

# Nonenzymic polycyclisation of analogues of oxidosqualene with a preformed C-ring†

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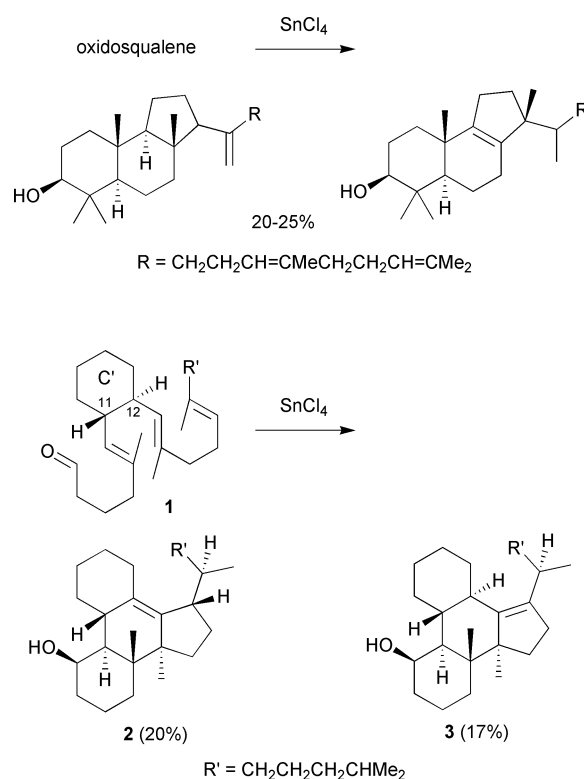
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Some nonenzymic epoxide-initiated polyolefin cyclisations are reported. The presented molecules are partially constrained analogues of (3*S*)-oxidosqualene, the natural substrate to many important cyclase enzymes. These model compounds feature a preformed C-ring with built-in stereochemical information. The experimental results allow for an instructive comparison with the enzymic processes, particularly those of the cyclases in steroid biosynthesis (*i.e.* lanosterol synthase).

## Introduction

From a synthetic point of view, the natural polycyclisation reactions of (3*S*)-2,3-oxidosqualene (OS) are arguably the most efficient single-step transformations known to man. The enzymes involved, which give rise to a broad range of complex tetra- and pentacyclic triterpenoids, have been shown to cyclise their linear substrate into either of the shown possible tetracyclic cationic intermediates (dammarenyl or protosteryl cation, see graphical overview in Scheme 2).<sup>1</sup> Next to the remarkable product specificity and selectivity of these carbocationic processes, their most striking feature is the six-membered C-ring. All known nonenzymic cationic OS cyclisations afford complex mixtures of structures with at most three carbocyclic rings and, invariably, a five-membered C-ring (Scheme 1, top).<sup>2</sup> Furthermore, the stereochemical discrepancies between the protosteryl and dammarenyl cation are completely localised in the C-ring area (inversion of every stereocenter) and are explained by different folding patterns of the polyene chain inside the enzyme cavity. A chair–chair–chair conformation is required to reach the dammarenyl cation, whereas the protosteryl cation is the result of an alternative chair–boat–chair fold. This prerequisite, first postulated in the Zürich proposal,<sup>3</sup> has received convincing experimental support from various areas.<sup>1a,3b</sup>

Biomimetic cationic olefin polycyclisations, as pioneered by Stork,<sup>4,5</sup> offer a good opportunity to expose the fundamental issues an OS cyclase has to address. In particular, several synthetic groups have aimed to (partly) emulate these intriguing biogenetic reactions by designing polyenes with incorporated cation-



Scheme 1

stabilizing (C-S) groups and/or conformational restrictions.<sup>5</sup> These modifications can be regarded as mimics of the role of the cyclase enzymes, which is still unclear. Most noteworthy are the achievements of William S. Johnson's group in efficient cationic tetra- and pentacyclisations by using initiating groups other than epoxides and various C-S substituents on the reacting alkenes, which also ensure six-membered C-ring formation.<sup>6</sup> Our group on the other hand has reported a few examples of nonenzymic polyene cyclisations leading to the familiar 'natural' 6 : 5 CD-ring system.<sup>7</sup> Interestingly, this did not require the use of any C-S groups, but merely resulted from restraining the possible conformations of the central part of the polyene chain (see for example aldehyde **1**<sup>7a</sup> in Scheme 1).

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† Electronic supplementary information (ESI) available: Experimental procedures and spectroscopic data, 2D NMR data for compounds **20**, **21a** and **22** and structural elucidation thereof, copies of selected NMR spectra and table and figures on X-ray diffraction and computational analysis of compound **21a**. CCDC reference number 675998. For crystallographic data in CIF or other electronic format and ESI see DOI: 10.1039/b801670d

The cyclohexane ring (designated as C'-ring) grafted on the polyene backbone of the model aldehyde **1** was designed to enforce a chair-like fold of the pre-C-ring 1,5-diene.<sup>7b</sup> Regrettably, these studies had to be limited to polyenes with a simplified initiating part, as the extraneous C'-ring seemed to interfere with the early cyclisation stages in the case of a full OS structure.<sup>8</sup> The influence of the single localised conformational restriction (imposed torsion around the C11,C12-bond, using classical steroid numbering) on the course of BCD-cyclisation was, however, striking (see polycyclised structures **2** and **3**). We felt compelled to further evaluate the possible effect of this conformational restriction on the full course of OS cyclisation (epoxide initiated ABCD(E)-cyclisation). It should be clear that, at least in a concerted pathway, the preorganisation of the pre-C-ring polyene part in either of the possible chair-like folds (defining a helical constraint relative to the epoxide, see Scheme 2) could be sufficient to direct the early AB-ring system formation (chair–chair or chair–boat cyclisation). This would mean that the entire stereochemical course of the enzymic OS tetracyclisation is essentially caused by a relatively minor and localised conformational restriction (such as the C11,C12 torsional angle).

For the present study, which was focused on the AB-ring system formation stage, new model compounds **4** and **5** were designed (Scheme 2). They feature a preformed C-ring that should secure the desired preorganisation of the central part of the OS polyene chain, by controlling the configurations of the C-ring stereocenters relative to the epoxide function. A conjugated diene in the preformed ring was considered to be the terminating nucleophile of choice. It should make AB-ring cyclisation effectively irreversible, assuring a true kinetic polycyclisation result. Furthermore, this diene can act as a relay for the cationic reaction center towards the D-ring cyclising stage, allowing for the formation of interesting dammarane or protolanostane skeletons. The diene system should also increase overall polycyclisation efficiency as an internal C-S group. Finally, these models have the advantage that no unnatural—possibly sterically hindering—substituents are present.

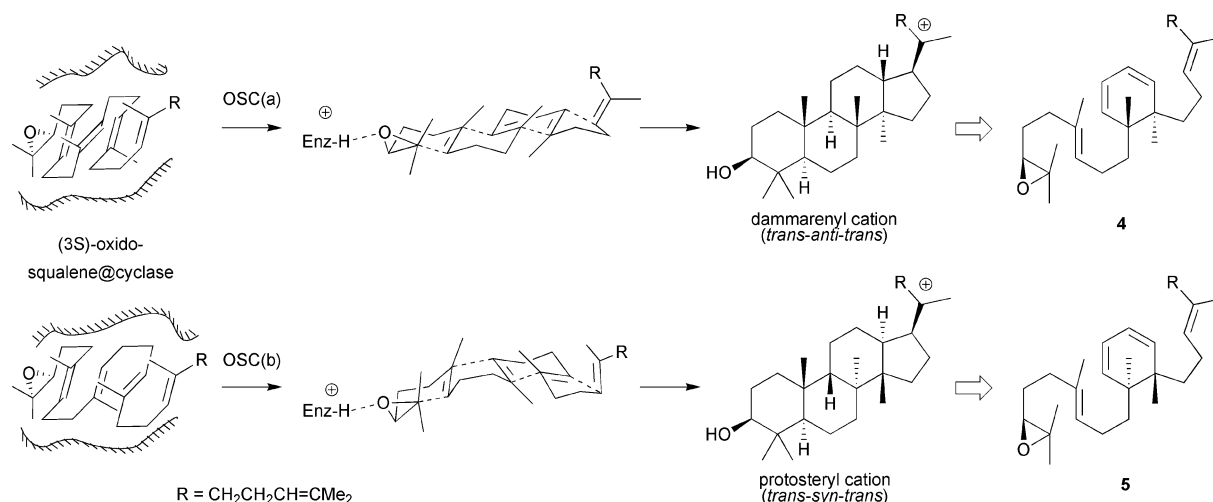
## Results and discussion

### Synthesis of model compounds

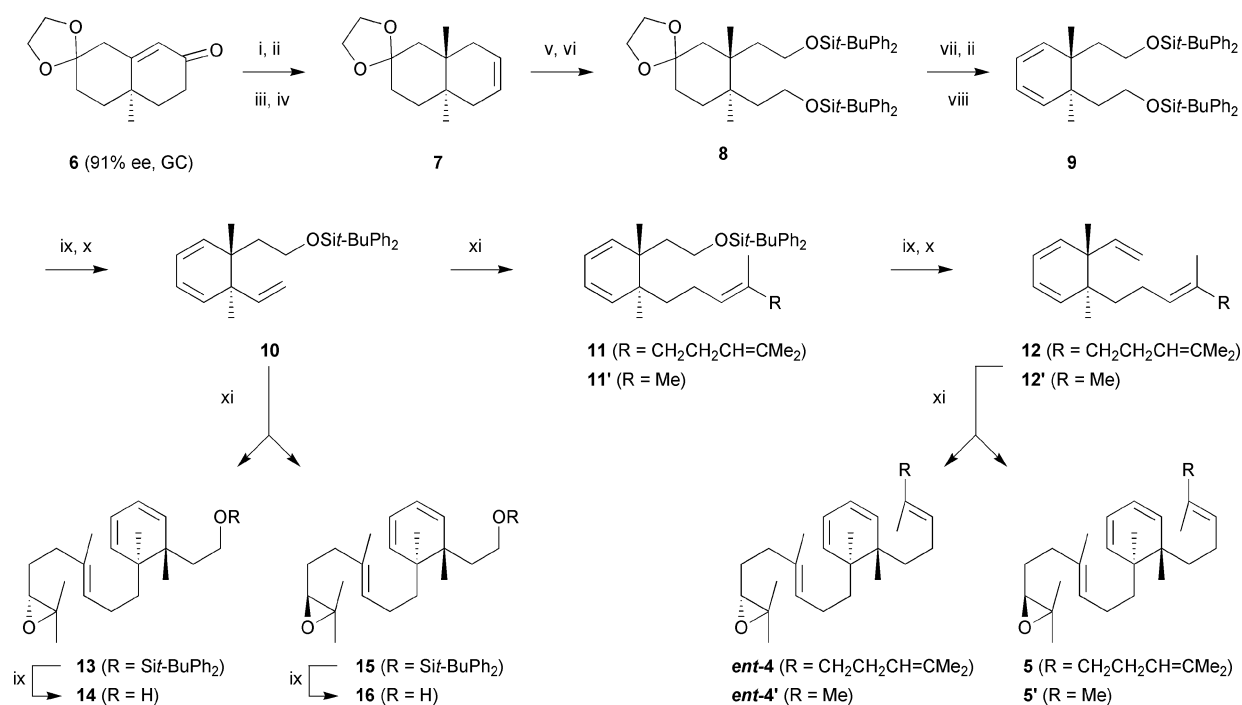
For the convergent synthesis of the target epoxypolyenes **4** and **5** (Scheme 3) we envisioned symmetrical disconnections. These allowed for a general strategy to introduce the required side chain fragments on the more challenging 1,6-difunctionalised central part, which was obtained using a reconnection strategy *via* the synthetically more attractive *trans*-fused decalin systems.

The easily accessible (*R*)-octalone **6** was recognized as a convenient enantiomerically enriched starting point.<sup>9</sup> Following Ireland's strategy for introducing an angular methyl group in the *trans*-position (Nagata hydrocyanation followed by nitrile reduction),<sup>10</sup> octalone **6** was converted to the ketal **7**. Prior removal of the ketone function *via* Shapiro reaction was essential for the success of the nitrile reduction. The non-nucleophilic base lithium hydride gave the best results for this highly regioselective reaction. Ozonolysis of the double bond followed by reductive work-up and silylation then gave the 1,6-bissilyl ether **8** as an advanced intermediate. Finally, the required cyclohexadiene building block **9** was obtained *via* Shapiro reaction giving the corresponding alkene which was then converted to the conjugated diene *via* a bromination–elimination sequence. As no conclusive evidence for the *trans*-fusion in these intermediates was obtained so far, we were pleased to observe a large optical rotation for the C<sub>2</sub>-symmetrical compound **9** ( $[\alpha]_D^{20} +110$  (*c* 1.36 in CHCl<sub>3</sub>)), which excludes the possibility of the achiral S<sub>1</sub>-symmetrical *cis*-isomer.

After some experimentation, we developed a general and direct strategy to introduce the initiating and terminating OS side chain fragments, based on the *B*-alkyl Suzuki–Miyaura cross-coupling, as has been reported in a preliminary communication.<sup>11</sup> The alkylborane reactants were prepared *in situ* *via* hydroboration of the terminal alkene **10**, easily obtained from **9** *via* monodesilylation and Grieco–Sharpless elimination. Consecutive coupling of the appropriate iodo alkene and (*S*)-epoxy iodo alkene in this manner, gave the target epoxy polyene **5**. Polyene **12** was coupled with



**Scheme 2** Enzymic polycyclisation of (3*S*)-oxidosqualene and proposed model compounds: pathway (a) is followed in plants for biogenesis of many tetra- and pentacyclic triterpenes, pathway (b) is followed for formation of lanosterol, cycloartenol or parkeol as steroid precursors.



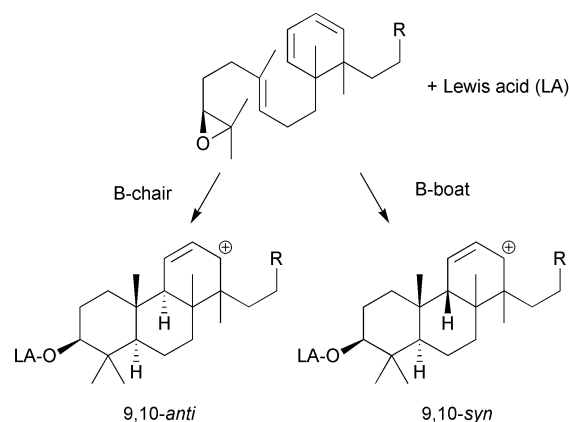
**Scheme 3** Reagents and conditions: i, 3 equiv.  $\text{Et}_2\text{AlCN}$ , THF–PhMe,  $-5^\circ\text{C}$  to rt, 80%; ii, (a)  $\text{TsN}_2\text{H}_3$ ,  $\text{TsOH}$ , mol. siev., THF,  $\Delta$ , (b) 5 equiv.  $\text{LiH}$ , THF–PhMe,  $\Delta$ , 80–85%; iii,  $i\text{-Bu}_2\text{AlH}$ , PhMe,  $0^\circ\text{C}$  to rt, quant.; iv,  $\text{N}_2\text{H}_4 \cdot x\text{H}_2\text{O}$ ,  $\text{N}_2\text{H}_4 \cdot \text{HCl}$ , TEG,  $135^\circ\text{C}$ , then excess  $\text{KOH}$ , rt to  $210^\circ\text{C}$  (distill.), 95%; v,  $\text{O}_3$ ,  $\text{CH}_2\text{Cl}_2$ , MeOH,  $-78^\circ\text{C}$ , then  $\text{NaBH}_4$ ,  $-78^\circ\text{C}$  to  $-15^\circ\text{C}$ ; vi, 4 equiv.  $t\text{-BuPh}_2\text{SiCl}$ , imidazole, DMAP, DMF, rt, 80% (v + vi); vii,  $\text{TsOH}$ ,  $\text{H}_2\text{O}$ , acetone,  $\Delta$ , quant.; viii, (a)  $\text{NBS}$ , bpo,  $\text{CCl}_4$ ,  $\Delta$ , (b)  $\text{KOt-Bu}$ , THF,  $\Delta$ , (c)  $\text{SnBu}_3\text{H}$ , AIBN, THF,  $\Delta$ , 70% (a + b + c); ix, TBAF,  $\text{Me}_4\text{NOH}$ , THF, 75% (from 9), 80–90% (from 11 or 11'), 65% (from 13 or 15); x, (a) 2 equiv.  $o\text{-NO}_2\text{PhSeCN}$ , 2 equiv.  $\text{PMe}_3$ , THF, (b) 2 equiv.  $\text{H}_2\text{O}_2$ , THF, 70–80%; xi, (a) 1.5 equiv.  $\text{Cy}_2\text{BH}$ , THF,  $0^\circ\text{C}$  to rt, then  $\text{H}_2\text{O}$ , (b)  $\text{Cs}_2\text{CO}_3$ ,  $\text{Ph}_3\text{As}$ ,  $\text{PdCl}_2(\text{dppf})$ , 0.96 equiv. (*E*)-1-iodo-2,6-dimethyl-hepta-1,5-diene or 1-bromo-2-methyl-propene or (3*S*)- or (3*R*)-(4-iodo-3-methyl-but-3-enyl)-2,2-dimethyl-oxirane, DMF, rt, 70–80% (from 10), 50% (from 12 or 12').

the enantiomeric epoxide-bearing side chain fragment to afford epoxypolyene **ent-4** which is the mirror image of the originally proposed target structure **4** and was subsequently used in its stead.

Early cyclisation experiments with these models quickly showed it would be desirable to have analogues with simplified terminating chains. The Suzuki–Miyaura coupling based strategy allowed for a straightforward synthesis of the truncated analogues **ent-4'** and **5'** using 1-bromo-2-methylpropene as the terminating side chain fragment (Scheme 3). Furthermore, by coupling silyloxypolyene **10** directly with the initiating side chains and simply deprotecting the silyl ether, the epoxy polyenols **14** and **16** were obtained. In these cases, the hydroxyl group was expected to function as a terminating nucleophile for the polycyclisation, affording tetracycles with a tetrahydrofuran type D-ring.

### Cyclisation experiments

Following the Stork–Eschenmoser postulate,<sup>12</sup> which advocates stereocontrolled antiperiplanar additions on both the epoxide and reacting alkenes, and reasonably assuming a chair-like fold for formation of the A-ring, only a limited number of stereochemical outcomes are expected for the AB-ring system upon polycyclisation of our model compounds (Scheme 4). The two alternative AB-cyclisation results for each of the examined diastereomers are distinguished by either a 9,10-*anti* or a 9,10-*syn* stereochemical relation (classical steroid numbering is used), which would reflect a B-chair or B-boat fold of the polyene, respectively. *A priori*, it was expected that a relaxed B-chair fold would only be



**Scheme 4**

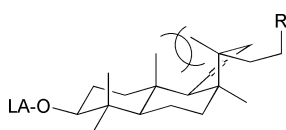
possible in the models corresponding to the dammarenyl-type configuration (**14**, **ent-4**, **ent-4'**), because a B-chair conformation in the diastereomeric protosteryl-type models (**16**, **5**, **5'**) would suffer from a severe *syn*-diaxial relation of the C-10 methyl substituent and the very bulky C-14 quaternary center (see Fig. 1). It was hoped that in this case B-boat cyclisation and thus a 9,10-*syn* stereochemistry would result, a pathway which only very rarely has been observed in nonenzymic reactions.<sup>13</sup>

The easily accessible models **14** and **16** were used to screen the most commonly used conditions to achieve cationic polycyclisation of epoxy polyenes. The results for the protosteryl type

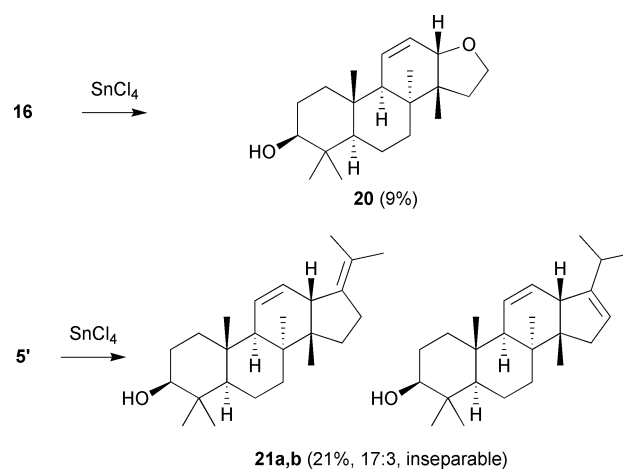
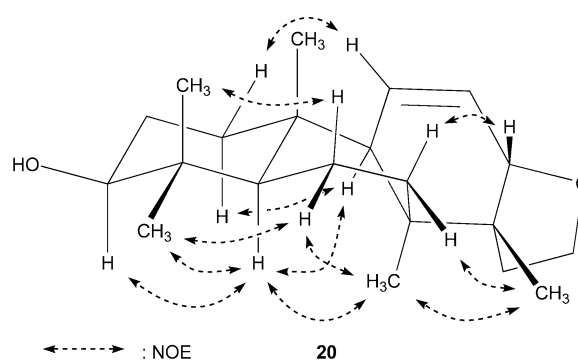
**Table 1** Cyclisation of protosteryl type model compounds **16** and **5'**

Reaction conditions	Yield of			
	17	18	19	
(i) <b>16</b> , 1.0 equiv. picric acid, CH <sub>3</sub> NO <sub>2</sub> , rt	—	10%	—	(80%) <sup>a</sup>
(ii) <b>16</b> , 1.5 equiv. MeAlCl <sub>2</sub> , CH <sub>2</sub> Cl <sub>2</sub> , −78 to −15 °C	—	25%	35%	30% <sup>b</sup>
(iii) <b>16</b> , 1.0 equiv. SnCl <sub>4</sub> , CH <sub>2</sub> Cl <sub>2</sub> , −78 to −25 °C	—	40%	30%	10% <sup>b</sup>
(iv) <b>16</b> , 0.25 equiv. SnCl <sub>4</sub> , CH <sub>2</sub> Cl <sub>2</sub> , 10 °C	9% <sup>c</sup>	15%	17%	3% <sup>b</sup>
(v) <b>5'</b> , 0.25 equiv. SnCl <sub>4</sub> , CH <sub>2</sub> Cl <sub>2</sub> , 10 °C	21% <sup>d</sup>	30%	24%	12% <sup>b</sup>

<sup>a</sup> Isolated yield of a complex fraction which contains diol **19a** as a major isomer (NMR). <sup>b</sup> Isolated fraction contains minor amounts (10–20%) of regioisomeric alkenes and HCl addition products thereof (NMR). <sup>c</sup> Isolated yield of tetracyclic alcohol **20** (Scheme 5). <sup>d</sup> Isolated yield of a 17 : 3 mixture of protolanostane-type alcohols **21a** and **21b** (Scheme 5).

**Fig. 1**

model **16** can be viewed in Table 1. Sharpless' protic conditions (entry (i))<sup>14</sup> using picric acid in nitromethane gave a very complex mixture of mostly dihydroxylic material with an intact conjugated diene system. The mild methylaluminium dichloride Lewis acid introduced by Corey as an efficient epoxypolyene cyclisation promoter (entry (ii))<sup>15</sup> gave two major monocyclised products **17a** and **18a** next to some more polar impure fractions of the type **19a**. Use of stannic chloride (entry (iii)), as originally introduced by Stork,<sup>4</sup> gave similar results. Sharpless and van Tamelen have optimised the nonenzymic polycyclisation of OS (Scheme 1) and found that maximal polycyclised (tricyclic) material was obtained using substoichiometric amounts of stannic chloride at moderate reaction temperatures.<sup>2</sup> In our case (entry (iv)), this procedure gave a quite complex mixture of rearranged or monocyclised material (as judged by the presence of an intact conjugated diene system in NMR spectra) of which only the main compounds **17a** and **18a** could be obtained in a pure form. However, this procedure uniquely yielded an easily separable, less polar fraction of solid material of the expected polycyclised kind. Furthermore, this fraction turned out to be a single diastereomer (the *trans-anti-cis-anti-cis*-tetracycle **20**, see Scheme 5). This configuration was unequivocally determined by detailed analyses of NMR spectra (APT, HSQC, HMBC, COSY and NOESY). As discussed earlier, the 9,10-*anti* relation should indicate a chair-like fold for B-ring formation (Scheme 4). Interestingly, the observed NOE relations in this *trans-anti-cis*-tetracycle **20** did not support a chair-like conformation of the B-ring (which, as discussed, would be a very strained system, *cf.* Fig. 1), but instead revealed a (twist-)boat-like conformation, as can be reached by a *cis*-decalin type ring flip of the BC-ring system (see structure with some diagnostic NOE relations in Fig. 2). Also the C-ring clearly adopts an unexpected conformation, with both methyl substituents in a pseudo-equatorial position. Regrettably,

**Scheme 5****Fig. 2**

our attempts to grow a suitable single crystal of compound **20** failed, so this interesting conformation could not be confirmed by X-ray diffraction analysis.

When conditions from entries (i)–(iii) were applied to the diastereomeric model compound **14** (corresponding to a mirrored dammarenyl type stereochemistry), roughly the same results were obtained and only monocyclised material of the type **17a**, **18a** and

**19a** was observed. Using the Sharpless–van Tamelen conditions (iv), no polycyclised compounds could be isolated.

Next, we applied the at least partially successful conditions (*cf.* entry (iv)) to the ‘full’ models **ent-4** and **5** with natural OS side chains. Again, mostly compounds of the previously obtained monocyclised type were isolated (*cf.* **17**, **18** and **19**). In both cases, a fraction was isolated which indicated the presence of polycyclised material (as judged by the absence of signals for the conjugated diene in NMR spectra). However, in the case of the dammarenyl type model **ent-4** this was only a very small (2%) and extremely complex mixture. In the case of the protosteryl-type model **5**, this fraction (12%) showed a major tetracyclic component but could not be sufficiently purified to be identified. Careful analysis of the NMR spectra did reveal some striking similarities with those obtained for tetracycle **20**. The signals that would correspond to the D-ring region, however, were complex and showed splitting indicative of a 1 : 1 mixture of isomers.

Fortunately, the product diversity could be greatly reduced by truncating the terminating polyene chain by one isoprenic unit (substrates **ent-4** and **5**). These models were synthesised and cyclised on a relatively large scale (80 and 120 mg respectively).

In the case of the protosteryl type model **5**, three major fractions were isolated (entry (v), Table 1), one of which contained only polycyclised material. The major constituent of this tetracyclic fraction (**21a**, 85%(NMR)) was shown to possess the (9 $\alpha$ ,13 $\beta$ )- $\Delta$ 17,20-protolanostane skeleton (Scheme 5) *via* detailed NMR analyses (APT, HSQC, HMBC, COSY, NOESY). The inseparable minor isomer (**21b**, 15%(NMR)) was tentatively assigned a (9 $\alpha$ ,13 $\beta$ )- $\Delta$ 16,17-protolanostane structure based on the resolved signals (mainly D-ring region). Both other major fractions contained a single isomer of the previously obtained abortive A-ring monocyclisation type (cyclic ether **17b** and ketone **18b**). Furthermore, five minor fractions were isolated: a complex apolar hydrocarbon fraction (6%), two fractions of the cyclohexenolic A-ring monocyclised type (8 and 4%, structures **19b**) and two very minor polycyclised fractions, one complex (2.5%) and one which could be assigned the unusual spirocyclic structure **22** (2%, Fig. 3) *via* detailed analysis of NMR spectra. In total, 97% of the mass balance was isolated.

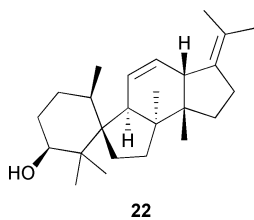


Fig. 3

Although obtaining sufficient 2D-NMR correlations for tetracycle **21a** was complicated by various overlaps and by the presence of the minor regioisomer **21b**, the data clearly showed the conformation of this protolanostane skeleton to be essentially the same as that established for tetracycle **20** (Fig. 2). Attempts at growing single crystals only afforded very thin (<10  $\mu$ m) plate-like crystals not suitable for single crystal X-ray diffraction on conventional diffractometers. This crystal morphology is probably due to the difficulty of growing a crystal along a long axis ( $c > 50$  Å, see Experimental section), where the packing forces are very weak.

Therefore the crystals were analysed exploiting the high brilliance of a synchrotron radiation source and the collected data allowed solving and refining of the structure. The results obtained, together with 2D-NMR analyses, allowed the full characterisation of the configurational and conformational features of compound **21a**.

Because of the employed wavelength and of the presence of light atoms only, the absolute structure could not be determined by diffraction only. However, among the two possible enantiomorph molecules compatible with the X-ray data, the configuration shown in Fig. 4 was chosen because it is the one in agreement with the adopted synthetic strategy. In fact, it shows the correct absolute configuration of the quaternary centres in the C-ring, which are preformed by enantioselective synthesis and can be unequivocally “back-tracked” to the original chiral starting material of known absolute configuration (compound **6**). Furthermore, the absolute configuration of the initiating (*S*)-epoxide is expected to give a hydroxyl group with the configuration as shown in Fig. 4.

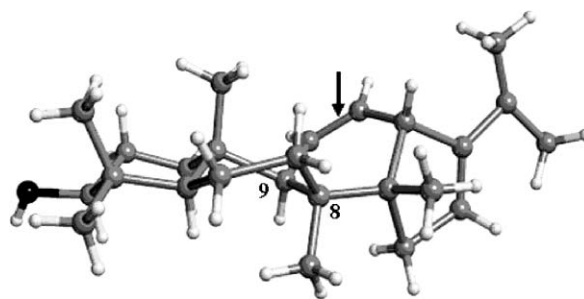


Fig. 4

The crystal structure of **21a** contains two molecules adopting very similar conformations, with just small differences, as shown in the superposition in Fig. SM1 in the supplementary material.† As illustrated in Fig. 4, the A-ring adopts a regular chair conformation, while the B-ring has a distorted twist-boat conformation (endocyclic torsional angles in the two conformers, starting from the C8 to C9 bond and then further around the ring in that direction:  $-23.6$ ,  $64.4$ ,  $-37.8$ ,  $-28.9$ ,  $66.2$ ,  $-39.9$  and  $-2.7$ ,  $44.8$ ,  $-29.8$ ,  $-30.6$ ,  $71.1$ ,  $-53.4$ ). The C-ring adopts a flattened half-chair (or “sofa”) conformation, as expected by the presence of the endocyclic double bond (black arrow in Fig. 4). Finally, another interesting feature is the almost planar envelope conformation of the D-ring (with four  $sp^3$  and one  $sp^2$  carbon atoms), despite the absence of an endocyclic double bond.

The quantum mechanic (QM) geometry optimisations were carried out to demonstrate that these peculiar conformational features are due to the electronic structure of the molecule rather than the unusual crystal packing (two almost identical conformers packed along a long axis) and that these features are retained also for the isolated molecule. After geometry optimisation at the B3LYP/6-31G(d,p) level, the overall conformations of the two molecules in the asymmetric unit converged to the same. The (distorted) twist-boat conformation of the B-ring and the almost planar conformation of the D-ring are retained, thus confirming that the crystal structure is a minimum energy conformation also as isolated molecule. The distortion of the B-ring can be ascribed to the double bond in the C-ring (indicated by the black arrow in Fig. 4). In other words, a perfect boat or twist-boat conformation of the B-ring would distort the C-ring too much.



The almost planar envelope conformation of the D-ring is imposed by its exocyclic double bond and again the constraints due to the double bond in the C-ring. To verify the relative stability of the crystal structure conformation with its unusual B-ring twist-boat, other conformations of compound **21a** adopting more conventional chair or boat conformations were generated by the MOLDRAW software and optimised at the B3LYP/6-31G(d,p) level. The hypothetical conformation with the B-ring in a standard boat conformation did not represent an energy minimum. The conformation with the B-ring assuming a chair conformation (see Fig. SM2 in the supplementary material) resulted in a less stable energy minimum (+8.2 kJ mol<sup>-1</sup>), and this instability explains the preference for the (distorted) twist-boat B-ring conformation in the crystal structure.

Upon attempted cyclisation of the dammarenyl-type model **ent-4'**, major amounts of the monocyclic product type (40% cyclic ether and 32% ketonic material) were isolated. Unfortunately, only minor and very complex (possibly) tetracyclic fractions (6% total yield) were obtained next to some small hydrocarbon and monocyclised fractions. Here, 98% of the mass balance was isolated.

Our analysis of the possible underlying cyclisation mechanisms is presented in Fig. 5.

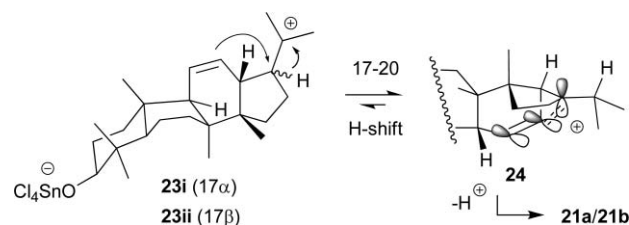
For the models **14**, **ent-4** and **ent-4'**, that were endowed with the mirrored dammarenyl-type stereochemistry (for clarity, the "natural" enantiomers are shown in Fig. 5), an explanation should be found for their apparent inability to undergo a cyclisation beyond the A-ring formation stage. Upon evaluating the required all-chair polyene fold (ccc), a strong steric interaction of the 1,3-*syn*-diaxial type is obvious between methyl substituents at C-8 and C-10 (classical steroid numbering). Because of the sp<sup>3</sup> hybridisation at C-8, this effect is indeed expected to be much more pronounced than in the natural OS dammarenyl-type fold. This steric effect could thus prevent C-9 and C-10 from coming within binding range. Alternatively, this might be achieved if the B-ring part of the polyene chain would adopt a (twisted) boat-like fold. However, this boat-type conformation seems unlikely because it requires the sterically demanding quaternary substituent (C-14) in an unaccommodating flagpole (boat) or axial (twist-boat) position.

For the models **16**, **5** and **5'**, having the protosteryl-type stereochemistry, at least one possible polyene fold should allow for an extended polycyclisation reaction. The conformation corre-

sponding to that assumed for the enzymic OS cyclisation (cbc) is clearly not followed, as no tetracycles are observed with a 9,10-*syn* relation. Based on the observed conformations of the tetracyclic products **20** and **21a** (cf. Figs. 2 and 4), we propose a polycyclisation process *via* the alternative cbc' conformation, in which the preformed C-ring adopts the flipped half-chair conformation (following a 120° rotation around the C-8,C-14 bond). Alternatively, the observed *trans-anti-cis* stereochemistry might be reached through an all-chair conformation (*i.e.* ccc). But, as previously noted for the resulting polycyclic systems (Fig. 1) and irrespective of the C-ring conformation, this is a very unlikely polyene fold due to the pronounced *syn*-diaxial relation of the methyl substituent at C-10 and the voluminous C-14 quaternary group, which is unlikely to adopt an axial orientation.

The prevalence of the cbc' fold over the envisioned (biomimetic) cbc fold, that would afford the natural protosteroid stereochemistry, might be explained by the closer proximity of the reacting  $\pi$ -systems (endocyclic diene and olefins in the pendant side chains) in the case of axially oriented polyene chains (relative to the preformed C-ring half-chair).

As for the D-ring formation stage, the second unnatural *cis*-fusion is also explained by the inverted half-chair conformation of the preformed C-ring. Unfortunately, the observed polycyclic products do not allow us to distinguish between formation of an initial tetracyclic cation with an  $\alpha$ - or  $\beta$ -oriented side chain (**23i** or **23ii**, Scheme 6). In enzymic reactions (and even in the absence of an enzyme)<sup>16</sup> the protosteryl cation is known to undergo an elaborate cascade of 1,2-hydride and -methyl shifts. Here this cascade apparently stops after the first (also naturally occurring) 17–20 hydride shift (Scheme 6). The subsequent 13–17 hydride shift could be prevented by a conformationally rigid  $\pi$ -cation interaction with the C-ring alkene (structure **24**), which would also explain the driving force for the first shift.



Scheme 6

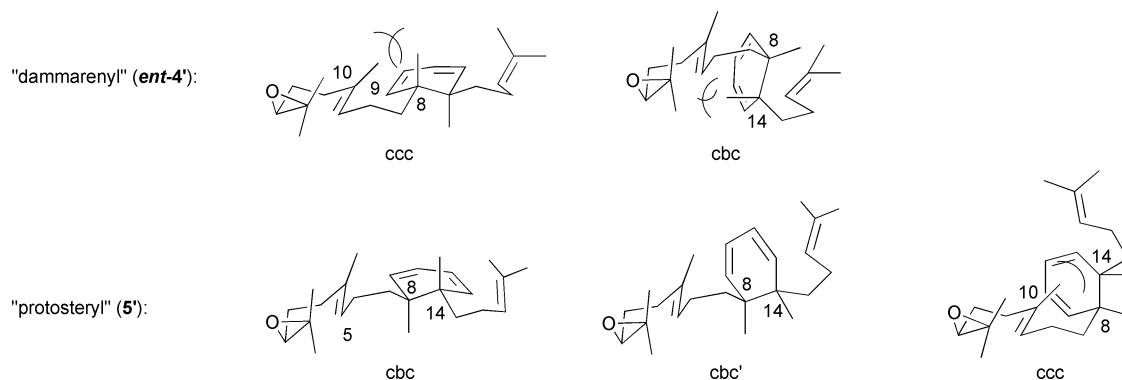


Fig. 5

The unusual AB-spiro-fused tetracycle **22** can be regarded as a polycyclised derivative of an intermediate cation *en route* to the major ketonic rearrangement product **18b**. This structure again shows the preference of our model systems for the formation of *cis*-fusions to the C-ring.

## Conclusions

Due to the complex nature of these cationic cyclisation experiments, it is unwise to draw straightforward conclusions with relevance to their biological counterparts. As expected, all cyclisation experiments gave complex mixtures of compounds. Furthermore, the polycyclised structures that were produced in sufficient quantities to be isolated and characterised, all possessed very unnatural *cis*-fusions to the C-ring. However, it should be clear that (i) where extended polycyclisation occurs in our model systems, the ABC-ring system stereochemistry is adequately controlled by the combination of epoxide and (preformed) C-ring chair configurations, (ii) a remarkable preference for a (distorted) twist-boat conformation exists for the B-ring in the observed tetracyclic lanostane-type compounds **20** and **21a**, indicated by crystal structure, 2D NMR analyses and DFT calculations, (iii) this preference can be rationalised by severe intramolecular steric compression in the case of a B-ring chair conformation and similar effects are reasonably expected to control the polyene fold during the nonenzymic polycyclisation of the examined model compounds and (iv) this provides a model for how the elusive B-ring (twist-)boat conformation might be achieved in steroid biogenesis, in this case by preorganising the C-ring polyene part in a specific chair-like fold. Such a conformational restriction has been shown to effect biomimetic (six membered) C-ring formation in a previous model study.<sup>7</sup>

## Experimental

### Synthesis and cyclisation of the model substrates

**Compounds 5, 6–12 and 15.** Procedures and characterisation as described previously in reference 11.

**Compounds *ent*-4, *ent*-4', 5', 11', 12' and 13.** Synthetic procedures and spectral data for these compounds are provided as electronic supplementary data.<sup>†</sup>

**Cyclisation of compounds 16, *ent*-4' and 5'.** Synthetic procedures and characterisation data (including a detailed analysis of 2D NMR data for polycyclised compounds **20**, **21a,b** and **22**) are provided as electronic supplementary data.<sup>†</sup>

### Crystal structure determination of compound 21a

Single crystal diffraction data on compound **21a** were collected at 100 K at the BM1a station (SNBL) of the ESRF synchrotron facility,<sup>17</sup> employing a monochromatised ( $\lambda = 0.7114$  Å) radiation. Absorption correction was performed using SADABS.<sup>18</sup> The structure was solved by direct methods employing the SIR2002 software,<sup>19</sup> and refined by full-matrix least-squares with SHELX-97.<sup>20</sup>

The data collection was carried out at 100 K on a crystal of  $0.120 \times 0.040 \times 0.005$  mm<sup>3</sup>. The space group was P2<sub>1</sub>2<sub>1</sub>2<sub>1</sub> with unit

cell parameters:  $a = 6.429(1)$  Å,  $b = 13.631(2)$  Å,  $c = 50.896(6)$  Å. 3482 reflections were collected with  $R(\text{int}) = 0.0381$  and the final agreement factor was  $R = 0.20$ . Full crystal data and details of data collections and refinements of compound **21a** are given in Table SM1 as supplementary material. Complete crystallographic information on compound **21a** has been deposited by the CCDC with deposition number CCDC 675998.<sup>†</sup>

The crystal morphology (very thin plates with thickness <10 µm), surely related to the long  $c$  axis, prevented us from obtaining good crystal and good agreement factors during the refinement. Because of these problems, the resulting crystal structure showed some disorder and a few restraints (see cif file for details) on the distances involving the C(CH<sub>3</sub>)<sub>2</sub> groups were necessary to have a stable refinement, and none of the atoms could be refined anisotropically. Indeed the space group attribution was correct with the expected systematic absences, as indicated by the reconstruction of the experimental reciprocal lattice (See Fig. SM3, 4 and 5 in the supplementary information). The conformational features of the crystal structure from X-ray data were confirmed by first principle QM calculations. Graphical manipulations to obtain ordered model structures from the disordered crystal structures were carried out employing the XP<sup>21</sup> and MOLDRAW<sup>22</sup> software.

The observed intermolecular contacts involving the methanol molecule were consistent with O–H...O hydrogen bonds and indicated the presence of disorder, with both atoms in the solvent moiety capable of acting as oxygen atoms. Therefore it was not possible to establish, by geometric considerations alone, the atomic species of the two solvent atomic positions. Nevertheless, the values of the ADP parameters [0.088(6) and 0.18(2) for O3 and C51 respectively] suggested the adopted assignment. It is worth noting that this hydrogen bond network allowed the formation of an hydrophilic layer, formed by OH groups of methanol and **21a**, and of an hydrophobic layer, constituted by the remaining part of the **21a** molecules.

### Computational details

DFT molecular orbital calculations on the different conformations of compound **21a** were performed using the Gaussian03 program<sup>23</sup> on a 16-node linux cluster. The initial geometries were those derived from X-ray crystal structure and were manipulated by the MOLDRAW<sup>22</sup> software and optimised employing the DFT method based on Becke's three parameters hybrid functional and Lee–Yang–Parr's gradient-corrected correlation functional (B3LYP),<sup>24,25</sup> using the 6-31 G(d,p) basis set<sup>26</sup> as implemented in the Gaussian03 software.

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