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Synthetic Studies on Natural Diterpenoid Glyceryl Esters

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Abstract—Synthesis of natural bicyclic and tricyclic diterpenoid diacylglycerols has been performed by regioselective coupling of terpenoid acid with glycerol at 1′-*sn* position. This method may be considered a general approach to obtain optically active acylglycerols. The preparation of ^{13}C-labelled geranylgeranoic acid glyceryl esters is also described here. © 2000 Elsevier Science Ltd. All rights reserved.

Introduction

Terpenoid acylglycerols represent an interesting group of natural bioactive molecules, which could be considered the chemical marker of marine dorid nudibranchs belonging to the related genera *Anisodoris*, *Archidoris*, *Austrodoris*, *Doris* and *Sclerodoris*.[1–3] After the first report of farnesyl glyceryl esters from the skin of *Archidoris odhneri*,[4] an increasing number of terpenoid diacylglycerols have been isolated from the mantle of several dorid species in the last years.[5–15] These molecules, which are supposed to be involved in the chemical defense of nudibranchs, shell-less molluscs apparently unprotected from predation, display deterrent and ichthyotoxic properties.[6,7] Promising physiological activities, such as the activation of protein kinase C in vitro and morphogenetic effects in the regenerative test in vivo with the fresh water hydrozoan *Hydra vulgaris*, have been also reported.[16] The majority of natural terpenoid acylglycerols are structurally characterized by the presence of a terpenoid acid residue esterified at C-1′ of glycerol, which is further linked to an acetyl group at C-2′ or C-3′. However, a few glyceryl esters with the terpene moiety linked to the secondary carbon of glycerol have been also isolated.[10,12,17] The *S* absolute configuration at C-2′ of glycerol has been determined for several 1,3-diacylglycerols by using different chemical methods[18,19] as well as by synthesis.[14,15] Surprisingly, the opposite *R* stereochemistry at C-2′ has been recently reported for two 1,3-diacylglycerols occurring in an Antarctic nudibranch.[19]

The terpenoid structure is quite variable. However, so far, the majority of diacylglycerols display either bicyclic labdane,[8,19] halimane,[13,19] and copalane[14] skeletons, or tricyclic isocopalane[5,6,9] and *ent*-isocopalane[7,9,14,15,17] skeletons, including rearranged and functionalized structures.

Terpenoid acylglycerols are likely biosynthesized de novo by the nudibranch, as has been recently demonstrated in some Pacific dorid species.[20]

In the course of our research on marine molluscs, in the last few years we have directed our interest towards dorid nudibranchs containing terpenoid acylglycerols. Several new bicyclic and tricyclic molecules have been isolated from different dorid species. Among these, an interesting pair of diastereoisomeric diacylglycerols, exhibiting enantiomeric terpenoid skeletons (isocopalane and *ent*-isocopalane structure), has been also reported.[9] The promising biological activities of terpenoid diacylglycerols have also prompted synthetic studies with the aim of either confirming the proposed structures or obtaining larger quantities of compounds for pharmacological studies. A series of linear and cyclic optically active terpenoid glyceryl esters have been prepared. Accounts of this synthetic work, including the assignment of the absolute stereochemistry of some natural sesquiterpenoid diacylglycerols, have already been published.[14,15,21–23]

Herein, we report the full experimental data on the synthesis of the natural glyceryl ester derivatives of bicyclic and tricyclic diterpenoid acids, with the aim at presenting a survey on our recent studies in this field. In particular, the preparation of the natural diacylglycerols **1e**, **2e**, **2i**, **3e** and **3i**, previously isolated from some dorid species, as indicated in Fig. 1, is described here.

Furthermore, the synthesis of the mixture of diastereoisomeric glyceryl derivatives of *rac*-isocopalic acid (**2a**/**3a**), obtained by superacidic cyclization of geranylgeranoic acid, is reported. Finally, this synthetic method has been also applied to prepare glyceryl derivatives of geranylgeranoic acid labelled with ^{13}C isotope, in order to achieve

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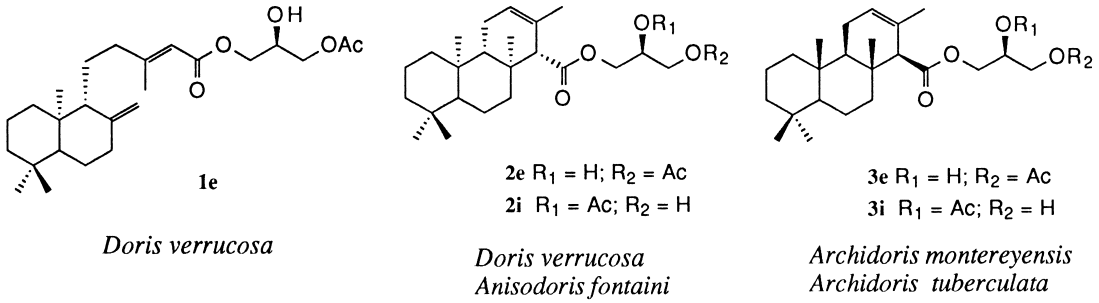


Figure 1.

suitable precursors for biosynthetic studies of diacylglycerols in dorid nudibranchs. The synthesis of the monoglyceryl and the 1,3-diacylglyceryl esters of 3,20-$^{13}C_2$-(all *E*)-geranylgeranoic acid (**4a**) is also described here.

Results and Discussion

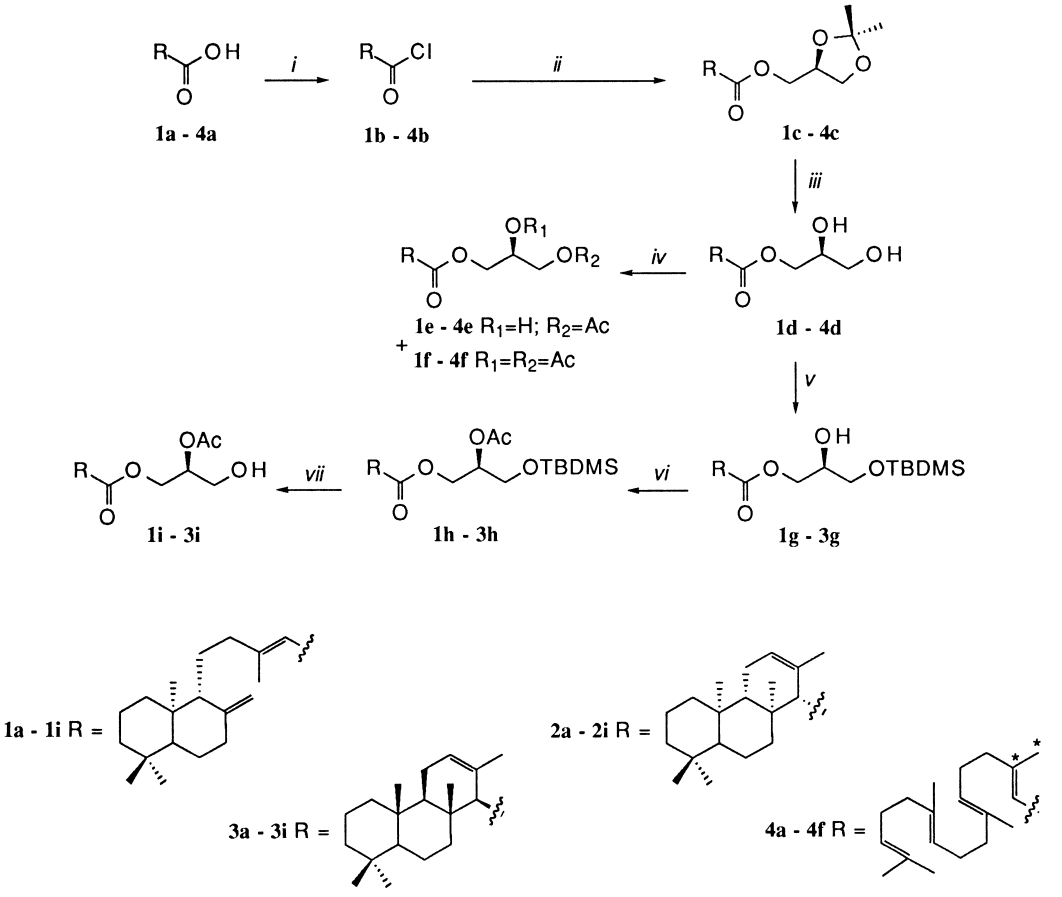
Synthetic strategy

All diterpenoid acylglycerols were synthesized using a general strategy, which was based on three sequential steps:

a) preparation of the starting diterpenoid acid;
b) regioselective coupling of the diterpenoid acid chloride with glycerol at 1′-*sn* position;[21]
c) regioselective acetylation at C-2′ or C-3′.[22]

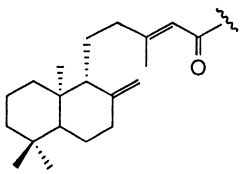
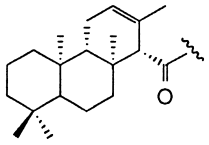
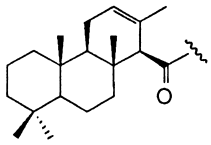
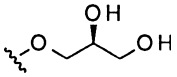
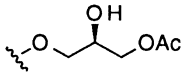
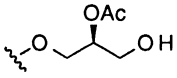
Steps *b* and *c*, which are common for all compounds synthesized, are illustrated in Scheme 1, whereas the preparation of the starting acids is described below. Overall yields obtained in the synthesis of natural acylglycerols are reported in Table 1.

The starting diterpenoid acids (**1a**–**4a**) were first transformed into the corresponding chlorides (**1b**–**4b**) by



Scheme 1. Reagents and conditions: (*i*) $(COCl)_2$, C_6H_6, room temperature (2 h), 45°C (0.5 h); (*ii*) (−)-2,3-*O*-isopropylidene-*sn*-glycerol, NaH, dry CH_2Cl_2, 0°C (20 min), room temperature (10 min); (*iii*) H_2SO_4, MeOH, room temperature (4 h); (*iv*) *N*-acetylimidazole, DBU, C_6H_6, room temperature (1 h); (*v*) 1.1 equiv. TBDMSCl, dry Pyr, room temperature (12 h); (*vi*) Ac_2O, dry Pyr, room temperature (12 h); (*vii*) ca. 1.1 equiv. $PdCl_2(MeCN)_2$, Me_2CO, room temperature (1 h).

Table 1. Coupling scheme of terpenoid acylglycerols. Overall yields (from starting acids **1a**–**3a**) are reported in brackets

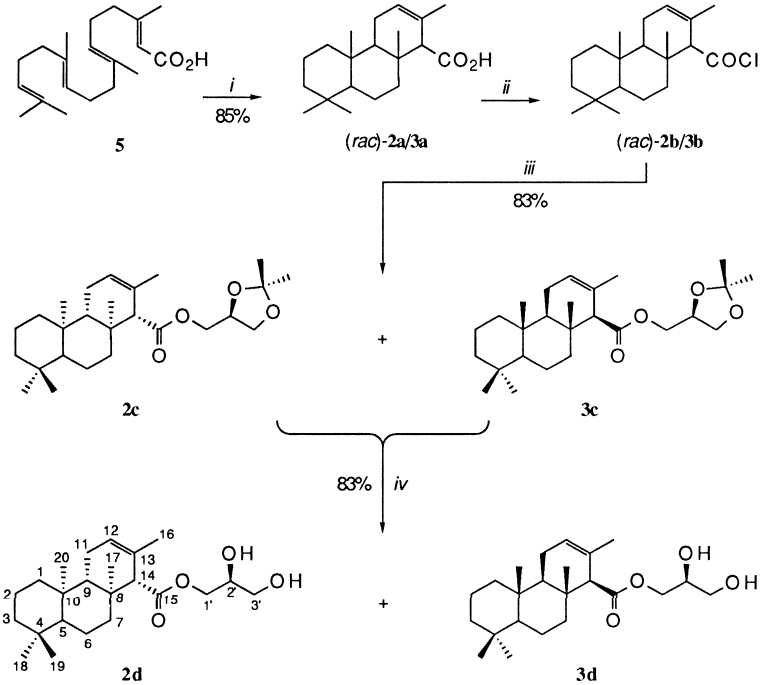
			
	**1d** (85%)	**2d** (66%)	**3d** (70%)
	**1e** (55%)	**2e** (44%)	**3e** (47%)
	**1i** (56%)	**2i** (40%)	**3i** (49%)

treatment with $(COCl)_2$ in dry C_6H_6, at room temperature for 2 h and at 45°C for 30 min. The crude chlorides, without purification, were immediately coupled with (−)-2,3-*O*-isopropylidene-*sn*-glycerol, by NaH in CH_2Cl_2, to give the acetonides **1c**–**4c**. These latter compounds were subsequently deprotected by 0.006 M H_2SO_4 in CH_3OH to afford the corresponding monoacylglycerols **1d**–**4d**.[21] Acetylation of **1d**–**4d** by *N*-acetylimidazole (DBU, C_6H_6) yielded the 1,3-diacyl glycerols **1e**–**4e**, as main products, along with the diacetyl derivatives **1f**–**4f** (ratio ca. 3:1). The 1,2-diacyl glyceryl esters **1i**–**3i** were also synthesized by selective protection with TBDMSCl of the primary hydroxyl group (compounds **1g**–**3g**), followed by acetylation of the secondary –OH (compounds **1h**–**3h**) and subsequent removal of TBDMS group by $PdCl_2(CH_3CN)_2$.[22]

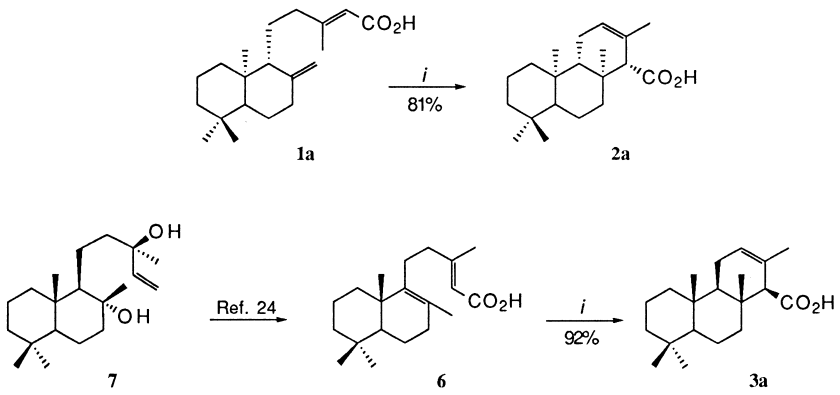
Preparation of starting diterpenoid acids

Bicyclic acylglycerols. Copalic acid (**1a**) was easily obtained by hydrolysis of the corresponding methyl ester, which was isolated from commercial 'Copaiva Balsam', as already reported.[14]

Tricyclic acylglycerols. The synthetic procedure in Scheme 1 was first carried out using as substrate (*rac*)-isocopalic acid (**2a**/**3a**) (Scheme 2), which was prepared by superacidic cyclization of geranylgeranoic acid (**5**), according to the literature.[24] In order to get the diastereoisomeric pairs **2e**–**3e** and **2i**–**3i**, the mixture of epimeric monoacylglycerols **2d** and **3d** obtained, was submitted to different chromatographic techniques. Every attempt to separate the two compounds failed, so it was decided to perform the



Scheme 2. Reagents and conditions: (*i*) FSO_3H (5 equiv.), *i*-PrNO2, −78°C, 40 min, then Et_3N; (*ii*) $(COCl)_2$, C_6H_6, room temperature (2 h), 45°C (0.5 h); (*iii*) (−)-2,3-*O*-isopropylidene-*sn*-glycerol, NaH, dry CH_2Cl_2, 0°C (20 min), room temperature (10 min); (*iv*) H_2SO_4, MeOH, room temperature (4 h).



Scheme 3. Reagents and conditions: (*i*) FSO_3H (5 equiv.), *i*-PrNO2, −78°C, 40 min, then Et_3N.

synthesis starting from both optically active isocopalic acid (**2a**) and *ent*-isocopalic acid (**3a**). Compound **2a** was prepared in high yields by superacidic cyclization of natural copalic acid (**1a**), whereas **3a** was obtained in high yields[21] by superacidic cyclization of the bicyclic diterpene acid **6**, previously synthesized from sclareol (**7**) in five steps[24] (Scheme 3).

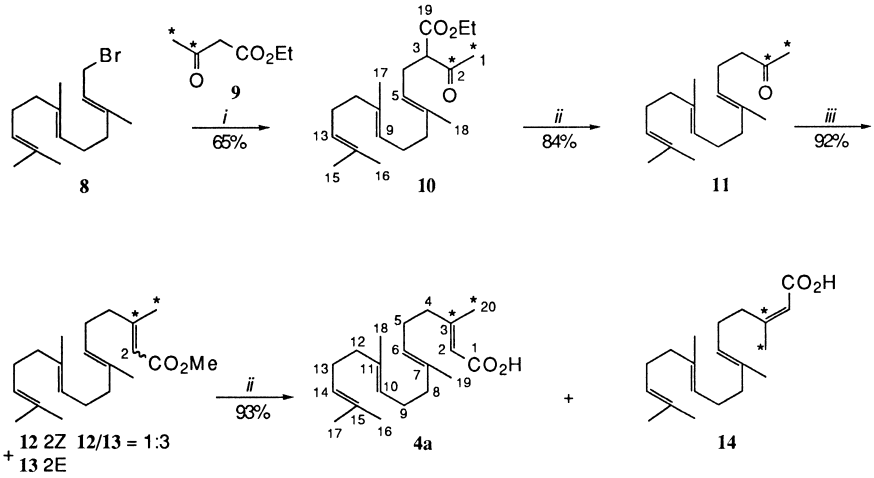
Synthesis of 3,20-$^{13}C_2$-(all *E*)-geranylgeranoic acid (4a). The 1,3-diacylglyceryl derivative of (all *E*)-geranylgeranoic acid (**5**) was found in the Mediterranean *Doris verrucosa* along with a series of bicyclic and tricyclic acylglycerols, which were suggested to be biogenetically correlated.[14] Unfortunately, preliminary incorporation in vivo of labelled mevalonate[25] gave ambiguous results. Therefore, in order to get suitable precursors for further biosynthetic studies in vivo in *D. verrucosa*, the synthesis of the monoglyceryl and the 1,3-acetylglyceryl derivatives of 3,20-$^{13}C_2$-(all *E*)-geranylgeranoic acid (**4a**), obtained as reported in Scheme 4, has been also planned.

According to the literature[24,26] for (all *E*)-geranylgeranoic acid (**5**), (*E*,*E*)-farnesylbromide (**8**) was coupled with [3,4-$^{13}C_2$]-ethyl acetoacetate (**9**), by Na metal in toluene, to give [1,2-$^{13}C_2$]-3-ethylcarboxy-(*E*,*E*)-farnesylacetone (**10**), which was decarboxylated by KOH/EtOH to obtain [1,2-$^{13}C_2$]-(*E*,*E*)-farnesylacetone (**11**). This compound was submitted to Wittig reaction with trimethylphosphonoacetate/MeONa, in C_6H_6, to afford a mixture of the two isomeric esters **12** (Δ^2 *Z*) and **13** (Δ^2 *E*). Finally, the ester mixture was hydrolyzed by KOH/EtOH to give, after chromatographic purification on Si-gel, pure (all *E*) isomer **4a**.

Labelled compounds **10**, **11**, **4a**–**4f** were characterized by MS and NMR data. In particular, ^{1}H NMR spectra were identical with those of the corresponding natural abundance molecules, except for the signal of the labelled methyl which resonates as a double doublet (J_1∼100–140 Hz and J_2∼6.0–7.0 Hz) for heteronuclear geminal and vicinal couplings (see Experimental). On the other side, ^{13}C NMR spectra were characterized by the presence of two very intense apparent doublet signals attributed to the geminal labeled carbons, each other coupled. In addition, expected ^{13}C–^{13}C couplings through two or three bonds[27] were also detected (see Experimental).

Conclusions

In conclusion, we have described a general synthetic method to prepare in good yield optically active diterpenoid



Scheme 4. Reagents and conditions: (*i*) **9**, Na metal, toluene, reflux 2 h; (*ii*) KOH, aq. EtOH, reflux 2 h; (*iii*) $(MeO)_2P(O)CH_2CO_2Me$, MeONa, C_6H_6, reflux 1.5 h.

acylglycerols. It has been reported that many of these natural compounds show potent PKC stimulatory effects in vitro and PKC morphogenetic properties in vivo in the *Hydra* test, suggesting their use as molecular probes for cell development studies.[16] The synthetic approach here described opens a very easily accessible way to better investigate such interesting pharmacological activities.

Experimental

General procedures

Melting points were measured on a Kofler apparatus and are uncorrected. The IR spectra were taken on a Bio-Rad FTS 7 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ on Bruker WM 500, Bruker AM 400 and Bruker WM 300 spectrometers; chemical shifts are reported in ppm and are referred to $CHCl_3$ as internal standard (δ 7.26 for proton and δ 77.0 for carbon). Optical rotations were measured in $CHCl_3$ on a Jasco DIP 370 polarimeter, using a 10-cm cell. EIMS spectra were recorded on a Carlo Erba TRIO 2000 spectrometer, coupled with an INTEL computer. HREIMS spectra of labelled compounds were obtained on a VG 70–70 EQ-HF instrument with manual peak-matching technique, using the appropriate PKF peaks as internal reference. Commercial Merck Si gel 60 (70–230 mesh ASTM) was used for column chromatography, and Merck precoated Si gel plates were used for TLC. The chromatograms were sprayed with 0.1% $Ce(SO_4)_2$ in 2N H_2SO_4 and heated at 80°C for 5 min to detect the spots. The work-up of the reaction mixtures in organic solvents included exhaustive extraction with diethyl ether and washing with water, up to neutral reaction, drying over anhydrous Na_2SO_4, filtration, and removal of the solvent in vacuo. Acid solutions were also washed with saturated $NaHCO_3$ water solutions and water, up to neutral reaction, and dried over anhydrous Na_2SO_4. [3,4-$^{13}C_2$]-ethyl acetoacetate (^{13}C 99%) was purchased by Trimital s.r.l. Co. (Milan, Italy).

Preparation of starting acids

Copalic acid 1a. Copalic acid methyl ester (220 mg, 0.692 mmol) was isolated from commercial 'Copaiva Balsam' as reported in Ref. 14. The ester was treated with 5% ethanolic solution of NaOH (5.5 ml), and the mixture was heated under reflux for 1.5 h. The crude product (256 mg), obtained after the usual work up, was purified on a silica gel column (5.5 g, petr. ether–Et_2O, 93:7) to give 152 mg (72%) of pure copalic acid **1a**: colorless oil; $[\alpha]_D$=+7.2° (*c* 0.31, $CHCl_3$), lit.[28] $[\alpha]_D$=+6.9° (*c* 1.15, $CHCl_3$).

(+)-Isocopalic acid 2a. A solution of copalic acid **1a** (35 mg, 0.115 mmol) in *i*-$PrNO_2$ (0.7 ml) was cooled at −78°C and treated with FSO_3H (58 mg, 0.58 mmol) in *i*-$PrNO_2$ (0.3 ml), under stirring. After 40 min, the reaction was stopped by adding a solution of Et_3N (1 ml) in petroleum ether (1 ml). The usual work up gave 35 mg of a crude residue, which was purified on a silica gel column (0.6 g) (petr. ether–Et_2O, 97:3) to give 28.4 mg (81%) of (+)-isocopalic acid **2a**: colorless crystals, mp 176–177.5°C (from petr. ether); $[\alpha]_D$=+7.2° (*c* 0.11, $CHCl_3$); IR: ν_{max} (liquid film) 3500, 1715 cm^{-1}; ^{1}H NMR (400 MHz, selected values) δ_H: 0.81 (3H, s, H_3-18), 0.86 (3H, s, H_3-19), 0.91 (3H, s, H_3-20), 0.97 (3H, s, H_3-17), 1.67 (3H, bs, H_3-16), 2.93 (1H, bs, H-14), 5.54 (1H, bs, H-12); ^{13}C NMR (100 MHz) δ_C: 177.1 (C-15), 128.4 (C-13), 124.4 (C-12), 62.1 (C-14), 56.4 (C-5), 54.2 (C-9), 41.8 (2C, C-3 and C-7), 39.9 (C-1), 37.4 (C-8), 36.4 (C-10), 33.4 (C-19), 33.1 (C-4), 22.6 (C-11), 21.6 (C-18), 21.2 (C-16), 18.6 (C-2 or C-6), 18.5 (C-6 or C-2), 15.8 (C-20), 15.5 (C-17); HRMS calcd for $C_{20}H_{32}O_2$ (M^+) *m/z* 304.2402, found 304.2410.

(−)-*ent*-Isocopalic acid 3a. Using the procedure above described and as already reported in Ref. 21, acid **6** (152 mg, 0.50 mmol) was dissolved in *i*-$PrNO_2$ (3 ml), cooled at −78°C and treated with FSO_3H (260 mg, 2.60 mmol) in *i*-$PrNO_2$ (1.5 ml), under stirring. After 40 min, a solution of Et_3N (3 ml) in petroleum ether (1.5 ml) was added. The reaction mixture was extracted and after removing of the solvent, the residue was chromatographed on a silica gel column (2.5 g, petr. ether–Et_2O, 97:3) to give 140 mg (92%) of (−)-*ent*-isocopalic acid **3a**: colorless crystals, mp 177–178°C (from petr. ether); $[\alpha]_D$=−9.1° (*c* 0.30, $CHCl_3$); IR, ^{1}H and ^{13}C NMR data were identical with those of **2a**.

(*rac*)-Isocopalic acid 2a/3a. A solution of FSO_3H (330 mg, 3.30 mmol) in *i*-$PrNO_2$ (1.9 ml) was added at −78°C, under stirring, to 200 mg (0.66 mmol) of *E*,*E*,*E*-geranylgeranoic acid **5** in *i*-$PrNO_2$ (4 ml), according to the above procedure. The usual work up yielded 199 mg of a crude residue, which was purified on a silica gel column (4.5 g, petr. ether–Et_2O, 97:3) to give 171 mg (85%) of (*rac*)-isocopalic acid **2a**/**3a**: colorless crystals, mp 174–176°C (from petr. ether); IR, ^{1}H and ^{13}C NMR data were identical with those of isocopalic acid **2a** and *ent*-isocopalic acid **3a**.

3,20-$^{13}C_2$-*E*,*E*,*E*-geranylgeranoic acid 4a. *(a) 1,2-$^{13}C_2$-3-ethylcarboxy-E,E-farnesylacetone **10**:* To a solution of 3,4-$^{13}C_2$-ethyl acetoacetate (**9**, 1.0 g, 7.58 mmol) in dry toluene (15 ml), 176 mg (7.65 equiv.) of sodium were added, under Ar atmosphere. After complete dissolving of sodium, the solution was treated with *E*,*E*-farnesylbromide (**8**, 1.9 g, 6.67 mmol) in dry toluene (9.3 ml). The reaction mixture was refluxed for 2 h and then stopped. The usual work-up yielded 2.1 g of a crude residue, which was purified on a silica gel column (45 g) (petr. ether–Et_2O, 97:3) to give 1.45 g (65%) of 1,2-$^{13}C_2$-3-ethylcarboxy-*E*,*E*-farnesylacetone (**10**): colorless oil; IR: ν_{max} (liquid film) 1730, 1684 cm^{-1}; ^{1}H NMR (400 MHz, selected values) δ_H: 1.26 (3H, t, *J*=7.1 Hz, CH_2CH_3), 1.58 (3H, s, H_3-17 or H_3-18), 1.59 (3H, s, H_3-18 or H_3-17), 1.62 (3H, s, H_3-16), 1.67 (3H, s, H_3-15), 2.21 (3H, dd, J_{CH}=128 and 6 Hz, H_3-1*), 4.17 (2H, q, *J*=7.1 Hz, CH_2–CH_3), 5.05 (3H, m, H-5, H-9 and H-13); ^{13}C NMR (100 MHz) δ_C: 203.1 (apparent d, *J*=42 Hz, C*-2), 172.0 (C-19), 138.4 (C-6), 135.2 (C-10), 131.3 (C-14), 124.3 (C-13 or C-9), 123.8 (C-9 or C-13), 119.6 (C-5), 61.2 (CH_2CH_3), 59.6 (dd, *J*=38 and 13 Hz, C-3), 39.7 (2C, C-7 and C-11), 29.1 (apparent d, *J*=42 Hz, C*-1), 26.9 (C-12 or C-8 or C-4), 26.7 (C-8 or C-4 or C-12), 26.5 (C-4 or C-8 or C-12), 25.7 (C-15), 18.4 (C-17), 17.6 (C-16), 16.0 (C-18, d, *J*=3.8 Hz), 14.1 (CH_2CH_3); MS, *m/z* (%): 336 (M^+, 14), 291 (9), 275 (8),

249 (10), 212 (15), 199 (42), 175 (30), 161 (63), 155 (100), 137 (78); HRMS calcd for $C_{19}{}^{13}C_2H_{34}O_3$ (M^+) *m/z* 336.2575, found 336.2561. *(b) 1,2-$^{13}C_2$-E,E-farnesylacetone **11**:* 1,2-$^{13}C_2$-3-Ethylcarboxy-*E*,*E*-farnesylacetone (**10**, 1.44 g, 4.29 mmol) was dissolved in 5.5 ml of EtOH and 8.5 ml of 10% KOH/EtOH solution were added. The reaction mixture was refluxed for 2 h. After the usual work-up, 1.11 g of a crude residue was obtained and purified on a silica gel column (23 g) (petr. ether–Et_2O, 7:3) to give 952 mg (84%) of 1,2-$^{13}C_2$-*E*,*E*-farnesylacetone (**11**): colorless oil; IR: ν_{max} (liquid film) 1675 cm^{-1}; ^1H NMR (400 MHz, selected values) δ_H: 1.58 (3H, s, H_3-17 or H_3-18), 1.59 (3H, s, H_3-18 or H_3-17), 1.62 (3H, s, H_3-16), 1.67 (3H, s, H_3-15), 2.13 (3H, dd, J_{CH}=127 and 6 Hz, H_3-1*), 5.08 (3H, m, H-5, H-9 and H-13); ^{13}C NMR (100 MHz) δ_C: 208.9 (apparent d, *J*=40 Hz, C*-2), 136.4 (C-6 or C-10), 133.0 (C-10 or C-6), 131.2 (C-14), 124.3 (C-13), 124.0 (C-9), 122.5 (C-5), 43.7 (dd, *J*=39 and 13 Hz, C-3), 39.7 (C-11 or C-7), 39.6 (C-7 or C-11), 29.9 (apparent d, *J*=40 Hz, C*-1), 26.7 (C-12 or C-7 or C-4), 26.6 (C-8 or C-12 or C-4), 26.5 (C-4 or C-8 or C-12), 25.7 (C-15), 17.7 (C-16), 16.0 (2C, C-17 and C-18); MS, *m/z* (%): 264 (M^+, 5), 225 (40), 209 (41), 207 (88), 169 (8), 142 (42), 97 (100), 83 (26), 69 (22), 57 (68); HRMS calcd for $C_{16}{}^{13}C_2H_{30}O$ (M^+) *m/z* 264.2364, found 264.2369. *(c) 3,20-$^{13}C_2$-E,E,E-geranylgeranoic acid **4a**:* A solution of sodium methoxide in methanol [248 mg (10.78 equiv.) of sodium metal in 6.1 ml of methanol] was slowly added to a stirred solution of ketone **11** (940 mg, 3.56 mmol) and trimethylphosphonoacetate (1.97 g, 10.82 mmol) in benzene (80 ml). After refluxing 1.5 h, the mixture was cooled, treated with ice-water and extracted with Et_2O. The residue (1.11 g), obtained after the usual work-up, contained a mixture of the isomeric esters **12** and **13** and was used in the next step without any purification. The mixture of the esters **12** and **13** (1.11 g) was dissolved in 4 ml of EtOH and 7 ml of 10% KOH/EtOH solution (7.0 ml) were added. The reaction mixture was refluxed for 2 h. The usual work-up yielded 1.01 g of crude reaction product, which was purified by SiO_2 chromatography (petr. ether–Et_2O gradient as eluent) to give 470 mg of a mixture containing the *E*,*E*,*E*-isomer acid **4a** and the *Z*,*E*,*E*-isomer acid **14**, along with 280 mg of pure **4a**: colorless oil; IR: ν_{max} (liquid film) 1683, 1614, 1236 cm^{-1}; ^1H NMR (400 MHz, selected values) δ_H: 1.60 (6H, bs, H_3-16 and H_3-18), 1.61 (3H, bs, H_3-19), 1.68 (3H, bs, H_3-17), 2.20 (3H, dd, J_{CH}=108 and 7 Hz, H_3-20), 5.09 (3H, m, H-6, H-10 and H-14), 5.69 (1H, d, J_{CH}=8 Hz, H-2); ^{13}C NMR (100 MHz) δ_C: 171.7 (C-1), 162.9 (apparent d, *J*=40 Hz, C*-3), 136.3 (C-7), 135.1 (C-11), 131.2 (C-15), 124.4 (C-14), 124.1 (C-10), 122.7 (C-6), 115.1 (d, *J*=72 Hz, C-2), 41.2 (d, *J*=39 Hz, C-4), 39.7 (2C, C-8 and C-12), 26.8 (C-13 or C-9), 26.6 (C-9 or C-13), 25.7 (2C, C-5 and C-17), 19.1 (apparent d, *J*=40 Hz, C*-20), 17.7 (C-16), 16.0 (2C, C-18 and C-19). MS, *m/z* (%): 306 (M^+, 6), 263 (5), 220 (5), 205 (18), 191 (12), 177 (11), 149 (21), 136 (100), 123 (95), 109 (52); HRMS calcd for $C_{18}{}^{13}C_2H_{32}O_2$ (M^+) *m/z* 306.2469, found 306.2447.

Preparation of acetonides 1c–4c

Compound 1c. In a typical procedure, copalic acid (**1a**, 76 mg, 0.25 mmol) was dissolved in 0.8 ml of dry C_6H_6 and the solution was added to 0.11 ml (1.26 mmol) of $(COCl)_2$ in 0.8 ml of dry C_6H_6, under Ar atmosphere. The reaction mixture was stirred at room temperature, for 2 h and at 45°C, for 30 min. Both the solvent and the unreacted oxalyl chloride were removed in vacuo to give 80.4 mg of crude chloride **1b**, which was used in the next step without any purification. A suspension of NaH (27 mg, 0.92 mmol, 80% dispersed in mineral oil) and 0.1 ml of dry pyridine were added to a solution of (−)-2,3-*O*-isopropylidene-*sn*-glycerol (120 mg, 0.91 mmol) in 3.3 ml of anhydrous CH_2Cl_2, at 0°C, under an Ar atmosphere. The mixture was stirred for 10 min and then crude **1c** (80.4 mg, 0.25 mmol) in 1.6 ml of anhydrous CH_2Cl_2 was added. The reaction mixture was stirred at 0°C for 20 min and at room temperature for 10 min. After the usual work-up, the crude residue (101.4 mg) was purified on a silica gel column (petr. ether–Et_2O, 93:7) to give 96.2 mg (92%) of acetonide **1c**: colorless oil; $[\alpha]_D$=−10.8° (*c* 0.65, $CHCl_3$); IR: ν_{max} (liquid film) 1723, 1382, 1228, 1145, 1066, 890, 850 cm^{-1}; ^1H NMR (300 MHz, selected values) δ_H: 0.67 (3H, s, H_3-20), 0.80 (3H, s, H_3-18), 0.87 (3H, s, H_3-19), 1.37 (3H, s, H_3-acetonide), 1.43 (3H, s, H_3-acetonide), 2.16 (3H, bs, H_3-16), 3.75 (1H, m, H-3′a), 4.09 (1H, m, H-3′b), 4.06–4.22 (2H, m, H_2-1′), 4.33 (1H, m, H-2′), 4.47 (1H, bs, H-17a), 4.83 (1H, bs, H-17b), 5.69 (1H, bs, H-14); ^{13}C NMR (100 MHz) δ_C: 166.5 (C-15), 162.3 (C-13), 148.3 (C-8), 114.6 (C-14), 109.9 [$(O)_2C(CH_3)_2$], 106.3 (C-17), 73.8 (C-2′), 66.5 (C-1′), 63.9 (C-3′), 56.2 (C-9), 55.5 (C-5), 42.1 (C-3), 39.9 (C-12), 39.7 (C-10), 39.0 (C-1), 38.3 (C-7), 33.6 (2C, C-4 and C-19), 26.7 (Me-acetonide), 25.4 (Me-acetonide), 24.4 (C-6), 21.7 (C-18), 21.5 (C-11), 19.4 (C-2), 19.1 (C-16), 14.5 (C-20); MS, *m/z* (%): 418 (M^+, 5), 403 (25), 360 (29), 345 (48), 286 (16), 271 (19), 244 (63), 169 (83), 156 (100); HRMS calcd for $C_{26}H_{42}O_4$ (M^+) *m/z* 418.3083, found 418.3075.

Compound 2c. Following the procedure above reported for **1c**, 27.4 mg (0.09 mmol) of isocopalic acid **2a** were treated with 43.6 mg (0.33 mmol) of (−)-2,3-*O*-isopropylidene-*sn*-glycerol to give, after purification, 30.4 mg (81%) of acetonide **2c**: colorless oil; $[\alpha]_D$=−4.5° (*c* 0.07, $CHCl_3$); IR: ν_{max} (liquid film) 1735, 1636, 1236, 1050, 841 cm^{-1}; ^1H NMR (400 MHz, selected values) δ_H: 0.81 (3H, s, H_3-18), 0.86 (3H, s, H_3-19), 0.90 (3H, s, H_3-20), 0.94 (3H, s, H_3-17), 1.37 (3H, s, H_3-acetonide), 1.43 (3H, s, H_3-acetonide), 1.60 (3H, d, *J*=1.4 Hz, H_3-16), 2.94 (1H, bs, H-14), 3.77 (1H, dd, *J*=6.3 and 8.5 Hz, H-3′a), 4.08 (1H, m, H-3′b), 4.10–4.18 (2H, m, H_2-1′), 4.31 (1H, m, H-2′), 5.51 (1H, bs, H-7); ^{13}C NMR (100 MHz) δ_C: 124.1 (C-12), 106.3 [$(O)_2C(CH_3)_2$], 73.8 (C-2′), 66.7 (C-1′), 64.2 (C-3′), 62.5 (C-14), 56.4 (C-5), 54.3 (C-9), 41.8 (C-3), 41.7 (C-7), 39.9 (C-1), 38.3 (C-10), 37.4 (C-8), 33.4 (C-19), 33.2 (C-4), 26.8 (Me-acetonide), 25.4 (Me-acetonide), 22.7 (C-11), 21.7 (C-18), 21.2 (C-16), 18.6 (C-2), 18.4 (C-6), 15.6 (C-20), 15.5 (C-17), (C-13 and C-15 were not detected); MS, *m/z* (%): 418 (M^+, 10), 403 (61), 360 (100), 345 (30), 286 (51), 271 (33), 258 (35), 192 (90), 177 (95), 156 (99); HRMS calcd for $C_{26}H_{42}O_4$ (M^+) *m/z* 418.3083, found 418.3077.

Compound 3c. See Ref. 21.

Mixture of compounds 2c+3c. Following the procedure above described for **1c**, 150 mg (0.49 mmol) of (*rac*)-iso-

copalic acid (**2a**/**3a**) reacted with 225 mg (1.70 mmol) of (−)-2,3-*O*-isopropylidene-*sn*-glycerol to give 172 mg (83%) of an unseparable diastereoisomeric mixture of acetonides **2c**+**3c**.

Compound 4c. According to the procedure above reported for **1c**, 160 mg (0.52 mmol) of 3,20-$^{13}C_2$-*E*,*E*,*E*-geranylgeranoic acid (**4a**) were treated with 248.5 mg (1.88 mmol) of (−)-2,3-*O*-isopropylidene-*sn*-glycerol to give 190.7 mg (87%) of acetonide **4c**: colorless oil; IR: ν_{max} (liquid film) 1714, 1197 cm^{-1}; ^{1}H NMR (400 MHz, selected values) δ_H: 1.37 (3H, s, H_3-acetonide), 1.43 (3H, s, H_3-acetonide), 1.59 (9H, bs, H_3-16, H_3-18 and H_3-19), 1.67 (3H, bs, H_3-17), 2.16 (3H, dd, J_{CH}=134 and 6 Hz, H_3-20), 3.75 (1H, dd, *J*=8.2 and 6.3 Hz, H-3′a), 4.11–4.07 (2H, m, H-1′a and H-3′b), 4.18 (1H, dd, *J*=11.5 and 4.7 Hz, H-1′b), 4.33 (1H, m, H-2′), 5.09 (3H, m, H-6, H-10 and H-14), 5.71 (1H, d, J_{CH}=8 Hz, H-2); ^{13}C NMR (100 MHz) δ_C: 166.4 (C-1), 161.2 (apparent d, *J*=40 Hz, C*-3), 136.2 (C-7), 135.0 (C-11), 131.2 (C-15), 124.3 (C-14), 124.0 (C-10), 122.8 (C-6), 114.4 (dd, *J*=109 and 72 Hz, C-2), 109.7 [$(O)_2C(CH_3)_2$], 73.8 (C-2′), 66.5 (C-1′), 63.9 (C-3′), 41.0 (d, *J*=39 Hz, C-4), 39.7 (2C, C-8 and C-12), 26.7 (C-13 or C-9), 26.6 (C-9 or C-13), 25.9 (C-5), 25.6 (C-17), 25.4 (Me-acetonide), 25.2 (Me-acetonide), 18.9 (apparent d, *J*=40 Hz, C*-20), 17.7 (C-16), 16.0 (2C, C-18 and C-19); MS, *m*/*z* (%): 420 (M$^+$, 7), 405 (78), 362 (27), 293 (38), 239 (12), 225 (43), 200 (40), 171 (35), 158 (100), 136 (91); HRMS calcd for $C_{24}{}^{13}C_2H_{42}O_4$ (M$^+$) *m*/*z* 420.3150, found 420.3113.

Preparation of diols 1d–4d

Compound 1d. In a typical procedure, compound **1c** (83.7 mg, 0.20 mmol) was dissolved in 0.4 ml of MeOH and 0.006 M H_2SO_4/MeOH (3.7 ml) was added at room temperature in Ar atmosphere. The mixture was stirred at room temperature for 4 h. The usual work up gave 77.2 mg of a crude residue, which was purified on a silica gel column (petr. ether–Et_2O, 1:2) to give 70.8 mg (94%) of diol **1d**: colorless oil; $[\alpha]_D$=−9.1° (*c* 0.91, $CHCl_3$); IR: ν_{max} 3410, 1711, 1390, 1224, 1150, 1050, 890, 850 cm^{-1}; ^{1}H NMR (300 MHz, selected values) δ_H: 0.68 (3H, s, H_3-20), 0.80 (3H, s, H_3-18), 0.87 (3H, s, H_3-19), 2.16 (3H, bs, H_3-16), 3.66 (2H, m, H_2-3′), 3.94 (1H, m, H-2′), 4.20 (2H, m, H_2-1′), 4.48 (1H, bs, H-17a) and 4.84 (1H, bs, H-17b), 5.68 (1H, bs, 1H, H-14); ^{13}C NMR (75 MHz) δ_C: 167.2 (C-15), 163.2 (C-13), 148.3 (C-8), 114.3 (C-14), 106.3 (C-17), 70.4 (C-2′), 64.5 (C-1′), 63.4 (C-3′), 56.2 (C-9), 55.5 (C-5), 42.1 (C-3), 40.0 (C-12), 39.7 (C-10), 39.1 (C-1), 38.3 (C-7), 33.6 (2C, C-4 and C-19), 24.4 (C-6), 21.7 (C-18), 21.5 (C-11), 19.4 (C-2), 19.2 (C-16), 14.5 (C-20); MS, *m*/*z* (%): 378 (M$^+$, 5), 363 (36), 347 (7), 305 (12), 286 (21), 271 (53), 258 (54), 244 (92), 174 (87), 137 (100); HRMS calcd for $C_{23}H_{38}O_4$ (M$^+$) *m*/*z* 378.2770, found 378.2778.

Compound 2d. According to the procedure above described for **1d**, 21 mg (0.05 mmol) of acetonide **2c** afforded, after deprotection by 0.006 M H_2SO_4/MeOH (0.9 ml), 15.5 mg (82%) of diol **2d**: colorless crystals, mp 132–134°C (from Et_2O–petr. ether); $[\alpha]_D$=+7.6° (*c* 0.36, $CHCl_3$);‡ IR: ν_{max} (liquid film) 3421, 1730, 1636, 1390, 1166, 1050 cm^{-1}; ^{1}H NMR (400 MHz, selected values) δ_H: 0.81 (s, 3H, H_3-18), 0.86 (s, 3H, H_3-19), 0.90 (s, 3H, H_3-20), 0.94 (s, 3H, H_3-17), 1.60 (s, 3H, H_3-16), 2.95 (1H, bs, H-14), 3.60–3.72 (2H, m, H_2-3′), 3.94 (1H, bs, H-2′), 4.13-4.26 (2H, m, H_2-1′), 5.53 (1H, bs, H-12); ^{13}C NMR (100 MHz) δ_C: 173.5 (C-15), 128.4 (C-13), 124.4 (C-12), 70.3 (C-2′), 65.1 (C-3′), 63.5 (C-1′), 62.5 (C-14), 56.4 (C-5), 54.2 (C-9), 41.8 (2C, C-3 and C-7), 39.8 (C-1), 37.4 (C-8), 36.6 (C-10), 33.4 (C-19), 33.1 (C-4), 22.6 (C-11), 21.6 (C-18), 21.2 (C-16), 18.6 (C-2 or C-6), 18.5 (C-6 or C-2), 15.7 (C-20), 15.6 (C-17); MS, *m*/*z* (%): 378 (M$^+$, 5), 360 (10), 345 (5), 286 (27), 258 (18), 192 (85), 177 (100), 149 (63), 12; HRMS calcd for $C_{23}H_{38}O_4$ (M$^+$) *m*/*z* 378.2770, found 378.2761.

Compound 3d. See Ref. 21.

Mixture of compounds 2d+3d. Following the procedure reported for **1d**, 100 mg (0.24 mmol) of the mixture of acetonides **2c**+**3c** afforded, after deprotection by 0.006 M H_2SO_4/MeOH (4.3 ml), 74 mg (83%) of an unseparable diastereoisomeric mixture of diols **2d**+**3d**.

Compound 4d. According to the procedure above reported for **1d**, 140 mg (0.33 mmol) of acetonide **4c** afforded, after deprotection by 0.006 M H_2SO_4/MeOH (6.0 ml), 102 mg (81%) of diol **4d**: colorless oil; IR: ν_{max} (liquid film) 1707, 1197 cm^{-1}; ^{1}H NMR (400 MHz, selected values) δ_H: 1.59 (6H, bs, H_3-16 and H_3-18), 1.60 (3H, bs, H_3-19), 1.67 (3H, bs, H_3-17), 2.16 (3H, dd, J_{CH}=133 and 6 Hz, H_3-20), 3.59–3.71 (2H, m, H_2-3′), 3.94 (1H, m, H-2′), 4.17 (1H, dd, *J*=6.1 and 11.7 Hz, H-1′a), 4.22 (1H, dd, *J*=4.8 and 11.7 Hz, H-1′b), 5.09 (3H, m, H-6, H-10 and H-14), 5.70 (1H, d, J_{CH}=7.9 Hz, H-2); ^{13}C NMR (100 MHz) δ_C: 167.1 (C-1), 162.0 (apparent d, *J*=40 Hz, C*-3), 136.3 (C-7), 135.1 (C-11), 131.3 (C-15), 124.3 (C-14), 124.0 (C-10), 122.7 (C-6), 114.1 (dd, *J*=100 and 72 Hz, C-2), 70.4 (C-2′), 64.5 (C-1′or C-3′), 63.3 (C-3′or C-1′), 41.0 (d, *J*=39 Hz, C-4), 39.7 (2C, C-8 and C-12), 26.7 (C-13 or C-9), 26.6 (C-9 or C-13), 25.9 (C-5), 25.7 (C-17), 19.0 (apparent d, *J*=40 Hz, C*-20), 17.6 (C-16), 16.0 (2C, C-18 and C-19); MS, *m*/*z* (%): 380 (M$^+$, 5), 288 (10), 245 (8), 219 (11), 205 (18), 191 (21), 176 (38), 136 (90), 123 (92), 81 (100); HRMS calcd for $C_{21}{}^{13}C_2H_{38}O_4$ (M$^+$) *m*/*z* 380.2837, found 380.2847.

Preparation of 1,3 diacyl derivatives 1e–4e

Selective acetylation of diol 1d. In a typical procedure 1,8-diazabicyclo-[5.4.0]-undec-7-ene (DBU, 3.1 mg, 0.02 mmol) and *N*-acetylimidazole (9.6 mg, 0.09 mmol) were added to a solution of diol **1d** (30.3 mg, 0.08 mmol) in dry C_6H_6 (0.5 ml). The mixture was stirred under Ar atmosphere at room temperature for 1 h, then the reaction was stopped by adding 1 ml of H_2O. After usual work-up the solvent was removed in vacuo and the residue (30.1 mg) was

‡ $[\alpha]_D$ of compound **2d** was obviously opposite in sign to that of compound *ent*-**2d** (Ref. 21), but surprisingly differed also in its absolute value. This apparent conflictual result is due to the wrong report in Ref. 21 of the $[\alpha]_D$ of compound *ent*-**2d**: $[\alpha]_D$=−5.2 (*c* 0.25, $CHCl_3$), reported value −51.8 (*c* 0.25, $CHCl_3$).

chromatographed on SiO_2 column by elution with petr. ether/Et_2O gradient giving, in order of increasing polarity, 6.9 mg (22%) of diacetate **1f**, 18.7 mg (64%) of 1,3-diacylglycerol **1e**, which showed spectral data identical with those of natural verrucosin-5,[14] and 4.2 mg (14%) of starting diol **1d**.

1f. Colorless oil; $[\alpha]_D=-15.2°$ (*c* 0.41, $CHCl_3$); IR: ν_{max} (liquid film) 1750, 1710, 1646, 1373, 1223, 1142, 1058, 888 cm^{-1}; ^1H NMR (300 MHz) δ_H: 0.69 (3H, s, H_3-20), 0.80 (3H, s, H_3-18), 0.87 (3H, s, H_3-19), 2.07 (3H, s, OAc), 2.09 (3H, s, OAc), 2.15 (3H, s, H_3-16), 4.10-4.35 (4H, m, H_2-3′ and H_2-1′), 4.49 (1H, bs,H-17a), 4.85 (1H, bs,H-17b), 5.28 (1H, m, H-2′), 5.64 (1H, bs, H-14); MS, *m*/*z* (%): 462 (M^+, 2), 447 (4), 387 (3), 305 (3), 286 (35), 271 (49), 258 (62), 198 (92), 159 (100), 137 (98).

1e. Colorless oil; $[\alpha]_D=-15.9°$ (*c* 0.27, $CHCl_3$); {$[\alpha]_D$ natural **1e** −12.6° (*c* 0.26; $CHCl_3$)}[14]; IR: ν_{max} (liquid film) 3421, 1733, 1722, 1637, 1228, 1150, 888 cm^{-1}; ^1H NMR (400 MHz, selected values) δ_H: 0.68 (3H, s, H_3-20), 0.80 (3H, s, H_3-18), 0.87 (3H, s, H_3-19), 2.11 (3H, s, OAc), 2.17 (3H, d, *J*=0.8 Hz, H_3-16), 2.51 (1H, d, *J*=5.0 Hz, −OH), 4.12–4.22 (5H, m, H_2-1′, H-2′ and H_2-3′), 4.48 (1H, bs, H-17a), 4.84 (1H, bs, H-17b), 5.68 (1H, bs, H-14); ^{13}C NMR (100 MHz) δ_C: 166.8 (C-15), 163.0 (C-13), 148.3 (C-8), 114.3 (C-14), 68.5 (C-2′), 65.4 (C-3′), 64.5 (C-1′), 56.3 (C-9), 55.5 (C-5), 42.1 (C-3), 40.0 (C-12), 39.7 (C-10), 39.1 (C-1), 38.3 (C-7), 33.6 (C-4), 24.4 (C-6), 21.5 (C-11), 20.8 (OAc), 19.4 (C-2).

Selective acetylation of diol 2d. According to the procedure above reported for **1d**, 16 mg (0.04 mmol) of diol **2d** afforded, after treatment with 1.6 mg (0.01 mmol) of DBU and 5.0 mg (0.05 mmol) of *N*-acetylimidazole, 15.7 mg of crude reaction product, which was purified by SiO_2 chromatography (petr. ether–Et_2O gradient as eluent) to give, in order of increasing polarity, diacetate **2f** (3.3 mg, 20%), 1,3-diacylglycerol **2e** (9.9 mg, 65%), which showed spectral data identical with those of natural **2e**,[9] and starting diol **2d** (2.3 mg, 14%).

2f. Colorless oil; $[\alpha]_D=+54.5°$ (*c* 0.07, $CHCl_3$); IR: ν_{max} (liquid film) 1749, 1375, 1223 cm^{-1}; ^1H NMR (400 MHz, selected values) δ_H: 0.81 (3H, s, H_3-18), 0.86 (3H, s, H_3-19), 0.90 (3H, s, H_3-20), 0.93 (3H, s, H_3-17), 1.58 (3H, s, H_3-16), 2.08 (6H, s, 2OAc), 2.93 (1H, bs, H-14), 4.16–4.32 (4H, m, H_2-1′ and H_2-3′), 5.26 (1H, m, H-2′), 5.53 (1H, m, H-12); MS, *m*/*z* (%): 286 ($M^+-C_7H_{12}O_5$, 77), 271 (14), 258 (15), 243 (8), 192 (85), 177 (90), 159 (100), 123 (65), 109 (48); HRMS calcd for $C_{20}H_{30}O$ ($M^+-C_7H_{12}O_5$) *m*/*z* 286.2297, found 286.2289.

2e. Colorless oil; $[\alpha]_D=+13.9°$ (*c* 0.30, $CHCl_3$) {$[\alpha]_D$ natural **2e** +21.9° (*c* 0.22, $CHCl_3$)}[9]; IR: ν_{max} (liquid film) 3444, 1738, 1266, 1158 cm^{-1}; ^1H NMR (400 MHz, selected values) δ_H: 0.81 (3H, s, H_3-18), 0.86 (3H, s, H_3-19), 0.90 (3H, s, H_3-20), 0.96 (3H, s, H_3-17), 2.11 (3H, s, OAc), 2.96 (1H, bs, H-14), 4.12–4.24 (5H, m, H_2-1′, H-2′ and H_2-3′), 5.53 (1H, m, H-12).

Selective acetylation of diol 3d. According to the procedure above reported for **1d**, 34.8 mg (0.09 mmol) of diol **3d**[21] afforded after treatment with 3.6 mg (0.02 mmol) of DBU, and 11.0 mg (0.10 mmol) of *N*-acetylimidazole, 35.5 mg of crude reaction product, which was purified by SiO_2 chromatography (petr. ether–Et_2O gradient as eluent) to give, in order of increasing polarity, diacetate **3f** (8.1 mg, 21%), 1,3-diacylglycerol **3e** (22.8 mg, 67%), which showed spectral data identical with those of natural **3e**,[6] and starting diol **3d** (4.1 mg, 12%).

3f. Colorless oil; $[\alpha]_D=-42.0°$ (*c* 0.13, $CHCl_3$); IR: ν_{max} (liquid film) 1749, 1375, 1224 cm^{-1}; ^1H NMR (400 MHz, selected values) δ_H: 0.81 (3H, s, H_3-18), 0.87 (3H, s, H_3-19), 0.90 (3H, s, H_3-20), 0.93 (3H, s, H_3-17), 1.58 (3H, bs, H_3-16), 2.08 (6H, s, 2OAc), 2.93 (1H, bs, H-14), 4.16–4.34 (4H, m, H_2-1′ and H_2-3′), 5.26 (1H, m, H-2′), 5.53 (1H, m, H-12); MS, *m*/*z* (%): 286 ($M^+-C_7H_{12}O_5$, 83), 271 (23), 258 (25), 243 (9), 192 (79), 177 (89), 159 (100), 149 (39), 123 (76); HRMS calcd for $C_{20}H_{30}O$ ($M^+-C_7H_{12}O_5$) *m*/*z* 286.2297, found 286.2306.

3e. Colorless crystals, mp 116.5–119°C (from Et_2O–petr. ether) lit.[6] mp 117–119°C (hexane–Et_2O); $[\alpha]_D=-47.7°$ (*c* 0.21; $CHCl_3$) {$[\alpha]_D$ natural **3e** −53.7° (*c* 0.13, $CHCl_3$)};[6] IR: ν_{max} (liquid film) 1738, 1266, 1158 cm^{-1}; ^1H NMR (300 MHz, selected values) δ_H: 0.82 (3H, s, H_3-18), 0.87 (3H, s, H_3-19), 0.91 (3H, s, H_3-20), 0.95 (3H, s, H_3-17), 1.57 (3H, s, H_3-16), 2.11 (3H, s, OAc), 2.96 (1H, bs, H-14), 4.09–4.19 (5H, m, H_2-1′, H-2′ and H_2-3′), 5.53 (1H, m, H-12).

Selective acetylation of diol 4d. Following the procedure described for **1d**, 70 mg (0.18 mmol) of diol **4d** afforded, after treatment with 7.0 mg (0.05 mmol) of DBU, and 21.9 mg (0.20 mmol) of *N*-acetylimidazole, 68.5 mg of crude reaction product, which was purified by SiO_2 chromatography (petr. ether–Et_2O gradient as eluent) to give, in order of increasing polarity, diacetate **4f** (12 mg, 16%), 1,3-diacylglycerol **4e** (43.4 mg, 65%), and starting diol **4d** (10.1 mg, 14%).

4f. Colorless oil; IR: ν_{max} (liquid film) 1745 (broad), 1220 cm^{-1}; ^1H NMR (400 MHz, selected values) δ_H: 1.59 (6H, bs, H_3-16 and H_3-18), 1.60 (3H, bs, H_3-19), 1.67 (3H, bs, H_3-17), 2.07 (3H, s, OAc), 2.09 (3H, s, OAc), 2.15 (3H, dd, J_{CH}=118 and 6 Hz, H_3-20), 4.14–4.21 (2H, m, H-1′a and H-3′a), 4.27–4.33 (2H, m, H-1′b and H-3′b), 5.09 (3H, m, H-6, H-10 and H-14), 5.27 (1H, m, H-2′), 5.68 (1H, d, J_{CH}=7.5 Hz, H-2); ^{13}C NMR (100 MHz) δ_C: 170.5 (CH_3CO), 170.1 (CH_3CO), 166.0 (C-1), 161.6 (apparent d, *J*=40 Hz, C*-3), 136.3 (C-7), 135.0 (C-11), 131.2 (C-15), 124.3 (C-14), 124.0 (C-10), 122.7 (C-6), 114.3 (dd, *J*=73.5 and 73.9 Hz, C-2), 69.3 (C-2′), 62.4 (C-3′ or C-1′), 61.3 (C-1′ or C-3′), 41.0 (d, *J*=39 Hz, C-4), 39.7 (2C, C-8 and C-12), 26.7 (C-13 or C-9), 26.6 (C-9 or C-13), 26.0 (C-5), 25.7 (C-17), 20.9 (CH_3CO), 20.7 (CH_3CO), 19.0 (apparent d, *J*=40 Hz, C*-20), 17.6 (C-16), 16.0 (2C, C-18 and C-19); MS, *m*/*z* (%): 464 (M^+, 8), 288 (16), 245 (10), 219 (15), 200 (28), 177 (32), 159 (100), 149 (45), 136 (98), 95 (92); HRMS calcd for $C_{25}{}^{13}C_2H_{42}O_6$ (M^+) *m*/*z* 464.3048, found 464.3067.

4e. Colorless oil; IR: ν_{max} (liquid film) 1738, 1714, 1259, 1197 cm^{-1}; ^1H NMR (300 MHz, selected values) δ_H: 1.59 (6H, bs, H_3-16 and H_3-18), 1.60 (3H, bs, H_3-19), 1.67 (3H,

bs, H_3-17), 2.09 (3H, s, OAc), 2.17 (3H, dd, overlapped, H_3-20), 4.10 (1H, m, H-2′), 4.10–4.20 (4H, m, H_2-1′ and H_2-3′), 5.09 (3H, m, H-6, H-10 and H-14), 5.70 (1H, d, J_{CH}=7.9 Hz, H-2); ^{13}C NMR (75 MHz) δ_C: 171.0 (CH_3CO), 166.7 (C-1), 161.9 (apparent d, J=40 Hz, C*-3), 136.3 (C-7 or C-11), 135.0 (C-11 or C-7), 131.2 (C-15), 124.3 (C-14), 124.0 (C-10), 122.69 (C-6), 114.6 (d, J=73 Hz, C-2), 68.4 (C-2′), 65.3 (C-3′), 64.5 (C-1′), 41.9 (d, J=39 Hz, C-4), 39.7 (C-12 or C-8), 39.6 (C-8 or C-12), 26.7 (C-9 or C-13), 26.6 (C-13 or C-9), 25.9 (C-5), 25.7 (C-16), 20.8 (CH_3CO), 19.0 (apparent d, J=40 Hz, C*-20), 17.6 (C-17), 16.0 (2C, C-18 and C-19); MS, m/z (%): 422 (M^+, 6), 288 (12), 245 (8), 219 (12), 205 (32), 200 (21), 177 (23), 149 (48), 136 (85), 117 (100); HRMS calcd for $C_{23}{}^{13}C_2H_{40}O_5$ (M^+) m/z 422.2943, found 422.2958.

Preparation of 1,2 diacyl derivatives 1i–3i

Compound 1g. In a typical procedure, *t*-butyl-dimethylsilyl chloride (TBDMSCl, 16.0 mg, 0.11 mmol) was added to a solution of diol **1d** (35 mg, 0.09 mmol) in dry pyridine (1.5 ml), under Ar atmosphere. The reaction mixture was stirred at room temperature for 12 h. Then 15 ml of 10% aqueous solution of H_2SO_4 was added. Usual work-up gave a crude reaction product (39.6 mg), which was purified on SiO_2 column (petr. ether/Et_2O, 97:3 as eluent) to afford 37.5 mg (82%) of 1-acyl-3-(*t*-butyldimethylsilyl)-glycerol **1g**: colorless oil; $[\alpha]_D$=−24.3° (*c* 0.07, $CHCl_3$); IR: ν_{max} (liquid film) 3460, 1722, 1645, 1212, 1142, 890 cm^{-1}; ^{1}H NMR (400 MHz, selected values) δ_H: 0.07 [6H, s, (CH_3)$_2$Si], 0.67 (3H, s, H_3-20), 0.79 (3H, s, H_3-18), 0.86 (3H, s, H_3-19), 0.90 [9H, s, C(CH_3)$_3$], 2.16 (3H, bs, H_3-16), 3.63 (1H, dd, J=5.6 and 10.1 Hz, H-3′a), 3.68 (1H, dd, J=4.7 and 10.1 Hz, H-3′b), 3.90 (1H, m, H-2′), 4.12 (1H, dd, J=6.1 and 11.5 Hz, H-1′a), 4.17 (1H, dd, J=4.7 and 11.5 Hz, H-1′b), 4.48 (1H, bs, H-17a), 4.84 (1H, d, J=0.6 Hz, H-17b), 5.68 (1H, bs, H-14); ^{13}C NMR (100 MHz) δ_C: 166.9 (C-15), 162.0 (C-13), 148.3 (C-8), 114.7 (C-14), 106.3 (C-17), 70.2 (C-2′), 64.5 (C-1′), 63.8 (C-3′), 56.2 (C-9), 55.5 (C-5), 42.1 (C-3), 39.9 (C-12), 39.7 (C-10), 39.1 (C-1), 38.3 (C-7), 33.6 (2C, C-4 and C-19), 25.9 [C(CH_3)$_3$], 24.4 (C-6), 21.7 (C-18), 21.5 (C-11), 19.4 (C-2), 19.0 (C-16), 18.3 [C(CH_3)$_3$], 14.5 (C-20), −5.5 [Si(CH_3)$_2$]; MS, m/z (%): 435 (M^+−C_4H_9, 12), 361 (7), 347 (7), 288 (30), 287 (98), 271 (62), 259 (90), 177 (61), 69 (100).

Compound 1h. A solution of 28.1 mg (0.06 mmol) of *t*-butyldimethylsilylglyceryl ester **1g** in 1.5 ml of dry pyridine was treated with 0.06 ml of Ac_2O. The reaction mixture was stirred at room temperature for 12 h. After usual work-up, the crude reaction product (31.2 mg) was chromatographed by SiO_2 column (petr. ether/Et_2O, 99:1) to give 28.1 mg (92%) of 1,2-diacyl-3-(*t*-butyldimethylsilyl)-glycerol **1h**: colorless oil; $[\alpha]_D$=−8.5° (*c* 0.07, $CHCl_3$); IR: ν_{max} (liquid film) 1745, 1722, 1637, 1235, 890, 842 cm^{-1}; ^{1}H NMR (400 MHz, selected values) δ_H: 0.05 [6H, s, (CH_3)$_2$Si], 0.68 (3H, s, H_3-20), 0.79 (3H, s, H_3-18), 0.86 (3H, s, H_3-19), 0.88 [9H, s, C(CH_3)$_3$], 2.07 (3H, s, OAc), 2.14 (3H, d, J=1.2 Hz, H_3-16), 3.74 (1H, d, J=5.2 Hz, H_2-3′), 4.20 (1H, dd, J=6.0 and 12.0 Hz, H-1′a), 4.32 (1H, dd, J=3.9 and 12.0 Hz, H-1′b), 4.48 (1H, bs, H-17a), 4.84 (1H, d, J=1.2 Hz, H-17b), 5.08 (1H, m, H-2′), 5.64 (1H, bs, H-14); ^{13}C NMR (100 MHz) δ_C: 170.4 (CH_3CO), 166.4 (C-15), 161.8 (C-13), 148.3 (C-8), 114.8 (C-14), 106.3 (C-17), 72.2 (C-2′), 61.8 (C-1′), 61.5 (C-3′), 56.2 (C-9), 55.5 (C-5), 42.1 (C-3), 39.9 (C-12), 39.7 (C-10), 39.1 (C-1), 38.3 (C-7), 33.6 (2C, C-4 and C-19), 25.9 [C(CH_3)$_3$], 24.4 (C-6), 21.7 (C-18), 21.5 (C-11), 21.1 (CH_3CO), 19.4 (C-2), 19.0 (C-16), 18.2 [C(CH_3)$_3$], 14.5 (C-20), −5.5 [Si(CH_3)$_2$]; MS, m/z (%): 477 (M^+−C_4H_9, 45), 361 (23), 287 (43), 271 (62), 231 (92), 191 (35), 177 (76), 117 (100), 95 (88).

Compound 1i. See Ref. 22.

Compound 2g. Following the procedure above reported for **1g**, diol **2d** (37.1 mg, 0.10 mmol) reacted with TBDMSCl (17 mg, 0.11 mmol) to afford 42.0 mg of crude reaction product, which, after chromatographic purification (SiO_2, petr. ether/Et_2O, 97:3), gave 39.4 mg (82%) of 1-acyl-3-(*t*-butyldimethylsilyl)-glycerol **2g**: colorless oil; $[\alpha]_D$=+56.3° (*c* 0.05, $CHCl_3$); IR: ν_{max} (liquid film) 3460, 1745, 1251, 1166, 1112 cm^{-1}; ^{1}H NMR (300 MHz, selected values) δ_H: 0.08 [6H, s, (CH_3)$_2$Si], 0.81 (3H, s, H_3-18), 0.86 (3H, s, H_3-19), 0.90 [12H, s, C(CH_3)$_3$ and H_3-20], 0.95 (3H, s, H_3-17), 1.61 (3H, bs, H_3-16), 2.96 (1H, bs, H-14), 3.60–3.71 (2H, m, H_2-3′), 3.90 (1H, m, H-2′), 4.10–4.21 (2H, m, H_2-1′), 5.52 (1H, m, H-12); ^{13}C NMR (75 MHz) δ_C: 173.1 (C-15), 128.8 (C-13), 124.1 (C-12), 70.1 (C-2′), 64.7 (C-3′), 63.9 (C-1′), 62.6 (C-14), 56.4 (C-5), 54.3 (C-9), 41.8 (2C, C-3 and C-7), 39.9 (C-1), 37.4 (C-8), 36.6 (C-10), 33.4 (C-19), 33.2 (C-4), 25.8 [C(CH_3)$_3$], 22.7 (C-11), 21.4 (C-18), 21.2 (C-16), 18.6 (C-2), 18.5 (2C, C-6 and [C(CH_3)$_3$], 15.7 (C-17), 15.5 (C-20), -5.45 [Si(CH_3)$_2$]; MS, m/z (%): 435 (M^+−C_4H_9, 8), 361 (6), 287 (83), 260 (77), 259 (99), 243 (21), 191 (76), 177 (97), 135 (98), 109 (100).

Compound 2h. A solution of 26 mg (0.05 mmol) of *t*-butyldimethylsilylglyceryl ester **2g** in 1.5 ml of dry pyridine was treated with 0.05 ml of Ac_2O. The reaction mixture was stirred at room temperature for 12 h. After usual work-up, the crude reaction product (28.3 mg) was chromatographed by SiO_2 column (petr. ether/Et_2O, 99:1) to give 25.8 mg (91%) of 1,2-diacyl-3-(*t*-butyldimethylsilyl)-glycerol **2h**: colorless oil; $[\alpha]_D$=+1.1° (*c* 0.13, $CHCl_3$); IR: ν_{max} (liquid film) 1745, 1637, 1390, 1236 cm^{-1}; ^{1}H NMR (400 MHz, selected values) δ_H: 0.05 [6H, s, (CH_3)$_2$Si], 0.81 (3H, s, H_3-18), 0.86 (3H, s, H_3-19), 0.88 [9H, s, C(CH_3)$_3$], 0.91 (3H, s, H_3-20), 0.93 (3H, s, H_3-17), 1.59 (3H, bs, H_3-16), 2.03 (3H, s, OAc), 2.94 (1H, bs, H-14), 3.71–3.74 (2H, m, H_2-3′), 4.11–4.35 (2H, m, H_2-1′), 5.10 (1H, m, H-2′), 5.51 (1H, m, H-12); MS, m/z (%): 477 (M^+−C_4H_9, 70), 361 (23), 287 (25), 259 (30), 243 (12), 231 (21), 191 (30), 177 (30), 131 (42), 117 (100).

Compound 2i. See Ref. 22.

Compound 3g. According to the procedure described for **1g**, diol **3d** (46.9 mg, 0.12 mmol) reacted with TBDMSCl (21.4 mg, 0.14 mmol) to obtain 55.6 mg of crude reaction product, which was purified (SiO_2, petr. ether/Et_2O, 97:3), giving 52.3 mg (86%) of 1-acyl-3-(*t*-butyldimethylsilyl)-glycerol **3g**: oil; $[\alpha]_D$=−31.7° (*c* 0.25, $CHCl_3$); IR: ν_{max} (liquid film) 3475, 1735, 1251, 1112, 1019 cm^{-1}; ^{1}H NMR (400 MHz, selected values) δ_H: 0.08 [6H, s, (CH_3)$_2$Si], 0.81 (3H, s, H_3-18), 0.86 (3H, s, H_3-19), 0.90

[12H, s, C(CH_3)$_3$ and H_3-20], 0.94 (3H, s, H_3-17), 1.61 (3H, bs, H_3-16), 2.95 (1H, bs, H-14), 3.62–3.71 (2H, m, H_2-3′), 3.87 (1H, m, H-2′), 4.12–4.19 (2H, m, H_2-1′), 5.52 (1H, m, H-12); ^{13}C NMR (100 MHz) δ_C: 174.0 (C-15), 128.8 (C-13), 124.1 (C-12), 70.1 (C-2′), 64.7 (C-3′), 63.9 (C-1′), 62.6 (C-14), 56.5 (C-5), 54.3 (C-9), 42.1 (C-7), 41.8 (C-3), 39.9 (C-1), 37.4 (C-8), 36.6 (C-10), 33.4 (C-19), 33.2 (C-4), 25.8 [C(CH_3)$_3$], 22.7 (C-11), 21.7 (C-18), 21.2 (C-16), 18.6 (C-2), 18.5 (C-6), 18.3 [C(CH$_3$)$_3$], 15.7 (C-17), 15.5 (C-20), −5.5 [Si(CH_3)$_2$]; MS, *m*/*z* (%): 435 ($M^+-C_4H_9$, 7), 361 (4), 287 (91), 259 (100), 243 (15), 191 (29), 177 (68), 163 (70), 135 (75), 109 (95).

Compound 3h. A solution of 35.0 mg (0.07 mmol) of *t*-butyldimethylsilylglyceryl ester **3g** in 1.5 ml of dry pyridine was treated with 0.04 ml of Ac_2O. The reaction mixture was stirred at room temperature for 12 h. After usual work-up, the crude reaction product (37.5 mg) was chromatographed by SiO_2 column (petr. ether/Et_2O, 99:1) to give 35.1 mg (92%) of 1,2-diacyl-3-(*t*-butyldimethylsilyl)-glycerol **3h**: colorless oil; $[\alpha]_D=-27.0°$ (*c* 0.12, $CHCl_3$); IR: ν_{max} (liquid film) 1745, 1382, 1236 cm^{-1}; ^{1}H NMR (400 MHz, selected values) δ_H: 0.05 [6H, s, (CH_3)$_2$Si], 0.81 (3H, s, H_3-18), 0.86 (3H, s, H_3-19), 0.88 [9H, s, C(CH_3)$_3$], 0.90 (3H, s, H_3-20), 0.93 (3H, s, H_3-17), 1.59 (3H, bs, H_3-16), 2.03 (3H, s, OAc), 2.92 (1H, bs, H-14), 3.74 (2H, d, J=5.4 Hz, H_2-3′), 4.17 (1H, dd, J=5.5 and 11.9 Hz, H-1′), 4.37 (1H, dd, J=3.7 and 11.9 Hz, H-1′), 5.05 (1H, m, H-2′), 5.51 (1H, m, H-12); MS, *m*/*z* (%): 478 ($M^+-C_4H_8$, 100), 435 (6), 361 (43), 287 (68), 259 (51), 231 (22), 191 (31), 177 (52), 117 (99), 109 (62).

Compound 3i. Using a procedure already reported in the literature for synthesis of **1i** and **2i**,[22] 1,2-diacyl-*sn*-*t*-butyldimethylsilylglyceryl ester **3h** (21 mg, 0.04 mmol) in dry acetone (4.5 ml) was treated with 11.1 mg (0.04 mmol) of $PdCl_2(CH_3CN)_2$. The reaction mixture was stirred at room temperature for 1 h and then 7 ml of H_2O were added. The usual work up gave a residue (16.7 mg) which was purified by SiO_2 column chromatography (petr. ether/Et_2O gradient) affording, in order of increasing polarity, starting ester **3h** (1.1 mg, 5%), and 1,2-diacylglycerol **3i** (13.5 mg, 86%), which showed spectral data (MS, IR, ^{1}H and ^{13}C NMR) identical with those of natural **3i**.[6]

3i. Colorless crystals, mp 75–76°C (from petr. ether); $[\alpha]_D=-41.8°$ (*c* 0.57, $CHCl_3$) {$[\alpha]_D$ natural **3i** −33.0° (*c* 0.83; $CHCl_3$)};[6] IR: ν_{max} (liquid film) 3482, 1740, 1238, 1161, 1050 cm^{-1}; ^{1}H NMR (500 MHz) δ_H: 0.82 (3H, s, H_3-18), 0.86 (3H, s, H_3-19), 0.91 (s, 3H, H_3-20), 0.94 (3H, s, H_3-17), 1.60 (3H, s, H_3-16), 2.10 (3H, s, OAc), 2.94 (1H, bs, H-14), 3.76 (d, J=5.5 Hz, 2H, H_2-3′), 4.27 (1H, dd, J=5.1 and 12.1 Hz, H-1′a) and 4.34 (1H, dd, J=4.4 and 12.1 Hz, H-1′b), 5.07 (1H, m, H-2′), 5.53 (1H, m, H-12).

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