**, *67*, 761. (b) Ulubelen, A.; Birman, H.; Óksüz, S.; Topçu, G.; Kolak, U.; Barla, A.; Voelter, W. *Planta Medica* **2002**, *68*, 821. (c) Ulubelen, A.; Öksüz, S.; Kolak, U.; Voelter, W. *Planta Medica* **2000**, *66*, 627.
9. (a) Tada, M.; Okuno, K.; Chiba, K.; Ohnishi, E.; Yoshii, Y. *Phytochemistry* **1994**, *35*, 539. (b) Batista, O.; Simoes, M. F.; Duarte, A.; Valdeira, M. L.; De la Torre, M. C.; Rodriguez, B. *Phytochemistry* **1995**, *38*, 167.
10. Awale, S.; Tezuka, Y.; Banskota, A. H.; Adnyana, I. K.; Kadota, S. *J. Nat. Prod.* **2003**, *66*, 255.
11. Yajima, A.; Mori, K. *Eur. J. Org. Chem.* **2000**, *65*, 4079.
12. (a) For a comprehensive review on the synthesis of diterpenes, see: ApSimon, J. In *The Total Synthesis of Natural Products*, *Vol. 8*; Wiley: New York, 1990. See also: (b) Ling, T.; Chowdhury, C.; Kramer, B. A.; Vong, B. G.; Palladino, M. A.; Theodorakis, E. A. *J. Org. Chem.* **2001**, *66*, 8843. (c) Yahima, A.; Mori, K.; Yabuta, G. *Tetrahedron Lett.* **2004**, *45*, 167. (d) Zambrano, J. L.; Rosales, V.; Nakano, T. *Tetrahedron Lett.* **2003**, *44*, 1859. (e) Li, A.; She, X.; Zhang, J.; Wu, T.; Pan, X. *Tetrahedron* **2003**, *59*, 5737. (f) Lee, H.-J.; Ravn, M. M.; Coates, R. M. *Tetrahedron* **2001**, *57*, 6155. (g) Ling, T.; Krameer, B. A.; Palladino, M. A.; Theodorakis, E. A. *Org. Lett.* **2000**, *14*, 2073. (h) Gan, Y.; Li, A.; Pan, X.; Chan, A. S. C.; Yang, T.-K. *Tetrahedron: Asymmetry* **2000**, *11*, 781. (i) Yahima, A.; Mori, K. *Tetrahedron Lett.* **2000**, *41*, 351. (j) Yamamura, I.; Fujiwara, Y.; Yamato, T.; Irie, O.; Shishido, K. *Tetrahedron Lett.* **1997**, *38*, 4121. (k) Danheiser, R. L.; Casebier, D. S.; Firooznia, F. *J. Org. Chem.* **1995**, *60*, 8341. (l) Weibel, J.-M.; Heisller, H. *Synlett* **1993**, 391. (m) Yee, N. K. N.; Coates, R. M. *J. Org. Chem.* **1992**, *57*, 4598. (n) Chu, M.; Coates, R. M. *J. Org. Chem.* **1992**, *57*, 4590. (o) Abad, A.; Arno, M.; Peiro, M.; Zaragozá, R. J. *Tetrahedron* **1991**, *47*, 3829.
13. Pascual Teresa, J. de; Urones, J. G.; Marcos, I. S.; Díez, D.; Alvarez, V. *Phytochemistry* **1986**, *25*, 711.
14. (a) Marcos, I. S.; Moro, R. F.; Carballares, S.; Urones, J. G. *Synlett* **2000**, 541. (b) Urones, J. G.; Marcos, I. S.; Pérez, B. G.; Lithgow, A.; Díez, D.; Gómez, P. M.; Basabe, P.; Garrido, N. M. *Tetrahedron* **1995**, *51*, 1845. (c) Urones, J. G.; Díez, D.; Gómez, P. M.; Marcos, I. S.; Basabe, P.; Moro, R. F. *J. Chem. Soc., Perkin Trans. 1* **1997**, 1815. (d) Urones, J. G.; Díez, D.; Gómez, P. M.; Marcos, I. S.; Basabe, P.; Moro, R. F. *Nat. Prod. Lett.* **1998**, *11*, 145. (e) Urones, J. G.; Marcos, I. S.; Pérez, B. G.; Lithgow, A.; Díez, D.; Basabe, P.; Gómez, P. M. *Tetrahedron Lett.* **1994**, *35*, 3781. (f) Urones, J. G.; Marcos, I. S.; Pérez, B. G.; Díez, D.; Lithgow, A.; Gómez, P. M.; Basabe, P.; Garrido, N. M. *Tetrahedron* **1994**, *37*, 10995.
15. Marcos, I. S.; Moro, R. F.; Carballares, S.; Urones, J. G. *Tetrahedron Lett.* **1999**, *40*, 2615.
16. Marcos, I. S.; Laderas, M.; Díez, D.; Basabe, P.; Moro, R. F.; Garrido, N. M.; Urones, J. G. *Tetrahedron Lett.* **2003**, *44*, 5419.
17. Marcos, I. S.; Moro, R. F.; Carballares, S.; Urones, J. G. *Synlett* **2002**, 458.
18. Marcos, I. S.; Cubillo, M. A.; Moro, R. F.; Díez, D.; Basabe, P.; Sanz, F.; Urones, J. G. *Tetrahedron Lett.* **2003**, *44*, 8831.
19. (a) Haines, A. H. Methods for the Oxidation of Organic Compounds. In *Alkanes, Alkenes, Alkynes and Arenes*; Academic: London, 1985; p 73. (b) VanRheenen, V.; Kelly, R. C.; Cha, D. Y. *Tetrahedron Lett.* **1976**, 1973. (c) VanRheenen, V.; Cha, D. Y.; Hartley, W. M. *Org. Synth.* **1979**, *58*, 43. (d) Ray, R.; Matteson, D. S. *Tetrahedron Lett.* **1980**, *21*, 449. (e) Schroder, M. *Chem. Rev.* **1980**, *80*, 187. (f) Schmaker, J. M.; Travis, B. R.; Borhan, B. *Org. Lett.* **2003**, *5*, 3089. (g) Ainai, T.; Wang, Y. G.; Tokoro, Y.; Kobayashi, Y. *J. Org. Chem.* **2004**, *69*, 655.
20. (a) Wolf, F. J.; Weijlard, J. *Org. Synth., Coll.* **1963**, *4*, 12. (b) Hale, K. J.; Hough, L.; Richardson, A. C. *Tetrahedron Lett.* **1987**, *28*, 891. (c) Tanaka, T.; Ozawa, M.; Miura, T.; Inazu, T.; Tsuji, S.; Kajimoto, T. *Synlett* **2002**, 1487.
21. (a) Paquette, L. A. In *Reagents for Organic Synthesis*, *Vol. 6*; Wiley: New York, 1995; p 4162. (b) Nishimura, S. Handbook of Heterogeneous Catalytic Hydrogenation for Organic Syntesis; Wiley: New York, 2001.
22. (a) Winterfeldt, E. *Synthesis* **1975**, 617. (b) Yoon, N. M.; Gyoung, Y. S. *J. Org. Chem.* **1985**, *50*, 2443.
23. (a) Sasaki, M.; Murae, T.; Takahashi, T. *J. Org. Chem.* **1990**, *55*, 528. (b) Govindan, S. V.; Fuchs, P. L. *J. Org. Chem.* **1988**, *53*, 2593. (c) Kubo, H.; Ishii, K.; Koshino, H.; Toubetto, K.; Naruchi, K.; Yamasaki, R. *Eur. J. Org. Chem.* **2004**, 1202.
24. (a) Collins, J. C.; Hess, W. W.; Frank, F. J. *Tetrahedron Lett.* **1968**, 3363. (b) Ratcliffe, R.; Rodehorst, R. *J. Org. Chem.* **1970**, *35*, 4000. (c) Collins, J. C.; Hess, W. W. *Org. Synth.* **1972**, *52*, 5. (d) Schwartz, M. A.; Crowell, J. D.; Musser, J. H. *J. Am. Chem. Soc.* **1972**, *94*, 4361.
25. Paquette, L. A. In *Reagents for Organic Synthesis*, *Vol. 7*; Wiley: New York, 1995; p 4593.
26. Paquette, L. A. In *Reagents for Organic Synthesis*, *Vol. 4*; Wiley: New York, 1995; p 2926.
27. (a) Ohloff, G.; Vial, C. *Helv. Chim. Acta* **1980**, *63*, 1932. (b) Kiruchi, T.; Kadota, S.; Suehara, H.; Namba, T. *Tetrahedron Lett.* **1981**, *22*, 465. (c) Harding, K. E.; Clement, K. S. *J. Org. Chem.* **1984**, *49*, 2049. (d) He, J.-F.; Wu, Y.-L. *Tetrahedron* **1988**, *44*, 1933. (e) Nussbaumer, C.; Frater, G.; Kraft, P. *Helv. Chim. Acta* **1999**, *82*, 1016.
28. (a) Brownbridge, P. *Synthesis* **1981**, 1. (b) Corey, E. J.; Gross, A. W. *Tetrahedron Lett.* **1984**, *25*, 495.
29. (a) Marcos, I. S.; Moro, R. F.; Carballares, S.; Urones, J. G. *Tetrahedron* **2001**, *57*, 713. (b) Schröder, M. *Chem. Rev.* **1980**, *80*, 187. (c) Lohray, B. B. *Tetrahedron: Asymmetry* **1992**, *3*, 1317. (d) For a review, see: Haines, A. H. Addition Reactions with Formation of Carbon–Oxygen Bonds: (iii) Glycol Forming Reactions. In *Comprehensive Organic Synthesis*; Trost, B. M., Fleming, I., Eds.; Pergamon: Oxford, 1991; Vol. 7; p 437. (e) Thomas, A. F.; Vial, C.; Ozainne, M.; Ohloff, G. *Helv. Chim. Acta* **1973**, *56*, 2270.

30. (a) Shioiri, T.; Aoyama, T.; Mori, S. In Freeman, J. P., Ed.; Org. Synth. Coll.; Wiley: New York, 1993; Vol. VIII; p 612. (b) Miwa, K.; Aoyama, T.; Shioiri, T. *Synlett* **1994**, 107. (c) Paquette, L. A. Encyclopedia of Reagents for Organic Synthesis; Wiley: New York, 1995; p 5248. (d) Bergamin, P.; Bortolini, O.; Costa, E.; Pringle, P. G. *Inorg. Chim. Acta* **1996**, *252*, 33.
31. Ley, S. V.; Norman, J.; Griffith, W. P.; Marsden, S. P. *Synthesis* **1994**, 639.
32. X-ray for **15**. CCDC 213752.
33. (a) Elliott, M. C.; Gist, M. J.; Binns, F.; Jones, R. G. *Tetrahedron Lett.* **2004**, *45*, 2899. (b) Kumar, D. N.; Rao, B. V. *Tetrahedron Lett.* **2004**, *45*, 2227. (c) Guo, Z.; Schultz, A. G. *Tetrahedron Lett.* **2004**, *45*, 919. (d) Schäfer, A.; Schäfer, B. *Tetrahedron* **1999**, *55*, 12309. (e) Stork, G.; White, W. N. *J. Am. Chem. Soc.* **1959**, *78*, 4605.
34. (a) Concellón, J. M.; Bernard, P. L.; Huerta, M.; García-Granda, S.; Díaz, M. R. *Chem. Eur. J.* **2003**, *9*, 5343. (b) Concellón, J. M.; Rodriguez-Solla, H.; Bardales, E.; Huerta, M. *J. Org. Chem.* **2003**, 1775. (c) Shinohara, I.; Okue, M.; Yamada, Y.; Nagaoka, H. *Tetrahedron Lett.* **2003**, *44*, 4649. (d) Deng, Y.; Snyder, J. K. *J. Org. Chem.* **2002**, *67*, 2864. (e) Boiron, A.; Zillig, P.; Faber, D.; Giese, B. *J. Org. Chem.* **1998**, *63*, 5877. (f) Adinolfi, M.; Barone, G.; Iadonisi, A.; Mangoni, N. L. *Tetrahedron Lett.* **1998**, *39*, 2021. (g) Adinolfi, M.; Barone, G.; Iadonisi, A.; Mangoni, L.; Manna, R. *Tetrahedron Lett.* **1997**, *38*, 11767. (h) Kornienko, A.; Alarcao, M. *Tetrahedron Lett.* **1997**, *38*, 6497. (i) Molander;, G. A.; Harris;, C. R. *Chem. Rev.* **1996**, *96*, 307. (j) Chiara, J. L.; Martín-Lomas, M. *Tetrahedron Lett.* **1994**, *35*, 2969. (k) Uenishi, J.; Masuda, S.; Wakabayashi, S. *Tetrahedron Lett.* **1991**, *32*, 5097. (l) Chiara, J. L.; Cabri, W.; Hanessian, S. *Tetrahedron Lett.* **1991**, *32*, 1125. (m) Girard, P.; Namy, J. L.; Kagan, H. B. *J. Am. Chem. Soc.* **1980**, *9*, 2693. (n) Guidot, J. P.; Le Gall, T.; Mioskowski, C. *Tetrahedron Lett.* **1994**, *35*, 6671. (o) Howells, D. M.; Barker, S. M.; Watson, F. C.; Light, M. E.; Hursthouse, M. B.; Kilburn, J. D. *Org. Lett.* **2004**, *6*, 1943. (p) Patin, A.; Kanzawa, A.; Philouze, C.; Greene, A. E.; Muri, E.; Barreiro, E.; Costa, P. C. C. *J. Org. Chem.* **2003**, *68*, 3831. (q) Edmonds, D. J.; Johnston, D.; Procter, D. J. *Chem. Rev.* **2004**, *104*, 3371.
35. X-ray for **24**. A single crystal of compound **24** was subjected to X-ray diffraction studies on a Seifert 3003 SC rotating anode diffractometer with (Cu K_α) radiation (graphite monochromator) using 2θ–ω scans at 293(2) K. Crystal data for **24**: $C_{18}H_{32}O_2 \cdot 1/2H_2O$, $M=289.44$, orthorhombic, space group $C222_1$ (no. 20), $a=7.904(2)$ Å, $b=11.265(2)$ Å, $c=38.272(8)$ Å, $\alpha=\beta=\gamma=90°$. $V=3407.6(12)$ Å^3, $Z=8$, $D_c=1.128$ Mg/m^3, $m=$(Cu K_α)$=0.563$ mm^{-1}, $F(000)=1288$. A total of 2916 reflections were collected of which 1064 were assumed as observed by applying the condition $I>2\sigma(I)$. The structure was solved by direct method and the non-hydrogen atoms were refined anisotropically by full-matrix least squares. Hydrogen atoms were placed in calculated positions. The final R factors were $R_1=0.1064$ and ($R_2=0.2717$ for a total of 195 parameters. Crystallographic data (excluding structure factors) for this compound has been deposited at the Cambridge Crystallographic Data Centre as supplementary material no. CCDC 244343.
36. The two dimensional structure of **37** was built using the sketcher in Maestro v5.1.016, supplied by Schrodinger, Inc, Portland, OR, USA, coupled to Macromodel v. 8.1.031 from the same supplier. The structure of **37** was energy-minimised using TNCG optimisation with a maximum of 5000 iterations to default convergence, with the MMFF94S forcefield, and a conformational search was then set up using the Maestro automatic setup routine with default parameters and the MCMM/Lowmode mixed method, with 1000 trials and the same optimisation and potential settings as previously. A total of 84 unique conformations were found, of which the global minimum (absolute MMFF94s energy 380.94 kJ/mol, found 7 times) showed the all-chair conformation and the next minimum (0.88 kJ/mol higher in energy, found 7 times) showed the chair–boat–chair conformation. Analysis of the boat or chair conformations of rings B and C was performed by measurement of appropriate torsion angles, and graphing of these angles against the MMFF94s energy (relative to the minimum) observed for each conformer. Identical studies were carried out on structures derived from the minimum found for **37** by epimerisation at C8 only, at C8, C13 and C14, or at C14 only, using both the same in vacuo conditions as above and in separate runs with the CHCl3 GB/SA solvation model available in MacroModel. The dihedral angles between H8 and H14, and between H13 and H14, were measured in Maestro for all conformations within 50 kJ/mol of the global minimum for all four structures. With respect to this dihedral angle, the lowest-energy conformer, or in the case of **37** the two lowest-energy conformers (all chair and chair–boat–chair), were representative of all conformers with energy within 20 kJ/mol of the global minimum.
37. X-ray for **36**. Suitable single crystals were subjected to X-ray diffraction studies on a Seifert 3003 SC rotating anode diffractometer with (Cu K_α) radiation (graphite monochromator) using 2θ–ω scans at 293(2) K. Crystal data for **36**: $2(C_{17}H_{30}O_2)(H_2O$, $M=550.84$, orthorhombic, space group $P2_12_12_1$ (no. 19), $a=7.6676(3)$ Å, $b=10.7927(5)$ Å, $c=39.156(2)$ Å, $\alpha=\beta=\gamma=90°$, $V=3240.3(3)$ Å^3, $Z=4$, $D_c=1.129$ Mg/m^3, $m=$(Cu K_α) $=0.570$ mm^{-1}, $F(000)=1224$. 3358 reflections were collected, of which 3034 were considered to be observed with $I>2\sigma(I)$. The structure was determined by direct methods using the SHELXTL™ (1) suite of programs. Full-matrix least squares refinement based on F^2 with anisotropic thermal parameters for the non-hydrogen atoms led to agreement factors $R_1=0.0911$ and $\omega R_2=0.2819$. Crystallographic data (excluding structure factors) for this structure has been deposited at the Cambridge Crystallographic Data Centre as supplementary material no. CCDC 244342.
38. Mahn, D. D. K.; Fetizon, M.; Lazare, S. *Tetrahedron* **1978**, *34*, 1207.
39. (a) House, H.; Tefertiller, B.; Olmstead, H. *J. Org. Chem.* **1968**, *33*, 935. (b) House, H.; Bare, T. *J. Org. Chem.* **1968**, *33*, 943.
40. (a) Hattori, T.; Date, M.; Sakurai, K.; Morohashi, N.; Kosugi, H.; Miyano, S. *Tetrahedron Lett.* **2001**, *42*, 8035. (b) Ray, J. K.; Gupta, S.; Kar, G. K.; Roy, B. C.; Lin, J-M. ; Amin, S. *J. Org. Chem.* **2000**, *65*, 8134.
41. (a) Rastetter, W. H.; Nachbar, R. B.; Russo-Rodríguez, S.; Wattley, R. V. *J. Org. Chem.* **1982**, *47*, 4873. (b) Ando, M.; Wada, T.; Kusaka, H.; Takase, K.; Hirata, N.; Yanagi, Y. *J. Org. Chem.* **1987**, *52*, 4792. (c) Monsun, K.; Kawada, H.; Gross, R. S.; Watt, D. S. *J. Org. Chem.* **1990**, *55*, 504. (d) Urones, J. G.; Basabe, P.; Marcos, I. S.; Díez Martín, D.; Sexmero, M. J.; Peral, M. H. *Tetrahedron* **1992**, *48*, 10389. (e) Kim, J. H.; Lim, H. J.; Cheon, S. H. *Tetrahedron* **2003**, *59*, 7501.

42. (a) Theissen, R. J. *J. Org. Chem.* **1971**, *36*, 752. (b) Pelcman, B.; Gribble, G. W. *Tetrahedron Lett.* **1990**, *31*, 2381. (c) Nelson, P. H.; Nelson, J. T. *Synthesis* **1991**, 192.
43. Evans, G. B.; Furneaux, R. H.; Gravestock, M. B.; Lynch, G. P.; Scott, G. K. *Bioorg. Med. Chem.* **1999**, *7*, 1953.
44. (a) Paquette, L. A. In *Encyclopedia of Reagents for Organic Synthesis*, *Vol. 5*; Wiley: New York, 1995; p 3062. (b) Vidari, G.; Ferrino, S.; Grieco, P. A. *J. Am. Chem. Soc.* **1984**, *106*, 3539. (c) Wang, X.; Paquette, L. A. *Tetrahedron Lett.* **1993**, *34*, 4579.
45. Hodges, R. *J. Chem. Soc.* **1961**, 4247.
46. (a) Nakagaya, Y.; Irie, K.; Masuda, A.; Ohigashi, H. *Tetrahedron* **2002**, *58*, 2101. (b) Nicolaou, K. C.; Xu, J. Y.; Kim, S.; Pfefferkorn, J.; Ohshima, T.; Vourloumis, D.; Hosokawa, S. *J. Am. Chem. Soc.* **1998**, *120*, 8661. (c) Shing, T. K. M.; Zhu, X. Y.; Yeung, Y. Y. *Chem. Eur. J.* **2003**, *9*, 5489.
47. (a) Oliver, F. O.; Högenauer, K.; Simic, O.; Antonello, A.; Smith, M. D.; Ley, S. V. *Angew. Chem., Int. Ed.* **2003**, *42*, 5996. (b) Kumar, M. B. *Synlett* **2002**, 2125.
48. Marcos, I. S.; Pedrero, A. B.; Sexmero, M. J.; Díez, D.; Basabe, P.; Henández, F. A.; Urones, J. G. *Tetrahedron Lett.* **2003**, *44*, 369.
49. Gao, J. J.; Han, G. Q. *Phytochemistry* **1997**, *44*, 759.
50. (a) Dunetz, J. R.; Danheiser, R. L. *Org. Lett.* **2003**, *5*, 4011. (b) Klotz, P.; Mann, A. *Tetrahedron Lett.* **2003**, *44*, 1927.