Cyclization cascade of the C_{33}-bisnorheptaprenoid catalyzed by recombinant squalene cyclase†

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Received 5th January 2009, Accepted 10th February 2009
First published as an Advance Article on the web 11th March 2009
DOI: 10.1039/b823167b

The enzymatic cyclization reaction of polyprenoid C_{33} by squalene–hopene cyclase (SHC) was investigated with the intention of creating an unnatural hexacyclic compound. The enzymatic products consisted of mono-, bi-, tri-, tetra- and pentacyclic skeletons; however, hexacyclic products were not generated, contrary to our expectations. The absence of a hexacyclic skeleton indicated that the entire carbon chain of C_{33} polyprene could not be included in the reaction cavity. Formation mechanisms of the products having mono- to pentacycles were discussed. Both chair/chair/boat conformation and chair/chair/chair conformations were formed for a tricycle, and both chair/chair/chair/boat conformation and chair/chair/chair/chair structures were constructed for a tetracycle. The pentacyclic product was created from the chair/chair/chair/chair/boat conformation. Squalene was folded in an all pre-chair conformation inside the reaction cavity to form the hopene skeleton. Therefore, the formation of a boat structure during the polycyclization reaction indicated that the molecule of polyprene C_{33} was folded improperly due to incorrect arrangement/positioning in the reaction cavity. The creation of the hexacyclic core failed; however, it should be noted that SHC possessed great potential to tolerate the elongated squalene analog C_{33}, thus leading to the creation of novel compounds with C_{33}.

Introduction

Triterpenes are abundant in nature and have important biological functions. Polycyclic triterpenes including a steroid scaffold are biosynthesized *via* ring-forming reactions (polycyclization) of linear molecules of either squalene **1** or 2,3-oxidosqualene with chain length C_{30}.[1] The structural diversity of triterpene skeletons is remarkable:[2] lanosterol from vertebrates and fungi; cycloartenol and α/β-amyrin from plants; and hopene from prokaryotes are well known. The polycyclization reactions proceed with complete regio- and stereospecificity, leading to the formation of new C–C bonds and chiral centers, seven chiral centers and four C–C bonds for the lanostane skeleton and nine stereocenters and five C–C bonds for hopanoid. Site-directed mutations of squalene–hopene cyclase (SHC) cause early truncation of the polycyclization cascade[1a,b] and/or aberrant cyclization products whose stereochemistry is opposite to those of the normal cyclization intermediates; this strongly indicates that the stereochemical destiny during the polycyclization cascade is directed by the steric bulk size of the active site residues.[3] We isolated many aborted cyclization products from the various site-directed mutants in which the electronic and steric environments are altered.[1b,3,4] Based on the number of rings of the isolated enzymatic products, *i.e.* mono-, bi-, tri- and tetracyclic skeletons, we propose the cyclization pathway of **1** to pentacyclic hopene **2** and hopanol **3**, as shown in Scheme 1. Carbocationic intermediates are involved in multi-reaction steps, and the ring expansion processes (**7**→**8** and **9**→**10**) occur during hopene biosynthesis. We have succeeded in trapping all the tertiary cations (**4**, **5**, **6**, **7**, **9**) by using the squalene analogs with highly nucleophilic hydroxyl group(s).[3b,4b,5b,c] The final deprotonation reaction (**11**→**2**) exclusively occurs from the terminal *Z*-methyl group but not from the *E*-methyl group.[6]

We have reported a several studies on the enzymatic conversions of squalene analogs by SHC.[3b,5–7] Studies conducted on the norsqualenes that lack a methyl group from squalene backbone have provided some important information related to the polycyclization pathway: (1) an isopropylidene moiety at the terminal side is essential for the initiation of the polycyclization;[6,7a] (2) the central methyl group at C(10) plays a crucial role in the normal polycyclization pathway, and C(10)-norsqualene is converted into the unprecedented carbocyclic skeleton(s) with a 6/5 + 5/5 + (6) ring system(s);[7b] (3) loss of one methyl group in the alternative terminal position creates a 6/6/6/6/6-fused pentacyclic ring system (tetrahymanol skeleton).[6,7a] Thus, the methyl groups at both the left and right terminal sides and the central methyl group at C(10) are indispensable for the formation of the hopanoid skeleton. The SHC can also accept truncated analogs with carbon-chain lengths of C_{15}–C_{25},[5a] yielding sesqui-, di- and sesterterpene skeletons; however, C_{10} analog, *i.e.* geraniol, cannot be recognized as the substrate.[5a] Recently, enzymatic conversions of a C_{20} isoprene unit containing an indole[8] or pyrrole ring[9] were reported. In addition, elongated squalene analogs were tested for the enzymatic reactions. The C_{31} analogs with a methylidene appendage were examined to test whether or not they were converted into the cyclized product(s) or acted as irreversible

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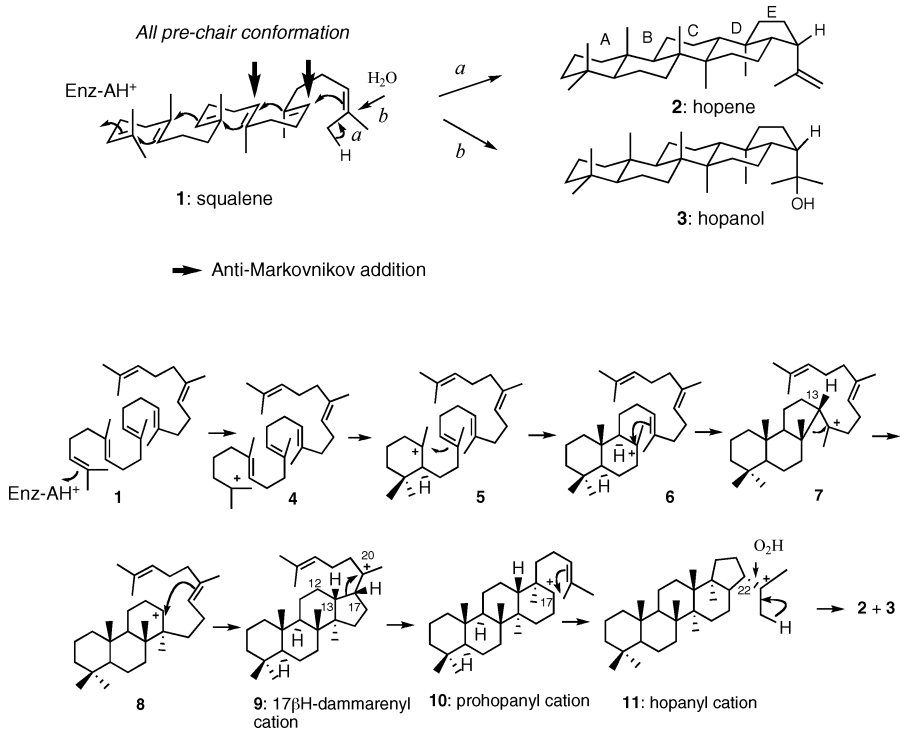
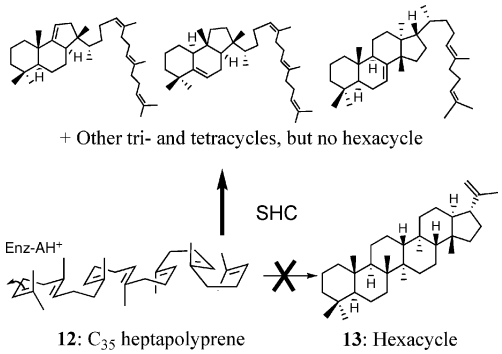
† Electronic supplementary information (ESI) available: Synthetic details, NMR data. See DOI: 10.1039/b823167b



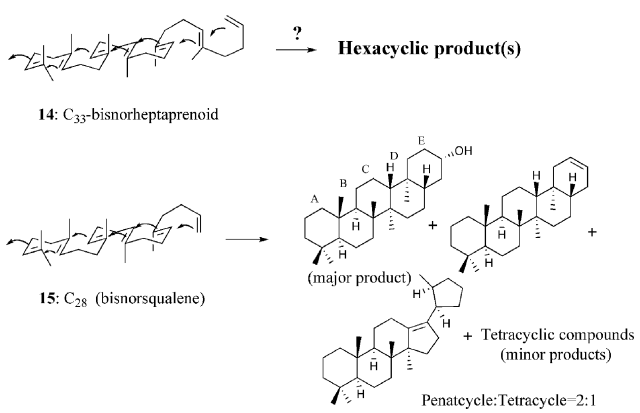
Scheme 1 Cyclization pathway of squalene **1** to hopene **2** and hopanol **3**.

inhibitors for the SHC activity.[10] More recently, Abe *et al.* reported that the elongated C_{35}-analog **12** was accepted as the substrate of the SHC, yielding product **13** with a novel 6/6/6/6/6/5-fused hexacyclic ring system in a 10% yield as a single product.[11] This report is very interesting because the hexacyclic skeleton has never been found in nature. However, our review study on its enzymatic reaction demonstrated that the formation of hexacyclic scaffold **13** from **12** is impossible; instead, a large amount of tri- and tetracyclic skeletons are produced (Scheme 2).[12]



Scheme 2 Polycyclization products of heptapolyprenoid C_{35} catalyzed by SHC. Large amounts of tri- and tetracyclic products are generated, but hexacyclic product **13** is not produced.[12]

The formation of tri- and tetracyclic compounds without the production of hexacyclic compound suggests that the entire carbon framework of analog **12** is not accommodated in the reaction cavity of SHC. Because compounds comprising a multi-ring system larger than a pentacycle have not yet been found in nature, the enzymatic creation of larger cycles such as hexa- and heptacycles is very fascinating. We designed substrate **14** with C_{33} as the candidate for generating a novel hexacyclic compound. The appendage of allyl group (C_3) to **1** is essential to the construction of hexacyclic scaffold when considering the cyclization mechanism, as shown in the upper part of Scheme 3. Analog **14** is the bisnor-compound of **12**, suggesting that the entire carbon chain of the less bulky **14** is likely to be incorporated into the reaction cavity of SHC. Previously, we have reported that SHC created a six-membered *E*-ring in a high yield (60%) from bisnorsqualene **15** (C_{28}) with a vinyl group (bottom part of Scheme 3).[6] Therefore, it is likely that the terminal vinyl group of **14** is subjected to the annulation reaction to create a 6/6/6/6/6/6-fused hexacyclic skeleton (Scheme 3). We report herein the enzymatic products obtained by incubating **14** with SHC. Mono-, bi-, tri-, tetra- and pentacyclic products were generated. However, hexacyclic



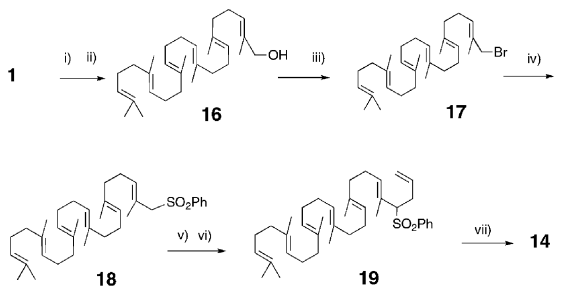
Scheme 3 Cyclization pathway of C_{33}-polyprene **14** and bisnorsqualene **15** with C_{28}.

products were not formed, contrary to our expectations. This result suggests that the C_3 appendage cannot be arranged and folded in the correct orientation and/or at the accurate position that enable(s) the construction of the hexacyclic skeleton. The polycyclization mechanisms of **14** are discussed in detail.

Results and discussion

Synthesis of 14

The synthesis method is shown in Scheme 4. Allylic alcohol **16** was prepared according to the published method.[13] Treatment of squalene **1** with the mixture of salicylic acid, selenium dioxide and 70% *t*-BuOOH in CH_2Cl_2 yielded **16**, which was converted into **17** by using PBr_3. Bromide **17** was transformed into phenylsulfone **18** by reacting with $PhSO_2Na$. The coupling reaction of **18** with allyl bromide occurred in the presence of *n*-BuLi to yield C_{33}-phenylsulfone **19**, followed by the desulfonylation reaction with the aid of $LiBEt_3H$ to yield the desired **14**.



Scheme 4 Synthetic scheme of **14**.

GC profile and the isolation of the enzymatic products from 14

Fig. 1 shows the gas chromatogram of the incubation mixture prepared by incubating **14** (1.0 mg) with the cell-free homogenates (2.0 ml) of *E. coli* clone (pET3a) encoding the native SHC from *Alicyclobacillus acidocaldarius*.[4a,c] The incubation was carried out for 16 h under optimal catalytic conditions (60 °C and pH 6.0) in the presence of Triton X-100 (under which **1** was fully converted into **2** and **3**). To the reaction mixture, 5% KOH–MeOH was added, and the products were extracted with hexane. The Triton X-100 included in the hexane was removed with a short SiO_2 chromatography column eluting with a mixture of hexane–EtOAc (100 : 20), and then the eluted fraction was subjected to GC analysis. A large number of enzymatic products, up to 16, were obtained in a high conversion yield (98%), and the yield of unreacted **14** was 2.0%.

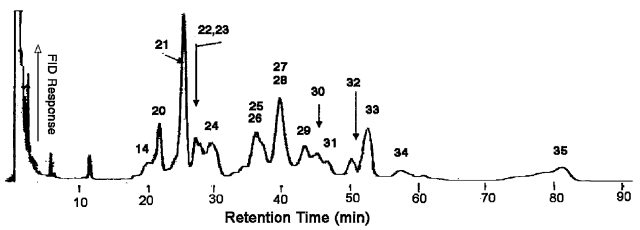


Fig. 1 Gas chromatogram of the hexane extract from the incubation mixture of analog **14** with the wild-type SHC. Triton X-100 included in the incubation mixture was removed by a short SiO_2 column eluting with hexane–EtOAc (100 : 20).

To isolate products **20**–**35**, a large-scale incubation was conducted by using 330 mg of **14** and 1 L of the cell-free homogenates from 20-L culture of the cloned *E. coli*. The hexane extract from the incubation mixture was subjected to a short SiO_2 column in order to remove excess Triton X-100. The eluents were analyzed by using SiO_2 TLC. The TLC of the low polar fraction (performed using hexane) yielded 9 spots that were named in the descending order of their R_f values as Fraction 1–9. The TLC of the high polar fraction (performed using hexane–EtOAc = 100 : 5) yielded two spots, which was named in a descending order as Fraction 10 and Fraction 11. The unreacted substrate **14** was found between Fr. 9 and Fr. 10 in SiO_2 TLC. Each of the fractions and **14** were separated by SiO_2 column chromatography with hexane as the eluent; commercially available SiO_2 was dried in an oven at 200 °C for 2 h in order to increase its adsorbability prior to use. Fr. 1 contained product **32** (solid), which was isolated in the pure state by the repeated SiO_2 column chromatography eluting with hexane. Fr. 2 was further subjected to SiO_2 chromatography (5% $AgNO_3$) with step-wise elution (hexane: EtOAc = 100 : 0.5–100 : 5), yielding pure product **24** (oil). SiO_2 chromatographies (5% $AgNO_3$) with step-wise elution (hexane–EtOAc = 100 : 0.5–100 : 5) of Fr. 3 and Fr. 4 yielded **28** (oil) and **31** (oil), respectively, in the pure state. Separated Fr. 5 was subjected to SiO_2 chromatography (5% $AgNO_3$) with step-wise elution (hexane–EtOAc= 100 : 1–100 : 5), yielding pure product **27** (oil) and a mixture of **27** and **33**. A complete separation of **27** and **33** was performed by using an HPLC (column: Inertsil ODS-3, solvent system: THF: CH_3CN: H_2O = 6 : 4 : 2), yielding a pure **33** (oil) and **27**. Partially purified Fr. 6 contained two products **29** and **21**; the separation was achieved by means of argentation column chromatography (5% $AgNO_3$) in a manner similar to the method described above, thus yielding pure products **29** (oil) and **21** (oil). Fr. 7 contained **23** (oil) and **26** (oil); the separation was achieved by means of argentation column chromatography. Fr. 8 and 9 contained products **22** (oil) and **20** (oil), respectively. Fr. 10 was subjected to a thorough SiO_2 column chromatography (5% $AgNO_3$) procedure with step-wise elution (hexane–EtOAc= 100 : 3–100 : 10), yielding pure products **30** (oil) and **35** (oil), Similarly, Fr. 11 was subjected to SiO_2 chromatography (5% $AgNO_3$) with step-wise elution (hexane–EtOAc= 100 : 3–100 : 10), yielding pure products in the following elution order: **34** (oil) and then **25** (oil).

Structures of enzymatic products 20–35

The structures of all the enzymatic products were determined by means of detailed NMR analyses including DEPT, ^{1}H-^{1}H COSY, NOESY, HMQC and HMBC spectra (see the ESI†). All the NMR spectra were measured in C_6D_6. The C_{33} analog **14** possessed seven allylic methyl and one vinyl group. Product **20** consisted of four allylic methyl protons (δ_H 1.77, 3H, s; 1.75, 3H, s; 1.73, 3H, s; 1.67, 3H, s) and vinyl protons, suggesting that **20** is a monocyclic compound. The presence of exomethylene group (H-29: δ_H 5.00, s; 4.82, s; C-29: δ_C 109.4, t) in **20** was confirmed by the following HMBC correlations: H-29/C-1 (δ_C 32.76, t), H-29/C-5 (δ_C 53.84, d) and H-29/C-6 (δ_C 149.4). Thus, the structure of **20**

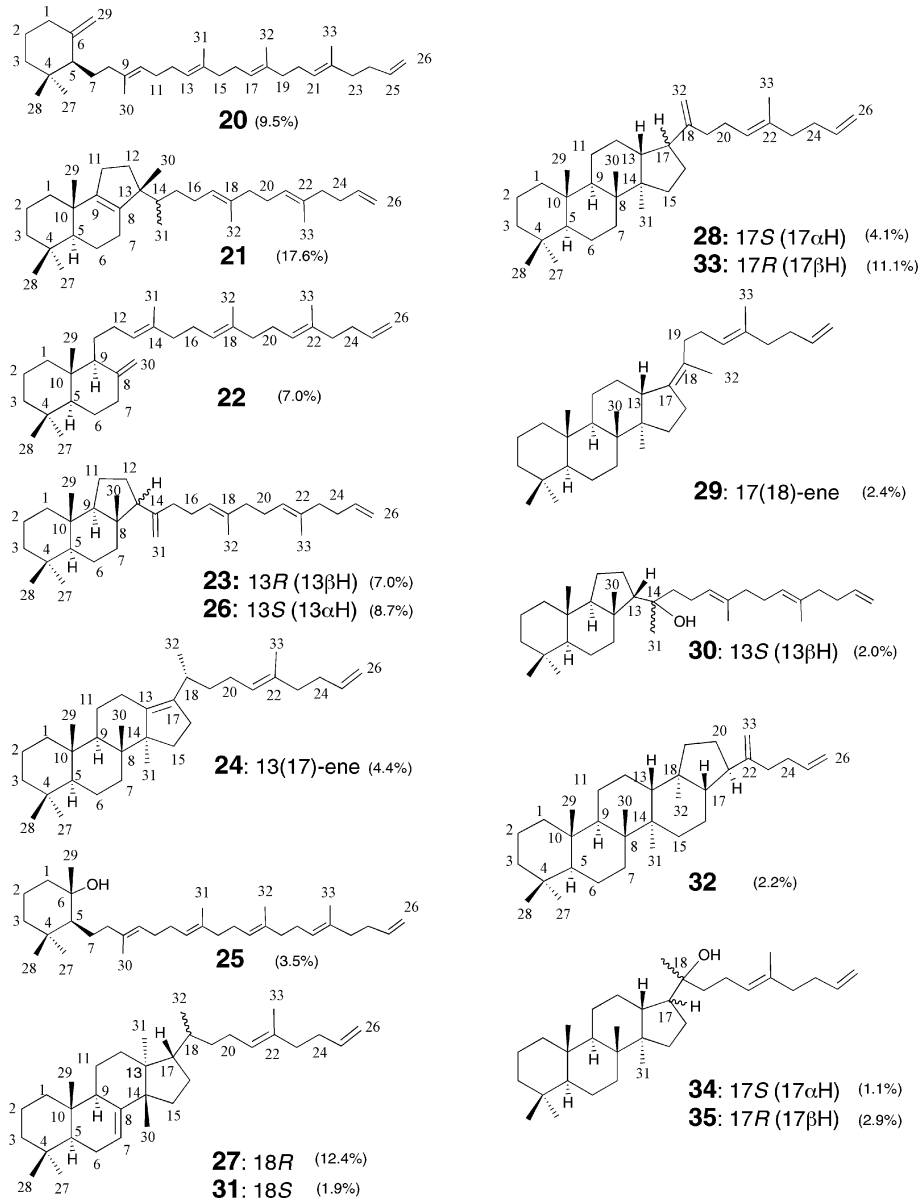


Fig. 2 Structures of the enzymatic products. Production amounts are shown in parentheses.

was determined to have the core of 3-deoxyachilleol A, as shown in Fig. 2. In **21**, two allylic methyl protons (δ_H 1.77, 3H, s; 1.68, 3H, s) and a doublet methyl group (δ_H 1.00, d, $J = 6.0$ Hz) were found together with a vinyl group, which is indicative of the tricyclic core. HMBC cross peaks of Me-29 (δ_H 1.13, 3H, s)/C-9 (δ_C 144.4, s) and Me-30 (δ_H 1.21, 3H, s)/C-8 (δ_C 138.1, s) confirmed that the double bond was located at C-8 and C-9, as shown in Fig. 2. A prominent ion peak of m/z 231 in the MS spectrum (ESI†) further supported the tricyclic skeleton. The tricyclic core of **21** was identical to that of podiodatriene triterpene. The C(14)-stereochemistry cannot be determined by means of NMR analyses; however, the cyclization mechanism discussed later in the next section suggests the 14*S*-configuration.

In **22**, three allylic methyl groups (δ_H 1.74, 3H, s; 1.71, 3H, s; 1.64, 3H, s) were found, suggesting a bicyclic skeleton for **22**. The involvement of exomethylene group (H-30: δ_H 5.06, s; 4.80, s; C-30; δ_C 106.7, t) in **22** was validated by the following HMBC correlations: H-30/C-8 (δ_C 148.8, s), H-30/C-9 (δ_C 56.43, d) and H-30/C-7 (δ_C 38.74, t). The cyclic structure of **22** was identical to that of α-polypodatetraene. The detailed HMBC analyses of **23** (ESI†) assigned the tricyclic scaffold for **23**. A strong NOE between Me-30 (δ_H 1.10, 3H, s) and H-13 (δ_H 2.31, m), indicated 13β-H orientation. The position of a vinylidene moiety (H-31: δ_H 4.90, s; 5.16, s; C-31: δ_C 109.3, t; C-14: δ_C 154.6, s) was determined by the HMBC cross peaks of H-31/C-13 (δ_C 56.98, d), H-13/C-14 and H-13/C-31. The entire structure of **23** is illustrated in Fig. 2. Detailed HMBC analyses of **26** indicated that **26** had the same tricyclic structure as **23**. A strong NOE between H-13 (δ_H 2.20, m) and H-9 (δ_H 1.28, m) indicated 13α-H configuration. Thus, the stereochemistry at C-13 was opposite to that of **23**. The tricyclic skeletons of **23** and **26** were identical to that of malabaricane triterpene. In **24**, the presence of an allylic methyl group (Me-33: δ_H 1.71, 3H, s) and a doublet methyl group (Me-32: δ_H 1.14, 3H, d, $J = 6.7$ Hz) indicated a tetracyclic skeleton. The detailed HMBC

analyses (ESI†) showed that the cyclic core was a dammarane skeleton. The double bond position was determined to be at C-13-C17, because the HMBC correlations were clearly observed between Me-32 and C-17 (δ_C 135.1, s) and between Me-31 (δ_H 1.31, 3H, s) and C-13 (δ_C 139.6, s). To determine the stereochemistry at C-18, the chemical shift of Me-32 (δ_H 0.908, d, $J = 6.8$ Hz) measured in $CDCl_3$ was very close to the published value (δ_H 0.910, d, $J = 6.9$ Hz)[14] of the corresponding methyl group in 20*R*-dammara-13(17)-24-diene, indicating that the stereochemistry at C-18 of **24** was *R*. Thus, the entire structure of **24** is shown in Fig. 2. Product **25** had a monocyclic compound similar as **20**, but this product had a hydroxyl group (δ_C 73.51, s). In **27**, one allylic Me (Me-33: δ_H 1.74, 3H, s) and doublet Me (Me-32: δ_H 1.06, d, $J = 6.5$ Hz) was found, indicating that **27** possesses a tetracyclic skeleton. In the HMBC spectrum, Me-31 (δ_H 1.07, 3H, s) showed correlations with C-17 (δ_C 53.73, d), C-13 (δ_C 43.95, s) and C-14 (δ_C 51.76, s), and Me-30 (δ_H 1.18, 3H, s) exhibited cross peaks with C-13, C-14 and C-8 (δ_C 146.1, s); thus, Me-31 and Me-30 were positioned at C-13 and C-14, respectively. Me-32 (δ_H 1.06, 3H, d, $J = 6.5$ Hz) also showed HMBC correlation with C-17. The double bond position in the cyclic core was determined to be at C-7-C-8 by the following HMBC cross peaks: H-7 (δ_H 5.48, bs)/C-5 (δ_C 51.76, d) and H-7/C-9 (δ_C 49.56, d). A definitive NOE between Me-30 and H-17 (δ_H 1.64, m) indicated 17β-H stereochemistry. These findings suggest that the cyclic core of **27** is identical to that of euphane triterpene. The chemical shift of Me-32 (δ_H 0.840, d, $J = 6.5$ Hz) in $CDCl_3$ was very close to the published value (δ_H 0.835, d, $J = 6.8$ Hz)[14] of the corresponding Me in 20*R*-euph-7(8)-ene, indicating 18*R*-configuration for **27**. Product **31** had a similar tetracyclic core as that of **27**, which was revealed by analyzing the HMBC spectrum (ESI†); however, the stereochemistry at C-18 was opposite to that of **27**, because the chemical shift of Me-32 (δ_H 0.870, d, $J = 6.5$ Hz) in $CDCl_3$ was almost identical to that of the published value (δ_H 0.865, d, $J = 6.7$ Hz)[14] of the corresponding methyl group in tirucall-7(8)-ene with 20*S*-stereochemsitry. Thus, the structure of **31** was analogous to that of tirucall-7(8)-ene, and the entire structure is shown in Fig. 2. Detailed HMBC analyses of **28** (ESI†) indicated the tetracyclic core. The vinylidene protons (δ_H 5.10, s; 5.05, s) had a HMBC correlation with C-17 (δ_C 48.28). Thus, **28** had a dammarene cyclic skeleton. A strong NOE between Me-30 (δ_H 1.11, 3H, s) and H-13 (δ_H 1.91, m) demonstrated 13β-H orientation. Further, a clear NOE between Me-31 (δ_H 0.971, 3H, s) and H-17 (δ_H 2.41, m) indicated 17α-H orientation. Therefore, the entire structure of **28** can be depicted as shown in Fig. 2. Product **33** exhibited a similar dammarene cyclic skeleton, as revealed by analyzing the HMBC data. A definitive NOE was found between H-13 (δ_H 2.06, m) and H-17 (δ_H 2.74, m), indicating that H-17 was arranged in β-orientation. Thus, the structural difference between **28** and **33** was found only in the stereochemistry at C-17. In **29**, two allylic methyl groups were found: Me-32 (δ_H 1.83, 3H, bs) and Me-33 (δ_H 1.74, 3H, s). Detailed HMBC analyses (ESI†) indicated the tetracyclic core. In the HMBC spectrum, Me-32 correlated with C-17 (δ_C 137.1, s), C-18 (δ_C 126.7, s) and C-19 (δ_C 34.13, t), indicating that Me-32 was situated on the tetrasubstituted double bond. A strong NOE between Me-32 and H-16 (δ_H 2.25, m; 2.36, m) demonstrated that the C-17–C-18 double bond had *Z*-configuration. Thus, the structure of **29** had the cyclic core of dammara-17(18)-ene (Fig. 2). The involvement of two allylic methyl groups (δ_H 1.76, s and δ_H 1.66, s) in **30** and the detailed HMBC analyses allowed us to assign the tricyclic skeleton for **30**. Product **30** was suggested to be a hydroxylated compound, because the R_f value of **30** was smaller than that of substrate **14**. Me-31 (δ_H 1.31, 3H, s) exhibited HMBC correlations with C-14 (δ_C 75.49, s) and C-13 (δ_C 59.11, d). Furthermore, H-13 (δ_H 1.76, m) exhibited a HMBC cross peak for C-14. Thus, a hydroxyl group was located at C-14. The observation of NOE between Me-30 (δ_H 1.07, 3H, s) and H-13 verified the β-orientation of H-13. Thus, **30** had the cyclic skeleton of malabaricane triterpene (Fig. 2). For product **32**, no allylic methyl group was found in the ^{1}H-NMR; instead, vinylidene protons (δ_H 5.00, bs; 5.07, bs) appeared. In addition, vinyl protons (δ_H 5.90, m; 5.20, bd, $J = 17.1$ Hz; 5.13 bd, $J = 10.5$ Hz) had remained without undergoing the cyclization reaction. These findings suggest that **32** is a pentacyclic compound. The stereochemistries of chiral centers determined by the NOESY spectrum were consistent with the hopene skeleton; however, the stereochemistry at C-21 (21α-H) was opposite to that of natural hopene skeleton **2**, which was confirmed by the strong NOE between Me-32 (δ_H 0.845, 3H, s) and H-21 (δ_H 2.41, m). Product **34** had a hydroxyl group, which was confirmed by observing the signal of δ_C 74.77 (s). The detailed HMBC analyses (ESI†) indicated a tetracyclic core for **34**. A strong NOE between Me-31 (δ_H 1.02, 3H, s) and H-17 (δ_H 1.80, m) verified the α-arrangement of H-17. Product **35** had the same skeleton as that of **34**, but only the stereochemistry at C-17 was different; H-17 was arranged in the β-orientation because of the absence of NOE between Me-31 (δ_H 1.22, 3H, s) and H-17 (δ_H 2.10, m).

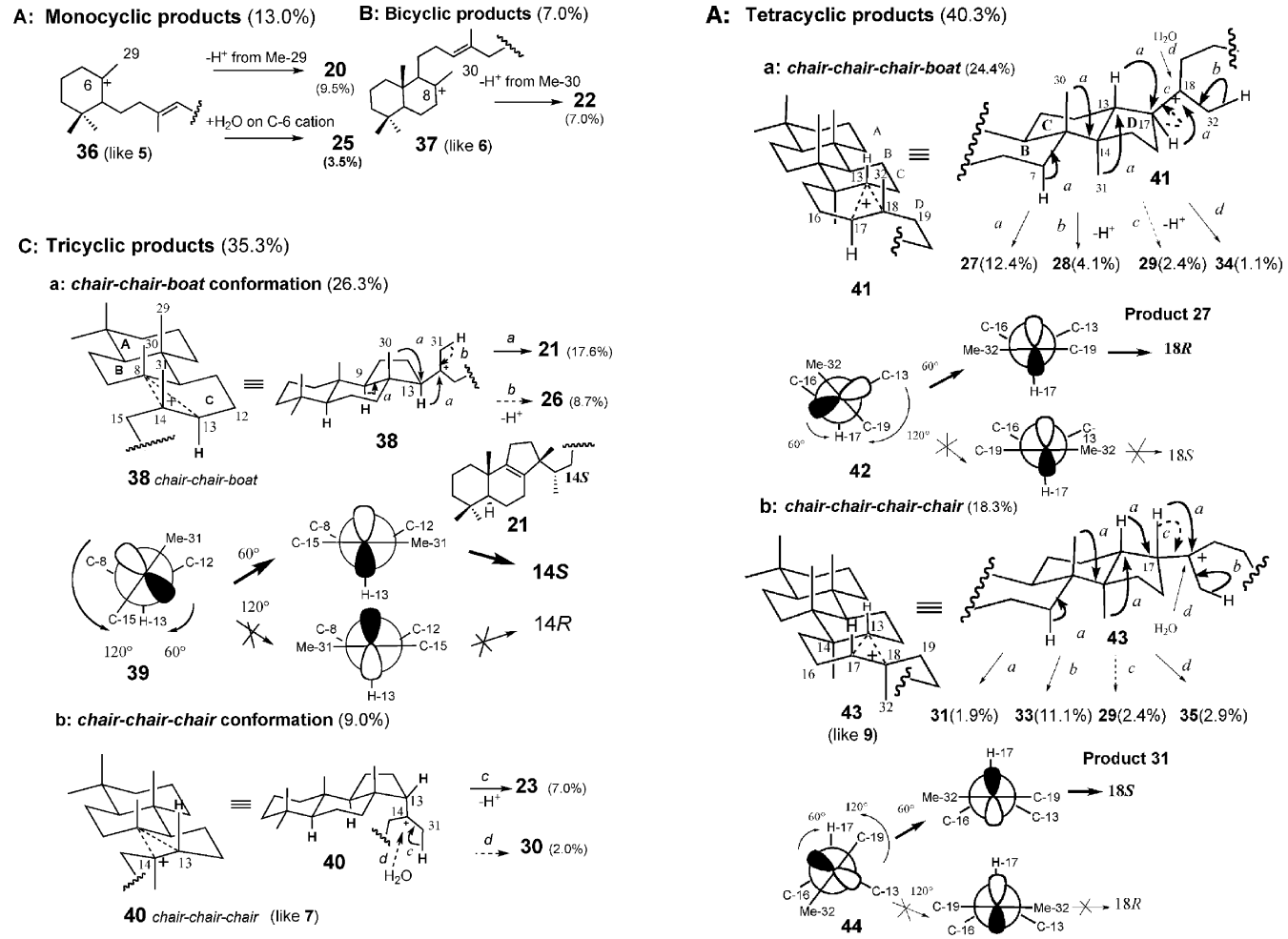
Production amounts (%) of the enzymatic products are depicted in the parentheses of Fig. 2. Mono-, bi-, tri-, tetra- and pentacyclic products were produced, but no hexacyclic skeleton was detected. In particular, the yields of tri- (35.3%) and tetracyclic skeletons (40.3%) were significantly high. No product other than **20**–**35** was found during isolating these enzymatic products **20**–**35** by SiO_2 column chromatography. Furthermore, prolonged GC for 240 min showed that no other peak appeared after the peak **35** (see Fig. 1). These findings indicate that product(s) other than **20**–**35** was not generated from **14**.

Cyclization mechanism of 14 into products 20–35 by SHC

Analog **14** has isopropylidene and vinyl moieties at the both the termini. All the products **20**–**35** possessed a vinyl moiety in the side chain, indicating that the polycyclization reaction did not initiate from the vinyl group site. Previously, we clarified that the isopropylidene group was essential to the initiation of the polycyclization.[6,7a] Thus, the cyclization reaction started from the isopropylidene site.

The enzymatic cyclization of **14** yielded mono-, bi-, tri-, tetra- and pentacyclic products. Analog **14** was cyclized to form monocyclic cation **36** (like **5**, a chair form, Scheme 5A). Deprotonation from Me-29 yielded **20**, and water attack on the C-6 cation generated **25**. Intermediate **36** was further cyclized to bicyclic cation **37** (like **6**) with a chair–chair conformation (Scheme 5B). The proton elimination from Me-30 of **37** could yield product **22**.

The bicyclic cation **37** was further cyclized into tricyclic cations **38** and **40** (like **7**). Intermediate **38** had a chair–chair–boat conformation (Scheme 5C-a), and **40** possessed a chair–chair–chair structure (Scheme 5C-b). In **38**, 1,2-shifts (H-13α →C-14,



Scheme 5 Formation mechanisms of mono- (A), bi- (B) and tricyclic products (C).

Scheme 6 Formation mechanisms of tetracyclic (A) and pentacyclic products (B).

Me-30→C-13), followed by the deprotonation of H-9, occurred in antiparallel fashion to yield **21** (path *a*). The Newman projection through the C13–C14 axis of **38** is depicted in **39**. A small rotation (*ca.* 60°) forms the 14*S*-configuration, and a large rotation (*ca.* 120°) yields 14*R*-stereochemistry. The least motion is preferable to the large movement inside the enzyme cavity, because the substrate is tightly constricted by the cyclase.[5b,c,12] Thus, product **21** is presumed to have 14*S*-stereochemistry. The deprotonation from Me-31 of **38** yielded **26** (path *b*). In **40**, the proton elimination from Me-31 yielded **23** (path *c*), and the attack of water molecule onto the C-14 cation yielded **30** (path *d*).

As shown in Scheme 6A, the folding of **14** into a chair–chair–chair–boat conformation could lead to the tetracyclic cation **41** with 17α-H (Scheme 6A-a), and the organization of a chair–chair–chair–chair conformation could yield the tetracyclic cation **43** having 17β-H (Scheme 6A-b). The production ratio of **41** to **43** was 1.3 : 1. Successive 1,2-shifts of hydrides and methyl groups (H-17α→C-18, H-13→C-17, Me-31→C-13 and Me-30→C-14), followed by deprotonation of H-7, yielded **27** and **31**, which were produced from **41** and **43** (like **9**), respectively (path *a*). The production amount of **27** was significantly higher by *ca.* 6-fold as compared to that of **31**. This could be attributed to the preference of antiparallel migration of H-13→C-17 in **41**, but its migration in **43** cannot be promoted due to the parallel orientation of H-13 and H-17. The deprotonation from Me-32 yielded **28** and **33** (path *b*). The elimination of H-17 of **41** and/or **43** led to the introduction of C17–C18 double bond, yielding **29** (path *c*). The production of **29** having *Z*-configuration, but not *E*-geometry, indicated that the enzymatic reaction proceeded kinetically to form an energetically unfavourable product. A water attack to C-18 cation of **41** and **43** yielded **34** and **35**, respectively (path *d*). The 18*R*-configuration in **27** could be attained *via* a least motion pathway involving only a small rotation (60°) about the C17–C18 axis (see Newman projection **42**). As described above, a large motion (120°) leading to 18*S* was prohibited. Based on the same idea as in the case of **27**, product **31** had 18*S*-stereochemistry due to a small motion (60°) as depicted in Newman projection **44**.

Further cyclization of intermediate **43** into a chair–chair–chair–chair–boat conformation yielded 6/6/6/6/5-fused pentacyclic cation **45**. The deprotonation from Me-33 yielded **32**. It should be noted that **45** was different from hopanyl cation **11** because the C-22 stereochemistry of **45** was opposite to that of **11**.

Conclusion

In this study, mono-, bi-, tri-, tetracyclic and pentacyclic skeletons were produced.

Abrogation of the polycyclization cascade at mono- (13%) and bicyclic stages (7%) suggested that **14** was inappropriately arranged around the formation sites of A and B rings. The tri- and tetracyclic intermediates **40** and **43** were analogous to **7** and **9**, respectively, including the stereochemistries. Thus, **40** and **43** were true cyclic intermediates. However, **38** and **41** were false intermediates, because the stereochemistries of C-13 and C-17 of these cyclic cations were opposite to those of **7** and **9**. In addition, the false intermediates were generated in higher yields as compared to those obtained for the true intermediates; for the tricyclic core, the false intermediate **38** possessed 26.3% yield, while the true intermediate **40** comprised 9.0%. In the case of tetracyclic skeleton, 24.4% was obtained for the false intermediate **41**, and 18.3% was obtained for the true intermediate **43**. The significantly higher production of the false tri- and tetracyclic intermediates indicated that substrate **14** was improperly folded inside the reaction cavity. Pentacyclic skeleton **32** was produced in a manner similar to that for squalene substrate **1**; however, the C-22 stereochemistry of *E*-ring was opposed to that of hopene **2**, indicating that cationic intermediate **45** was a false intermediate. The formation of these false intermediates was attributed to the appendage of C_3 to squalene (C_{30}). The introduction of extra C_3 unit into **1** would have prohibited the correct placement of the C_{33} molecule **14** across the entire A–E ring formation sites of the reaction cavity, leading to the failure of the participation of vinyl group in the polycyclization reaction. Thus, no hexacyclic scaffold could be constructed. Pentacyclic compounds were not produced from **12**[12] but generated from **14**, albeit in small amounts (2.2%). In addition, mono- and bicyclic skeletons were not generated from **12**; however, these cores were produced in 20% yield from **14**. Thus, the product distribution was significantly different between **12** and **14**. This difference would have occurred because the molecular orientation of **14** inside the reaction cavity differs extensively from that of **12**. Substrate **14** lacks two methyl groups of **12**. It was surprising that the presence of two methyl groups at the terminal, albeit a subtle change between these substrate structures, significantly influenced the substrate placement inside the reaction cavity, which could have led to distinct polycyclization pathways between **12** and **14**. Furthermore, no production of a hexacyclic compound from **14** indicated that the reaction cavity was not sufficiently large to accept appropriately the elongated carbon chain lengths of C_{33}. Contrary to our expectations, it can be concluded that hexacyclic terpenoid(s) cannot be created from the elongated C_{33} molecule **14** as well as from C_{35} **12**.[12] However, the present and the previous studies[12] show that the elongated C_{33} and C_{25} analogs can be recognized as substrates to yield new cyclic products with C_{33} and C_{35} in a high conversion yield (*ca.* 98%). Thus, SHC exhibits admirable plasticity to tolerate a variety of truncate and elongated substrate analogs.

Experimental

Analytical method

NMR spectra were measured in C_6D_6 on a Bruker DMX600 or DPX400 spectrometer, the chemical shifts being given in ppm 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 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. GC analyses were performed on a Shimadzu GC-8A chromatograph equipped with flame ionization detector (DB-1 capillary column, 0.53 mm × 30 m). GC-MS spectra were on a JEOL SX 100 spectrometer under electronic impact at 70 eV with a DB-1 capillary column (0.32 mm × 30 m), the oven temperature being elevated from 220 to 290 °C (3 °C min^{-1}). A Hitachi HPLC apparatus was used that was composed of L-7100 pump, L-7400 UV detector and D-2500 chromato-integrator (flow rate, 1 ml min^{-1}; detected at 210 nm). HR-EIMS was measured by direct inlet system. Specific rotation values were recorded at 25 °C with a Horiba SEPA-300 polarimeter.

Synthesis of (6*E*, 10*E*, 14*E*, 17*E*, 21*E*)-5,9,13,18, 22,26-hexamethyltricosa-1,5,9,13, 17,21-hexanene 14

The synthetic experiments are described in the ESI.†

Incubation of 14 with the wild-type SHC from *A. acidocaldarius*

The culture condition of the cloned *E. coli* encoding the native SHC (pET3a vector) and the preparation of the cell-free extract were performed according to the method described previously.[4–7] The incubation was done at the optimal catalytic conditions that were described in the previous papers.[4]

Spectroscopic data of products 20–35

Product 20. ^{1}H-NMR (400 MHz, C_6D_6): $\delta = 0.972$ (Me-28, 3H, s), 1.075 (Me-27, 3H, s), 1.27 (H-3, 1H, m), 1.52 (H-3, 1H, m), 1.61 (H-2, 2H,m), 1.64 (H-7, m), 1.671 (Me-33, 3H, s), 1.731 (Me-32, 3H, s), 1.746 (Me-31, 3H, s), 1.770 (Me-30 & H-7, 4H, s), 1.86 (H-5, 1H, dd, $J = 11.4$, 3.0 Hz), 2.05 (H-8, m). 2.10 (H-1, m), 2.17 (H-23, 2H,m), 2.22 (H-1, H-15, H-19 & H-24, 7H, m), 2.23 (H-8, m), 2.27 (H-11 & H-12, 4H,m), 2.30 (H-16 & H-20, 4H,m), 4.82 (H-29, 1H, bs), 5.00 (H-29, 1H, bs), 5.12 (H-26b, 1H, bd, $J = 10$ Hz), 5.16 (H-26a, 1H, bdd, $J = 16.4$, 1.6 Hz), 5.36 (H-21, 1H, t, $J = 6.8$ Hz), 5.42 (H-17, 1H, t, $J = 6.8$ Hz), 5.47 (H-10 & H-13, 2H, m), 5.93 (H-25, 1H, m); ^{13}C NMR (100 MHz, C_6D_6): $\delta = 16.01$ (C-33, q), 16.10 (C-32, q), 16.19 (C-31, q), 16.28 (C-30, q), 24.07 (C-2, t), 25.19 (C-7, t), 26.51 (C-28, q), 27.03 (C-20, t), 27.11 (C-16, t), 28.57 (C-27, q), 28.77 (C-11 & C-12, 2C, t), 32.76 (C-1, t), 32.76 (C-24, t), 34.96 (C-4, s), 36.48 (C-3, t), 38.68 (C-8, t), 39.45 (C-23, t), 40.16 (C-19, t), 40.24 (C-15, t), 53.84 (C-5, d), 109.4 (C-29, t), 114.5 (C-26, t), 124.6 (C-10 & C-17, 2C, d), 124.9 (C-13, d), 125.0 (C-21, d), 134.4 (C-22, s), 134.9 (C-18, s), 135.2 (C-14, s), 135.7 (C-9, s), 138.8 (C-25, d), 149.4 (C-6, s). The following ^{13}C NMR signals are indistinguishable from each other, due to very close chemical shifts: C-31/C-32, C-16/C-20 and C-15/C-19. EIMS: *m/z* (%): 81(80), 95(46), 109(100), 149(34), 177(21), 341(10), 435(4), 450(M^+, 6). HRMS (EI): *m/z*: calcd for $C_{33}H_{54}$

(M^+) 450.4226, found 450.4218. $[\alpha]_D^{25}$= +8.99 (EtOH, c = 0.2). Oil.

Product 21. ^{1}H-NMR (600 MHz, C_6D_6): δ = 1.000 (Me-31, 3H, d, J = 6.0 Hz), 1.010 (Me-28, 3H, s), 1.043 (Me-27, 3H, s), 1.132 (Me-29, 3H, s), 1.206 (Me-30, 3H, s), 1.23 (H-12 & H-15, 2H, m), 1.26 (H-1 & H-3, 2H, m), 1.29 (H-3, 1H, m), 1.33 (H-5, 1H, bd, J = 10.7 Hz), 1.49 (H-2 & H-6, 2H, m), 1.54 (H-3, 1H, m), 1.63 (H-14, m), 1.679 (Me-33, 3H, s), 1.72 (H-1, 1H, m), 1.773 (Me-32, 3H, s), 1.82 (H-15, 1H, m), 1.85 (H-12, 1H, m), 1.97 (H-7, 1H, m), 2.12 (H-7, 1H, m), 2.15 (H-16, m), 2.20 (H-23, 2H,m), 2.20 (H-24, 1H, m), 2.25 (H-19, 2H, m), 2.25 (H-24, 1H, m), 2.27 (H-11, 1H, m), 2.31 (H-16, m)), 2.33 (H-20, 2H, m), 2.36 (H-11, 1H, m), 5.12 (H-26b, 1H, bd, J = 10.1 Hz), 5.17 (H-26a, 1H, bdd, J = 17.1, 1.6 Hz), 5.38 (H-21, 1H, t, 6.6), 5.46 (H-17, 1H, t, J = 6.6 Hz), 5.94 (H-25, 1H, m); ^{13}C NMR (150 MHz, C_6D_6): δ = 15.05 (C-31, q), 16.03 (C-33, q), 16.14 (C-32, q), 19.34 (C-2, t), 19.54 (C-6, t), 19.69 (C-29, q), 21.75 (C-28, q), 23.75 (C-7, t), 25.52 (C-30, q), 27.04 (C-20, t), 27.16 (C-16, t), 28.31 (C-11, t), 31.28 (C-12, t), 32.27 (C-15, t), 32.78 (C-24, t), 33.27 (C-4, s), 33.56 (C-27, q), 36.29 (C-10, s), 37.83 (C-1, t), 38.86 (C-14, d), 39.46 (C-23, t), 40.19 (C-19, t), 42.39 (C-3, t), 52.87 (C-5, d), 52.99 (C-13, s), 114.5 (C-26, t), 125.1 (C-21, d), 125.6 (C-17, d), 134.5 (C-22, s), 134.7 (C-18, s), 138.1 (C-8, s), 138.9 (C-25, d), 144.4 (C-9, s). The following ^{13}C NMR signals are indistinguishable from each other, due to very close chemical shifts: C-2/C-6 and C-20/C-16. EIMS: m/z (%): 81(12), 95(10), 109(21), 147(8), 231(100), 341(10), 435(1), 450(M^+, 1). HRMS (EI): m/z: calcd for $C_{33}H_{54}$ (M^+) 450.4226, found 450.422. $[\alpha]_D^{25}$= -28.08 (c = 0.073, EtOH). Oil.

Product 22. ^{1}H-NMR (400 MHz, C_6D_6): δ = 0.831 (Me-29, 3H,s), 0.892 (Me-28, 3H, s), 0.939 (Me-27, 3H, s), 1.05 (H-1, 1H, m), 1.10 (H-5, 1H, dd, J = 12.8, 2.4 Hz), 1.27 (H-3, 1H, m), 1.39 (H-6, 1H, m), 1.72 (H-6, 1H, m), 1.47 (H-3, 1H, m), 1.53 (H-2, 1H, m), 1.60 (H-2, 1H, m), 1.63 (H-11, 1H, m), 1.64 (Me-33, 3H, s), 1.71 (Me-32, 3H, s), 1.74 (Me-31, 3H, s), 1.76 (H-1, H-9 & H-11, 3H, m), 2.08 (H-12, 1H m), 2.17 (H-23, 2H, m), 2.18 (H-7, 1H, m), 2.21 (H-15 & H-19, 4H, m), 2.23 (H-24, 2H, m), 2.31 (H-16 & H-20, 4H, m), 2.40 (H-12, 1H, m), 2.49 (H-7, 1H, m), 4.80 (H-30, 1H, s), 5.06 (H-30, 1H, s), 5.11 (H-26b, 1H, bd, J = 10.0), 5.16 (H-26a, 1H, bdd, J = 16.6, 1.6), 5.33 (H-21, 1H, t, J = 6.8 Hz), 5.40 (H-17, 1H, m), 5.42 (H-13, 1H, m), 5.90 (H-25, 1H, m); ^{13}C NMR (100 MHz, C_6D_6): δ = 14.76 (C-29, q), 16.04 (C-31, q), 16.14 (C-32, q), 16.19 (C-33, q), 19.77 (C-2, t), 21.92 (C-28, q), 24.22 (C-11, t), 24.77 (C-6, t), 27.06 (C-20, t), 27.14 (C-16, t), 27.37 (C-12, t), 32.77 (C-24, t), 33.67 (C-4, s), 33.75 (C-27, q), 38.74 (C-7, t), 39.29 (C-1, t)), 39.46 (C-23, t), 39.82 (C-10, s), 40.21 (C-19, t), 40.26 (C-15, t), 42.43 (C-3, t), 55.65 (C-5, d), 56.43 (C-9, d), 106.7 (C-30, t), 114.5 (C-26, t), 124.9 (C-17, d), 125.0 (C-21, d), 125.7 (C-13, d), 134.4 (C-22, s), 134.9 (C-14, s, & C-18, s), 138.8 (C-25, d), 148.8 (C-8, s). The following ^{13}C NMR signals are indistinguishable from each other, due to very close chemical shifts: C-15/C-19, C-12/C-19/C-20, C-13/C-17/C-21 and C-14/C-18/C-22. EIMS: m/z (%): 69(48), 81(62), 95(48), 109(65), 123(25), 137(33), 149(100), 163(16), 177(21), 217(10), 231(10), 279(10), 341(4), 435(14), 450(M^+, 28). HRMS (EI): m/z: calcd for $C_{33}H_{54}$ (M^+) 450.4226, found 450.4227. $[\alpha]_D^{25}$= +17.00 (c = 0.20, EtOH). Oil.

Product 23. ^{1}H-NMR (400 MHz, C_6D_6): δ = 0.92 (H-5, 1H, m), 0.945 (Me-27, 3H, s), 0.969 (Me-28, 3H, s), 0.994 (Me-29, 3H, s), 1.08 (H-1, 1H, m), 1.101 (Me-30, 3H, s), 1.25 (H-3, 1H, m), 1.44 (H-7, 1H, m), 1.48 (H-3. 1H, m), 1.49 (H-2, 1H, m), 1.49 (H-6, 1H, m), 1.56 (H-11, 1H, m), 1.59 (H-1, 1H, m), 1.65 (H-12, 1H, m), 1.65 (H-9, 1H, m), 1.670 (Me-33, 3H, s), 1.68 (H-6, 1H, m), 1.70 (H-7, 1H, m), 1.721 (Me-32, 3H, s), 1.76 (H-2, 1H, m), 1.84 (H-12, 1H, m), 2.15 (H-12, 1H, m), 2.18 (H-23, 2H, m), 2.20 (H-15, 1H, m), 2.20 (H-19, 2H, m), 2.27 (H-20, 2H, m), 2.27 (H-24, 2H, m), 2.30 (H-16, 1H, m), 2.31 (H-13, 1H, m), 2.34 (H-15, 1H, m), 2.40 (H-16, 1H, m), 4.90 (H-31, 1H, s), 5.11 (H-26b, bd, J = 10.4 Hz), 5.16 (26Ha, 1H, bd, J = 16.8), 5.16 (H-31, 1H, s), 5.35 (H-21, 1H, t, J = 6.4 Hz), 5.42 (H-17, 1H, t, J = 6.4 Hz), 5.93 (H-25, 1H, m); ^{13}C NMR (100 MHz, C_6D_6): δ = 15.80 (C-29, q), 16.04 (C-33, q), 16.12 (C-32, q), 18.83 (C-2, t), 19.63 (C-6, t), 21.06 (C-11, t), 21.63 (C-28, q), 25.01 (C-30, q), 27.00 (C-20, t), 27.38 (C-16, t), 28.17 (C-12, t), 32.77 (C-24, d), 33.14 (C-4, s), 33.62 (C-27, q), 37.05 (C-10, s), 37.10 (C-7, t), 39.45 (C-23, d), 39.81 (C-15, t), 40.13 (C-19, t), 40.74 (C-1, t), 42.68 (C-3, t), 45.86 (C-8, s), 55.84 (C-9, d), 56.98 (C-13, d), 57.25 (C-5, d), 109.3 (C-31, q), 114.5 (C-26, t), 124.8 (C-21, d), 125.0 (C-17, d), 134.5 (C-22, s), 135.1 (C-18, s), 138.8 (C-25, d), 154.6 (C-14, s). The following ^{1}H- and ^{13}C NMR signals are indistinguishable from each other, due to very close chemical shifts: H-2/H-6 and C-2/C-6. EIMS: m/z (%): 81(55), 95(36), 109(51), 123(19), 137(19), 149(15), 163(12), 177(12), 191(100), 205(14), 231(28), 341(5), 435(5), 450(M^+, 10). HRMS (EI): m/z: calcd for $C_{33}H_{54}$ (M^+) 450.4226, found 450.4227. $[\alpha]_D^{25}$= +1.42 (c = 0.2, EtOH). Oil.

Product 24. ^{1}H-NMR (600 MHz, C_6D_6): δ = 0.94 (H-5, 1H, m), 0.95 (H-1, 1H, m), 0.972 (Me-28, 3H, s), 1.67 (H-7, 1H, m), 1.017 (Me-29, 3H, s), 1.03 (Me-27, 3H, s), 1.105 (Me-30, 3H, s), 1.144 (Me-32, 3H, d, J = 6.7 Hz), 1.27 (H-3, 1H, m), 1.310 (Me-31, 3H, s), 1.43 (H-11, 1H, m), 1.45 (H-2, 1H, m), 1.46 (H-6, 1H, m), 1.50 (H-3, H-19 & H-7, 3H, m), 1.60 (H-9, 1H, bd, J = 12.2 Hz), 1.67 (H-6, 1H, m), 1.70 (H-2 & H-11, 2H, m), 1.79 (H-1, 1H, m), 1.82 (H-19, 1H, m), 2.05 (H-12, 1H, m), 2.11 (H-15, 2H,m), 2.18 (H-20, 1H, m), 2.23 (H-23, 2H.m), 2.27 (H-24, 2H,m), 2.30 (H-16, 1H, m), 2.33 (H-20, 1H, m), 2.48 (H-16, 1H, m), 2.62 (H-12, 1H, m), 2.67 (H-18, 1H, m), 5.14 (H-26b, 1H, bd, J = 9.7 Hz), 5.23 (H-26a, 1H, bd, J = 17.1 Hz), 5.44 (H-21, 1H, bt, J = 7.1 Hz), 5.97 (H-25, 1H, m); ^{13}C NMR (150 MHz, C_6D_6): δ = 16.03 (C-33, q), 16.67 (C-29, q), 17.67 (C-30, q), 18.99 (C-2, t), 19.08 (C-6, t), 20.21 (C-32, q), 21.91 (C-28, q), 22.19 (C-11, t), 23.13 (C-31, q), 23.41 (C-12, t), 29.49 (C-16, t), 31.09 (C-15, t), 32.27 (C-18, d), 32.63 (C-20, t), 32.77 (C-24, t), 33.53 (C-4, s), 33.68 (C-27, q), 35.43 (C-19, t), 35.85 (C-7, t), 38.10 (C-10, s), 39.52 (C-23, t), 40.96 (C-1, t), 41.67 (C-8, s), 42.41 (C-3, t), 52.25 (C-9, d), 57.00 (C-14, s), 57.38 (C-5, d), 114.3 (C-26, t), 125.8 (C-21, d), 134.2 (C-22, s), 135.1 (C-17, s), 137.6 (C-25, d), 139.6 (C-13, s). The following ^{1}H- and ^{13}C NMR signals are indistinguishable from each other, due to very close chemical shifts: H-2/H-6 and C-2/C-6. EIMS: m/z (%): 69(67), 81(53), 95(43), 109(27), 121(22), 135(20), 149(48), 161(30), 191(100), 297(34), 341(34), 450(M^+, 33). HRMS (EI): m/z: calcd for $C_{33}H_{54}$ (M^+) 450.4226, found 450.4219. $[\alpha]_D^{25}$= -35.67 (c = 0.1, EtOH). Oil.

Product 25. ^{1}H-NMR (600 MHz, C_6D_6): δ = 0.872 (Me-28, 3H, s), 1.056 (Me-27, 3H, s), 1.15 (H-5, 1H, m). 1.203 (Me-29, 3H, s), 1.22 (H-3, 1H, m), 1.23 (H-1, 1H, ddd, J = 12.5, 12.5, 3.8 Hz),

1.35 (H-3, 1H, m), 1.41 (H-2, 1H, m), 1.49 (H-2, 1H, m), 1.57 (H-7, m), 1.673 (Me-33, 3H, s), 1.72 (H-1, 1H, m), 1.734 (Me-32, 3H, s), 1.764 (Me-31, 3H, s), 1.843 (Me-30, 3H, s), 1.86 (H-7, m), 2.23 (H-15, H-19, H-23 & H-24, 8H, m), 2.30 (H-11 & H-12, 4H, m), 2.32 (H-20, 2H, m), 2.33 (H-8 & H-16, 3H, m), 2.47 (H-8, m), 5.12 (H-26b, 1H, bd, $J = 9.3$ Hz), 5.16 (H-26a, 1H, bd, $J = 16.6$ Hz), 5.37 (H-21, 1H, t, $J = 6.6$ Hz), 5.42 (H-17, 1H, t, $J = 6.4$ Hz), 5.47 (H-13, 1H, m), 5.54 (H-10, 1H, m), 5.93 (H-25, 1H, m); ^{13}C NMR (150 MHz, C_6D_6): $\delta = 16.02$ (C-33, q), 16.11 (C-32, q), 16.21 (C-31, q), 16.35 (C-30, q), 20.85 (C-2, t), 21.58 (C-28, q), 23.50 (C-29, q), 25.35 (C-7, t), 27.04 (C-20, t), 27.14 (C-16, t), 28.76 (C-12, t), 28.79 (C-11, t), 32.76 (C-24, t), 32.95 (C-27, q), 35.60 (C-4, s), 39.45 (C-23, t), 40.16 (C-15, t), 40.25 (C-19, t), 41.77 (C-3, t), 43.41 (C-8, t), 43.97 (C-1, t), 56.86 (C-5, d), 73.51 (C-6, s), 114.5 (C-26, t), 124.6 (C-10, d), 124.9 (C-13, d), 124.9 (C-17, d), 125.1 (C-21, d), 134.4 (C-22, d), 134.9 (C-18, s), 135.2 (C-14, s), 136.6 (C-9, s), 138.8 (C-25, d). The following ^{13}C NMR signals are indistinguishable from each other: C-11/C-12, C-14/C-18, C-15/C-19, C-16/C-20 and C-31/C-32. EIMS: *m/z* (%): 69(90), 81(49), 95(58), 109(60), 119(38), 131(35), 137(31), 149(50), 169(25), 191(100), 231(21), 281(11), 281(12), 301(12), 341(17), 381(8), 435(8), 450(M^+-H_2O, 28). HRMS (EI): *m/z*: calcd for $C_{33}H_{54}$ (M^+-H_2O) 450.4226, found 450.4228. $[\alpha]_D^{25} = +33.1$ ($c = 0.036$, EtOH). Oil.

Product 26. ^{1}H-NMR (400 MHz, C_6D_6): $\delta = 0.847$ (Me-30, 3H, s), 0.88 (H-5, 1H, m), 0.94 (H-1, 1H, m), 0.962 (Me-29, 3H, s), 0.975 (Me-28, 3H, s), 1.020 (Me-27, 3H, s), 1.27 (H-7, 1H, m), 1.28 (H-3, H-6 & H-9, 3H, m), 1.40 (H-6, 1H, m), 1.45 (H-11, 1H, m), 1.49 (H-2, 1H, m), 1.52 (H-1 & H-3, 2H, m), 1.60 (H-11, 1H, m), 1.667 (Me-33, 3H, s), 1.74 (H-2, 1H, m), 1.756 (Me-32, 3H, s), 1.82 (H-12, 1H, m), 1.92 (H-7, 1H, m), 1.95 (H-12, 1H, m), 2.19 (H-23, 2H, m), 2.20 (H-13, 1H, m), 2.22 (H-19, 2H, m), 2.25 (H-15, 1H, m), 2.26 (H-24, 2H, m), 2.30 (H-20, 2H, m), 2.32 (H-15, 1H, m), 2.41 (H-16, 2H, m), 5.03 (H-31, 1H, s), 5.12 (H-26b, bd, $J = 10.0$ Hz), 5.18 (H-26a, 1H,bdd, $J = 16.8$, 1.6 Hz), 5.21 (H-31, 1H, s), 5.36 (H-21, 1H, t, $J = 6.0$ Hz), 5.46 (H-17, 1H, t, $J = 6.4$ Hz), 5.93 (H-25, 1H, m); ^{13}C NMR (100 MHz, C_6D_6): $\delta = 15.38$ (C-30, q), 15.62 (C-29, q), 16.05 (C-33, q), 16.17 (C-32, q), 18.77 (C-2, t), 19.81 (C-11, t), 19.81 (C-6, t), 21.50 (C-28, q), 25.80 (C-12, t), 26.99 (C-20, t), 27.53 (C-16, t), 32.78 (C-24, t), 33.17 (C-4, s), 33.74 (C-27, q), 37.39 (C-10, s), 38.12 (C-15, t), 39.45 (C-23, t), 40.15 (C-1, t), 40.26 (C-19, t), 41.47 (C-7, t), 42.90 (C-3, t), 43.99 (C-8, s), 57.57 (C-5 & C-13, 2C, d), 63.36 (C-9, d), 110.6 (C-31, t), 114.5 (C-26, t), 124.9 (C-17 & C-21, 2C, d), 134.5 (C-22, s), 135.0 (C-18, s), 138.8 (C-25, d), 149.2 (C-14, s). The assignments of C-1 and C-19 may be exchangeable. EIMS: *m/z* (%): 81(33), 95(35), 109(62), 123(22), 137(25), 149(15), 163(12), 177(12), 191(100), 203(10), 217(12), 231(74), 435(6), 450(M^+, 12). HRMS (EI): *m/z*: calcd for $C_{33}H_{54}$ (M^+-H_2O) 450.4226, found 450.4224. $[\alpha]_D^{25} = +19.04$ ($c = 0.2$, EtOH). Oil.

Product 27. ^{1}H-NMR (600 MHz, C_6D_6): $\delta = 0.955$ (Me-29, 3H, s), 0.964 (Me-27, 3H, s), 1.031(Me-28, 3H, s), 1.055(Me-32, 3H, d, $J = 6.5$ Hz), 1.06(H-1, m), 1.072(Me-31, 3H, s), 1.183(Me-30, 3H, s), 1.28(H-3, 1H, m), 1.38(H-16, 1H, m), 1.48 (H-5, 1H, m), 1.50(H-11, 1H, m), 1.52(H-19, 1H, m), 1.55(H-3, 1H, m), 1.58(H-18, 1H, m), 1.62(H-15, 1H, m), 1.64(H-17, 1H, m), 1.65(H-2, 2H,m), 1.66(H-11, 1H, m), 1.735(Me-33, 3H, s), 1.75(H-1, m), 1.76(H-15, 1H, m), 1.78(H-12, 1H, m), 1.89(H-19, 1H, m), 1.95(H-12, 1H, m), 1.98(H-6, 1H, m), 2.04(H-16, 1H, m), 2.18(H-20, 1H, m), 2.23 (H-23, 2H,m), 2.25(H-6, 1H, m), 2.26 (H-24, 2H,m), 2.33(H-20, 1H, m), 2.43(H-9, 1H, m), 5.11(H-26b, 1H, bd, $J = 9.2$ Hz), 5.17(H-26a, 1H, bd, $J = 17.1$ Hz), 5.44(H-21, 1H,bt, $J = 6.9$ Hz), 5.48(H-7, 1H, bs), 5.93(H-25, 1H, m); ^{13}C NMR (150 MHz, C_6D_6): $\delta = 13.36$ (C-29, q), 16.06 (C-33, q), 18.55 (C-2, t), 18.82 (C-32, q), 19.47 (C-11, t), 21.51 (C-28, q), 22.48 (C-31, q), 24.76 (C-6, t), 25.68 (C-20, t), 27.62 (C-30, q), 28.80 (C-16, t), 32.79 (C-24, t), 33.16 (C-27, q), 33.32 (C-4, s), 34.44 (C-12 & C-15, 2C, t), 35.46 (C-10, s), 35.65 (C-19, t), 36.09 (C-18, d), 39.36 (C-1, t), 39.54 (C-23, t), 42.77 (C-3, t), 43.95 (C-13, s), 49.56 (C-9, d), 51.76 (C-5, d & C-14, s), 53.73 (C-17, d), 114.5 (C-26, t), 118.5 (C-7, d), 125.8 (C-21, d), 134.2 (C-22, s), 138.8 (C-25, d), 146.1 (C-8, s). EIMS: *m/z* (%): 69(52), 81(46), 95(37), 109(48), 121(26), 149(25), 161(11), 191(12), 243(6), 297(10), 311(9), 435(100), 450(M^+, 20). HRMS (EI): *m/z*: calcd for $C_{33}H_{54}$ (M^+-H_2O) 450.4226, found 450.4231. $[\alpha]_D^{25} = -19.80$ ($c = 0.1$, EtOH). Oil.

Product 28. ^{1}H-NMR (400 MHz, C_6D_6): $\delta = 0.872$ (H-1, 1H, ddd, $J = 12.0$, 12.0, 4.1 Hz), 0.911 (H-5, 1H,dd, $J = 12.0$, 2.0 Hz), 0.971 (Me-31, 3H, s), 0.984 (Me-28, 3H, s), 1.014 (Me-29, 3H, s), 1.030 (Me-27, 3H, s), 1.109 (Me-30, 3H, s), 1.24 (H-15, 1H, m), 1.27 (H-3, 1H, m), 1.28 (H-11, 1H, m), 1.30 (H-12, 1H, m), 1.37 (H-7, 1H, m), 1.47 (H-9, 1H, m), 1.50 (H-2, H-3 & H-6, 3H, m), 1.63 (H-2, H-6 & H-16, 3H, m), 1.65 (H-11, 1H, m), 1.697 (Me-33, 3H, s), 1.72 (H-1 & H-7. 3H, m), 1.76 (H-15, 1H, m), 1.90 (H-12, 1H, m), 1.91 (H-13, 1H, m), 2.08 (H-16, 1H, m), 2.18 (H-23, 2H,m), 2.22 (H-24, 2H, m), 2.25 (H-19, 2H, m), 2.38 (H-20, 2H, m), 2.41 (H-17, 1H, m), 5.05 (H-32, 1H, s), 5.10 (H-32, 1H, s), 5.12 (H-26b, 1H, bd, $J = 11.6$ Hz), 5.17 (H-26a, 1H, bdd, $J = 17.2$, 1.6 Hz), 5.41 (H-21, 1H, t, $J = 6.4$ Hz), 5.93 (H-25, 1H, m); ^{13}C NMR (100 MHz, C_6D_6): $\delta = 15.91$ (C-33, q), 16.09 (C-30,q), 16.19 (C-31, q), 16.43 (C-29, q), 18.98 (C-2, t), 19.08 (C-6, t), 21.61 (C-11, t), 21.79 (C-28, q), 25.49 (C-12, t), 27.39 (C-20, t), 29.40 (C-16, t), 31.72 (C-15, t), 32.74 (C-24, t), 33.53 (C-4, s), 33.65 (C-27, q), 34.75 (C-19, t), 35.78 (C-7, t), 37.77 (C-10, s), 39.44 (C-23, t), 40.92 (C-1, t), 40.97 (C-8, s), 42.42 (C-3, t), 45.69 (C-13, d), 48.28 (C-17, d), 49.78 (C-14, s), 51.43 (C-9, d), 57.31 (C-5, d), 108.2 (C-32, t), 114.6 (C-26, t), 125.1 (C-21, d), 134.6 (C-22, s), 138.8 (C-25, d), 152.5 (C-18, s). The assignments of C-2 and C-6 are exchangeable. EIMS: *m/z* (%): 81(60), 95(57), 109(45), 123(24), 137(25), 149(34), 161(15), 177(11), 191(100), 205(10), 231(13), 244(10), 258(7), 301(7), 367(5), 435(4), 450(M^+, 32). HRMS (EI): *m/z*: calcd for $C_{33}H_{54}$ (M^+-H_2O) 450.4226, found 450.4224. $[\alpha]_D^{25} = +26.59$ ($c = 0.135$, acetone). Oil.

Product 29. ^{1}H-NMR (600 MHz, C_6D_6): $\delta = 0.88$ (H-1, 1H, m), 0.91 (H-5, 1H, bd, $J = 11.8$ Hz), 0.984 (Me-28, 3H, s), 0.991 (Me-29, 3H, s), 1.021 (Me-31, 3H, s), 1.029 (Me-27, 3H, s), 1.102 (Me-30, 3H, s), 1.25 (H-15, 1H, m), 1.27 (H-3, 1H, m), 1.28 (H-11, 1H, m), 1.43 (H-7, 1H, m), 1.47 (H-2, H-3 & H-6, 2H, m), 1.49 (H-9, 1H, m), 1.63 (H-2, H-6 & H-7, 3H, m), 1.65 (H-12 & H-15, 2H, m), 1.68 (H-11, 1H, m), 1.736 (Me-33, 3H, s), 1.75 (H-1, 1H, m), 1.826 (Me-32, 3H, bs), 2.21 (H-23, 2H, m & H-24, 1H, m), 2.25 (H-16, 1H, m), 2.29 (H-24, 1H, m), 2.30 (H-20, 1H, m), 2.36 (H-16, 1H, m), 2.38 (H-20, 1H, m), 2.41 (H-19, 2H, m), 2.48 (H-12, 1H, m), 2.50 (H-13, 1H, m), 5.12 (H-26b1H, bd, $J = 10.0$ Hz), 5.18 (H-26a, 1H, bd, $J = 17.1$), 5.45 (H-21, 1H, m), 5.94 (H-25, 1H, m); ^{13}C NMR (150 MHz, C_6D_6): $\delta = 15.92$ (C-30, q), 16.08 (C-33,

q), 16.58 (C-29, q), 16.90 (C-31, q), 18.93 (C-2, t), 19.05 (C-6, t), 21.08 (C-32, q), 21.74 (C-28, q), 21.78 (C-11, t), 25.49 (C-12, t), 28.39 (C-20, t), 30.05 (C-16, t), 30.53 (C-15, t), 32.77 (C-24, t), 33.51 (C-4, s), 33.59 (C-27, q), 34.13 (C-19, t), 35.89 (C-7, t), 37.62 (C-10, s), 39.53 (C-23, t) \, 40.36 (C-8, s), 40.87 (C-1, t), 42.44 (C-3, t), 47.27 (C-13, d), 50.25 (C-14, s), 51.08 (C-9, d), 57.33 (C-5, d), 114.5 (C-26, t), 125.3 (C-21, d), 126.7 (C-18, s), 134.3 (C-22, s), 137.1 (C-17, s), 138.8 (C-25, d). The assignments of C-2 and C-6 are exchangeable. EIMS: *m/z* (%): 69(100), 81(30), 95(31), 109(36), 119(42), 131(43), 149(43), 169(27), 181(28), 191(54), 205(12), 231(18), 281(12), 341(75), 381(7), 450(M^+, 20). HRMS (EI): *m/z*: calcd for $C_{33}H_{54}$ (M^+) 450.4226, found 450.4240. $[\alpha]_D^{25}$= -13.05 ($c = 0.082$, EtOH). Oil.

Product 30. ^{1}H-NMR (400 MHz, C_6D_6): $\delta = 0.975$ (Me-28, 3H, s), 0.982 (Me-29, 3H, s), 0.99 (H-5, 1H, m), 1.002 (Me-27, 3H, s), 1.04 (H-1, 1H, m), 1.068 (Me-30, 3H, s), 1.25 (H-3, 1H, m), 1.308 (Me-31, 3H, s), 1.47 (H-2 & H-11, 2H, m), 1.50 (H-3, 1H, m), 1.52 (H-6, 1H, m), 1.54 (H-9, 1H, m), 1.58 (H-1, 1H, m), 1.65 (H-11, 1H, m), 1.662 (Me-33, 3H, s), 1.68 (H-12, 1H, m), 1.70 (H-15, 1H, m), 1.73 (H-2 & H-6, 2H, m), 1.760 (H-13, 1H, m & Me-32, 3H, s), 1.94 (H-7 & H-12, 2H, m), 2.10 (H-15, 1H, m), 2.12 (H-19 & H-23, 3H, bt, $J = 7.0$ Hz), 2.12 (H-7, 1H, m), 2.27 (H-16 & H-20, 4H, m), 2.34 (H-24, 2H, m), 5.13 (H-26b, 1H, bd, $J = 10.0$ Hz), 5.21 (H-26a, 1H, bdd, $J = 16.8$, 2.0 Hz), 5.42 (H-17 & H-21, 2H, m), 5.94 (H-25, 1H, m); ^{13}C NMR (100 MHz, C_6D_6): $\delta = 15.93$ (C-33, q), 16.04 (C-32, q), 16.57 (C-29, q), 18.89 (C-2, t), 20.17 (C-6, t), 21.54 (C-28, q), 21.70 (C-11, t), 23.23 (C-16, t), 24.71 (C-12, t), 26.08 (C-31, q), 26.63 (C-20, t), 26.83 (C-30, q), 32.70 (C-24, t), 33.16 (C-4, s), 33.68 (C-27, q), 37.48 (C-10, s), 38.32 (C-7, t), 39.59 (C-23, t), 39.67 (C-19, t), 41.13 (C-1, t), 42.31 (C-15, t), 42.69 (C-3, t), 45.04 (C-8, s), 57.13 (C-5, d), 59.11 (C-13, d), 60.39 (C-9, d), 75.49 (C-14, s), 114.3 (C-26, t), 121.9 (C-21, d), 125.4 (C-17, d), 135.0 (C-18, s), 136.5 (C-22, s), 137.6 (C-25, d). The signals of C-15 and C-31 were significantly small. EIMS: *m/z* (%): 69(66), 81(78), 95(62), 109(35), 123(28), 137(28), 149(36), 191(100), 205(15), 231(41), 341(15), 435(5), 450(M^+-H_2O, 18). HRMS (EI): *m/z*: calcd for $C_{33}H_{54}$ (M^+-H_2O) 450.4226, found 450.4240. $[\alpha]_D^{25}$= -70.80 ($c = 0.05$, EtOH). Oil.

Product 31. ^{1}H-NMR (400 MHz, C_6D_6): $\delta = 0.965$ (Me-29, 3H, s), 0.971 (Me-27, 3H, s), 1.034 (Me-28, 3H, s), 1.05 (H-1, 1H, m), 1.058 (Me-31, 3H, s), 1.107 (Me-32, 3H, d, $J = 6.0$ Hz), 1.186 (Me-30, 3H, s), 1.27 (H-19, 1H, m), 1.29 (H-3, 1H, m), 1.40 (H-16, 1H, m), 1.50 (H-5, 1H, m), 1.55 (H-3 & H-11, 2H, m), 1.58 (H-18, 1H, m), 1.59 (H-19, 1H, m), 1.60 (H-2, 1H, m), 1.63 (H-17, 1H, m), 1.65 (H-15, 1H, m), 1.68 (H-2, 1H, m), 1.69 (H-11, 1H, m), 1.730 (Me-33, 3H, s), 1.75 (H-1, 1H, m), 1.78 (H-12 & H-15, 2H, m), 1.89 (H-12, 1H, m), 1.97 (H-6, 1H, m), 2.12 (H-16, 1H, m), 2.17 (H-20, 1H, m), 2.24 (H-23, 2H, m), 2.25 (H-6, 1H, m), 2.31 (H-20, 1H, m), 2.43 (H-9, 1H, m), 5.12 (H-26b, 1H, d, $J = 9.5$), 5.18 (H-26a, 1H, bd. $J = 17.2$), 5.42 (H-21, 1H, t, $J = 6.4$ Hz), 5.47 (H-7, 1H, bs), 5.94 (H-25, 1H, m); ^{13}C NMR (100 MHz, C_6D_6): $\delta = 13.36$ (C-29, q), 15.99 (C-33, q), 18.49 (C-2, t), 18.63 (C-32, q), 19.44 (C-11, t), 21.50 (C-28, q), 22.26 (C-31, q), 24.71 (C-6, t), 25.36 (C-20, t), 27.56 (C-30, q), 28.62 (C-16, t), 32.75 (C-24, t), 33.16 (C-27, q), 33.29 (C-4, s), 34.16 (C-12, t), 34.46 (C-15, t), 35.40 (C-10, s), 36.29 (C-18, d), 36.58 (C-19, t), 39.27 (C-1, t), 39.50 (C-23, t), 42.69 (C-3, t), 43.84 (C-13, s), 49.51 (C-9, d), 51.60 (C-14, s), 51.67 (C-5, d), 53.43 (C-17, d), 114.6 (C-26, t), 118.5 (C-7, d), 125.9 (C-21, d), 134.1 (C-22, s), 138.8 (C-25, d), 146.0 (C-8, s). The assignments of the proton and carbon at 12 and 15 positions may be exchangeable. EIMS: *m/z* (%): 69(75), 81(55), 95(44), 109(55), 119(17), 135(15), 149(15), 243(10), 257(10), 436(100), 450(M^+, 17). HRMS (EI): *m/z*: calcd for $C_{33}H_{54}$ (M^+) 450.4226, found 450.4231. $[\alpha]_D^{25}$= −15.00 ($c = 0.064$, EtOH). Oil.

Product 32. ^{1}H-NMR (600 MHz, C_6D_6): $\delta = 0.845$ (Me-32, 3H, s), 0.87 (H-1, 1H, m), 0.91 (H-5, 1H, m), 0.979 (Me-29, 3H, s), 0.989 (Me-28, 3H, s), 1.037 (Me-27, 3H, s), 1.106 (Me-30, 3H, s), 1.114 (Me-31, 3H,s), 1.19 (H-19, 1H, m), 1.25 (H-3, 1H, m), 1.28 (H-17, 1H, m), 1.30 (H-11, 1H, m), 1.33 (H-15, 1H, m), 1.35 (H-7, 1H, m), 1.41 (H-9, 1H, m), 1.42 (H-2 & H-11, 2H, m), 1.51 (H-3 & H-6, 2H, m), 1.54 (H-15, 1H, m), 1.55 (H-12, 1H, m), 1.58 (H-13 & H-20, 2H, m), 1.61 (H-7, 1H, m), 1.63 (H-2 & H-19, 2H, m), 1.64 (H-12, 1H, m), 1.65 (H-16, 1H, m), 1.72 (H-6 & H-16, 1H, m), 1.78 (H-1, 1H, m), 2.06 (H-20, 1H, m), 2.24 (H-23, 2H, t, $J = 7.7$ Hz), 2.37 (H-24, 2H, m), 2.41 (H-21, 1H, m), 5.00 (H-33, 1H, bs), 5.07 (H-33, 1H, bs), 5.13 (H-26b, 1H bd,10.5 Hz), 5.20 (H-26a, 1H, bd, $J = 17.1$ Hz), 5.96 (H-25, 1H, m); ^{13}C NMR (150 MHz, C_6D_6): $\delta = 15.42$ (C-32, q), 16.13 (C-29, q), 16.96 (C-31, q), 17.03 (C-30, q), 19.10 (C-2, t), 19.13 (C-6, t), 21.38 (C-11, t), 21.42 (C-16, t), 21.85 (C-28, q), 24.35 (C-12, t), 29.03 (C-20, t), 32.96 (C-24, t), 33.14 (C-15, t), 33.44 (C-4, s), 33.62 (C-27, q), 33.80 (C-7, t), 34.43 (C-23, t), 37.76 (C-10, s), 40.47 (C-19, t), 40.71 (C-1, t), 42.27 (C-14, s), 42.45 (C-3, t), 42.63 (C-8, s), 44.55 (C-18, s), 47.46 (C-21, d), 49.20 (C-13, d), 50.98 (C-9, d), 55.12 (C-17, d), 56.60 (C-5, d), 108.2 (C-33, t), 114.7 (C-26, t), 138.8 (C-25, d), 151.9 (C-22, s). The following ^{13}C NMR signals are indistinguishable from each other, due to very close chemical shifts: C-2/C-6, 11/16 and 30/31. EIMS: *m/z* (%): 69(52), 81(56), 95(51), 109(27), 121(21), 137(20), 149(16), 161(17), 191(100), 229(80), 367(19), 435(23), 450 (M^+, 18). HRMS (EI): *m/z*: calcd for $C_{33}H_{54}$ (M^+) 450.4226, found 450.4213. $[\alpha]_D^{25}$= −52.39 ($c = 0.046$, EtOH). Solid.

Product 33. ^{1}H-NMR (600 MHz, C_6D_6): $\delta = 0.88$ (H-1, 1H, m), 0.910 (H-5, 1H, bd, $J = 12.1$ Hz), 0.989 (Me-28, 3H, s), 1.003 (Me-29, 3H, s), 1.017 (Me-27, 3H, s), 1.077 (Me-30, 3H, s), 1.131 (Me-31, 3H, s), 1.25 (H-3, 1H, m), 1.27 (H-11, 1H, m), 1.34 (H-15, 1H, m), 1.40 (H-7, 1H, m), 1.45 (H-12, 1H, m), 1.48 (H-2, H-6 & H-9, 3H, m), 1.50 (H-3, 1H, m), 1.65 (H-2 & H-6, 2H, m), 1.68 (H-15, 1H, m), 1.699 (Me-33, 3H, s), 1.70 (H-11, 1H, m), 1.72 (H-7, 1H, m), 1.75 (H-1, 1H, m), 1.83 (H-12, 1H, m), 1.93 (H-16, 1H, m), 2.74 (H-17, 1H, m), 2.03 (H-16, 1H, m), 2.06 (H-13, 1H, m), 2.20 (H-19 & H-23, 2H, m), 2.21 (H-24, 1H,m), 2.27 (H-24, 1H,m), 2.35(H-20, 1H, m), 2.36 (H-19 & H-23, 2H, m), 2.42 (H-20, 1H, m), 5.12 (H-26b, 1H, d, $J = 10.1$ Hz), 5.17 (H-26a, 1H,bd, $J = 16.9$ Hz), 5.19 (H-32, 1H,s), 5.21 (H-32, 1H, s), 5.41 (H-21, 1H, bs), 5.93 (H-25, 1H, m); ^{13}C NMR (150 MHz, C_6D_6): $\delta = 16.09$ (C-30, q), 16.20 (C-33, q), 16.47 (C-29, q), 17.24 (C-31, q), 19.11 (C-2, t, & C-6 t), 21.79 (C-28, q), 22.26 (C-11, t), 25.52 (C-12, t), 27.68 (C-20, t), 28.82 (C-16, t), 32.76 (C-24, t), 33.46 (C-15, t), 33.56 (C-4, s), 33.60 (C-27, q), 35.48 (C-7, t), 37.81 (C-10, s), 39.05 (C-19, t), 39.47 (C-23, t), 40.98 (C-1), 41.41 (C-8, s), 42.50 (C-3, t), 44.51 (C-17, d), 45.12 (C-13, d), 50.27 (C-14, s), 51.46 (C-9, d), 57.39 (C-5, d), 109.5 (C-32, t), 114.5 (C-26, t), 125.2 (C-21, d), 134.6 (C-22, s), 138.8 (C-25, d), 152.2 (C-18, s). EIMS: *m/z* (%): 69(49), 81(54), 95(51), 109(35), 123(21), 137(23), 149(32), 191(100), 231(15), 258(8), 299(8), 341(8), 435(4),

450(M^+, 23). HRMS (EI): *m/z*: calcd for $C_{33}H_{54}$ (M^+) 450.4226, found 450.4229. $[\alpha]_D^{25} = -51.64$ ($c = 0.056$, EtOH). oil.

Product 34. ^{1}H-NMR (600 MHz, C_6D_6): $\delta = 0.88$ (H-1, 1H, m), 0.93 (H-5, 1H, dd, $J = 12.0$,1.6 Hz), 0.978 (Me-29, 3H, s), 0.988 (Me-28, 3H, s), 1.022 (Me-31, 3H, s), 1.029 (Me-27, 3H,s), 1.103 (Me-30, 3H, s), 1.19 (H-15, 1H, m), 1.226 (Me-32, 3H, s), 1.27 (H-3 & H-11, 2H, m), 1.32 (H-16, 1H, m), 1.37 (H-7, 1H, m), 1.46 (H-9, 1H, m), 1.48 (H-2, H-3 & H-6, 3H, m), 1.61 (H-11, 1H, m), 1.62 (H-2, H-6 & H-15, 3H, m), 1.66 (H-12 & H-19, 1H, m), 1.68 (H-7, 1H, m), 1.72 (H-12, 1H, m), 1.729 (Me-333H, s), 1.75 (H-1, 1H, m), 1.80 (H-17, 1H, m), 1.81 (H-13, 1H, m), 2.03 (H-16, 1H, m), 2.19 (H-23, 2H, m), 2.22 (H-24, 2H, m), 2.26 (H-20, 2H, m), 5.13 (H-26b, 1H, bd, $J = 10.4$ Hz), 5.19 (H-26a, 1H, bd, $J = 17.2$ Hz), 5.40 (H-21, 1H, t, $J = 7.2$ Hz), 5.93 (H-25, 1H, m); ^{13}C NMR (150 MHz, C_6D_6): $\delta = 15.79$ (C-30, q), 16.03 (C-33, q), 16.43 (C-29, q), 16.73 (C-31, q), 19.00 (C-2, t), 19.09 (C-6, t), 21.76 (C-11, t & C-28, q), 22.93 (C-20, t), 25.18 (C-12, t), 25.82 (C-32, q), 28.02 (C-16, t), 31.55 (C-15, t), 32.70 (C-24, t), 33.52 (C-4, s), 33.63 (C-27, q), 35.64 (C-7, t), 37.70 (C-10, s), 39.44 (C-23, t), 40.89 (C-1, t & C-8, s), 40.98 (C-19, t), 42.48 (C-13, d & C-3, t), 50.40 (C-17, d), 50.62 (C-14, s), 51.20 (C-9, d), 57.34 (C-5, d), 74.77 (C-18, s), 114.6 (C-26, t), 125.7 (C-21, d), 134.4 (C-22, s), 138.8 (C-25, d). The following ^{1}H- and ^{13}C NMR signals are indistinguishable from each other, due to very close chemical shifts: H-2/H-6, H-11/H-12/H-16, C-2/C-6, C-11/C-12/C-16, C-15/C-24, C-1/C-19. EIMS: *m/z* (%): 69(90), 81(48), 95(58), 109(60), 119(37), 131(35), 149(49), 169(25), 181(24), 191(100), 205(15), 219(12), 231(20), 281(12), 301(12), 341(15), 381(7), 435(7), 450(M^+-H_2O, 39). HRMS (EI): *m/z*: calcd for $C_{33}H_{54}$ (M^+) 450.4226, found 450.4247. $[\alpha]_D^{25} = +20.28$ ($c = 0.036$, EtOH). Oil.

Product 35. ^{1}H-NMR (400 MHz, C_6D_6): $\delta = 0.92$(H-1, 1H, m), 0.94(H-5, 1H,dd, $J = 12.0$, 2.0 Hz), 0.994 (Me-28, 3H, s), 1.008 (Me-29, 3H, s), 1.025 (Me-27, 3H, s), 1.092 (Me-30, 3H, s), 1.218 (Me-31, 3H, s) 1.22 (H-11, 1H, m), 1.26 (H-15, 1H, m), 1.308 (Me-32, 3H, s), 1.31 (H-3, 1H, m), 1.41 (H-7, 1H, m), 1.49 (H-2, 1H, m), 1.51 (H-3, 1H, m), 1.52 (H-6, 1H, m), 1.54 (H-9, 1H, m), 1.56 (H-15, 1H, m), 1.62 (H-19, 2H, m), 1.64 (H-12, 1H,m), 1.67 (H-2, 1H, m), 1.68 (H-11, 1H, m), 1.722 (Me-33, 3H, s), 1.73 (H-7, 1H, m), 1.75 (H-6, 1H, m), 1.77 (H-1 & H-16, 3H, m), 2.04 (H-12, 1H,m), 2.10 (H-17, 1H, m), 2.13 (H-13, 1H, m), 2.20 (H-23, 2H, m), 2.21 (H-20, 2H, m), 2.26 (H-24, 2H, m), 5.12 (H-26b,1H, bd, $J = 10.0$ Hz), 5.17 (H-26a, 1H, bd, $J = 16.8$ Hz), 5.37 (H-21, 1H, t, $J = 6.0$ Hz), 5.91 (H-25, 1H, m); ^{13}C NMR (100 MHz, C_6D_6): $\delta = 16.03$ (C-33, q), 16.17 (C-30, q), 16.50 (C-29, q), 17.16 (C-31, q), 19.11 (C-2, t), 19.15 (C-6, t), 21.78 (C-28, q), 22.88 (C-11, t), 23.42 (C-20, t), 26.42 (C-12, t), 26.75 (C-16, t), 27.69 (C-32, q), 32.72 (C-24, t), 32.76 (C-15, t), 33.54 (C-4, s), 33.62 (C-27, q), 35.73 (C-7, t), 37.71 (C-10, s), 39.43 (C-23, t), 40.88 (C-1, t), 41.19 (C-8, s), 42.49 (C-3, t), 42.67 (C-19, t), 44.07 (C-13, d), 48.38 (C-17, t), 49.48 (C-14, s), 51.14 (C-9, d), 57.34 (C-5, d), 74.59 (C-18, s), 114.6 (C-26, t), 125.6 (C-21, d), 134.4 (C-22, s), 138.8 (C-25, d); The following ^{1}H- and ^{13}C NMR signals are indistinguishable from each other, due to very close chemical shifts: H-2/H-6, H-11/H-12/H-16, C-2/C-6, C-11/C-12/C-16, C-15/C-24. EIMS: *m/z* (%): 69(71), 81(76), 95(71), 109(65), 123(31), 137(35), 149(53), 161(18), 181(28), 177(13), 191(100), 205(12), 231(19), 299(10), 341(32), 435(4), 450(M^+-H_2O, 22). HRMS (EI): *m/z*: calcd for $C_{33}H_{54}$ (M^+) 450.4226, found 450.4247. $[\alpha]_D^{25} = -18.21$ ($c = 0.2$, EtOH). Oil.

Acknowledgements

This work was supported by Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan (Nos.17-05429 and 18380001). One of the authors (J. C.) worked in this laboratory as a JSPS postdoctoral fellow for foreign researchers. We thank the Japanese Society for the promotion of Science (JSPS).

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