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Squalene–hopene cyclase: insight into the role of the methyl group on the squalene backbone upon the polycyclization cascade. Enzymatic cyclization products of squalene analogs lacking a 26-methyl group and possessing a methyl group at C(7) or C(11)†

Shin-ichi Nakano, Shumi Ohashi and Tsutomu Hoshino*

Department of Applied Biological Chemistry, Faculty of Agriculture, and Graduate School of Science and Technology, Niigata University, Ikarashi, Niigata 950-2181, Japan. E-mail: hoshitsu@agr.niigata-u.ac.jp; Fax: +81-25-262-6854; Tel: +81-25-262-6637

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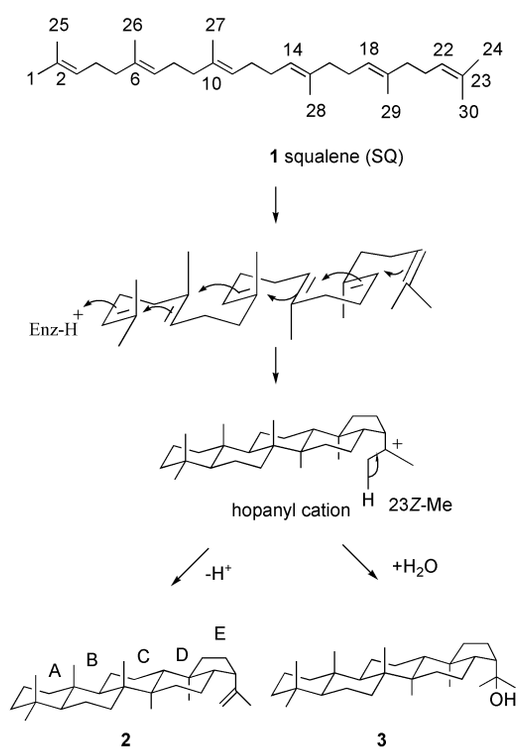
To provide deep insight into the polycyclization reaction of squalene, some analogs were synthesized and incubated with the cell-free homogenates of the recombinant *Escherichia coli* encoding the wild-type squalene cyclase. The presence of C(6)–Me leads to an efficient polycyclization cascade. Substitution of the C(14)–H and the C(18)–H with a methyl group halted the polycylization reaction at the tricyclic ring stage having a 6/6/6-fused ring system and the tetracycle with a 6/6/6/6-fused ring, respectively, both of which were produced according to a Markovnikov closure. Replacement of the C(7)–H and the C(11)–H with a methyl group led to no cyclization. These results, in conjunction with our previous reports, indicated that the methyl positions are important for bringing to completion of the normal polycylization reaction and further demonstrated that the precise steric bulk size at the methyl positions of squalene is critical to the correct folding and the strong binding of the substrate to the squalene cyclase.

Introduction

Linear squalene **1** is cyclized into the pentacyclic triterpene, hop-22(29)-ene **2** and hopan-22-ol **3** (*ca.* 5 : 1), which is mediated by squalene–hopene cyclase (SHC) from prokaryotic species (Scheme 1).[1–3] This polycyclization reaction proceeds with regio- and stereochemical specificity under precise enzyme control to form the 6/6/6/6/5-fused ring system and nine new chiral centers. SHC folds **1** into the all pre-chair conformation (a product-like conformation) inside the enzyme cavity, leading to the final hopanyl cation through sequential ring-forming reactions. The proton elimination occurs exclusively from the (23*Z*)-methyl group, but not from the *E*-methyl group, to form **2**.[1,4] A nucleophilic attack of water molecule on the cation affords **3**. Recent studies by three-dimensional X-ray crystallographic analyses[5] and by site directed mutagenesis experiments[1,2] have provided deeper insights into the polycyclization mechanism. The investigations by using modified substrates also have given important information. In previous papers,[4,6,7] we have shown that the methyl groups at the both terminal sides play crucial roles both in initiating the polycyclization and in constructing the five-membered E-ring,[4,6] and also established that the methyl group at C(10) of the central part has a critical role in forming the all pre-chair structure[7] (Scheme 2). In the case of the substrate analogs **4** and **5** lacking 25-Me and 1-Me of **1**, respectively, the polycyclization reaction started from the isopropylidene moiety, and not from the methyl-deficient side. This finding was further confirmed by bisnorsqualene lacking both 1-Me and 25-Me.[4a] As major product(s), tetrahymanol skeletons **6** and **7** having a six-membered E-ring were constructed instead of the five-membered E-ring of **2**.[6] When the substrate analog **9** lacking the 27-Me of **1** was incubated, two cyclization pathways *a* and *b* occurred (Scheme 2). Path *a* shows that **9** was recognized as C(10)-norsqualene, while path *b* as C(15)-norsqualene. C(10)-Norsqualene afforded novel carbocyclic skeletons **10** and **11** having the 6/5+5/5+(6) ring system.[7] To afford the novel skeleton, **9** must be folded in an unusual conformation,[7] completely different from the all pre-chair conformation. On the other hand, C(15)-norsqualene was folded in an all pre-chair conformation to give **12** and **13** (10.5 : 7.3) in a



Scheme 1 Polycyclization reaction of squalene **1** by hopene synthase: **1** is folded in all pre-chair conformation to give the hopanyl cation, leading to hopene **2** and hopanol **3** in a ratio of 5 : 1. The deprotonation from the hopanyl cation gives **2**, which occurs exclusively from the 23*Z*-Me of **1** to give **2**, while **3** is produced by a nucleophilic attack of a water molecule to the hopanyl cation.

† Electronic supplementary information (ESI) available: Additional characterizatrion data for products **34**–**45**. See http://www.rsc.org/suppdata/ob/b4/b404287e/

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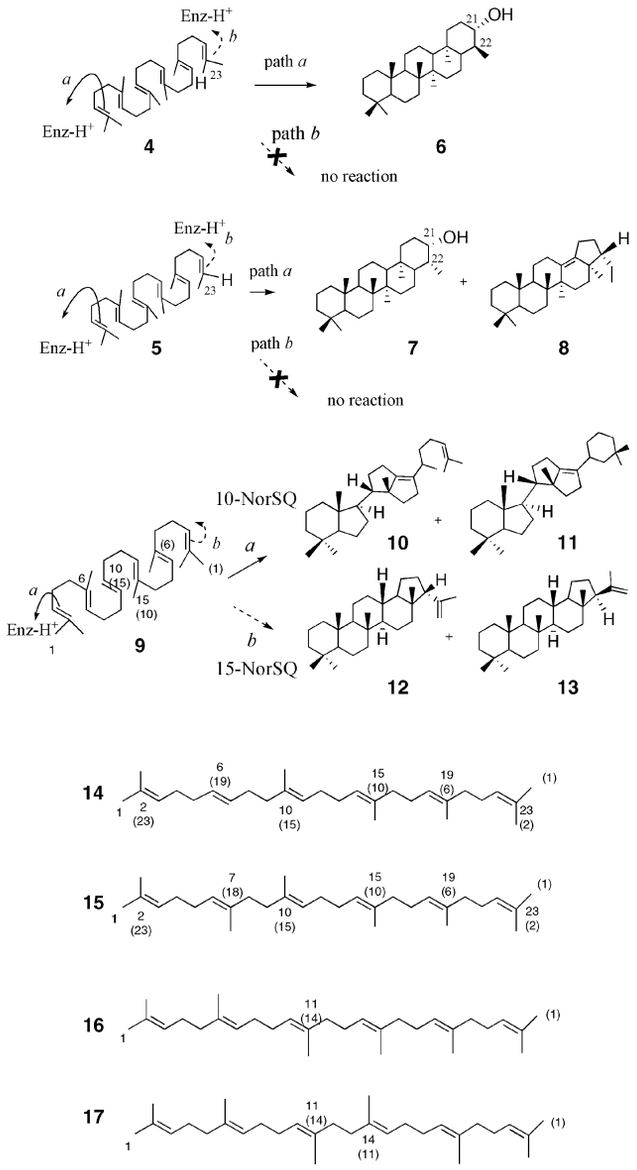


Scheme 2 The cyclization pathways of norsqualenes **4**, **5** and **9**, and the substrate analogs **14**–**17** employed in this investigation. Analogs **4** and **5** lack the methyl group at the terminal double bond of **1**. The (23*Z*)-Me of **1** is missing for **4**, while (23*E*)-Me is missing for **5**. The methyl group at the central part is absent in **9**. The both left and right sides have an isopropylidene moiety, thus **9** can undergo the polycyclization reaction from the left (path *a*, recognized as C(10)-norsqualene) and right sides (path *b* as C(15)-norsqualene). Unusual folding conformation, completely different from the all pre-chair conformation shown in Scheme 1, was adopted *via* path *a* to afford **10** and **11** having a novel carbocyclic skeleton of a 6/5 + 5/5 + (6) ring system.

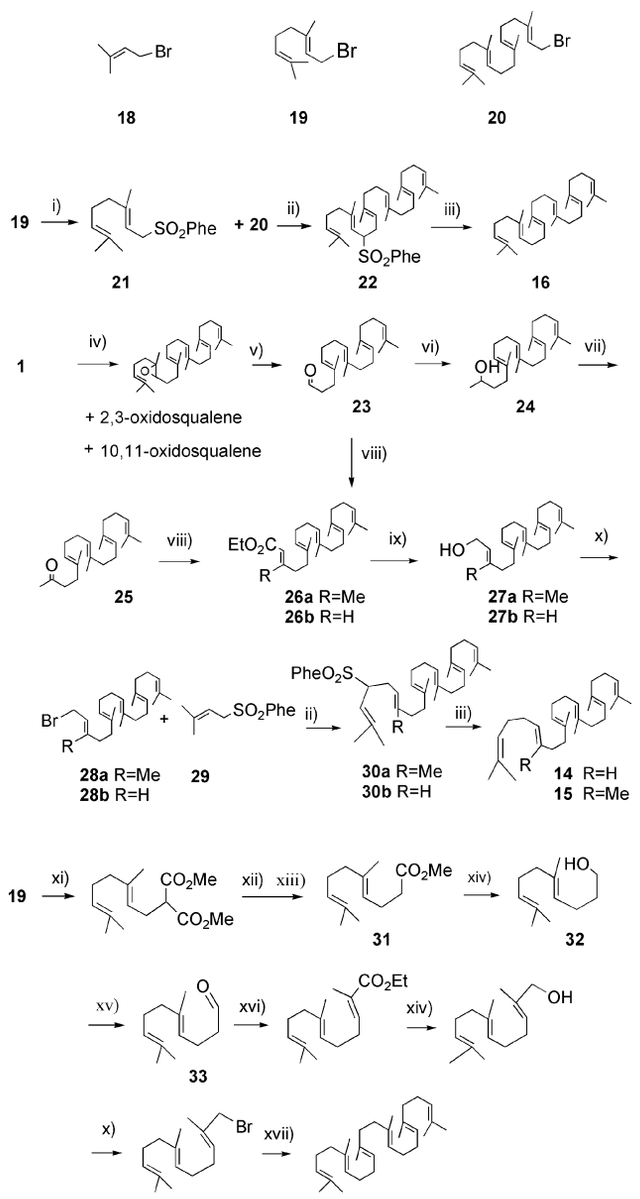
similar manner as the formation of **2**, but the stereochemistry at C(21) of **13** was different from those of natural type **12** and **2**.[7] Based on these experimental results, we have proposed that the binding or accepting sites of the methyl groups at the terminal and central sides are involved in the cyclase enzyme.

Squalene **1** is a symmetrical molecule and the methyl groups are arranged at regular intervals on the backbone. As described above, SHC has binding sites for the branching methyl group of **1**. To provide further insight into the binding sites of the methyl groups, analogs **14**–**17** were synthesized and incubated with the wild-type SHC. The 26-Me at C-6 of **1** is absent in **14**.[8] Compounds **15**–**17** are squalene analogs in which methyl groups are arranged at position(s) different from **1** (regio-isomers of methyl groups). Analog **15** has a methyl group at C-7 instead of C-6 of **1**. Analog **16** has a methyl group at C-11 instead of C-10. Analog **17** has methyl groups at both C-11 and C-14, instead of C-10 and C-15. We describe here the enzymatic products obtained from these analogs and discuss the role of the branching methyl group(s) upon the polycyclization cascade.

Results

Syntheses of analogs 14–17

Analogs **14**–**17** were synthesized according to Scheme 3 and described in detail in the Experimental section. Compound **16** was synthesized by the allyl coupling of a geranylgeranyl bromide **20** with geranyl phenylsulfone **21** as the key step. The cleavage of the carbon–sulfur bond was done with $LiBEt_3$ and a catalytic amount of $PdCl_2$(dppp) [dppp = 1,3-bis(diphenylphosphino)propane] in Et_2O.[9] Aldehyde **23**, obtained from **1**, was subjected to a Grignard reaction with CH_3I to give alcohol



Scheme 3 Synthetic scheme of squalene analogs **14**–**17**. *Reagents and conditions*: (i) $PheSO_2Na$/DMF; (ii) *n*-BuLi/THF–$[(CH_3)_2N]_3PO$ (4 : 1); (iii) $LiBEt_3H$, $PdCl_2$(dppp)/Et_2O at 0 °C; (iv) *m*-CPBA/CH_2Cl_2 at 0 °C; (v) H_5IO_6/Et_2O; (vi) CH_3I/Mg in Et_2O; (vii) CrO_3(py)/CH_2Cl_2; (viii) $(EtO)_2POCH_2CO_2Et$/NaH in THF; (ix) (*iso*-$C_4H_9)_2AlH$/Et_2O; (x) PBr_3/THF; (xi) $CH_2(CO_2Me)_2$/NaOEt in EtOH; (xii) 1 M KOH/MeOH, then HCl, heat; (xiii) 5% dry HCl/MeOH; (xiv) LAH/THF; (xv) PCC/CH_2Cl_2; (xvi) $Ph_3P^+CH_3CHBr^-CO_2Et$/NaOH; (xvii) pyrrolidine/*n*-BuLi/CuI/Et_2O.

24, which was then oxidized into **25** with Collin's reagent. Wadsworth–Emmons reaction of **25** with ethyl diethylphosphonoacetate gave the ester **26a**, which was reduced with DIBAL-H to afford alcohol **27a** and the allyl coupling of the bromide **28a**, prepared by treatment of **27a** with PBr_3, with phenylsulfone **29** gave **30a**. The deprotection of the phenylsulfone group from **30a** was carried out with $LiBEt_3H$ to afford the desired **15** in a similar manner as described above. Analog **14** was prepared with essentially the same method as in the synthesis of **15**, but **23** was directly subjected to Wadsworth–Emmons reaction to give **26b**. Analog **17** was synthesized as follows. Geranyl bromide **19** was condensed with methyl malonate to elongate by a C_2 unit, followed by decarboxylation and methylation to yield methyl ester **31**. Then the following five steps were carried out: the reduction of **31** with LAH, oxidation with PCC, the Wittig reaction with the ylide $Ph_3P{=}C(CH_3)CO_2Et$, reduction with LAH, and bromination with PBr_3. The C_{15}-bromide, thus prepared, was subjected to an allyl coupling reaction by using pyrrolidine/*n*-BuLi/CuI,[10] leading to the desired **17** after purification by $AgNO_3$–SiO_2 column chromatography.

GC analyses of the enzymatic products

Fig. 1 shows the gas chromatograms of the incubation mixtures prepared by incubating **1** and **14**–**17** with the cell-free homogenates of *E. coli* clone encoding the native SHC from *Alicyclobacillus acidocaldarius*. Identical incubation conditions were employed to compare the quantities and distribution pattern of enzymic products obtained from each substrate analog. Incubation conditions were as follows: substrate analog, 1.0 mg; the cell-free extract as the enzyme source, 1.5 cm^3, Triton X-100, 20 mg; optimal pH, 6.0; optimal temperature, 60 °C; incubation time, 16 h; total volume, 5 cm^3. To the reaction mixture, was added 5% KOH/MeOH and the products were extracted with hexane. Triton X-100 included in the hexane extracts was removed with a short SiO_2 chromatography column eluting with a mixture of *n*-hexane–EtOAc (100 : 20). Fig. 1(A) shows the product distribution pattern obtained by incubating **1**. No substrate remained in the incubation mixture. Three major products (**34**, **35** and **36**) from **14** were found in a good conversion ratio, as shown in Fig. 1(B). The yield of each product was estimated by the GC (Fig. 1(B)) to be as follows: 29.4, 19.8, 34.8 and 1.4% for **34**, **35**, **36** and unreacted **14**, respectively. The remaining products (total yield, 14.6%) comprised of some minor products, but the yield of each minor product was less than 2–4%. As shown in Fig. 1(C), many products were found from the incubation mixture of analog **15** in a good conversion ratio (*ca.* 98%) and only a small amount of **15** was recovered (1.4%). Analog **16** was converted into **45**, but in a small yield (7%, Fig. 1(D)). The conversion of **17** was low as shown in Fig. 1(E).

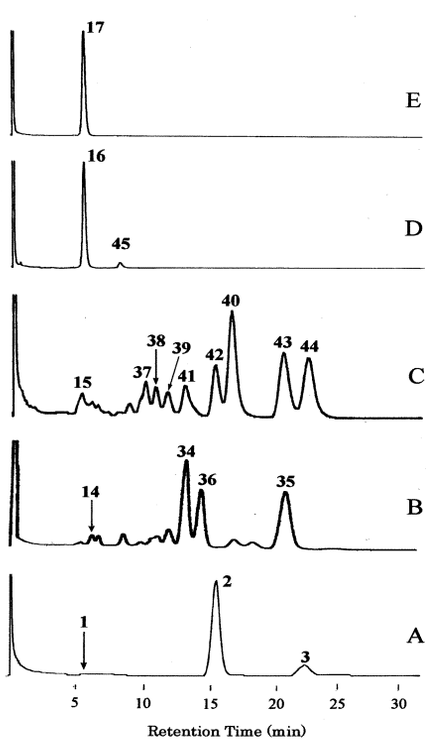


Fig. 1 Gas chromatograms of the reaction mixtures obtained by incubating **1** (A), **14** (B), **15** (C), **16** (D) and **17** (E) with the wild-type SHC. Triton X-100 was removed by a short SiO_2 column chromatogram.

Enzymatic products 34, 35 and 36 from 14

With the cell-free homogenates (150 cm^3) prepared from a 3 L culture of *E. coli* encoding the native SHC, 40 mg of **14** was incubated at optimum catalytic conditions (pH 6 and 60 °C). After lyophilizing the incubation mixture, the residues were extracted with hexane and passed through a short SiO_2 column to remove Triton X-100 by eluting with a mixed solvent of hexane–EtOAc (100 : 20). Three major peaks **34**–**36** were found in addition to some minor peaks and a small amount of recovered **14**, as shown in Fig. 1(B), but the analysis of SiO_2 TLC showed only two major spots, their R_f values being close to those of **2** and **3**; 0.81 with hexane for **2**, 0.57 with EtOAc–hexane = 100 : 20 for **3**. Column chromatography over SiO_2 eluting with hexane gave the low polar fraction, then elution with a mixed solvent of hexane–EtOAc (100 : 5) gave the more highly polar product **35**, which was further purified by normal phase HPLC (hexane–*iso*-PrOH = 100 : 0.05). The GC analysis showed that the low polar fraction was comprised of two products, **34** and **36**. Complete separation of **34** and **36** was successfully done by HPLC (reverse-phased C_{18} column) with THF–H_2O (55 : 45). The structures of **34**–**36** (Fig. 2) were determined by detailed analyses of the NMR spectra including DEPT, COSY-45, HOHAHA, NOESY, HMQC and HMBC. Products **34** and **35** had a pentacyclic hopane skeleton lacking a methyl group at C-18. In the HMBC spectra of **34** and **35**, the methyl signal (δ_H 1.01, s, Me-25) had strong correlations for both C-5 (δ_C 56.8) and C-9 (δ_C 51.3), indicating that this methyl group is positioned at C-10. On the other hand, the proton signal (δ_H 1.26, m, H-10) of **36** had clear HMBC cross peaks for both C-4 (δ_C 33.2) and C-8 (δ_C 41.0), indicating that a methyl group at C-10 was absent in **36**. The polar compound **35** had a tertiary hydroxy group (δ_C 73.9), the position of which was determined to be at C-22 from the HMBC cross peaks of Me-28/C-22 and Me-29/C-22. Compounds **34** and **35** were the products when the substrate **14** was recognized as C(19)-norsqualene (**14b** of Scheme 4), while **36** was produced from C(6)-norsqualene (**14a**). The produced amounts of **34** and **35** were *ca.* three times higher than that of **36**, indicating that the SHC recognized the C(19)-norsqualene **14b** over the C(6)-norsqualene **14a** as the substrate (Scheme 4).

Enzymatic products from substrate 15

Fig. 1(C) depicts the product distribution pattern of the reaction mixture obtained by incubating **15** at optimum catalytic conditions. Many products were detected. To isolate these products, 100 mg of **15** was incubated for 20 h with the cell-free homogenates (200 cm^3) from a 2 L culture of the cloned *E. coli*. The enzymic products consisted of a mixture of relatively high and low polar materials. SiO_2 column chromatography eluting with a step-wise elution (100% hexane to 10% EtOAc–hexane) afforded pure **40**, **43** and **44** (high polar compounds) in this elution order, but the separation of low polar materials was

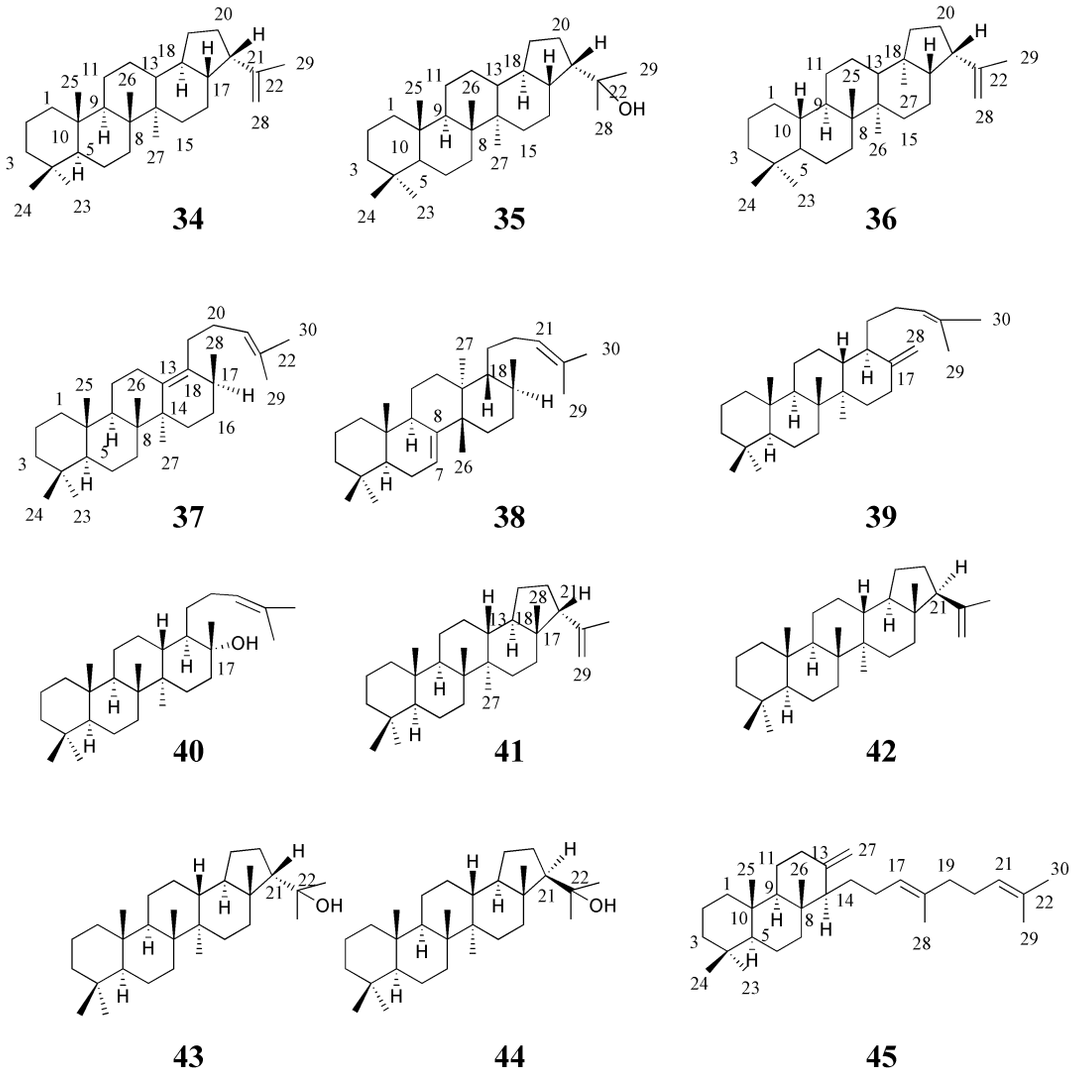
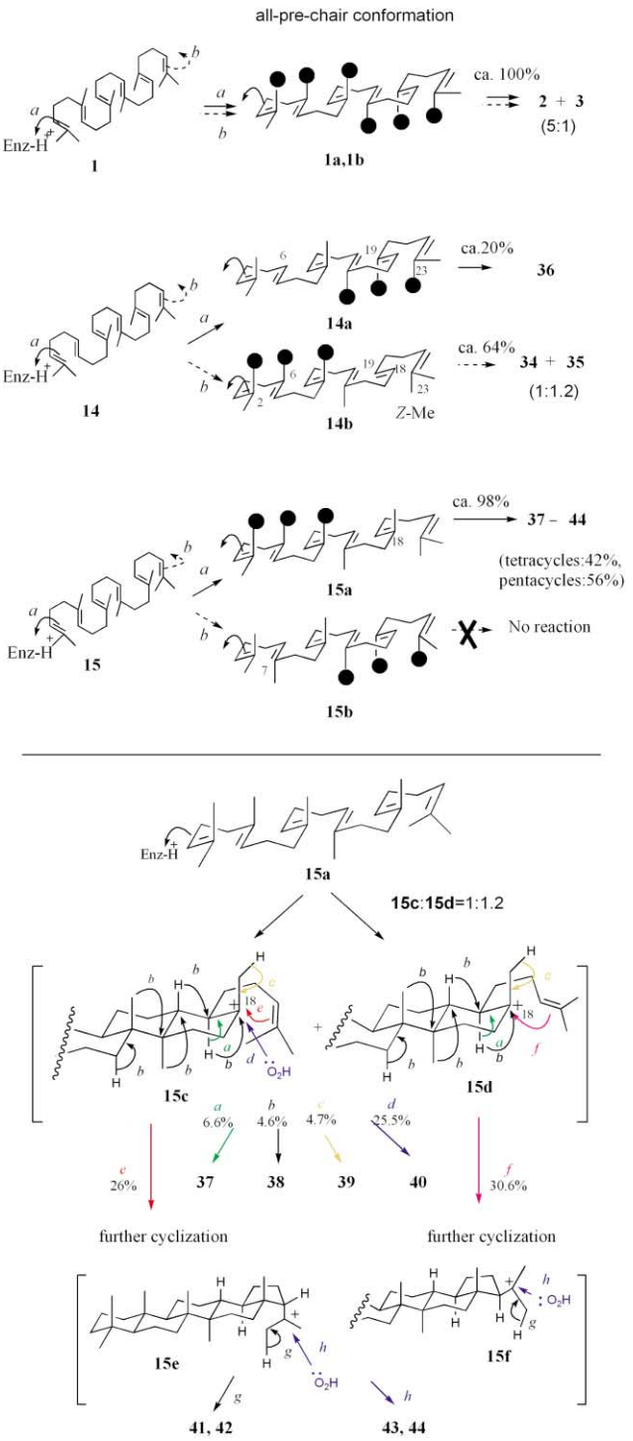


Fig. 2 Structures of products **34**–**45** obtained from analogs **14**–**17**.

unsuccessful. Repeated washing with cold MeOH succeeded in the separation of solid and oily materials. The solid fraction consisted of two enzymic products, each of which was purified by 10% $AgNO_3$–SiO_2 column chromatography eluting with hexane–EtOAc (100 : 0.02), giving pure products **41** and **42**. The mixture of oily materials contained three major products **37**–**39**, which were successfully separated by 10% $AgNO_3$–SiO_2 column chromatography (EtOAc–hexane = 0.05 : 100), and further purified by HPLC (reverse-phased C_{18} column) with THF–H_2O (70 : 30), affording pure products **37**–**39**. The structures of these products were unequivocally determined by detailed 2D NMR analyses. Product **37** had one olefinic proton (δ_H 5.38, br t, *J* 6.7), correlated with δ_C 125.3 in the HMQC, and had three quaternary sp^2 carbons (δ_C 130.9 s, C-22; 134.1 s, C-18; 135.0 s, C-13), indicating the presence of two double bonds. Two allyl methyl protons (δ_H 1.83 and 1.74, Me-30 and Me-29, respectively) that had the correlations with the two sp^2 carbons at C-21 and 130.9 were found, suggesting that one terminal isopropylidene moiety remained without undergoing cyclization, and thus **37** was a tetracyclic product. One doublet methyl signal was found (δ_H 1.24, d, *J* 7.0, Me-28). The remaining five methyl groups that appeared as singlets in the ^1H NMR spectrum was determined to be Me-23, -24, -25, -26 and -27 by HMBC and NOESY spectra. Strong HMBC cross peaks of Me-27 (δ_H 1.31)/C-13 and Me-28 (δ_H 1.24, d, *J* 7.0))/C-18 have established the position of another double bond. The clear NOE between Me-28 and Me-26 (δ_H 1.10, s) indicated that Me-28 was arranged in β-orientation. Thus, the structure of **37** was determined to have a 6/6/6/6-fused tetracyclic ring system, as shown in structure **37** of Fig. 2. Product **38** also showed two double bonds (δ_C 117.5 d, C-7; 125.9 d, C-21; 131.0 s, C-22; 145.9 s, C-8) and two olefinic protons (δ_H 5.61, br s, H-7 and 5.42, br t, *J* 7.0, H-21) according to the NMR spectra. The two allyl methyl groups (δ_H 1.85, s, Me-30 and 1.77, s, Me-29) were correlated with C-21 and C-22 in the HMBC spectrum. Thus, **38** also had a 6/6/6/6-fused tetracyclic ring system, but the position of the other double bond was different from that of **37**. Me-26 (δ_H 1.22, s) had a HMBC cross peak for C-8, indicating that the double bond position was at C7–C8. A strong NOE between Me-26 and H-18 (δ_H 1.12, m) was suggestive of β-orientation of H-18. Me-27 (δ_H 1.11, s) was oriented in α-disposition, since a clear NOE between Me-27 and H-9 (δ_H 2.38, m) was observed. A clear NOE between Me-28 (δ_H 1.10, d, *J* 5.8) and Me-26 definitively demonstrated the β-orientation for Me-28. Product **39** had an olefinic proton (δ_H 5.43, br t, *J* 6.7, H-21), which was correlated with both of two allyl methyl signals (δ_H 1.84, s, Me-30 and 1.75, s, Me-29) in the COSY-45 spectrum, suggesting that **39** also had a tetracyclic structure. The findings of seven methyl signals and the vinyl protons (δ_H 4.92, s and δ_H 5.05, s) in the ^1H NMR spectrum indicated that one of eight methyl groups involved in **15** underwent a deprotonation reaction from one methyl group. The vinyl protons had a strong cross peak for C-16 (δ_C 33.3) in the HMBC spectrum and showed clear correlations for H-16 (δ_H 2.32, 2H, dd, *J* 7.4, 4.6) and H-18 (δ_H 2.06, br t, *J* 9.4) in the COSY-45 spectrum, revealing that the vinyl group is positioned at C-17 and C-28. Observation of a strong NOE between Me-27 (δ_H 1.14, s) and H-18 (δ_H 2.06, br t, *J* 9.4) showed the



Scheme 4 All pre-chair conformation adopted during the polycyclization processes of **1**, **14** and **15** (top), and the reaction process of **15** leading to the enzymatic products **37**–**44** (bottom). Two cyclization pathways (*a* and *b*) are possible for **14** and **15**, but **1** is a symmetrical molecule, thus paths *a* and *b* lead to the identical all pre-chair conformation (**1a**,**b**). The symbol ● shows the steric hindrance due to 1,3-diaxial interactions between each of methyl groups.

α-orientation of H-18. Thus the structure of **39** was determined as shown in Fig. 2. The polar compound **40** had a tertiary alcoholic carbon (δ_C 73.4, C-17) and one olefinic proton (δ_H 5.47, br t, *J* 7.2, H-21), the latter being correlated with the two allyl methyl signals (δ_H 1.84, s, Me-30 and 1.78, s, Me-29) in the COSY-45 spectrum. Me-28 (δ_H 1.15, s) had HMBC correlations for C-17 and C-18 (δ_C 48.1). Strong NOEs of Me-28/H-13 (δ_H 1.41, m) and Me-27 (δ_H 1.05, s)/H-18 (δ_H 1.28, m) indicated that H-18 was of α-arrangement. Thus, **40** consisted of a 6/6/6/6-fused tetracyclic ring system having a hydroxy group. The vinyl protons (δ_H 4.84, s; δ_H 5.09, s, CH_2-29), which correlated with δ_C 110.6 (t, C-29) in the HMQC spectrum, were found in the ^{1}H NMR spectrum of **41**. The apparent HMBC cross peaks of the vinyl protons (H-29) for C-30 (δ_C 26.0), C-22 (δ_C 150.0) and C-21 (δ_C 56.4) indicated that an isopropenyl moiety was linked to C-21. In the HMBC spectrum, Me-28 (δ_H 0.97, s) had cross peaks for C-21 and C-18 (δ_C 42.6). In addition, Me-27 (δ_H 1.12, s) had a strong HMBC for H-13 (δ_H 1.68, m). The clear NOEs of Me-27/H-18 and Me-28/H-21 indicated an α-orientation for H-18 (δ_H 1.78, m) and β-orientation for H-21, respectively. These findings revealed that **41** had a 6/6/6/6/5-fused pentacyclic ring system like **2**, but the methyl group at C-18 of **2** was placed at C-17 for **41**. Product **42** also had a 6/6/6/6/5-fused pentacyclic skeleton according to the detailed NMR analyses, but the stereochemistry at C-21 was opposite to that of **41**; H-18 (δ_H 1.42, m) was placed in α-orientation, since clear NOEs of Me-27 (δ_H 1.05, s)/H-18 and H-18/H-21 (δ_H 2.15, t, *J* 9.4) were found, in addition to the absence of NOE between Me-28 and H-21. Polar compounds **43** and **44** contained a hydroxy group (δ_C 73.8 and 72.7, respectively), the position of which was determined to be at C-22 by the finding of strong HMBC cross peaks of Me-29 and Me-30 (δ_H 1.27, s and 1.25, s, for **43**; δ_H 1.35, s and 1.21, s, for **44**) for C-22. A strong NOE between Me-28 (δ_H 0.96, s) and H-21 (δ_H 1.75, m) was observed for **43**, but no NOE between them for **44**, suggesting β-orientation for H-21 of **43**, and α-orientation for that of **44**. The product distribution ratio (%) was determined by the GC analysis to be as follows: 6.6 : 4.6 : 4.7 : 7.3 : 10.9 : 25.5 : 18.7 : 19.7 for **37**, **38**, **39**, **41**, **42**, **40**, **43**, **44**, respectively, and 2.0 for the recovered **15**. It is noteworthy that all the tetracyclic skeletons of **37**–**40** consisted of a 6/6/6/6-fused ring system (six-membered D-ring), while a dammarane skeleton (a 6/6/6/5-fused tetracyclic ring system: five-membered D-ring) was not constructed, that is believed to be one of the intermediates in the bioconversion of **1** to **2**.[1] The tetracyclic ring systems of **37**–**40** were constructed according to a Markovnikov closure. The pentacyclic products **41**–**44** also were produced in a high yield (total yield, 56.6%). It is remarkable that **15** undergoes the polycyclization as **15a**, but not as **15b** (see Scheme 4). Thus, the cyclization pathway was directional.

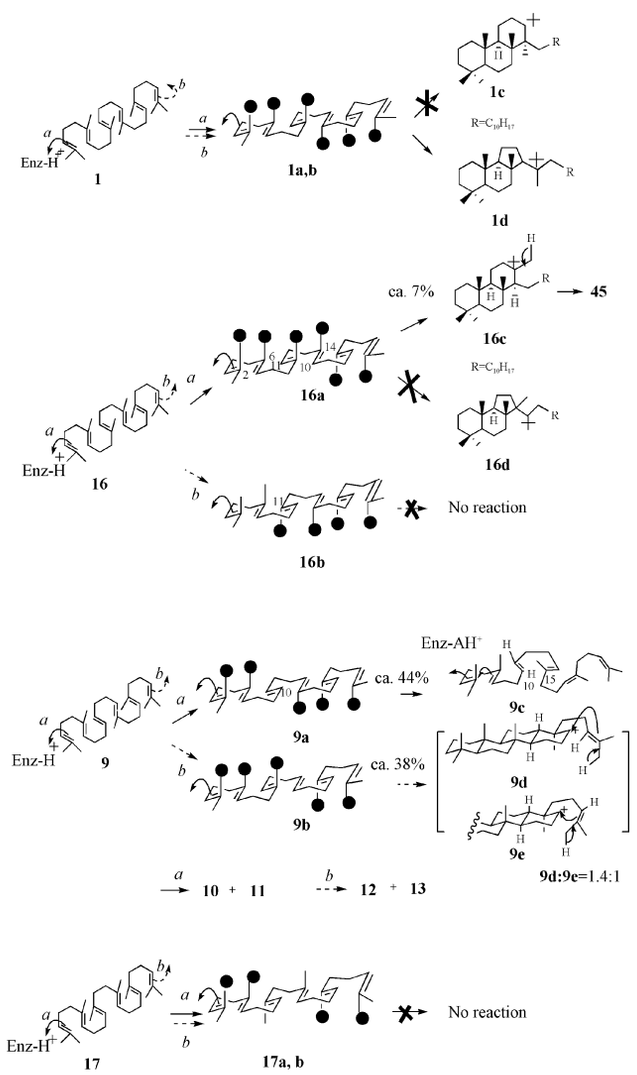
Enzymatic reaction of 16 and 17

The conversion from **16** was low (7%) and only one product **45** was found (Fig. 1(D)). Analog **16** (20 mg) was incubated with the cell-free homogenate (200 cm^3) from a 4 L culture of the cloned *E. coli* at the optimum catalytic conditions for 16 h. The quantity of the cell-free homogenates employed was about 6 times higher than that for **15** in order to isolate a sufficient amount of **45** for spectroscopic analyses. The hexane extracts were subjected to column chromatography over SiO_2 by eluting with hexane, giving pure **45** (yield, 2.3 mg). The presence of three allyl methyl groups (δ_H 1.70, s, Me-29; 1.78, s, Me-28; 1.82, s, Me-30) and two olefinic protons (δ_H 5.49, br t, *J* 6.9, H-17; 5.39, br t, *J* 6.8, H-21) suggests that **45** had a tricyclic skeleton. The vinyl protons (δ_H 4.84, s; 5.10, s: H-27) were also observed, that had correlations with C-14 (δ_C 56.7) and C-12 (δ_C 38.7) in the HMBC spectrum, demonstrating that the vinyl group was arranged as shown in the structure of **45** in Fig. 2. It is interesting that **45** consisted of a 6/6/6-fused tricyclic ring system, which was produced according to Markovnikov closure, but the yield was very low. Analog **17**, which has methyl groups at C-11 and C-14 on the squalene backbone, was incubated to test whether **17** can be recognized as a substrate. No detectable amount of the enzymic product was found and was marginal at best (Fig. 1(E)).

Discussion

As shown in Scheme 2, the methyl group(s) at the terminal double bond(s) plays crucial roles in forming a hopane skeleton[6] (see the cyclization products **6**–**8** from substrate

analogs **4** and **5**). In addition, the C(10)–Me at the central part of **1** has a pivotal role in adopting the all-chair conformation during the polycyclization reaction (path *a* of **9**).[7] Next, we investigated how the methyl group at C-6 of **1** has an influence upon the polycyclization cascade. The C(6)-norsqualene **14** showed a significantly high conversion (*ca*. 98%). Substrate **14** has two isopropylidene moieties at the both terminal sides, thus the polycyclization reaction can start from both the left (path *a*, *via* intermediate **14a**) and right sides (path *b* *via* **14b**). The structures of products **34**–**36** indicated that the polycyclization reactions of both **14a** and **14b** occurred with a folding of the all pre-chair conformation in a similar way as that of **1**. The absence of the methyl group at C-6 (**14a**) or C-19 (**14b**) showed no influence upon the construction of a hopane skeleton,[11] in contrast to the unusual conformation adopted for **9a** (Scheme 5). However, the amount of **34** + **35** produced (64%) was about three times higher than that of **36** (20%), suggesting that **14b** was more preferably recognized as the substrate than **14a** and that a presence of the methyl group at C-6 effectively leads to the formation of the hopane skeleton. This finding is very informative for the insight into the polycyclization mechanism; the 1,3-diaxial interactions between each of the β-arranged methyl groups at C-2, C-6 and C-10 are elevated for **1a** (**1b**) or **14b**, compared to that for **14a** lacking the methyl group at C-6, but the polycyclization reaction of **1a**,**b** or **14b** more efficiently proceeded than that of **14a**, suggesting that the cyclase enzyme has the binding site that can accept the methyl group at C-6. As an alternative explanation, a Markovnikov preference may have given rise to the efficient polycyclization reaction, because the tertiary cation can be formed at C-6 of **14b** during the polycyclization. In the case of substrate **1**, **2** is produced in a significantly higher yield, compared to **3** (**2** : **3** = 5 : 1), but **14b** gave a nearly equivalent amount of the hydroxylated product **35** relative to that of the deprotonation product **34** (**35** : **34** = 1.2 : 1). Now, it is well accepted that the "front water" acts as a catalytic base to abstract the proton from the (23*Z*)-Me of **1** to form **2**.[2,4,5a] The methyl group at C-19 of **1** may have a role in placing the (23*Z*)-Me at the correct position to facilitate the introduction of the double bond of **2**, but the decreased steric bulk size at C-19 of **14b** led to close proximity of the "front water" to the final hopanyl cation of **14b**. An optimal size at C-19 is a methyl group, whereas a hydrogen atom is small. Analog **15** also can undergo two cyclization pathways, *i.e.*, path *a* (**15a**) and *b* (**15b**). Compounds **15a** and **15b** have a methyl group at C-18 and C-7, respectively. Almost full conversion (98% yield) was found for **15a**, but no cyclization for **15b**. A difference between **15b** and **14a** is found only at C-7. An introduction of the methyl group at C-7 into **14a** completely stopped the polyclization reaction. A relatively high conversion occurred for **14a** (20%), but no cyclization product was found from **15b** (Scheme 4). This suggests that a chair conformation shown in **15b** would not have been organized around A- and B-rings during the polycyclization process due to the repulsive interaction between the (C)7-Me of **15b** and the substrate recognition site of the cyclase. As for the steric repulsion between each of methyl groups at C-2, C-6 and C-10, that for **15a** is greater than for **15b**. However, **15a** was efficiently cyclized (98%), while no reaction occurred for **15b**. Thus, it can be concluded from the substrate analogs **14**, **15**, **4** and **5** that three methyl groups at C-2, C-6 and C-10, which are arranged in β-orientation, are critical to the efficient polycyclization reaction, despite great steric hindrance between each methyl group involved. Introduction of a methyl group at C-18 of **14b**, *i.e.*, **15a**, afforded 6/6/6/6-fused tetracyclic products **37**–**40** and the 6/6/6/6/5-fused pentacyclic skeletons **41**–**44**. The abortive tetracyclic products were produced according to a Markovnikov closure (bottom of Scheme 4), the tertiary cation (**15c**,**d**) being formed at C-18. The stereochemistry at C-21 of **42** and **44** was opposite to that of **41** and **43** (natural type), suggesting that two different folding conformations (**15c** and **15d**) are involved in the polycyclization cascade, as shown in Scheme 4 (bottom). The deprotonation reactions from **15c** and/or **15d** gave **37** and **39**, respectively. A sequential reaction of 1,2-shifts of hydride and methyl group in an anti-parallel fashion gave **38**. Attack by water on **15c** and/or **15d** afforded **40**. A further cyclization gave pentacyclic cations **15e** and **15f**. The deprotonation reactions from the cations gave **41** and **42** and attack of water to the cations gave **43** and **44**. The difference between **15a** and **14b** is found only at C-18 position; a large methyl group is involved in **15a**, but a less bulky hydrogen atom in **14b**. Only the pentacyclic skeleton was found from **14b** as major products, but a significantly large amount of tetracyclic products (*ca*. 41%) was formed from **15a**, strongly indicating that the methyl group at C-18 interrupted the completion of the polycyclization reaction. Thus, a small hydrogen atom must be situated at C-18 for the full conversion of **1** into **2**. This interruption would have occurred due to the repulsive interaction between C(18)–Me of **15a** and the recognition site nearest to C-18, which is involved in the cyclase enzyme, and this repulsion also may have led to the improper folding conformation **15d**. The GC analysis (Fig. 2) showed that **15c** and **15d** were produced in a nearly equivalent amount [(**42** + **44**) : (**41** + **43**) = 1 : 1.2]. The experimental results obtained from **14b** and **15a** revealed that inappropriate bulk size around C-18 and C-19 disturbed the normal polycyclization pathway.



Scheme 5 Cyclization pathways of substrate analogs **9**, **16** and **17**. Analog **17** is a symmetrical molecule similarly to **1**, thus paths *a* and *b* result in an identical conformation as shown in **17a**,**b**. The symbol ● shows the steric hindrance due to 1,3-diaxial interactions between each of methyl groups.

Analog **16** was converted into **45** (6/6/6-fused tricyclic ring system) *via* the folding conformation **16a**, but the yield was low (7%). No detectable amount of the cyclization product *via* **16b** was found in the incubation mixture. The tricycle **45** was formed *via* the tertiary cation **16c** according to Markovnikov preference, but was not *via* the secondary cation **16d** (Scheme 5). When **1** was incubated with a variety of site-directed mutants, the 6/6/5-fused tricycle **1d** only was trapped without isolation of the 6/6/6-fused tricycle **1c** (Scheme 5).[1,12,13] Moreover, the trapping experiment of the secondary cation **1c** failed even by using the squalene analogs having a highly nucleophilic hydroxy group, and the tertiary cation **1d** only was trapped.[1,12*a*,*b*,14] These findings obtained from **1** and **16** may suggest that a stable tertiary cation having a significantly longer lifetime is involved during the polycyclization reaction.[15] Previously, we have reported the cyclization products of **9**.[7] The novel carbocyclic skeleton(s) consisting of a 6/5 + 5/5 + (6) ring system was constructed with a conformational change from **9a** into **9c** (*ca*. 44%), while hopane skeletons were formed *via* **9b** in high yields (38%). The difference between **16a** and **9b** is found only at C-14. A small hydrogen atom at C-14 (**9b**) led to complete polycyclization, but the introduction of a large methyl group at C-14 halted the polycyclization at the premature tricyclic stage (**45**) and the yield was low (7%). This finding suggested that the steric bulk size at C-14 is crucial to guide further cyclizations; the bulk size of a methyl group at C-14 is too large. The β-arranged methyl group at C-14 of **16a** may repulsively interact with the 10β-methyl group of **16a** and/or with the substrate recognition site nearest to C-14, giving the low yield (7%) of the Markovnikov product **45**. No cyclization of **16b** also indicated that the bulk size at C-11 must be small; a methyl group is too large to form a chair conformation around A/B-rings. Indeed, **9a** having a small hydrogen at C-11 underwent the polycyclization reaction in a high yield (44%) with an unusual conformation being adopted (Scheme 5). The importance of the accurate size of the attached group at C-11 was further demonstrated by the incubation experiment of analog **17**; no cyclization product was found as shown in Fig. 1(E). In the all pre-chair conformation, the 1,3-diaxial repulsive interactions found in **15b** and **17** are diminished, compared with that found in **1a**,**b**, but no cyclization occurred for **15b** and **17**. This finding further indicated that the methyl groups repulsively interacted with the substrate binding pockets nearest to C-7 and C-11.

In conclusion, the substrate analogs used in this study clearly demonstrated that substrate binding pockets are involved which accept the methyl groups of **1**. The methyl groups at C-2, C-6 and C-10, which are orientated in the β-face, are strongly captured by the cyclase to afford chair conformations around the A/B-ring formation, despite great repulsions due to the occurrence of 1,3-diaxial interactions (see **1a**,**b** of Schemes 4 and 5). The involvement of the thermodynamically stable Markovnikov cations generated during the polycyclization process would further overcome the 1,3-diaxial steric repulsions. The methyl group at C-2 is indispensable for the cyclization initiation.[4*a*,6] As shown in Scheme 5, **9a** lacking C(10)–Me was folded in an unusual conformation in the enzyme cavity. The less bulky hydrogen at C(10) of **9a** could not precisely interact with the binding site of C(10)–Me; in turn, the C(15)–Me could be strongly captured by the binding pocket intrinsic to C(10)–Me of **1** through the folding conformation shown in **9c**.[7] Thus, the C(15)–Me of **9a** could have a β-orientation as shown in **10** and **11**. In addition, a small hydrogen atom at C-6 of **14a** also could not correctly interact with the binding pocket inherent to C(6)–Me of **1**, leading to lower production of **14a** compared with that of **14b**. The methyl groups at C-15 and C-19 along with the *Z*-Me at C-23, which are arranged in α-face (**1a**,**b**), have a crucial role in assembling a half chain of the squalene molecule ranging from C-15 to C-23 into the exact position in the reaction cavity, leading to the correct folding conformation around D/E-ring formation. Substitution with a small hydrogen atom at C-15 (**9b**) gave the stereochemistry of 21α-H in a significantly high yield (**9d**), which is opposite to that of **9e** given by the normal polycyclization pathway (**9d** : **9e** = 1.4 : 1). The importance of appropriate steric bulk size is also true of **14b**, which gave a higher production of the hydroxylated product **35**, compared to **1a**,**b**. Incorporation of a methyl group into C-18 (**15a**) also halted the polycyclization reaction at the tetracyclic ring stage. Replacement with a small hydrogen atom at C-23 led to an abnormal cyclization product having a six-membered E-ring.[4*a*,6] Replacement of the methyl group at different positions, C-7 (**15b**, **17a**,**b**) and C-11 (**16b**), led to no cyclization. An introduction of a methyl group at C-14 also interrupted the polycyclization. This study verified that the methyl positions are important for leading to completion of the normal polycylization reaction and further demonstrated that the precise steric bulk size at the methyl positions of **1** is critical to the correct folding and the strong binding of the substrate to the squalene cyclase.

Experimental

Analytical methods

NMR spectra were recorded in C_6D_6 on a Bruker DMX 600 or DPX 400 spectrometer, the chemical shifts being relative to the solvent peak δ_H 7.280 and δ_C 128.0 ppm as the internal reference for ^{1}H and ^{13}C NMR spectra, respectively. The NMR spectra of all the enzymatic products were measured in C_6D_6. Some synthetic intermediates were measured in $CDCl_3$. The chemical shifts in $CDCl_3$ solution were given according to the internal solvent peaks of δ_H 7.26 and δ_C 77.0 ppm. The coupling constants *J* are given in Hz. GC analyses were done on a Shimadzu GC-8A chromatograph equipped with a flame ionization detector (DB-1 capillary column (0.53 mm × 30 m); injection temperature, 290 °C; column temperature, 270 °C; N_2 carrier pressure, 1.0 kg cm^{-2}). GC-MS spectra were on a JEOL SX 100 spectrometer under electron impact at 70 eV with a DB-1 capillary column (0.32 mm × 30 m), the oven temperature being elevated from 220 to 270 °C (3 °C min^{-1}). HR-EIMS was performed using a direct inlet system. Specific rotation values were measured at 25 °C with a Horiba SEPA-300 polarimeter.

Incubation conditions

Standard incubation conditions were performed according to the published protocols.[16,17] The cell-free extract was prepared as follows. A 1 L culture of *E. coli* encoding the native SHC from *Alicyclobacillus acidocaldarius* was harvested by centrifugation and to the collected pellets was added 50 cm^3 of citrate buffer solution (pH 6.0). Ultrasonication was then carried out to disrupt the cells. The supernatant was used for the incubations after removing the cell debris by centrifugation. 1 cm^3 of the supernatant contains *ca.* 200 μg of the pure SHC, which had a specific activity of *ca*. 1900 nmol min^{-1} mg^{-1} for the substrate **1**.[16,17]

Preparation of isoprenyl bromides

1-Bromo-3-methylbut-2-ene **18**, geranyl bromide **19** and geranylgeranyl bromide **20** were prepared by treatment of the corresponding alcohols with PBr_3 in THF at 0 °C for 30 min. The reaction mixture was poured into ice-water. The product was extracted with *n*-hexane and dried over anhydrous Na_2SO_4 and used without further purification. ^{1}H NMR data (400 MHz, $CDCl_3$): **18**: δ_H 1.71 (3H, s), 1.76 (3H, s), 4.00 (2H, d, *J* 7.8), 5.50 (br t, *J* 7.8). **19**: δ_H 1.60 (3H, s), 1.68 (3H, s), 1.73 (3H, s), 2.1–2.0 (4H, m), 4.01 (2H, d, *J* 8.4), 5.05 (1H, br t, J 6.8), 5.53 (1H, t, *J* 8.4). **20**: δ_H 1.60 (6H, s), 1.68 (3H, s), 1.73 (3H, s), 2.2–1.95 (14H, m), 4.08 (2H, d, *J* 8.4), 5.09 (3H, m), 5.53 (1H, t, *J* 8.4).

Synthesis of (*E*,*E*,*E*,*E*)-2,6,10,14,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene 16

To a stirred solution of **19** (320 mg, 1.47 mmol) in DMF (5 cm^3), 240 mg of benzenesulfinic acid sodium salt (1.46 mmol) was added under N_2 atmosphere and stirred over night. The reaction mixture was poured into ice-water, extracted with *n*-hexane and dried over anhydrous Na_2SO_4. After evaporation of hexane, the residue was subjected to SiO_2 column chromatography eluting with a mixture of hexane and EtOAc (100 : 10) to give (3,7-dimethylocta-2,6-diene-1-sulfonyl)benzene **21** (380 mg, 92% yield) in a pure state. NMR data in $CDCl_3$: δ_H 1.31 (3H, s), 1.58 (3H, s), 1.68 (3H, s), 1.99 (3H, s), 3.80 (2H, d, *J* 8.0), 5.01 (1H, m), 5.20 (1H, t, *J* 8.0), 7.53 (2H, t, *J* 7.2), 7.63 (1H, br t, *J* 7.2), 7.86 (2H, br d, *J* 7.8); δ_C 17.7 (q), 25.7 (q), 26.2 (t), 39.6 (t), 56.1 (t), 110.3 (d), 123.4 (d), 128.6 (d, 2 × C), 128.9 (d, 2 × C), 132.1 (s), 133.5 (d), 138.7 (s), 146.3 (s). To a solution of **21** (380 mg, 1.37 mmol) dissolved in 5 cm^3 of THF and $[(CH_3)_2N]_3PO$ (4 : 1), 2.0 cm^3 (mmol) of *n*-BuLi (1.58 M) was added at −20 °C to give an orange color, and stirred for 20 min. The temperature was further lowered to −78 °C and 550 mg (1.56 mmol) of **20** in THF (1.5 cm^3) was added dropwise and stirred for 1 h. The reaction mixture was poured into ice-water and extracted with *n*-hexane and dried over anhydrous Na_2SO_4, followed by purification with SiO_2 column chromatography, to afford the pure sulfone derivative **22** (290 mg, yield 38.5%). NMR data of **22** (400 MHz, $CDCl_3$): δ_H 1.19 (3H, s), 1.56 (3H, s), 1.58 (6H, s), 1.59 (3H, s), 1.61 (3H, s), 1.68 (6H, s), 2.0–1.9 (16H, m), 2.34 (1H, m), 2.88 (1H, m), 3.73 (1H, dt, *J* 3.2, 10.4), 5.1–4.9 (6H, m), 7.50 (2H, t, *J* 8.0), 7.60 (1H, d, *J* 7.6), 7.84 (2H, d, *J* 7.6); δ_C 15.92 (q), 15.96 (q), 16.34 (q), 16.47 (q), 17.64 (q, 2 × C), 25.65 (2, 2 × C), 26.23 (t), 26.42 (t), 26.59 (t), 26.64 (t), 26.73 (t), 39.69 (t, 3 × C), 39.73 (t), 64.82 (d), 117.0 (d), 118.6 (d), 123.6 (d), 123.8 (d), 124.1 (d), 124.3 (d), 128.6 (d, 2 × C), 129.1 (d, 2 × C), 131.2 (s), 131.9 (s), 133.3 (d), 134.9 (s), 135.2 (s), 138.1 (s), 138.6 (s), 145.1 (s). The deprotection reaction of the phenylsulfone group was done with $LiBEt_3H$ and a catalytic amount (3 mg) of $PdCl_2$(dppp) in Et_2O. The ethereal solution of the sulfone (28.7 mg/5 cm^3) was cooled at 0 °C. To the solution, 2.0 cm^3 of 1.0 M $LiBEt_3H$ was added, and stirred for 1 h and poured into ice-water, and the hexane-extract was purified with SiO_2 column chromatography eluting with *n*-hexane to give pure **16** (19.0 mg yield, 89%). NMR data of **16** (400 MHz, C_6D_6): δ_H 1.69 (6H, s), 1.73 (12H, s), 1.80 (6H, s), ~2.3 (20H, m), ~5.4 (6H, m); δ_C 16.1 (q), 17.7 (q), 25.8 (q), 27.1 (t), 27.2 (t), 28.7 (t), 30.4 (q), 40.2 (t), 124.8 (d), 124.8 (d), 124.9 (d), 131.1 (s), 134.9 (s), 135.1 (s). EIMS: *m*/*z* 69 (100%), 91 (65), 137 (16), 410 (M^+, 3).

Synthesis of (*E*,*E*,*E*,*E*)-2,6,10,15,18,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene 15

The synthetic intermediate **24** was prepared as follows. Compound **1** (1.01 g, 2.46 mmol) dissolved in 25 cm^3 of CH_2Cl_2 was treated with *m*-CPBA (629 mg, 3640 mmol) at 0 °C for 2 h to give a mixture of 2,3-, 6,7 and 10,11-oxidosqualenes. The reaction mixture was washed with 20% aq. $NaHCO_3$ and brine, and the organic layer was dried over anhydrous Na_2SO_4 (yield, 960 mg). A mixture of the products (11.88 g, prepared by repeated experiments) was treated with H_5IO_6 (9.49 g), followed by purification with a reverse-phased open column (C_{18}) by stepwise elution using aq. CH_3CN (75% CH_3CN to 100% CH_3CN), giving the pure C_{22}-aldehyde **23** (3.1 g). ^1H NMR data of **23** (400 MHz, $CDCl_3$): δ_H 1.59 (15H, s), 1.60 (3H, s), 1.65 (3H, s), ~2.0 (12H, m), 2.30 (2H, t, *J* 6.8), 2.11 (2H, t, *J* 7.2), 2.23 (6H, m), 2.30 (2H, t, *J* 8.0), 2.50 (2H, t, *J* 8.0), 5.10 (4H, m), 9.74 (1H, s). EIMS: *m*/*z* 69 (100), 81 (100), 316 (M^+, 20). To a suspension of Mg metal (19.2 mg, 0.8 mmol) in 5 cm^3 of Et_2O, the solution of CH_3I (454 mg, 3.4 mmol) in Et_2O (5 cm^3) was added and refluxed for 30 min. To the Grignard reagent, a solution of **23** (100 mg) in Et_2O (5 cm^3) was slowly added and stirred for 30 min. Then, the reaction mixture was poured into aq. dilute HCl, the ether layer being washed with saturated Na_2CO_3 and dried over anhydrous Na_2SO_4, followed by purification with SiO_2 column chromatography eluting with a mixture of hexane–EtOAc (100 : 10) to afford pure **24** in a yield of 84.5 mg (80 %). ^1H NMR data of **24** (400 MHz, $CDCl_3$): δ_H 1.18 (3H, d, *J* 6.0), 1.60 (6H, s), 1.61 (3H, s), 1.67 (3H, s), ~2.0 (16H, m), 3.77 (1H, m), ~5.12 (4H, m). EIMS: *m*/*z* 69 (100), 81 (47), 109 (64), 137 (12), 333 (M^+, 3). To obtain **25** from **24**, Collin's reagent was prepared. To a solution of pyridine (1.5 g, 19.0 mmol) in CH_2Cl_2 (5 cm^3), 324 mg of CrO_3 (3.2 mmol) was added and stirred for 2 h, then the reaction flask was cooled to 0 °C. A solution of **24** (90 mg, 0.27 mmol) in CH_2Cl_2 (3 cm^3) was slowly added and stirred for 12 h, and then anhydrous $MgSO_4$ was added. The resultant precipitates were filtered off, followed by purification with SiO_2 column chromatography by eluting with a mixed solvent of hexane and EtOAc (100 : 5) to give **25** (yield, 60.5 mg, 68%). NMR data of **25** (400 MHz, $CDCl_3$): δ_H 1.59 (12H, s), 5.10 (4H, m), 1.67 (3H, s), ~2.0 (12H, m), 2.12 (3H, s), 2.23 (2H, t, *J* 7.6), 2.50 (2H, t, *J* 7.6); δ_C, 15.93 (q), 15.98 (q), 16.00 (q), 17.61 (q), 25.63 (q), 25.58 (t), 26.70 (t), 28.04 (t), 28.16 (t), 29.79 (q), 33.54 (t), 39.67 (2 × C, t), 42.37 (t), 124.0 (d), 124.2 (d), 124.3 (d), 124.9 (d), 131.2 (s), 133.5 (s), 134.8 (s), 135.2 (s). 208.7 (s, C=O). EIMS: *m*/*z* 69 (100%), 81 (62), 125 (41), 330 (M^+, 3). NaH (24 mg, 1.0 mmol), obtained after washing with hexane, was added to the THF solution of $(EtO)_2POCH_2CO_2Et$ (336 mg, 1.5 mmol/5 cm^3) and stirred for 30 min under N_2 atmosphere. To the solution, **25** (100 mg, 0.3 mmol) in 5 cm^3 of THF was added and stirred for 12 h. The reaction mixture was poured into ice-water and extracted with hexane. SiO_2 column chromatography eluting with a mixture of hexane and EtOAc (100 : 2) gave pure **26a** in a yield of 88.3 mg (74%). ^1H NMR data of **26a** (400 MHz, $CDCl_3$): δ_H 1.27 (3H, t, *J* 7.2), 1.57 (3H, s), 1.60 (12H, s), 1.68 (3H, s), ~2.0 (16H, m), 4.14 (2H, q, *J* 7.2), 5.11 (4H, m), 5.65 (1H, s). EIMS: *m*/*z* 69 (100%), 81 (85), 128 (51), 400 (M^+, 19). The ester **26a** was reduced with DIBAL-H as follows. To the solution of **26a** (100 mg, 0.25 mmol) dissolved in Et_2O (5 cm^3), 1.5 cm^3 of (*iso*-Bu)$_2$AlH (0.95 M in *n*-hexane) was slowly added and stirred for 1 h. The reaction mixture was poured into aq. EtOAc, stirred for 2 h and then aq. saturated NH_4Cl was added, the resultant precipitated salts being filtered off. After evaporation of the organic layer, the residues were subjected to SiO_2 column chromatography eluting with 20% EtOAc–hexane to yield **27a** (70 mg, 78%). ^1H NMR data of **27a** (400 MHz, $CDCl_3$): δ_H 1.60 (12H, s), 1.67 (6H, s), ~1.98 (8H, m), ~2.06 (8H, m), 4.12 (2H, *J* 6.8), 5.11 (4H, m), 5.40 (1H, t, *J* 6.4). EIMS *m*/*z* 69 (100%), 81 (82), 93 (40), 135 (29), 358 (M^+, 3). 3-Methylbut-2-en-1-ol and **27a** were treated with PBr_3 to prepare the corresponding bromides **18** and **28**, respectively. **18** was converted into **29** according to the method described in the preparation of **21**. ^1H NMR data of **29** (400 MHz, $CDCl_3$); δ_H 1.27 (3H, s), 1.66 (3H, s), 3.74 (2H, br d, *J* 7.6), 5.14 (br t, *J* 6.8), 7.50 (2H, t, *J* 7.2), 7.60 (1H, *J* 7.2), 7.82 (d, *J* 7.6). A solution of **29** (100 mg, 0.476 mmol), dissolved in 10 cm^3 of the mixed solvent of THF and $[(CH_3)_2N]_3PO$ (4 : 1), was cooled at −20 °C and then 2.0 cm^3 of *n*-BuLi (1.58 M in *n*-hexane) was added, the color changing to orange, and stirred for 20 min. The temperature was further lowered to −78 °C. To the cooled solution, **28a** (75 mg, 0.178 mmol) in THF (5 cm^3) was added and stirred for 1 h. The reaction mixture was poured into ice-water, extracted with hexane, and dried over anhydrous Na_2SO_4. Purification was done with SiO_2 column chromatography eluting with 10% EtOAc–hexane, giving pure **30a** in a yield of 52.6 mg (79%). NMR data of **30a** (400 MHz, $CDCl_3$): δ_H 1.16 (3H, s), 1.55 (3H, s), 1.58 (12H, s), 1.65 (3H, s), 1.66 (3H, s), ~2.0 (16H, m), 2.33 (1H, m), 2.83 (1H, m), 3.68 (1H, ddd, *J* 10.7, 10.7, 3.6), 4.95 (2H, t, *J* 7.2), 5.10 (4H, m), 7.50 (2H, t, *J* 7.6), 7.60 (1H, t, *J* 7.6), 7.82 (2H, d *J* 7.2). The phenylsulfone group was removed by using $LiBEt_3H$ and a catalytic amount of $PdCl_2$(dppp) in

Et_2O. Compound **30a** (50 mg, 0.09 mmol) was dissolved in 5 cm^3 of diethyl ether and was cooled at 0 °C. To the solution, 1.0 cm^3 of 1.0 M $LiBEt_3H$ was added and stirred for 30 min and poured into ice-water, and then extracted with hexane and purified with SiO_2 column chromatography by eluting with *n*-hexane to give pure **15** (28.1 mg yield, 75%). NMR data of **15** (400 MHz, C_6D_6): δ_H 1.69 (6H, s), 1.73 (12H, s), 1.80 (6H, s), ~2.3 (20H, m), ~5.4 (6H, m); δ_C 16.1 (q), 16.2 (q), 17.7 (q), 17.8 (q), 25.8 (q), 27.1 (t), 27.2 (t), 28.7 (t), 28.8 (t), 38.9 (t), 40.2 (t), 124.8 (d, 2 × C), 124.9 (d), 125.0 (d), 131.1 (s), 131.3 (s), 134.9 (s), 135.1 (s), 135.3 (s). EIMS: *m*/*z* 69 (100%), 81 (78), 95 (31), 137 (22), 410 (M^+, 4).

Preparation of (*E*,*E*,*E*,*E*)-2,6,10,15,23-pentamethyltetracosa-2,6,10,14,18,22-hexaene 14

The aldehyde **23** was subjected to a Wadsworth–Emmons reaction with $(EtO)_2POCH_2CO_2Et$ to obtain the desired ester **26b**. The preparation protocols from **26b** to **14** were essentially the same as those from **26a** to **15**, which was described above. ^1H NMR data of **26b** (400 MHz, $CDCl_3$): δ_H 1.27 (3H, t, *J* 7.2), 1.57 (12H, s, 4 × Me), 1.65 (3H, 1 × Me, s), 2.10–1.94 (16H, m), 2.28 (2H, dd, *J* 7.2), 4.16 (2H, q, *J* 7.2), 5.09 (4H, m), 5.79 (1H, d, *J* 15.5), 6.93 (1H, m). EIMS: *m*/*z* 69 (100%), 81 (52), 107 (32), 386 (M^+, 13). ^1H NMR data of **27b** (400 MHz, $CDCl_3$); δ_H 1.58 (12H, s), 1.73 (3H, s), ~2.0 (16H, m), 4.06 (2H, d, *J* 4.4), 5.09 (4H, m), 5.64 (2H, m). EIMS: *m*/*z* 69 (100%), 81 (100), 344 (M^+, 3). NMR data of **14** (400 MHz, $CDCl_3$): δ_H 1.58 (15H, s, 5 × Me), 1.66 (6H, s, 2 × Me), 2.10 (20H, m), 5.09 (5H, m), 5.34 (2H, br s); δ_C 15.99 (q), 16.03 (q, 2 × Me), 17.68 (q), 17.73 (q), 25.7 (q, 2 × C), 26.6 (t), 26.7 (t), 28.2 (t, 2 × C), 135.1 (s), 31.3 (t), 31.6 (t), 32.8 (t), 39.72 (t), 39.73 (t), 39.78 (t), 124.17 (d), 124.25 (d), 124.27 (d), 124.37 (d), 124.38 (d), 129.9 (d), 130.2 (d), 131.2 (s), 131.5 (s), 134.8 (s), 134.9 (s). EIMS: *m*/*z* 69 (100%), 81 (80), 327 (6), 353 (3), 396 (M^+, 7).

Synthesis of (*E*,*E*,*E*,*E*)-2,6,11,14,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene 17

To a solution of malonic dimethyl ester (13.56 g, 0.1 mol) in EtOH (150 cm^3), 64.1 cm^3 of 1.5 M NaOEt (96 mmol) was added under N_2 atmosphere and the reaction temperature was cooled to −20 °C. **19** (13.92 g, 64 mmol), dissolved in 50 cm^3 of EtOH, was slowly added and stirred for 1 h. The reaction mixture was poured into ice-water, extracted with hexane and dried over anhydrous Na_2SO_4. This crude material was stirred overnight in 1 M KOH/MeOH. The solution was diluted with water and acidified with 4 M aq. HCl and extracted with EtOAc (× 4), and dried over anhydrous Na_2SO_4. After evaporation, the residues were heated at 130 °C to undergo decarboxylation, followed by the methylation with dry 5% HCl–MeOH under reflux. Purification was done with SiO_2 column chromatography (hexane–EtOAc 100 : 2) to give pure **31** (yield 10 g). ^1H NMR data of **31** (400 MHz, $CDCl_3$): δ_H 1.57 (3H, s), 1.59 (3H, s), 1.65 (3H, s), 1.95 (2H, br t, *J* 6.8), 2.02 (2H, br t, *J* 6.8), 2.33 (2H, br s), 3.64 (3H, s), 5.05 (2H, m). EIMS: *m*/*z* 69 (100), 95 (58), 107 (41), 135 (23), 210 (M^+, 32). To a solution of **31** (5.20 g) in THF (150 cm^3), powdered $LiAlH_4$ was added in a small portion at 0 °C, the reaction being monitored by TLC, and the reaction mixture was poured into aq. EtOAc and stirred overnight. Filtration gave almost pure alcohol **32** (yield 4.4 g). ^1H NMR data of **31** (400 MHz, C_6D_6): δ_H 1.68 (6H, s), 1.79 (3H, s), ~2.2 (6H, m), 3.99 (2H, br s), 5.34 (2H, br m), 5.65 (1H, m). EIMS *m*/*z* 57 (100), 86 (30), 95 (16), 139 (7), 182 (M^+, 1). Next, the alcohol **32** was subjected to the oxidation reaction. To a stirred suspension of pyridinium chrorochromate (1.2 g, 0.54 mmol) in CH_2Cl_2 containing Celite and anhydrous NaOAc (300 mg each), a solution of **32** (500 mg) in CH_2Cl_2 (5 cm^3) was added. After further stirring for 30 min, the reaction mixture was passed through a short SiO_2 column to remove the salts by eluting with hexane–EtOAc (100 : 20) (yield, 380 mg, 76%). EIMS *m*/*z* of **33**: 69 (100), 81 (23), 93 (28), 137 (12), 180 (M^+, 7). **33** thus obtained was subjected to the Wittig reaction with $Ph_3P{=}C(CH_3)CO_2Et$ [18] under reflux in benzene to obtain 2,7,11-trimethyldodeca-2,6,10-trienoic acid ethyl ester; EIMS: *m*/*z* 69 (100%), 81 (43), 128 (35), 264 (M^+, 13). From 1.5 g of **33**, 1.4 g of the ethyl ester was obtained (yield, 64%). The ester was then reduced with LAH to the corresponding alcohol (yield, 68%), EIMS: *m*/*z* 69 (100%), 81 (40), 95(27), 137 (30), 204 (4), 222 (M^+, 3). The alcohol (400 mg) was then treated with PBr_3 to give the corresponding bromide, which was then subjected to allyl coupling reaction [10] to give the desired substrate **17** as follows: to the stirred solution of pyrrolidine (250 mg, 3.5 mmol) in dry Et_2O, was slowly added 2.1 cm^3 of *n*-BuLi (1.58 M in hexane) at 0 °C and the solution allowed to stand for 20 min under N_2 atmosphere. Powdered CuI (250 mg, 1.3 mmol) was added in small portions, the color changing to reddish brown. After 20 min, a solution of 100 mg of the C_{15}-bromide (0.35 mmol) in Et_2O (15 cm^3) was slowly added and stirred for 90 min. The reaction mixture was poured into ice-water and the product was extracted with hexane and dried over anhydrous Na_2SO_4. The desired product **17** was obtained by purifying with 5% $AgNO_3$–SiO_2 column chromatography. The coupling yield was small, but 6.8 mg was obtained from 100 mg of the C_{15}-bromide. Repeated coupling reaction experiments allowed us to obtain 90 mg of **17**. NMR data of **17** (400 MHz, C_6D_6): δ_H 5.45 (2H, br t, *J* 7.1), 5.42 (2H, br t, *J* 7.1), 5.37 (2H, br t, *J* 7.1), 2.35–2.20 (20H, m), 1.80 (6H, s), 1.74 (6H, s), 1.73 (6H, s), 1.69 (6H, s); δ_C 134.93 (s, 2 × C), 134.94 (s, 2 × C), 131.1 (s, 2 × C), 124.93 (d, 2 × C), 124.86 (d, 2 × C), 124.81 (d, 2 × C), 40.2 (t, 4 × C), 28.7 (t, 2 × C), 27.2 (t, 2 × C), 27.1 (t, 2 × C), 25.8 (q, 2 × C), 17.7 (q, 2 × C), 16.15 (q, 2 × C), 16.1 (q, 2 × C). EIMS: *m*/*z* 69 (100%), 81 (78), 95 (27), 137 (23), 410 (M^+, 4).

Spectroscopic data of products 34–45

Product 34 (solid). 600 MHz in C_6D_6: δ_H 0.89 (2H, m, H-1, H-5), 0.99 (3H, s, Me-24), 1.01 (3H, s, Me-25), 1.03 (3H, s, Me-23), 1.09 (3H, s, Me-27), 1.11 (m, H-19), 1.20 (m, H-17), 1.29 (m, H-3), 1.34 (m, H-12), 1.35 (m, H-16), 1.38 (m, H-13), 1.44 (m, H-15), 1.49 (m, H-2), 1.50 (2H, m, H-3, H-15), 1.52 (2H, m, H-9, H-10), 1.54 (2H, m, H-7), 1.59 (m, H-6), 1.60 (m, H-18), 1.62 (m, H-12), 1.64 (m, H-6), 1.71 (m, H-2), 1.74 (m, H-19), 2.04 (m, H-20), 2.70 (m, H-21), 4.85 (s, H-28), 5.03 (s, H-28); δ_C 15.15 (C-27), 16.13 (C-26), 16.45 (C-25), 18.99 (C-2), 19.07 (C-6), 21.29 (C-12), 21.79 (C-24), 24.44 (C-29), 24.54 (C-11), 27.56 (C-16), 29.62 (C-20), 29.98 (C-19), 32.40 (C-15), 33.44 (C-4), 33.57 (C-23), 34.63 (C-7), 37.81 (C-10), 40.64 (C-1), 40.93 (C-8), 42.09 (C-14), 42.32 (C-3), 42.48 (C-18), 44.87 (C-13), 48.25 (C-21), 50.86 (C-17), 51.30 (C-9), 56.79 (C-5), 110.70 (C-28), 149.42 (C-22), Assignments of C-2 and C-6 are exchangeable. EIMS: *m*/*z* 191 (100%), 353 (25), 381 (18), 396 (M^+, 63). HREIMS: *m*/*z* found 396.3713 (M^+, $C_{29}H_{48}$ requires 396.3756). $[a]_D^{25}$ (C_6H_6) 7.24 (*c* 0.12).

Product 35 (solid). 600 MHz in C_6D_6: δ_H 0.90 (2H, m, H-1, br d, 9.4, 1.7 Hz, H-5), 0.97, (m, H-19), 0.99 (3H, s, Me-24), 1.01 (3H, s, Me-25), 1.04 (3H, s, Me-23), 1.11 (3H, s, Me-26), 1.14 (3H, s, Me-27), 1.22 (4H, m, H-17, s, Me-29), 1.25 (3H, s, Me-29), 1.26 (m, H-13), 1.28 (m, H-3), 1.32 (m, H-11), 1.33 (m, H-12), 1.45 (2H, m, H-15), 1.48 (2H, m, H-18, H-20), 1.49 (2H, m, H-2), 1.50 (m, H-3), 1.51 (m, H-9), 1.55 (2H, m, H-7), 1.63 (2H, m, H-6), 1.64 (m, H-11), 1.74 (m, H-1), 1.75 (m, H-12), 1.84 (m, H-16), 1.89 (m, H-19), 1.90 (m, H-20), 1.95 (m, H-16), 2.10 (m, H-21); δ_C 15.16 (C-27), 16.07 (C-26), 16.62 (C-25), 18.99 (C-2), 19.07 (C-6), 21.32 (C-11), 21.80 (C-24), 25.56 (C-16), 27.53 (2 × C, C-12, C-20), 27.94 (C-28), 29.94 (C-29), 30.98 (C-19), 33.28 (C-15), 33.45 (C-4), 33.58 (C-23), 34.61 (C-7), 37.82 (C-10), 40.66 (C-1), 40.86 (C-8), 41.61 (C-14), 42.33 (C-3), 44.33 (C-18), 45.51 (C-13), 50.12 (C-17), 50.97

(C-21), 51.37 (C-9), 56.84 (C-5), 73.91 (C-22), Assignments of C-2 and C-6 are exchangeable. EIMS: *m/z* 191 (100%), 356 (44), 396 (33), 414 (M^+, 22). HREIMS: *m/z* found 414.3848 (M^+, $C_{29}H_{50}O$ requires 414.3862). $[a]_D^{25}$ (C_6H_6) +10.57 (*c* 0.11).

Product 36 (solid). 600 MHz in C_6D_6: δ_H 0.78 (2H, m, H-1, H-5), 0.99 (3H, s, Me-24), 1.01 (3H, s, Me-25), 1.03 (3H, s, Me-23), 1.09 (3H, s, Me-27), 1.11 (m, H-19), 1.20 (m, H-17), 1.29 (m, H-3), 1.34 (m, H-12), 1.35 (m, H-16), 1.38 (m, H-13), 1.44 (m, H-15), 1.49 (m, H-2), 1.50 (2H, m, H-3, H-15), 1.52 (2H, m, H-9, H-10), 1.54 (2H, m, H-7), 1.59 (m, H-6), 1.60 (m, H-18), 1.62 (m, H-12), 1.64 (m, H-6), 1.71 (m, H-2), 1.74 (m, H-19), 2.04 (m, H-20), 2.70 (m, H-21), 4.85 (s, H-28), 5.03 (s, H-28); δ_C 15.15 (C-27), 16.13 (C-26), 16.45 (C-25), 18.99 (C-2), 19.07 (C-6), 21.29 (C-12), 21.79 (C-24), 24.44 (C-29), 24.54 (C-11), 27.56 (C-16), 29.62 (C-20), 29.98 (C-19), 32.40 (C-15), 33.44 (C-4), 33.57 (C-23), 34.63 (C-7), 37.81 (C-10), 40.64 (C-1), 40.93 (C-8), 42.09 (C-14), 42.32 (C-3), 42.48 (C-18), 44.87 (C-13), 48.25 (C-21), 50.86 (C-17), 51.30 (C-9), 56.79 (C-5), 110.70 (C-28), 149.42 (C-22), Assignments of C-2 and C-6 are exchangeable. EIMS: *m/z* 285 (100%), 381 (35), 396 (M^+, 63). HREIMS: *m/z* found 396.3732 (M^+, $C_{29}H_{48}$ requires 396.3756). $[a]_D^{25}$ (C_6H_6) +5.67 (*c* 0.08).

Product 37 (oil). 600 MHz in C_6D_6: δ_H 0.92 (m, H-5), 0.95 (m, H-1), 0.98 (3H, s, Me-24), 1.01 (3H, s, Me-25), 1.05 (3H, s, Me-23), 1.10 (3H, s, Me-26), 1.20 (m, H-15), 1.24 (3H, d, *J* 7.0 Hz, Me-28), 1.29 (m, H-3), 1.31 (3H, s, Me-27), 1.40 (m, H-2), 1.44 (m, H-6), 1.45 (m, H-11), 1.51 (m, H-2), 1.52 (m, H-3), 1.55 (2H, m, H-16), 1.58 (2H, m, H-7), 1.63 (m, H-9), 1.65 (m, H-11), 1.67 (m, H-6), 1.74 (3H, s, Me-30), 1.79 (m, H-1), 1.83 (3H, s, Me-29), 1.93 (m, H-15), 2.11 (m, H-19), 2.16 (m, H-12), 2.18 (2H, m, H-20), 2.46 (m, H-19), 2.71 (m, H-12), 5.38 (t, *J* 7.2 Hz); δ_C 16.57 (C-25), 17.71 (C-30), 18.16 (C-26), 19.04 (C-6), 19.07 (C-2), 19.54 (C-28), 21.46 (C-27), 21.89 (C-11), 25.64 (C-12), 25.90 (C-29), 26.06 (C-15), 27.26 (C-16), 28.63 (C-20), 33.07 (C-19), 33.40 (C-4), 33.65 (C-23), 33.70 (C-17), 35.09 (C-7), 37.78 (C-10), 40.72 (C-1), 41.18 (C-8), 42.34 (C-3), 44.26 (C-14), 51.17 (C-9), 56.80 (C-5), 125.28 (C-21), 130.92 (C-22), 134.13 (C-18), 135.02 (C-13), 125.74 (C-17), 131.09 (C-22), 135.07 (C-18), 148.83 (C-13), Assignments of C-2 and C-6 are exchangeable. EIMS: *m/z* 69 (100%), 148 (82), 161 (67), 191 (93), 205 (70), 410 (M^+, 86). HREIMS: *m/z* found 410.3890 (M^+, $C_{30}H_{50}$ requires 410.3913). $[a]_D^{25}$ ($CHCl_3$) −25.23 (*c* 0.11).

Product 38 (oil). 600 MHz in C_6D_6: δ_H 0.96 (3H, s, Me-25), 0.97 (3H, s, Me-24), 0.92 (m, H-5), 1.03 (3H, s, Me-23), 1.04 (m, H-1), 1.10 (3H, d, *J* 5.8 Hz, Me-28), 1.11 (3H, s, Me-27), 1.12 (2H, m, H-18), 1.18 (m, H-19), 1.22 (3H, s, Me-26), 1.30 (m, H-3), 1.42 (m, H-17), 1.44 (m, H-16), 1.46 (m, H-2), 1.52 (m, H-5), 1.52 (2H, m, H-2), 1.57 (m, H-3), 1.58 (m, H-11), 1.67 (m, H-11), 1.68 (2H, m, H-16, H-19), 1.71 (2H, m, H-15), 1.77 (3H, s, Me-29), 1.78 (m, H-1), 1.80 (2H, m, H-12), 1.85 (3H, s, Me-30), 2.01 (m, H-6), 2.21 (m, H-20), 2.24 (m, H-6), 2.26 (m, H-20), 2.38 (2H, m, H-9), 5.42 (t, *J* 7.0 Hz, H-21), 5.61 (s, H-7); δ_C 13.38 (C-25), 17.46 (C-11), 17.84 (C-29), 19.47 (C-2), 21.22 (C-27), 21.50 (C-23), 21.93 (C-28), 24.19 (C-26), 24.88 (C-6), 25.83 (C-30), 30.50 (C-19), 31.45 (C-20), 31.71 (C-16), 33.12 (C-15), 33.19 (C-12), 33.26 (C-4), 33.38 (C-24), 34.40 (C-17), 35.56 (C-10), 37.79 (C-13), 39.09 (C-1), 41.38 (C-14), 42.63 (C-3), 49.18 (C-9), 50.19 (C-18), 51.23 (C-5), 117.53 (C-7), 125.93 (C-21), 131.05 (C-22), 145.97 (C-8), EIMS: *m/z* 231 (83%), 243 (61), 395 (100), 410 (M^+, 58). HREIMS: *m/z* found 410.3899 (M^+, $C_{30}H_{50}$ requires 410.3913). $[a]_D^{25}$ ($CHCl_3$) +22.08 (*c* 0.12).

Product 39 (oil). 400 MHz in C_6D_6; δ_H 0.87 (2H, m, H-1, H-5), 0.95 (3H, s, Me-25), 0.97 (3H, s, Me-24), 1.03 (3H, s, Me-23), 1.05 (3H, s, Me-26), 1.14 (3H, s, Me-27), 1.15 (m, H-12), 1.26 (m, H-3), 1.30 (m, H-11), 1.40 (2H, m, H-2, H-15), 1.47 (2H, m, H-7, H-9), 1.50 (2H, m, H-6), 1.51 (m, H-3), 1.60 (H, m, H-2), 1.61 (m, H-11), 1.63 (m, H-13), 1.70 (m, H-19), 1.71 (m, H-15), 1.72 (m, H-1), 1.75 (3H, s, Me-30), 1.84 (3H, s, Me-29), 1.86 (m, H-19), 1.92 (m, H-12), 2.07 (m, H-18), 2.30 (m, H-20), 2.32 (2H, dd, *J*7.4, 4.6, H-16), 2.41 (m, H-20), 4.92 (s, H-28), 5.02 (s, H-28), 5.43 (t, *J* 6.7 Hz, H-21); δ_C 15.59 (C-27), 16.01 (C-26), 16.42 (C-25), 17.83 (C-30), 18.92 (C-6), 19.05 (C-2), 21.27 (C-11), 25.55 (C-20), 25.87 (C-29), 26.66 (C-12), 29.16 (C-19), 33.26 (C-16), 33.41 (C-4), 33.57 (C-23), 34.08 (C-15), 34.38 (C-7), 37.67 (C-10), 40.59 (C-1), 41.30 (C-8), 41.87 (C-14), 42.36 (C-3), 43.10 (C-18), 43.17 (C-13), 50.87 (C-9), 56.81 (C-5), 105.51 (C-28), 125.79 (C-21), 130.93 (C-22), 152.12 (C-17). Assignments of C-2 and C-6 and those of C-13 and C-18 are exchangeable. EIMS: *m/z* 69 (100%), 191 (71), 231 (70), 395 (74), 410 (M^+, 58). HREIMS: *m/z* found 410.3940 (M^+, $C_{30}H_{50}$ requires 410.3913). $[a]_D^{25}$ ($CHCl_3$) +15.50 (*c* 0.1).

Product 40 (oil). 600 MHz in C_6D_6: δ_H 0.88 (m, H-5), 0.90 (m, H-1), 0.98 (3H, s, Me-25), 0.99 (3H, s, Me-24), 1.02 (3H, s, Me-26), 1.03 (3H, s, Me-23), 1.05 (3H, s, Me-27), 1.15 (3H, s, Me-28), 1.20 (m, H-19), 1.23 (2H, m, H-11, H-12), 1.28 (2H, m, H-3, H-18), 1.41 (m, H-13), 1.44 (m, H-6), 1.45 (2H, m, H-7), 1.48 (m, H-9), 1.51 (m, H-2), 1.52 (m, H-3), 1.61 (3H, m, H-12, H-15), 1.62 (2H, m, H-16), 1.63 (m, H-2), 1.72 (m, H-6), 1.76 (m, H-1), 1.78 (3H, s, Me-30), 1.80 (m, H-11), 1.84 (3H, s, Me-29), 2.24 (2H, m, H-20), 5.47 (t, *J* 7.2 Hz); δ_C 15.13 (C-27), 15.94 (C-26), 16.50 (C-25), 17.97 (C-30), 19.00 (2 × C, C-2, C-6), 21.05 (C-28), 21.52 (C-12), 21.77 (C-24), 25.86 (C-29), 26.82 (C-11), 28.93 (C-19), 29.31 (C-15), 30.55 (C-20), 33.43 (C-4), 33.57 (C-23), 34.31 (C-7), 37.68 (C-10), 38.89 (C-16), 40.65 (C-1), 41.01 (C-14), 41.73 (C-8), 42.02 (C-13), 42.38 (C-3), 48.06 (C-18), 50.95 (C-9), 56.83 (C-5), 73.41 (C-17), 125.99 (C-21), 130.83 (C-22), Assignments of C-2 and C-6 are exchangeable. EIMS: *m/z* 69 (100%), 95 (74), 191 (62), 205 (26), 328(21), 410 ($M^+ - H_2O$, 71). HREIMS: *m/z* found 410.3940 ($M^+ - H_2O$, $C_{30}H_{50}$ requires 410.3913). $[a]_D^{25}$ (EtOH) +10.65 (*c* 0.23).

Product 41 (solid). 400 MHz in C_6D_6: δ_H 0.87 (m, H-1), 0.88 (br d, 11.6, 2.0 Hz, H-5), 0.97 (3H, s, Me-28), 0.99 (3H, s, Me-24), 1.00 (3H, s, Me-25), 1.02 (3H, s, Me-23), 1.12 (3H, s, Me-27), 1.15 (3H, s, Me-26), 1.18 (2H, m, H-15), 1.25 (m, H-3), 1.32 (2H, m, H-19), 1.37 (m, H-12), 1.39 (m, H-11), 1.46 (2H, m, H-16), 1.48 (m, H-3), 1.49 (2H, m, H-2, H-6), 1.50 (m, H-9), 1.52 (2H, m, H-7), 1.62 (2H, m, H-6, H-12), 1.66 (m, H-11), 1.68 (m, H-13), 1.72 (m, H-2), 1.78 (2H, m, H-1, H-18), 1.82 (m, H-20), 1.86 (3H, s, Me-30), 2.12 (m, H-20), 2.35 (br d, 8.8, 1.8 Hz, H-21), 4.84 (s, H-29), 5.09 (s, H-29); δ_C 14.55 (C-27), 16.20 (C-26), 16.39 (C-25), 18.98 (C-2), 19.05 (C-6), 21.12 (C-28), 21.16 (C-11), 21.80 (C-24), 25.90 (C-19), 26.01 (C-30), 26.91 (C-12), 27.68 (C-15), 28.34 (C-20), 31.38 (C-16), 33.44 (C-4), 33.58 (C-23), 34.47 (C-7), 37.20 (C-13), 37.77 (C-10), 40.55 (C-1), 41.14 (C-14), 42.32 (C-3), 42.55 (C-8), 45.57 (C-17), 50.97 (C-9), 56.37 (C-21), 56.73 (C-5), 11.63 (C-29), 150.03 (C-22), Assignments of C-2 and C-6 are exchangeable. EIMS: *m/z* 191 (100%), 204 (35), 218 (28), 367 (9), 395 (9), 410 (M^+, 71). HREIMS: *m/z* found 410.3940 (M^+, $C_{30}H_{50}$ requires 410.3913). $[a]_D^{25}$ ($CHCl_3$) +21.44 (*c* 0.26).

Product 42 (solid). 400 MHz in C_6D_6: δ_H 0.79 (3H, s, Me-28), 0.90 (m, H-5), 0.91 (m, H-1), 0.99 (6H, s, Me-24, Me-25), 1.05 (6H, s, Me-23, Me-27), 1.12 (m, H-15), 1.13 (3H, s, Me-26), 1.23 (m, H-19), 1.28 (m, H-3), 1.37 (m, H-11), 1.42 (m, H-18), 1.49 (m, H-16), 1.51 (3H, m, H-2, H-6, H-9), 1.52 (m, H-3), 1.58 (4H, m, H-7, H-12), 1.62 (m, H-19), 1.63 (2H, m, H-2, H-11), 1.67 (m, H-13), 1.72 (m, H-6), 1.73 (m, H-15), 1.77 (m, H-16, H-18), 1.78 (m, H-1), 1.86 (2H, m, H-20), 1.91 (3H, s, Me-30), 2.15 (t, *J* 9.4 Hz, H-20), 5.01 (s, H-29), 5.13 (s, H-29); δ_C 12.83 (C-28), 14.68 (C-27), 16.17 (C-26), 16.33 (C-25), 19.00 (C-2), 19.06 (C-6), 21.07 (C-11), 21.79 (C-24), 24.53 (C-19), 24.97

(C-30), 25.77 (C-20), 26.27 (C-12), 27.88 (C-15), 33.46 (C-4), 33.63 (C-23), 34.46 (C-7), 35.67 (C-16), 37.00 (C-13), 37.78 (C-10), 40.59 (C-1), 41.11 (C-8), 42.37 (C-14), 42.40 (C-3), 44.25 (C-17), 49.31 (C-18), 51.00 (C-9), 56.83 (C-5), 57.68 (C-21), 111.15 (C-29), 145.61 (C-21). Assignments of C-2 and C-6 are exchangeable. EIMS: *m/z* 191 (100%), 204 (31), 218 (28), 410 (M^+, 72). HREIMS: *m/z* found 410.3899 (M^+, $C_{30}H_{50}$ requires 410.3913). $[a]_D^{25}$ ($CHCl_3$) +5.05 (*c* 0.70).

Product 43 (solid). 600 MHz in C_6D_6: δ_H 0.88 (m, H-1), 0.90 (m, H-5), 0.96 (3H, s, Me-28), 0.99 (3H, s, Me-24), 1.00 (3H, s, Me-25), 1.03 (3H, s, Me-23), 1.15 (3H, s, Me-26), 1.16 (3H, s, Me-27), 1.18 (2H, m, H-19), 1.21 (m, H-15), 1.25 (3H, s, Me-30), 1.27 (3H, s, Me-29), 1.28 (m, H-3), 1.33 (m, H-11), 1.46 (m, H-6), 1.49 (m, H-2), 1.51 (m, H-6), 1.54 (m, H-9), 1.64 (2H, m, H-2, H-11), 1.65 (m, H-6), 1.70 (2H, m, H-13, H-18), 1.71 (m, H-16), 1.72 (2H, m, H-7), 1.75 (m, H-21), 1.78 (m, H-12), 1.95 (2H, m, H-20), 1.98 (m, H-12), 2.21 (m, H-16); δ_C 14.93 (C-25), 16.13 (C-26), 16.41 (C-27), 19.03 (C-2), 19.03 (C-6), 21.21 (C-11), 21.81 (C-24), 22.89 (C-28), 25.82 (C-20), 26.39 (C-29), 26.99 (C-30), 28.01 (C-15), 32.29 (C-16), 33.45 (C-19), 33.59 (C-23), 33.59 (C-4), 34.48 (C-7), 36.97 (C-10), 37.40 (C-13), 37.80 (C-12), 40.59 (C-1), 41.14 (C-8), 42.16 (C-14), 42.35 (C-3), 45.11 (C-17), 45.57 (C-18), 51.04 (C-9), 56.80 (C-5), 59.91 (C-21), 73.80 (C-22). Assignments of C-2 and C-6 are exchangeable. EIMS: *m/z* 191 (100%), 204 (21), 367 (22), 395 (10), 410 ($M^+ - H_2O$, 28), 428 (M^+, 3). HREIMS: *m/z* found 410.3899 ($M^+ - H_2O$, $C_{30}H_{50}$ requires 410.3913). $[a]_D^{25}$ (EtOH) +6.00 (*c* 0.20).

Product 44 (solid). 400 MHz in C_6D_6: δ_H 0.89 (m, H-5), 0.91 (m, H-1), 1.00 (6H, s, Me-24, Me-25), 1.02 (3H, s, Me-28), 1.05 (3H, s, Me-23), 1.06 (3H, s, Me-27), 1.08 (m, H-15), 1.14 (3H, s, Me-26), 1.20 (m, H-19), 1.21 (3H, s, Me-30), 1.27 (m, H-3), 1.29 (m, H-18), 1.33 (m, H-11), 1.42 (m, H-16), 1.41 (t, *J* 10.0 Hz, H-11), 1.46 (2H, m, H-6), 1.49 (3H, m, H-2, H-7), 1.50 (2H, m, H-3, H-9), 1.55 (2H, m, H-12), 1.62 (m, H-19), 1.63 (m, H-11), 1.64 (m, H-2), 1.68 (m, H-15), 1.70 (m, H-13), 1.72 (m, H-20), 1.75 (m, H-1), 1.88 (m, H-20), 1.90 (m, H-16); δ_C 13.40 (C-28), 14.69 (C-27), 16.14 (C-26), 16.33 (C-25), 19.01 (C-6), 19.06 (C-2), 21.09 (C-11), 21.79 (C-24), 23.33 (C-20), 24.16 (C-19), 26.24 (C-12), 27.66 (C-15), 30.37 (C-29), 31.62 (C-30), 33.46 (C-4), 33.63 (C-23), 34.47 (C-7), 35.92 (C-13), 36.96 (C-16), 37.79 (C-10), 40.60 (C-1), 42.42 (C-3), 49.55 (C-18), 51.01 (C-9), 56.87 (C-5), 60.50 (C-21), 72.21 (C-22). Assignments of C-2 and C-6 are exchangeable. EIMS: *m/z* 191 (100%), 204 (18), 367 (17), 395 (13), 410 ($M^+ - H_2O$, 32), 428 (M^+, 11). HREIMS: *m/z* found 410.3940 ($M^+ - H_2O$, $C_{30}H_{50}$ requires 410.3913). $[a]_D^{25}$ ($CHCl_3$) +1.85 (*c* 0.50).

Product 45 (oil). 600 MHz in C_6D_6: δ_H 0.80 (m, H-1), 0.85 (m, H-5), 0.87 (3H, s, Me-26), 0.90 (3H, s, Me-25), 0.95 (3H, s, Me-24), 1.03 (3H, s, Me-23), 1.04 (m, H-9), 1.17 (m, H-7), 1.22 (m, H-3), 1.39 (m, H-6), 1.40 (m, H-11), 1.48 (2H, m, H-2), 1.62 (m, H-6), 1.65 (m, H-1), 1.66 (2H, m, H-15), 1.68 (m, H-2), 1.70 (3H, s, Me-29), 1.72 (m, H-11), 1.78 (3H, s, Me-28), 1.79 (m, H-14), 1.82 (3H, s, Me-30), 1.91 (m, H-7), 2.10 (m, H-12), 2.20 (m, H-16), 2.27 (2H, m, H-19), 2.32 (2H, m, H-20), 2.43 (m, H-16), 2.52 (m, H-12), 4.84 (s, H-27), 5.10 (s, H-27), 5.39 (t, *J* 6.9 Hz, H-21), 5.49 (t, *J* 6.9 Hz, H-17); δ_C 15.76 (C-26), 16.15 (C-28), 16.45 (C-25), 17.77 (C-29), 19.04 (C-2), 19.42 (C-6), 21.63 (C-24), 23.55 (C-11), 24.08 (C-15), 25.93 (C-30), 27.23 (C-20), 27.33 (C-16), 33.39 (C-4), 33.51 (C-23), 37.95 (C-10), 38.68 (C-12), 40.07 (C-8), 40.21 (C-1), 40.27 (C-20), 40.95 (C-7), 42.32 (C-3), 56.40 (C-5), 56.69 (C-14), 60.21 (C-9), 106.39 (C-27), 124.94 (C-21), 125.74 (C-17), 131.09 (C-22), 135.07 (C-18), 148.83 (C-13). Assignments of C-1 and C-20 are exchangeable. EIMS: *m/z* 69 (100%), 81 (33), 191 (18), 410 (M^+, 32). HREIMS: *m/z* found 410.3890 (M^+, $C_{30}H_{50}$ requires 410.3913). $[a]_D^{25}$ ($CHCl_3$) −3.00 (*c* 0.2).

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