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Selective Transformations of the Ca^{2+} Pump Inhibitor Thapsigargin

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The importance of the sesquiterpene lactone thapsigargin as a tool for studying Ca^{2+} homeostasis has created a need for structure–activity relationship studies and consequently for procedures for selective transformations of the functional groups in the molecule. Methods for the selective inversion of configuration at C-3 and C-8, selective acetylation, and selective cleavage of the ester groups at O-3, O-8 and O-10 are presented.

Since the secondary messenger Ca^{2+} is crucially involved in the regulation of a number of cell processes ranging from growth and transformation-related gene expression[1] to muscle contraction,[2] regulation of the cytoplasmic Ca^{2+} concentration is of utmost importance for the cell.[3,4] The two families of Ca^{2+}–ATPases, the *p*lasma-*m*embrane *C*a^{2+}–*A*TPase family (PMCA),[5] and the *s*arco-*e*ndoplasmic *r*eticulum *C*a^{2+}–*A*TPase (SERCA) family, which is found in intracellular membranes, are the enzymes that perform the fine tuning of the cytoplasmic Ca^{2+} concentration.[6] Neither the exact mechanism of action of these Ca^{2+} pumps[7] nor the relationship between the intra- and extra-cellular Ca^{2+} pools and the signalling Ca^{2+} inside the cells are well understood and both are, at present, subject to intensive research. Owing to its high affinity and specificity for the SERCA family[8] thapsigargin (**1**), a sesquiterpene lactone isolated from the Mediterranean umbelliferous plant *Thapsia garganica* L,[9,10] has become a very interesting tool for the molecular characterization of the ATPase mechanism (for some recent reviews see Refs. 11 and 12). Attempts to characterize the binding of thapsigargin to the ATPase have indicated that the ligand stoichiometrically titrates the enzyme at nanomolar concentrations.[13,14] At present no evidence for the site of binding exists. It has been suggested that the protecting effects of a high Ca^{2+} concentration against the inhibitory effects of thapsigargin[15] might indicate juxtaposed binding sites for the two ligands. An approach for elucidating the binding site is to map the excluded and essential volumes of the enzyme by structure–activity relationship studies.[16] A comparison of these volumes together with knowledge of the enzyme topology[17] might reveal possible binding sites. Such studies, however, require analogues of thapsigargin. Structural modifications of this sesquiterpene are complicated by the fact that, although heavily substituted, the guaianolide nucleus possesses only two different reactive substituents: tertiary alcohols and esters. In this paper we present methods for selective modifications of some of the functionalities of thapsigargin.

Results and discussion

Inversion of the configuration at C-8. Treatment of thapsigargin (**1**) with a methanolic solution of triethylamine afforded cleavage of the butanoate ester to give the derivative **2** (Scheme 1).[18] Anchimeric assistance from the 11-hydroxy group may account for the lability of this ester group. Oxidation of the secondary alcohol **2** with chromic acid afforded the ketone **3**. Appropriate choice of reducing agents enabled selective formation of either **2** or the epimeric alcohol **4**. When **3** was reduced with sodium borohydride only **2** was formed in detectable amounts, whereas only **4** was detected in the reaction mixture upon treatment of **3** with sodium triacetoxyborohydride. This finding is analogous to the case of some veratrum alkaloids, in which the hydroxy group in the β-hydroxy ketone complexes with triacetoxyborohydride, and the stereochemistry of this complex determines the stereochemistry of the secondary alcohol formed.[19] The secondary alcohol **4** is converted into the epimeric thapsigargin isomer

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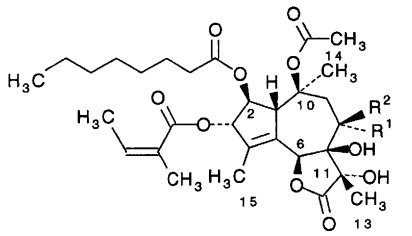


1 R^1= $OOC(CH_2)_2CH_3$, R^2= H

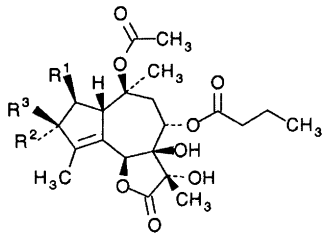
2 R^1= OH, R^2= H

3 R^1,R^2= O

4 R^1= H, R^2= OH

5 R^1= H, R^2= $OOC(CH_2)_2CH_3$

Scheme 1.



6 R^1= $OOC(CH_2)_6CH_3$, R^2= $OOCOCCH_3$, R^3= H

7 R^1= $OOC(CH_2)_6CH_3$, R^2= OH, R^3= H

8 R^1= $OOC(CH_2)_6CH_3$, R^2= H, R^3= OH

9 R^1= OH, R^2= H, R^3= $OOC(CH_2)_6CH_3$

10 R^1=R^3= $OOC(CH_2)_6CH_3$, R^2= H

11 R^1= $OOC(CH_2)_6CH_3$, R^2,R^3= O

12 R^1=R^2= $OOC(CH_2)_6CH_3$, R^3= H

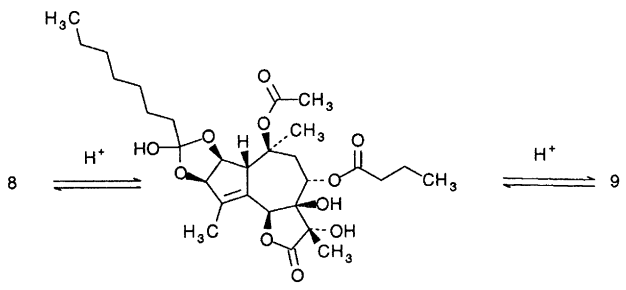
Scheme 2.

5 (neothapsigargin) upon treatment with butyric anhydride in the presence of 4-dimethylaminopyridine. The inverted stereochemistry at C-8 in **5** was confirmed by an analysis of the coupling pattern of the ABX-coupling system comprising H-8, H-9α, and H-9β. In **2** the two vicinal couplings both are approximately 3 Hz, indicating a torsion angle of about 60°, whereas the two couplings constant are 4.7 and 11.8 Hz, respectively, in the spectrum of **5**, in agreement with the assumption that one of the torsion angles approaches 180°.

Inversion of configuration at C-3. In contrast with the lability of the ester group at O-8, the α,β-unsaturated angelate ester at C-3 is the most solvolysis-resistant ester group of the molecule. In carbohydrate chemistry the sensitivity of double bond towards hydrazine has been used to advantage to convert α,β-unsaturated esters into 3-hydrazino derivatives, which, under mild conditions, can be converted into the corresponding pyrazolidin-3-ones with simultaneous cleavage of the ester bond.[20] Treatment of **1** with pyrazine yielded a complex mixture, probably due to the lability of the lactone towards this reagent. Instead oxidation of a toluene solution of **1** with potassium permanganate in the presence of a phase-transfer catalyst[21] afforded the pyruvate **6** (Scheme 2). Selective solvolysis of the pyruvate ester was quickly performed in a methanolic pyridine solution to give the secondary alcohol **7**. Treatment of **7** with trifluoromethanesulfonic anhydride afforded a reaction mixture containing two compounds in approximately equal amounts. Attempts to separate the compounds were complicated by an apparent interconversion of the two products. Anticipating that the interconversion proceeds through the ortho acid depicted in Scheme 3, the two products should possess the structures **8** and **9**. This hypothesis was confirmed by acylation of the mixture using octanoic anhydride as the reagent, which reaction afforded only one product, the dioctanoate **10**. Further confirmation of the structure of **10** was obtained by oxidation **7** with chromic acid to give **11**, which, upon reduction with sodium borohydride in the presence of cerium(III) chloride followed by acylation with octanoic anhydride, afforded a product identical with **10**. Attempts to invert the configuration of C-3 in **1** using the Mitsunobu approach[22] failed.

The dioctanoate **12** was prepared in order to have a reference compound for the evaluation of the enzyme inhibiting potency of **10**.

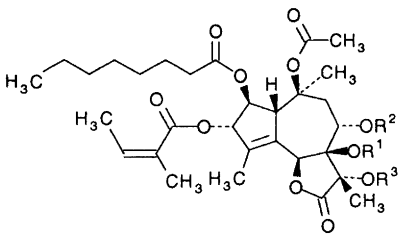
Selective acetylation of thapsigargin. Treatment of thapsigargin with acetic anhydride in the presence of 4-dimethylaminopyridine at room temperature led to acetylation at O-11 to give **13** (Scheme 4) and, to some extent, acetylation at O-11 as well as O-7. Selective acetylation of O-7 is possible taking advantage of the acetonide **14** formed by the acid-catalysed reaction between **2** and acetone. Acetylation of **14** with acetic anhydride afforded the acetate **15**, which upon acidic hydrolysis and butanoylation was converted into **17** via **16**.



Scheme 3.

Selective cleavage of the acetate ester of thapsigargin. Whereas treatment of thapsigargin with a methanolic triethylamine solution under mild conditions led to the debutanoylated derivative **2** treatment for prolonged time at increased temperature yielded a mixture in which the tri-deacylated compound **18** was a major product. Surprisingly no product corresponding to cleavage of only the two secondary esters at O-2 and O-8 were detectable. Acylation of the two secondary hydroxy groups in **18** took place at approximately the same rate making selective esterification of either of the two groups difficult. Instead the derivative **19** was prepared and advantage was taken of the lability of the O-8 ester for synthesizing the monooctanoate **20**. Selective butanoylation of the secondary alcohol group in **20** afforded deacetylthapsigargin **21** (Scheme 5).

Access to the epimeric thapsigargin analogues and the analogues in which reactive substituents have been selectively transformed now enables characterization of the binding site on the Ca^{2+}-ATPases through structure–activity relationships.



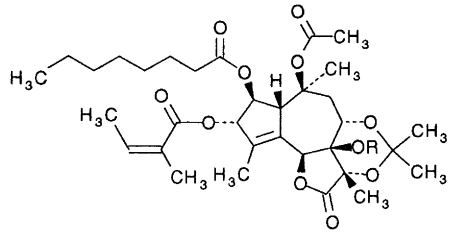
1 $R^1=R^3=$ H, R2= $OC(CH_2)_2CH_3$

2 $R^1=R^2=R^3=$ H

13 $R^1=$ H, $R^2=$ $OC(CH_2)_2CH_3$, $R^3=$ $OCCH_3$

16 $R^1=$ $OCCH_3$, $R^2=R^3=$ H

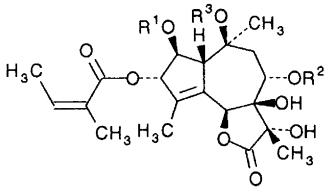
17 $R^1=$ $OCCH_3$, $R^2=$ $OC(CH_2)_2CH_3$, $R^3=$ H



14 R= H

15 R= $OCCH_3$

Scheme 4.



2 $R^1=$ $OC(CH_2)_6CH_3$, $R^2=$ H, $R^3=$ $OCCH_3$

18 $R^1=R^2=R^3=$ H

19 $R^1=R^2=$ $OC(CH_2)_6CH_3$, $R^3=$ H

20 $R^1=$ $OC(CH_2)_6CH_3$, $R^2=R^3=$ H

21 $R^1=$ $OC(CH_2)_6CH_3$, $R^2=$ $OC(CH)_2CH_3$, $R^3=$ H

Scheme 5.

Experimental

Column chromatography separations were performed using Merck SiO_2 60 (0.063–0.200 mm) or Merck SiO_2 60 (0.040–0.063 mm). Reversed-phase column chromatography was performed using Merck SiO_2 60 silanised (0.063–0.200 mm). Merck SiO_2 60 F254 precoated aluminium sheets were used for TLC and the spots were visualized by UV and by spraying with a 0.1% solution of naphthoresorcinol in 1 M sulfuric acid. Separations by preparative HPLC were performed using Waters 6000A pump, Shimadzu SPD 6A detector ($\lambda = 230$ nm) and LiChrosorb RP-18 column (5 μm, 16 × 240 mm, flow = 9.0 ml min^{-1}). Negative FAB-mass spectra were recorded on a JEOL JMS-AX 505 W instrument and HEDS was used as a matrix. NMR spectra were recorded with a Bruker AC 200, a Bruker AMX 400 or a Bruker AM 500 spectrometer using Me_4Si as an internal standard.

Neothapsigargin **5**. A solution of sodium triacetoxyborohydride was prepared by dissolving sodium borohydride (8.0 mg, 0.21 mmol) in glacial acetic acid and leaving the solution for 5 min. Ten μl of this solution were added to a solution of the ketone **3**[18] (10 mg, 17.3 μmol) in glacial acetic acid (0.5 ml) and the mixture was left for exactly 1.5 min at room temperature. Acetone (2 ml) and water (2 ml) were added. The residue was concentrated to half volume under vacuum and the residual mixture was partitioned between water (7 ml) and CH_2Cl_2 (7 ml). The aqueous phase was extracted with CH_2Cl_2 (7 ml). The combined CH_2Cl_2 phases were dried ($MgSO_4$) and concentrated under vacuum. The residue, 4-dimethylaminopyridine (50.1 mg, 0.41 mmol) and butyric anhydride (14 μl, 85.7 μmol) were dissolved in CH_2Cl_2 (6 ml). The reaction was stirred for 45 min at room temperature after which time 4 M HCl (5 ml) was added. The CH_2Cl_2 layer was dried ($MgSO_4$) and concentrated under vacuum to

give a gum from which **5** (3.5 mg, 5.4 μmol, 28.6%) was isolated by column chromatography (toluene–EtOAc 7:1). ^{1}H NMR ($CDCl_3$): the sesquiterpene skeleton: 5.71 (br s, 1 H, H-3), 5.46 (dd, 1 H, J = 3.4 Hz and 4.4 Hz, H-2), 5.33 (dd, 1 H, $J_{8,9'}$ = 4.7 Hz, $J_{8,9}$ = 11.8 Hz, H-8), 5.28 (br s, 1 H, H-6), 4.15 (br s, 1 H, H-1), 2.87 (t, 1 H, $J_{8,9} = J_{9,9'}$ = 12.6 Hz, H-9), 2.43 (dd, 1 H, $J_{8,9'}$ = 4.5 Hz, $J_{9,9'}$ = 12.6 Hz, H-9′), 1.84 (s, 3 H, H-15), 1.53 (s, 3 H, H-13), 1.36 (s, 3 H, H-14). ^{13}C NMR ($CDCl_3$): the sesquiterpene skeleton: 174.5 (C-12), 141.4 (C-4), 128.8 (C-5), 83.7 (C-3), 81.8 (C-11), 81.6 (C-7), 78.1 (C-6), 78.3 (C-2), 70.2 (C-8), 57.1 (C-1), 38.2 (C-9), 22.6 (C-14), 15.8 (C-13), 12.9 (C-15). The signals of the acyl groups were found as previously reported.[23] MS: 649 (100%, $M-1$).

Preparation of **7**. Thapsigargin (1.0 g, 1.54 mmol) was added in small portions to a stirred cooled mixture of toluene (90 ml), benzyl(triethyl)ammonium chloride (30.0 mg, 0.13 mmol), potassium permanganate (967.9 mg, 6.12 mmol) and water (180 ml) and the mixture was stirred for 7 h at room temperature under an argon atmosphere. The excess of permanganate was destroyed with sodium sulfite (1.1 g, 8.73 mmol). The reaction mixture was filtered and the filter was washed twice with toluene (20 ml) and water (20 ml). The filtrate was acidified with 2 M HCl (10 ml) and the aqueous phase was extracted with diethyl ether (3 × 100 ml). The combined organic phases were dried ($MgSO_4$) and concentrated under vacuum. The crude product was dissolved in CH_3OH (150 ml), pyridine (12 ml) and water (7.5 ml). The solution was refluxed for 7 h. The mixture was concentrated under vacuum and the residue purified by column chromatography (toluene–EtOAc 9:1 added 0.25% acetic acid, then toluene–EtOAc 8:1 added 0.25% acetic acid, toluene–EtOAc 5:1 added 1% acetic acid and toluene–EtOAc 3:1) to give **7** (402.4 mg, 0.708 mmol, 46%). ^{1}H NMR ($CDCl_3$): the sesquiterpene skeleton: 5.66 (m, 2 H, H-6 and H-8), 4.89 (t, 1 H, J = 2.8 Hz, H-2), 4.41 (br s, 1 H, H-3), 4.36 (br s, 1 H, H-1), 3.32 (dd, 1 H, $J_{8,9}$ = 3.4 Hz, $J_{9,9'}$ = 14.7 Hz, H-9), 2.20 (dd, 1 H, $J_{8,9}$ = 3.4 Hz, $J_{9,9'}$ = 14.8 Hz, H-9′), 1.92 (br s, 3 H, H-15), 1.46 (br s, 3 H, H-13), 1.38 (s, 3 H, H-14). ^{13}C NMR ($CDCl_3$): the sesquiterpene skeleton: 175.4 (C-12), 143.8 (C-4), 126.4 (C-5), 84.5 (C-10), 84.2 (C-3), 83.3 (C-6), 78.2 (C-7 and C-11), 76.8 (C-2), 65.8 (C-8), 54.9 (C-1), 37.8 (C-9), 22.9 (C-14), 15.8 (C-13), 12.5 (C-15). The signals of the acyl groups were found as previously reported.[23]

Preparation of compound **8** *and* **9** *via substitution.* A solution of **7** (32.9 mg, 57.9 μmol) and 4-dimethylaminopyridine (32.3 mg, 0.26 mmol) in dry CH_2Cl_2 (2.8 ml) was cooled to 0°C under dry argon, and trifluoromethanesulfonic anhydride (14 μl, 82.7 μmol) was added dropwise. After 5 h the solution was allowed to warm to room temperature. The reaction mixture was poured into an iced mixture of diethyl ether (10 ml) and 2 M HCl (5 ml). The aqueous phase was extracted with diethyl ether (3 × 10 ml). The organic layer was washed with 2 M HCl (5 ml). The combined organic phases were dried ($MgSO_4$) and concentrated under vacuum. Column chromatography of the residue (toluene–EtOAc 9:1 added 0.25% acetic acid) afforded a mixture of **8** and **9** (23.5 mg, 41.3 μmol, 21.9%). Analytical samples of **8** and **9** (21.5 mg) were obtained by preparative HPLC (CH_3OH–0.5% acetic acid 4:1). Because of interconversion between **8** and **9** ^{13}C NMR data are not available. ^{1}H NMR (CD_3OD) compound **8**: the sesquiterpene skeleton: 5.61 (br s, 1 H, H-6), 5.57 (t, 1 H, $J_{8,9} = J_{8,9'}$ = 3.5 Hz, H-8), 5.26 (dd, 1 H, J = 4.6 Hz, J = 7.0 Hz, H-2), 4.50 (br s, 1 H, H-3), 4.47 (br s, 1 H, H-1), 3.23 (dd, 1 H, $J_{8,9}$ = 3.5 Hz, $J_{9,9'}$ = 14.0 Hz, H-9), 2.19 (dd, 1 H, $J_{8,9}$ = 3.5 Hz, $J_{9,9'}$ = 14.0 Hz, H-9′), 1.93 (br s, 3 H, H-15), 1.33 (br s, 6 H, H-13 and H-14). Compound **9**: the sesquiterpene skeleton: 5.62 (br s, 1 H, H-6), 5.56 (t, 1 H, $J_{8,9'}$ = 3.5 Hz, H-8), 5.47 (d, 1 H, $J_{2,3}$ = 7.0 Hz, H-3), 4.38 (dd, 1 H, J = 4,6 Hz, $J_{2,3}$ = 7.0 Hz, H-2), 4.31 (br s, 1 H, H-1), 3.28 (dd, 1 H, $J_{8,9}$ = 3.5 Hz, H-9), 2.14 (dd, 1 H, $J_{8,9} = J_{8,9'}$ = 3.5 Hz, $J_{9,9'}$ = 14.0 Hz, H-9′), 1.87 (br s, 3 H, H-15), 1.33 (br s, 6 H, H-13 and H-14). The signals of the acyl groups were found as previously reported.[23]

Preparation of compound **8** *and* **9** *via reduction.* To a solution of **11** (30.4 mg, 53.6 μmol) in 0.4 M methanolic $CeCl_3$ (10 ml) was added sodium borohydride (5.0 mg, 0.13 mmol). The mixture was stirred for 1.5 h at room temperature and 1 M HCl (10 ml) was added. After concentration under vacuum to half volume the residue was extracted with diethyl ether (3 × 10 ml). The combined organic phases were dried ($MgSO_4$) and concentrated under vacuum. Column chromatography (toluene–EtOAc 7:1 added 0.25% acetic acid) afforded a mixture of **8** and **9** (12.2 mg, 40.1%). The ^{1}H NMR spectrum was identical with that of the product described above.

Preparation of **10**. To a solution of **8** and **9** (22.4 mg, 39.4 μmol) and 4-dimethylaminopyridine (22.8 mg, 0.19 mmol) in CH_2Cl_2 (2 ml) was added octanoic anhydride (60 μl, 0.20 mmol). The solution was stirred for 3 h at room temperature and CH_2Cl_2 (2 ml) and 1 M HCl (4 ml) was admixed. The organic layer was washed with 1 M Na_2CO_3 (4 ml) and 0.5 M HCl (4 ml) and dried ($MgSO_4$). The organic layer was concentrated under vacuum and chromatographed twice (CH_3OH–0.5% acetic acid 2:1 and 1.5:1) to give **10** (8.7 mg, 12.5 μmol, 31.8%). ^{1}H NMR ($CDCl_3$): the sesquiterpene skeleton: 5.69 (d, 1 H, $J_{2,3}$ = 6.2 Hz, H-3), 5.62 (t, 1 H, $J_{8,9} = J_{8,9'}$ = 3.6 Hz, H-8), 5.59 (d, 1 H, J = 1.4 Hz, H-6), 5.54 (dd, 1 H, J = 4.2 Hz, $J_{2,3}$ = 6.9 Hz, H-2), 4.41 (br s, 1 H, H-1), 3.10 (dd, 1 H, $J_{8,9}$ = 3.4 Hz, $J_{9,9'}$ = 14.8 Hz, H-9), 2.26 (m, 1 H, H-9′), 1.90 (s, 3 H, H-15), 1.48 (s, 3 H, H-13), 1.34 (s, 3 H, H-14). ^{13}C NMR ($CDCl_3$): the sesquiterpene skeleton: 175.6 (C-12), 142.1 (C-4), 130.8 (C-5), 84.7 (C-10), 78.6 (C-7), 77.7 (C-3), 77.2 (C-11), 77.0 (C-2), 70.3 (C-6), 66.1 (C-8), 57.9 (C-1), 22.8 (C-14), 16.2 (C-13), 13.7 (C-15). The octanoate group at C-3:

172.4 (C = O), 34.0, 31.8, 29.2, 24.9, 22.6, 14.1. The signals of the other acyl groups were found as previously reported.[23] MS: 693 (18%, $M-1$).

Preparation of the ketone **11**. To a solution of **7** (61.6 mg, 0.11 mmol) in acetone (12 ml) was added Jones R[24] (24 µl) three times: immediately, after 17 min and after 1 h. The mixture was stirred for 3.5 h at room temperature after the last addition. Isopropyl alcohol (4 ml) was added and the reaction mixture was stirred for 10 min before being filtered through a Celite pad. The pad was extracted with acetone and the filtrate was concentrated under vacuum. The crude product was recrystallized (CH_3OH–water) to give **11** (7.4 mg, 13.0 µmol, 12.0%). An additional amount of **11** (42.2 mg, 74.3 µmol, 69%) was obtained by column chromatography (toluene–EtOAc 4:1 added 1% acetic acid) of the residue of the mother liquor. ^{1}H NMR ($CDCl_3$): the sesquiterpene skeleton: 5.83 (s, 1 H, H-6), 5.68 (t, 1 H, $J_{8,9} = J_{8,9'} = 3.2$ Hz, H-8), 5.20 (d, 1 H, $J = 3.2$ Hz, H-2), 4.55 (br s, 1 H, H-1), 3.20 (dd, 1 H, $J_{8,9} = 3.4$ Hz, $J_{9,9'} = 15.1$ Hz, H-9), 2.31 (m, H-9 hidden under signals from the α protons of the acyl groups), 1.99 (br s, 3 H, H-15), 1.51 (s, 3 H, H-13), 1.38 (s, 3 H, H-14). ^{13}C NMR ($CDCl_3$): the sesquiterpene skeleton: 198.5 (C-3), 174.5 (C-12), 156.2 (C-4), 142.2 (C-5), 83.8 (C-10), 79.0 (C-11), 78.5 (C-6), 77.8 (C-7), 73.0 (C-2), 66.1 (C-8), 51.8 (C-1), 38.7 (C-9), 22.5 (C-14), 16.2 (C-13), 10.2 (C-15). The signals of the acyl groups were found as previously reported.[23] MS: 565 (25%, $M-1$).

Preparation of **12**. Compound **12** was prepared analogously to compound **10** by using **7** (37.0 mg, 65.1 µmol), 4-dimethylaminopyridine (36.9 mg, 0.30 mmol), CH_2Cl_2 (2 ml) and octanoic anhydride (100 µl, 0.34 mmol) as starting materials. Column chromatography (CH_3OH–0.5% acetic acid 2:1) afforded **12** (24.3 mg, 35.0 µmol, 53.7%). ^{1}H NMR ($CDCl_3$): the sesquiterpene skeleton: 5.63 (m, 3 H, H-3, H-6, H-8), 5.42 (t, 1 H, $J = 3.5$ Hz, H-2), 4.26 (br s, 1 H, H-1), 3.03 (dd, 1 H, $J = 3.5$ Hz, $J = 14.0$ Hz, H-9), 2.30 (m, H-9′ hidden under signals from the α protons of the acyl groups), 1.83 (br s, 3 H, H-15), 1.48 (s, 3 H, H-13), 1.40 (s, 3 H, H-14). ^{13}C NMR ($CDCl_3$): the sesquiterpene skeleton: 175.3 (C-12), 141.6 (C-4), 130.1 (C-5), 84.5 (C-10), 84.2 (C-3), 78.6 (C-7 and C-11), 77.7 (C-2), 76.9 (C-6), 66.2 (C-8), 57.4 (C-1), 38.3 (C-9), 22.5 (C-14), 16.2 (C-13), 12.9 (C-15). The octanoate group at C-3: 172.6 (C = O), 34.2, 31.7, 29.1, 28.9, 24.8, 22.5, 14.1 (CH_3). The signals of the other acyl groups were found as previously reported.[23] MS: 693 (43%, $M-1$).

Preparation of **13**. Compound **1** (59.7 mg, 91.7 µmol), 4-dimethylaminopyridine (48.5 mg, 0.40 mmol) and acetic anhydride (75 µl, 0.80 mmol) were dissolved in CH_2Cl_2 (7 ml). The mixture was stirred for 17 h and washed with 4 M HCl (7 ml). The water phase was extracted twice with CH_2Cl_2 (7 ml). The combined organic layers were dried ($MgSO_4$) and concentrated under vacuum. Compound **13** (22.4 mg, 32.3 µmol, 35.2%) was isolated by column chromatography (toluene–EtOAc 7:1) of the residue. ^{1}H NMR of **13** ($CDCl_3$): the sesquiterpene skeleton: 5.67 (br s, 1 H, H-6), 5.63 (br s, 1 H, H-3), 5.47 (m, 2 H, H-2 and H-8), 4.31 (br s, 1 H, H-1), 2.97 (dd, 1 H, $J = 3.0$ Hz, $J_{9,9'} = 15.1$ Hz, H-9), 2.41 (dd, 1 H, $J = 3.0$ Hz, $J_{9,9'} = 14.9$ Hz, H-9′), 1.89 (s, 3 H, H-15), 1.60 (s, 3 H, H-13), 1.33 (s, 3 H, H-14). The acetyl group: 2.01 (s, 3 H). ^{13}C NMR: the sesquiterpene skeleton: 172.5 (C-12), 142.0 (C-4), 130.3 (C-5), 84.2 (C-3), 82.6 (C-10), 78.8 (C-11), 77.5 (C-7), 77.3 (C-2), 76.7 (C-6), 68.2 (C-8), 58.0 (C-1), 37.4 (C-9), 22.8 (C-14), 16.7 (C-13), 13.1 (C-15). The acetyl group: 168.8 (C = O), 21.1 (CH_3). The signals of the other acyl groups were found as previously reported.[23] MS: 691 (30%, $M-1$).

Preparation of compound **15**. A solution of **14**[10] (9.0 mg, 14.5 µmol), acetic anhydride (50 µl, 0.53 mmol) and 4-dimethylaminopyridine (10.0 mg, 81.9 µmol) in CH_2Cl_2 (2 ml) was left for 7 days at room temperature. 0.2 M Na_2CO_3 (10 ml) and CH_2Cl_2 (10 ml) were added. The aqueous layer was extracted with CH_2Cl_2 (10 ml). The combined CH_2Cl_2 phases were dried ($MgSO_4$) and concentrated under vacuum. Column chromatography (toluene–EtOAc 9:1) of the residue afforded **15** (8.0 mg, 12.7 µmol, 87.4%).

Preparation of **17**. To a solution of **15** (26.7 mg, 40.2 µmol) in CH_3OH (25 ml) was added 2 M HCl (5 ml). The solution was left for 1 h at 55 °C. The mixture was cooled and water (5 ml) and EtOAc (10 ml) were added. The aqueous layer was extracted with EtOAc (10 ml). The combined EtOAc phases were dried ($MgSO_4$) and concentrated under vacuum. The residue was dissolved in CH_2Cl_2 (7.7 ml) and 4-dimethylaminopyridine (50.0 mg, 0.41 mmol) and butyric anhydride (58.3 µl, 0.36 mmol) were added to the solution. After 45 min at room temperature 4 M HCl (5 ml) was added. The CH_2Cl_2 layer was dried ($MgSO_4$) and concentrated under vacuum to give a gum from which **17** (8.4 mg, 12.1 µmol, 30%) was isolated by double column chromatography (first column: toluene–EtOAc 5:1, second column: toluene–EtOAc 9:1). ^{1}H NMR ($CDCl_3$): the sesquiterpene skeleton: 6.60 (t, 1 H, $J_{8,9} = J_{8,9'} = 3.2$ Hz, H-8), 5.78 (br s, 1 H, H-6), 5.69 (br s, 1 H, H-3), 5.44 (t, 1 H, $J = 2.4$ Hz, H-2), 4.29 (br s, 1 H, H-1), 2.96 (dd, 1 H, $J_{8,9} = 3.8$ Hz, $J_{9,9'} = 16.2$ Hz, H-9), 2.13 (dd, 1 H, $J_{8,9'} = 3.8$ Hz, $J_{9,9'} = 16.2$ Hz, H-9′), 1.89 (br s, 3 H, H-15), 1.51 (s, 3 H, H-13), 1.33 (s, 3 H, H-14). The acetyl group: 2.20 (s, 3 H). ^{13}C NMR ($CDCl_3$): the sesquiterpene skeleton: 173.9 (C-12), 141.7 (C-4), 130.2 (C-5), 87.9 (C-10), 84.2 (C-3), 83.5 (C-11), 78.5 (C-7), 77.4 (C-2), 76.1 (C-6), 64.3 (C-8), 56.4 (C-1), 38.9 (C-9), 22.0 (C-14), 15.9 (C-13), 13.0 (C-15). The acetyl group: 172.1 (C = O), 24.2 (CH_3). The signals of the other acyl groups were found as previously reported.[23] MS: 691 (10%, $M-1$).

Preparation of **18** *and* **2**. Compound **1** (500 mg, 0.77 mmol) was dissolved in 5% methanolic triethylamine (52.5 ml). The solution was left for 2 days in a sealed glass container at 75°C. The solution was concentrated under vacuum to give a gum from which **18** (156.4 mg, 0.38 mmol, 49%) and **2** (213.1 mg, 0.37 mmol, 48%) were isolated by column chromatography (EtOAc–acetone 7:1 added 1% acetic acid). ^{1}H NMR (CD_3OD) of the sesquiterpene skeleton of compound **18**: 5.78 (br s, 1 H, H-6), 5.62 (br s, 1 H, H-3), 4.29 (t, 1 H, $J = 4.4$ Hz, H-2), 4.26 (t, 1 H, $J_{8,9} = J_{8,9'} = 3.3$ Hz, H-8), 3.34 (br s, 1 H, H-1), 2.26 (dd, 1 H, $J_{8,9} = 3.3$ Hz, $J_{9,9'} = 14.2$ Hz, H-9), 1.85 (dd, 1 H, $J_{8,9'} = 3.4$ Hz, $J_{9,9'} = 14.2$ Hz, H-9′), 1.80 (s, 3 H, H-15), 1.38 (s, 3 H, H-14), 1.19 (s, 3 H, H-13). Compound **2**: The ^{1}H NMR spectrum was identical with that previously reported.[23] ^{13}C NMR (CD_3OD) of compound **18**: the sesquiterpene skeleton: 177.6 (C-12), 139.4 (C-4), 133.3 (C-5), 87.8 (C-3), 80.4 (C-11), 79.3 (C-6), 79.0 (C-7), 78.9 (C-2), 74.5 (C-10), 70.1 (C-8), 62.9 (C-1), 47.4 (C-9), 24.9 (C-14), 16.1 (C-13), 13.1 (C-15).

Preparation of **19**. To a solution of **18** (103.1 mg, 0.25 mmol) in pyridine (10 ml) were added octanoic anhydride (500 µl, 1.7 mmol) and 4-dimethylaminopyridine (100 mg, 0.82 mmol). The reaction was stirred for 1 h at room temperature after which 4 M HCl (50 ml) and diethyl ether (50 ml) were added. The organic layer was dried ($MgSO_4$) and concentrated under vacuum. **19** (155.9 mg, 0.23 mmol, 94%) was isolated from the residue by column chromatography (toluene–EtOAc 3:1). ^{1}H NMR ($CDCl_3$): the sesquiterpene skeleton: 5.76 (br s, 1 H, H-6), 5.70 (br s, 1 H, H-3), 5.56 (br s, 1 H, H-8), 5.23 (t, 1 H, $J < 3$ Hz, H-2), 3.58 (br s, 1 H, H-1), 2.34 (m, H-9 hidden under signals from the α protons of the ester moieties), 1.95 (dd 1 H, $J < 4$ Hz, $J_{9,9'} = 13.1$ Hz, H-9′), 1.87 (s, 3 H, H-15), 1.50 (s, 3 H, H-13), 1.18 (s, 3 H, H-14). ^{13}C NMR ($CDCl_3$): the sesquiterpene skeleton: 179.7 (C-12), 140.1 (C-4), 131.8 (C-5), 83.6 (C-3), 79.0 (C-11), 78.7 (C-6 and C-7), 77.2 (C-2), 73.1 (C-10), 66.4 (C-8), 61.4 (C-1), 44.2 (C-9), 24.5 (C-14), 16.1 (C-13), 12.9 (C-15). The octanoyl group at C-8: 173.0 (C = O), 34.7, 34.1, 31.7, 29.0, 24.5, 22.6, 14.1 (CH_3). The signals of the other acyl groups were found as previously reported.[23]

Preparation of **21**. Compound **19** (155.9 mg, 0.23 mmol) was dissolved in 5% methanolic triethylamine (16 ml). The solution was left for 5 h at room temperature, triethylamine (780 µl) was added and the mixture was heated to 60°C for 1.5 h. The mixture was partially concentrated under vacuum after addition of 4 M HCl (6 ml). The residue was extracted with diethyl ether (20 ml). The extract was dried ($MgSO_4$) and concentrated under vacuum. The residue was dissolved in CH_2Cl_2 (5 ml) and to the solution were added 4-dimethylaminopyridine (50 mg, 0.41 mmol) and butyric anhydride (100 µl, 0.61 mmol). The reaction was stirred for 1 h at room temperature after which 4 M HCl (10 ml) was added. The organic phase was dried ($MgSO_4$) and concentrated under vacuum. Column chromatography (toluene–EtOAc 5:1) of the residue afforded **21** (38.1 mg, 62.5 µmol, 26.7%). ^{1}H NMR ($CDCl_3$): the sesquiterpene skeleton: 5.75 (br s, 1 H, H-6), 5.69 (br s, 1 H, H-3), 5.55 (t, 1 H, $J < 4$ Hz, H-8), 5.24 (br s, 1 H, H-2), 3.50 (br s, 1 H, H-1), 2.30 (m, H-9 hidden under signals from the α protons of the acyl groups), 1.98 (dd, 1 H, $J = 2.4$ Hz, $J = 13.1$ Hz, H-9′), 1.90 (br s, 3 H, H-15), 1.50 (s, 3 H, H-13), 1.18 (s, 3 H, H-14). ^{13}C NMR ($CDCl_3$): the sesquiterpene skeleton: 174.9 (C-12), 141.0 (C-4), 131.3 (C-5), 83.5 (C-3), 79.1 (C-6), 78.9 (C-7), 78.6 (C-11), 76.8 (C-2), 72.8 (C-10), 66.4 (C-8), 62.0 (C-1), 44.6 (C-9), 24.8 (C-14), 15.9 (C-13), 13.0 (C-15). The signals of the acyl groups were found as previously reported.[23] MS: 607 (54%, $M - 1$).

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