Bioorganic & Medicinal Chemistry 18 (2010) 5634–5646

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Elucidation of the topography of the thapsigargin binding site in the sarco-endoplasmic calcium ATPase

Dorthe Mondrup Skytte [a], Jesper Vuust Møller [b], Huizhen Liu [a], Helle Østergren Nielsen [a], Louise Elsa Svenningsen [a], Christina Mernøe Jensen [a], Carl Erik Olsen [c], Søren Brøgger Christensen [a,*]

[a] Department of Medicinal Chemistry, University of Copenhagen, Universitetsparken 2, DK-2100 Copenhagen Ø, Denmark
[b] PUMPKIN, Department of Physiology and Biophysics, University of Aarhus, Ole Worms Alle blg 1185, DK-8000 Aarhus C, Denmark
[c] Department of Basic Sciences and Environment, University of Copenhagen, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark

ARTICLE INFO

Article history:
Received 20 April 2010
Revised 7 June 2010
Accepted 10 June 2010
Available online 16 June 2010

Keywords:
Thapsigargin
SERCA
Pharmacophore
Topography of binding cavity

ABSTRACT

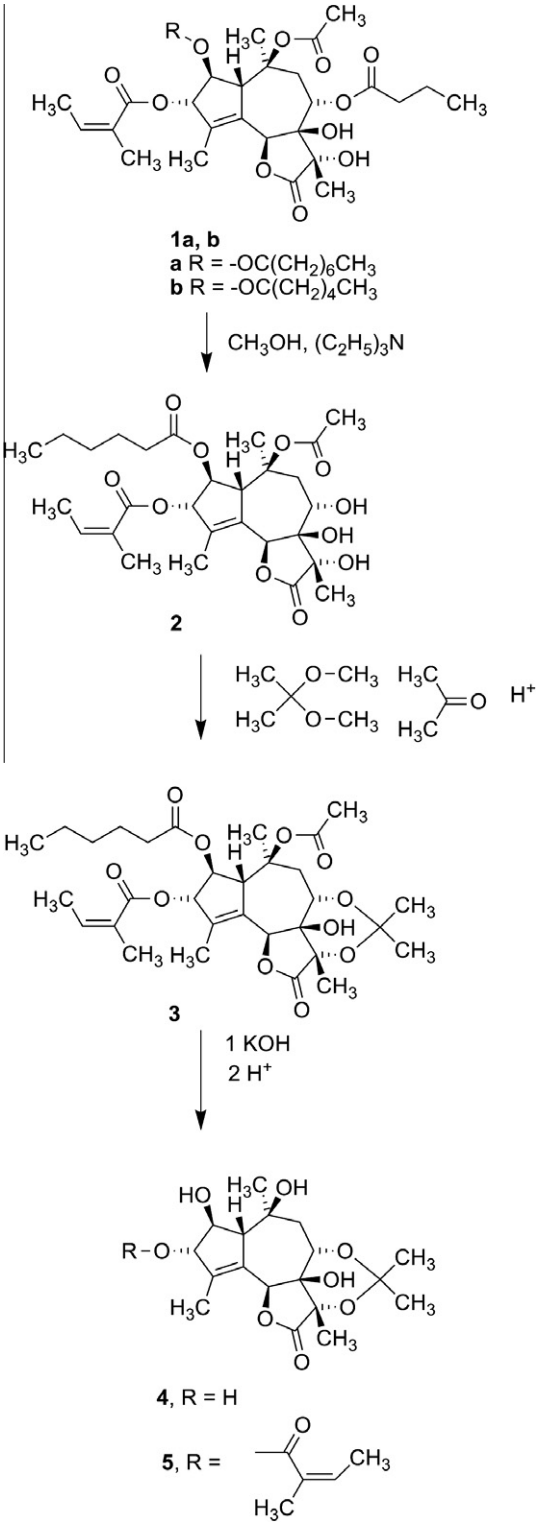
Removal of each of the acyl groups of thapsigargin at O-3, O-8 and O-10 significant reduces the affinity of the inhibitors to the SERCA1a pump. Replacement of the acyl groups at O-3 and O-10 with flexible residues could be performed with only a minor decrease of the affinity, whereas introduction of voluminous stiff residues caused dramatic reduction of the affinity. The results can be rationalized on the basis of the interactions of thapsigargin with the SERCA1a pump as revealed from 3D X-ray structural models of thapsigargin bound to the SERCA1a. In conclusion the results confirm and elaborate the previously suggested pharmocophore model of thapsigargin.

1. Introduction

The sarco- and endoplasmic Ca^{2+}-ATPases (SEERCA) are essential for the calcium homeostasis of cells. The SERCA family includes the products of three genes named SERCA1 (*ATP2A1*), SERCA2 (*ATP2A2*), and SERCA3 (*ATP2A3*), each giving rise to alternatively spliced mRNA.[1] Presently the SERCA family comprises 14 isoforms: two SERCA1 (1a and 1b), three SERCA2 (2a–2c), and in humans six SERCA3 (3a–3f). In some species (mice and rats) other specific isoforms of SERCA3 have been found.[1] Thapsigargin (Tg, **1a**) isolated from the Mediterranean umbelliferous plant *Thapsia garganica* L. was originately believed only to inhibit the endoplasmic Ca^{2+}-ATPases.[2] Later studies, however revealed that thapsigargin is a potent and specific inhibitor of all the subtypes of SERCA even though the inhibition constants towards the different subtypes vary from 0.2 to 12 nM.[2–7] Blockage of SERCA leads to malfunction of the calcium homeostasis in the cells and eventually apoptosis.[8–10] In contrast to chemotherapeutics Tg provokes apoptosis at all stages of the cell cycle.[8–10] Slow proliferation of prostate cancer cells prevents treatment of prostate cancer by ordinary chemotherapeutics since these attack the cells during the mitotic phase of the cell cycle.[11] Treatment with Tg will overcome this problem but the ubiquitous presence of SERCA in all kind of cells precludes the use of Tg as per se. By taking advantage of expression of characteristic proteolytic enzymes such as humane glandular kallikrein 2 (hK2) and prostate specific antigen [PSA also known as human glandular kallikrein 3 (hK3)] Tg has been targeted towards cells of different tissues.[12–15] The strategy is based on the assumption that conjugation of an amino-containing derivative of Tg to a peptide yields a prodrug, which does not penetrate the cell membrane, but after cleavage of the peptide moiety by a tissue characteristic proteolytic enzyme a drug is formed, which can reach and block the intracellular located SERCA and eventually induce apoptosis.[16] The concept of selectively targeting prostate cancer cells has been proven valid in a mouse model, where systemic administration of a Tg derived prodrug selectively controlled the growth of prostate cancer cells.[17] Conversion of Tg into a derivative, which can be used for conjugation with a peptide, necessitates introduction of a long linker moiety.[14] Introduction of the linker into a critical position might afford an inactive derivative. Consequently, extensive studies of the topography of the Tg binding site in SERCA have been performed[14,18–21] and reviewed.[11,22] Empirical structure–activity relationships reveal that a large flexible linker is allowed at O-8 and at O-2 (for numbering see Scheme 1) the lactone carbonyl at C-12 can be replaced with a methylene group, and the hydroxyl group at C-11 can be alkylated without loss of SERCA inhibitory activity. A GRID analysis of the Tg binding site in SERCA as revealed by X-ray structures of SERCA1a stabilized with Tg[23] or with Tg, in which the O-8 group has been replaced with a *N*-Boc-12-aminododecanoate[21] to some extent rationalized these findings.[24] This pharmacophore model suggests lipophilic interactions between

* Corresponding author. Tel.: +45 3533 6253; fax +45 3533 6401.
E-mail address: sbc@farma.ku.dk (S.B. Christensen).

0968-0896/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2010.06.032



Scheme 1.

SERCA and the acetyl group at O-10, the angeloyl group at O-3, and the butanoyl group at O-8. In addition a minor interaction between the methyl-15 and the pump is suggested. The octanoyl group at O-2 might stabilize the complex by interacting with the lipophilic membrane. Surprisingly no hydrophilic interactions were revealed[24] even though a X-ray crystal structure of the SERCA-Tg complex indicates that a potential hydrogen bond between Ile829 and the carbonyl group of the butanoyl moiety might have a minor importance.[25] The present study was undertaken in order to elaborate on the topographies of the binding cavity around the angeloyl group at O-3, the acetyl group at O-10, and of the hydroxyl group at O-11.

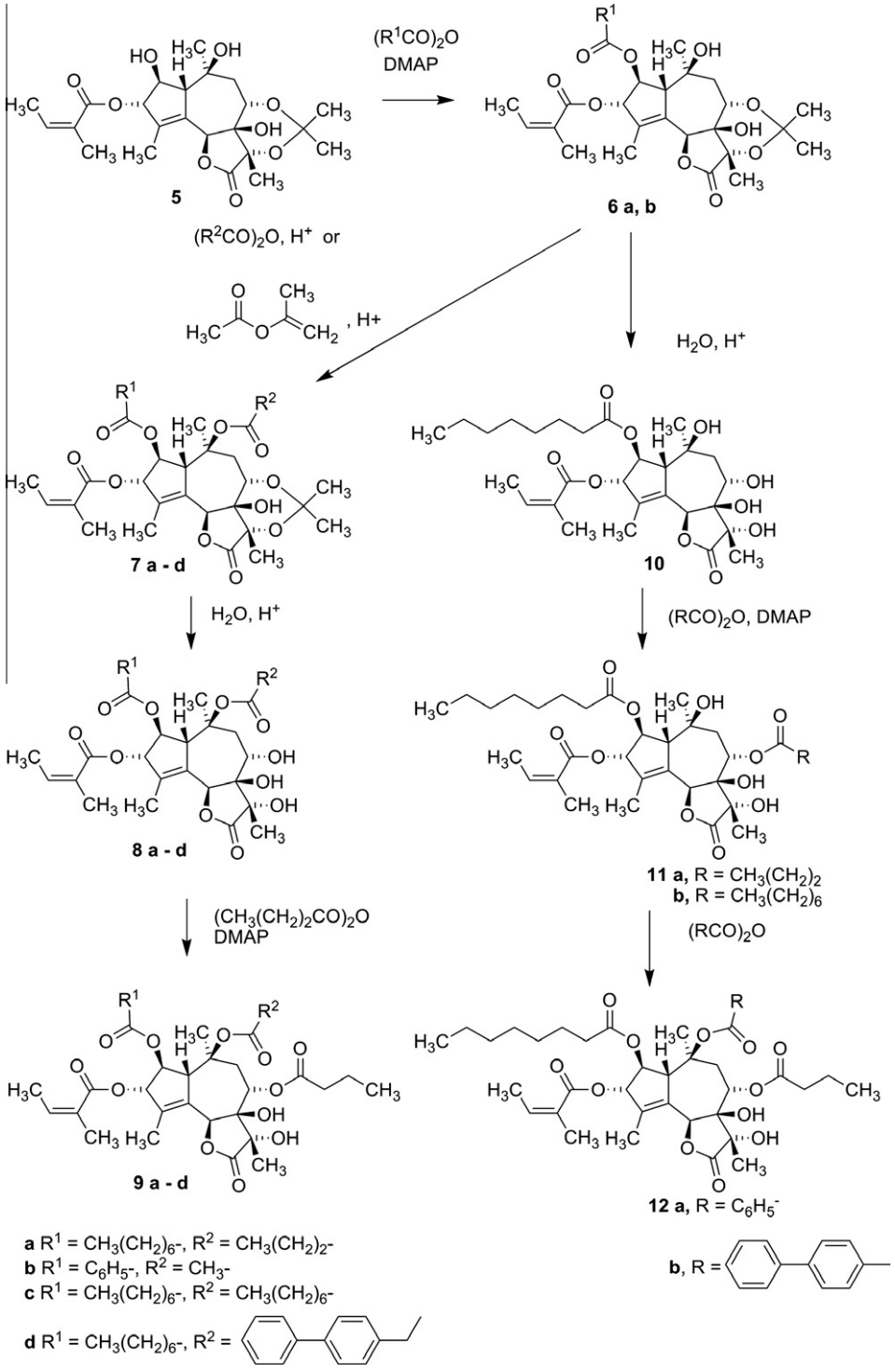
2. Results

2.1. Chemistry

To save our stock of thapsigargin (**1a**) thapsigargicin (**1b**, Scheme 1) obtained from *Thapsia garganica* as previously described[26] was used as starting material for the model compounds, in which hexanoate during the reaction sequence later was replaced with octanoic acid. Thus, the use of thapsigargicin only had importance for the synthetic procedure. The described methods[27–30] for removing the acyl groups at O-2, O-8, and O-10 and for selective protection of O-8 and O-11 were used to get the isopropylidene diol **5** (Scheme 1). Selective acylation at O-2 was obtained by reaction of **5** with octanoic or benzoic anhydride in the presence of 4-*N*,*N*-dimethylaminopyridine (DMAP) to give **6a** and **6b**, respectively (Scheme 2). Acetylation at O-10 to give **7b** proceeded smoothly when isopropenyl acetate and *p*-toluenesulfonic acid (*p*-TsOH) were used as reagents. If, however, larger esters of isopropenol like butyric or octanoic esters were used to acylate **6a** significant amounts of side products like **13a** and **14** were observed beside **7a** or **7c** (Scheme 3). Since formation of the isopropenyl esters by reesterification of isopropenyl acetate with the appropriate acid is known to give acid anhydrides as side products,[31] it was assumed that the formed acid anhydrides caused the side products. Surprisingly, however, treatment of **7a** with anhydrides like butyric, 4-biphenylacetic or octanoic acid anhydride in the presence of *p*-TsOH smoothly and selectively afforded the O-10 esters **7a**, **7c**, and **7d**. Consequently, this method was used for preparation of these compounds. Acidic hydrolysis of the isopropylidene acetal followed by selective esterification of O-8 was performed analogous to published procedures.[21] Acylation of O-10 in **6a** by reaction with benzoic acid anhydride and *p*-TsOH was not possible, apparently according to steric hindrance from the isopropylidene acetal. Alternatively, the isopropylidene acetal of **6a** was hydrolyzed under acidic conditions to give **10** (Scheme 2). Selective acylation of O-8 with butyric acid anhydride and DMAP gave **11a** which subsequently was acylated at O-10 by reaction with benzoic acid anhydride and *p*-TsOH under prolonged reaction time to give **12**. Advantage was taken of the availability of the side products **13a** and **14** to test the importance of substituents at O-7 and O-11. Furthermore, the reaction was used to prepare the O-10 octanoic or butanoic acid esters **7a**, **9a**, **9c**, and **14** using **5** as starting material. For replacement of the angeloyl residue advantage of the trisubstituted double bond was taken. Selective oxidative cleavage of this double bond was obtained using osmium tetraoxide and periodate as reagents to give the pyruvate, which selectively could be hydrolyzed to give the 3-hydroxy derivative **15** (Scheme 4). The secondary hydroxy group was selectively esterified using either acid anhydrides and DMAP as catalyst or DCC and DMAP promoted esterification of free acids to give analogues **16a**–**16f**.

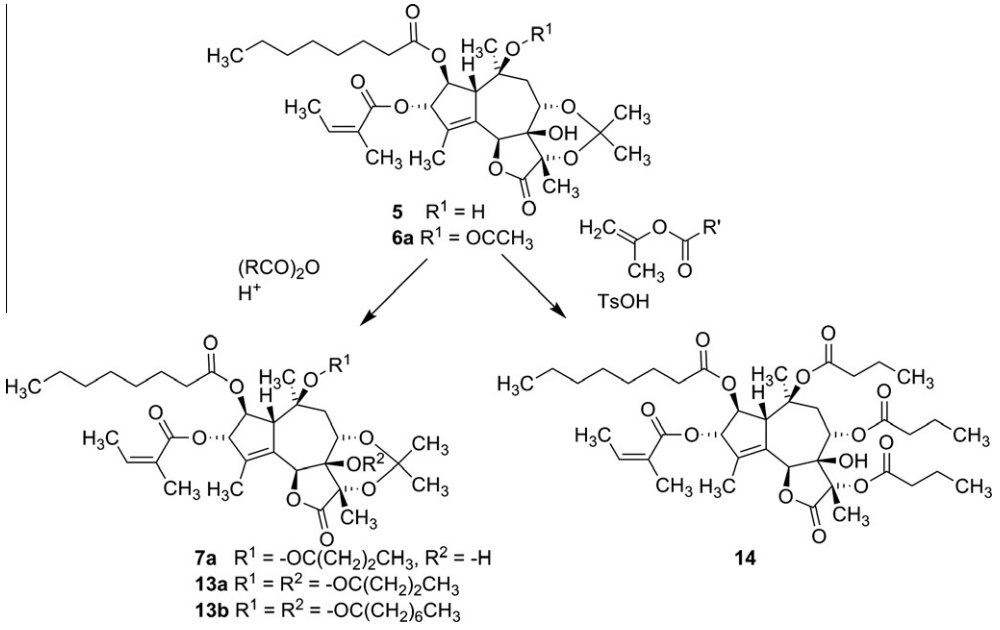
2.2. SERCA inhibitory activity

The activity of SERCA was measured spectrophotometrically with a NADH coupled and ATP regenerating assay medium.[32] For this an assay protocol was established in which SERCA1a (purified from sarcoplasmic reticulum vesicles of rabbit skeletal muscle by treatment with a low concentration of deoxycholate) at a protein concentration of 1 mg/mL was pre-equilibrated with



Scheme 2.

different inhibitor concentrations for 2.5–6 min in a medium containing 1 mM EGTA, 1 mM Mg^{2+}, 10 mM Tes (pH 7.5), and 100 mM KCl. Activities were measured from the rate of absorption decrease at 340 nm. Following addition of 10 μL of the pre-equilibrated mixture to a cuvette, containing 3 mL of 0.1 mM Ca^{2+}, 1 mM Mg^{2+}, 5 mM MgATP, 1 mM phosphoenolpyruvate, 0.15 mM NADH, phosphoenolpyruvate kinase, and lactate dehydrogenase. The inhibitor (thapsigargin or thapsigargin derivative), solubilized in DMSO, was added cumulatively to the ATPase pre-equilibration mixture in increments of usually 0.2–0.4 mol/mg protein. We found that the gradual increase in inhibitor concentration in the cuvette sample, effectuated by the incremental addition, facilitated the attainment of binding equilibrium, which often was a slow process, requiring up to 6 min preincubation. The assay was often carried out with glassware instead of plastic to safeguard as much as possible against unspecific adsorption of the inhibitor by the side walls of containers, pipettes, and cuvettes. The inhibition of activity is given as IC_{50} values in Table 1. Thapsigargin was used as a positive control in all experiments.



Scheme 3.

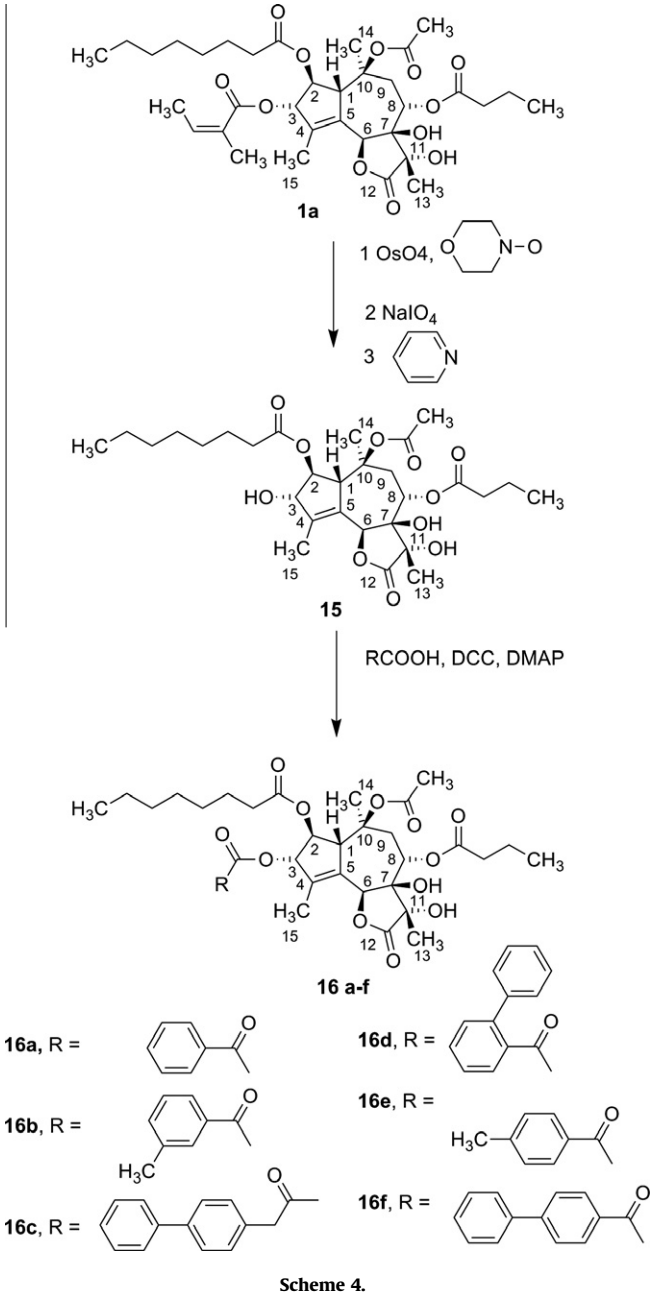
3. Discussion

The pharmacophore model for Tg[24] suggests extensive hydrophobic interactions between SERCA and the angeloyl, the butanoyl, and the acetyl moieties of Tg. These interactions are localized to the 3rd, 5th, and 7th (M3, M5, and M7) transmembrane helices of SERCA which form the bottom and sides of the binding cavity, while the front of the bound Tg molecule is exposed towards the cytoplasmic leaflet of the lipid phase (Fig. 1). The pharmacophore model implies that removal of the three mentioned side chains should cause a significant reduction in the affinity for SERCA. This prediction was confirmed by measuring the inhibitory effect on enzyme activity of Tg analogues with removed side chains. Thus, the debutanoylated thapsigargin derivative possesses an IC_{50} value of 10 nM (Table 1, compound **9e**), compared to an IC_{50} value of 0.2 nM estimated for the unmodified Tg. Analoguously, the desacetylated analogues **11a** and **11b** give rise to IC_{50} values of 20 nM. All of these analogues, and in particular the desangeloylated **15**, confirm the importance of the hydrophobic interactions between the acyl group and the pump. In continuation of these findings we have substituted the angeloyl and acetyl groups of thapsigargin with other acyl groups in order to explore the topography of the binding cavity.

Replacement of the acetyl group at O-10 with flexible acyl groups like butanoyl (**9a**), octanoyl (**9c**), the non-flexible benzoyl (**12a**), and even the large bisphenylacetyl group (**9d**) reduced the IC_{50} values, but not nearly as much as expected for a 'lock and key' mechanism. This suggests flexibility at the O-10 binding site for accommodation of large ligands. From a structural perspective this calls attention to Phe 834 which closely covers and interacts with the acetyl group of Tg.[21] Comparisons of E2 conformations of ATPase, with and without bound Tg, indicate a slight retraction of Phe 834 upon Tg bind in accordance with the idea that the 834 phenyl group is flexible and by rotation adjusts to the volume size of the O-10 side chain. Furthermore, Tyr 837 upon Tg binding undergoes a dramatic 110 degree rotation out of the binding cavity to make room for accommodation of Tg. There is thus clear evidence that this part of the binding site which is localized between the M5 and M7 helices has induced-fit characteristics (Fig. 1B). We also found that in contrast to the more flexible bisphenylacetyl (**9d**). The stiff and equally voluminous 4-phenylbenzoate (**12b**) failed to exert SERCA inhibitory activity in the concentration range studied. This suggests that as expected the O-10 acetyl pocket is not large enough to contain the 4-phenylbenzoyl group (**12b**). Consequently the high affinity of the bisphenylacetyl (**9d**) must be ascribed to the flexibility retained, due to rotation of the two single bonds attaching the methylene group to the carbonyl- and the bisphenyl group, respectively. This flexibility enables part of the side chain either to avoid sterical interaction with the backbone of the transmembrane segments or to engage in interaction with other parts of the ATPase or with the lipid phase.

Very similar characteristics as with the O-10 acetyl group was observed by replacement of the angeloyl residue with voluminous acyl groups. The benzoyl analogue **16a** is bound with almost the same affinity as Tg. The *meta*-methylbenzoyl analogue **16b**, even the very voluminous, but flexible *para*-bisphenylacetate **16c** and *ortho*-phenylbenzoyl analogue **16d** (an *ortho*-phenylbenzoyl) only to a limited extent displayed reduced IC_{50} values. In contrast introduction of a *para*-methylbenzoate (**16e**) and even more pronounced the stiff 4-phenylbenzoate (**16f**) dramatically reduced the affinities. These different binding properties can also be accounted for on the basis of the interactions between Tg and the SERCA pump revealed by the X-ray diffraction data. In the Tg bound pump the angeloyl side chain is located in an internal cavity between M3, M4, and M5, the bottom of which is formed by the proline residue of the A(306)IPEGL(311) motif in M4, forming part of one of the Ca^{2+} binding sites. This cavity would be sufficiently large for accommodation of a benzoyl group; it can also adjust to the **16b** *meta*-methyl, but not so well to the **16e** *para*-methyl derivative, because the length of the substituent exceeds the length of the cavity. However, the cavity is too small to accept the bulkier substituents. Furthermore, the stiff phenylbenzoyl group (**16f**) is bound with a low, but perceptible affinity ($\sim$0.1 μM). We therefore conclude that the side chains with bulky flexible and stiff substituents at O-2 probably are bound differently than in the internal cavity of the angeloyl moiety, but without disrupting other interactions of the ligand with the ATPase.

Remaining data in Table 1 deal with Tg analogues close to the lactone ring.



Scheme 4.

The IC_{50} value of the isopropylidene derivative **7a** was 19 nM and comparable to the affinity of the debutanoylated analogue (**9e**), which may be accounted for by assuming that the loss of affinity compared to Tg is caused by the absence of the butanoyl group and that the isopropylidene group does not interact with the SERCA pump. Furthermore, docking of **7a** with the guaianolide skeleton of 12ADT (structure 2BY4)[21] reveals that the two methyl groups of the isopropylidene group are placed distant from the helices of the pump. The pharmacophore model does not predict the importance of the two hydroxyl groups of Tg and previous data have revealed that acetylation of O-7 or O-11 only moderately reduces the affinity for the SERCA pump approximately 2.5 times, whereas acetylation of both hydroxy groups reduces the affinity 15 times.[19] Introduction of a large acyl groups at O-11 as in **14** confirms the previous findings by showing that this only causes some reduction in affinity. Substituents at O-7 as in compound **13** reveal that substituents in this position have importance for the affinity. Substitution in this position, however, from a chemical synthetic as well as from a medicinal chemical aspect appears to be complicated and will be a subject of a future communication.[21,25,33]

4. Conclusions

Selective acylation of OH-10 in the presence of free hydroxyl groups at O-7 in a thapsigargin analogue can be obtained using an acid anhydride as reagent and *p*-TsOH as a catalyst. The assumed crucial effect of a lipophilic acyl group at O-3, O-8 and O-10 has been confirmed. Introduction of flexible voluminous groups at O-3 and O-10 only to a minor extent reduces the affinity for the analogues to the pump. In contrast introduction of the voluminous and stiff 4-phenylbenzoic acid at O-3 dramatically reduces the ability to inhibit the pump. Our data suggest that stiff benzoyl and 4-methylbenzoyl derivatives can be used as rulers to estimate available space in the binding pockets of the side chains, for example, the latter substituent at O-3 (with a distance from carbonyl carbon atom to the carbon atom of the methyl group 0.58 nm) dramatically reduces the affinity compared to introduction of benzoic acid (distance from carbonyl carbon atom to para carbon atom 0.43 nm). The present findings substantiate the previously suggested pharmacophore model.[24]

5. Experimental

5.1. General methods for chemistry

^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a Varian Mercury spectrometer or a Varian Gemini 2000 spectrometer using $CDCl_3$ as a solvent. 2D NMR spectra were recorded using standard pulse sequences. Splitting patterns are described as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), broad singlet (br s), double doublet (dd), double double double doublet (dddd), double quartet (dq) or quartet of quartets (qq). High-resolution MS was recorded on a Q-Tof1 (Micromass). Column chromatography was performed on Silica Gel 60 (Merck 107734). Reversed phase chromatography was performed over LiChroprep RP-18 (Merck 1.13900).

Octanoic anhydride: Dicyclohexylcarbodiimide (DCC, 6.5 g, 31.6 mmol) was added to a solution of octanoic acid (10 mL, 63.1 mmol) in CH_2Cl_2. The mixture was stirred at room temperature for 1½ h, filtered and concentrated in vacuo.

^{1}H NMR ($CDCl_3$) δ (ppm): 2.44 (4H, t, J = 7.5 Hz, 2 × –OCOCH_2CH$_2$–); 1.66 (4H, dddd, J = 7.5 Hz, 2 × –OCOCH$_2$CH_2CH$_2$–); 1.28–1.37 (16H, m, 4 × –CH$_2$–); 0.88 (6H, t, J = 6.9 Hz, 2 × –CH$_2$CH_3).[28]

4-Biphenylacetic anhydride: 4-Biphenylacetic acid (2.00 g, 9.4 mmol) was dissolved in (60 mL) and DCC (0.97 g, 4.7 mmol) was added. The reaction mixture was stirred at room temperature over night, filtered and concentrated in vacuo. The product was recrystallized from heptane and EtOAc. White solid (1.32 g, 69%). Melting point 145–147 °C. ^{1}H NMR ($CDCl_3$) δ (ppm): 7.51–7.56 (m, 8H, H-8, H-9, H-11, and H-12); 7.39–7.42 (m, 4H, H-3 and H-5); 7.34 (dddd, J = 2.4 and 7.5 Hz, 2H, H-10); 7.27 (d, J = 8.1 Hz, 4H, H-2 and H-6); 3.78 (s, 4H, COCH_2-Ph).

5.1.1. Debutanoyl thapsigargicin (2)

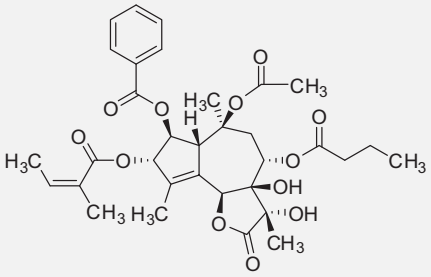
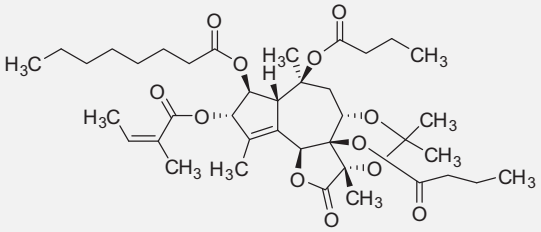
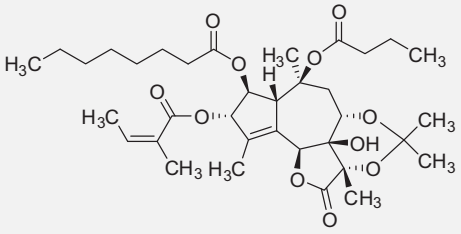
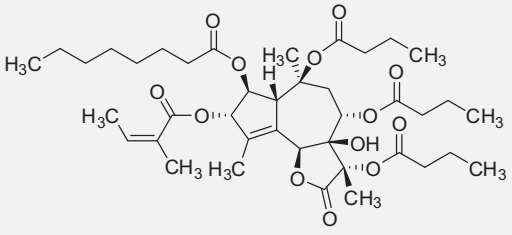
Thapsigarcin (**1b**) (1.5 g, 2.4 mmol) was dissolved in dry MeOH (150 mL). Et_3N (6.8 mL, 48 mmol) was added and the mixture was stirred at room temperature for 4½ h. The reaction was quenched with saturated aqueous NH_4Cl-solution (200 mL) and extracted five times with EtOAc (100 mL). The combined organic layer was dried over Na_2SO_4, filtered and concentrated in vacuo. Compound **2** (1.11 g, 83%) was purified by column chromatography using heptanes–EtOAc (3:2) as an eluent.

^{1}H NMR ($CDCl_3$) δ (ppm): 5.81 (br s, 1H, H-6); 5.69 (br s, 1H, H-3); 5.45 (dd, J = 3.6 Hz, 1H, H-2); 5.00 (s, 1H, OH); 4.36 (br m, 1H, H-8); 4.22 (br s, 1H, H-1); 4.13 (s, 1H, OH); 3.53 (s, 1H, OH); 2.84 (dd, J = 2.1 Hz and 14.1 Hz, 1H, H-9a); 2.47 (dd, J = 3.3 Hz and 14.1 Hz, 1H, H-9b), 1.90 (br s, 3H, H-15), 1.49 (3H, s, H-13); 1.44 (3H, s, H-14). Acetate 1.90 (s, 3H, H-2). Angeloate 6.12 (1H, qq, J = 1.5 and 7.2 Hz, 1H, H-3), 1.98 (3H, dq, J = 1.5 Hz; 7.2 Hz, 3H, H-4), 1.84 (s, 3H, Me-2). Hexanoate 2.20–2.38 (2H, m, H-2), 1.60 (m, 2H, H-3); 1.30 (m, 4H, H-4 and H-5); 0.88 (t, J = 6.9 Hz, 3H, H-6).

5.1.2. Isopropylidene (3)

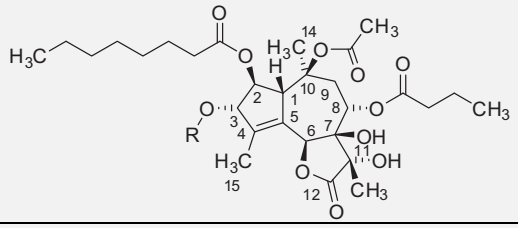
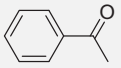
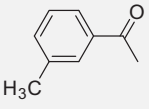
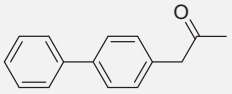
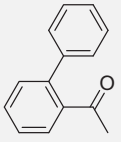
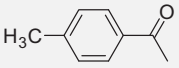
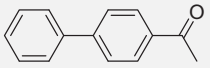
Compound **2** (1.09 g, 1.97 mmol) was dissolved in acetone (16 mL) and 2,2-dimethoxypropane (30 mL). *p*-Toluenesulfonic acid monohydrate (*p*-TsOH, 0.055 g, 0.29 mmol) was added and the mixture was stirred at room temperature over night. The reaction was quenched with a saturated aqueous $NaHCO_3$-solution (100 mL) and extracted three times with EtOAc (100 mL). The combined organic layer was washed with brine (100 mL), dried over Na_2SO_4, filtered and concentrated in vacuo. Compound **3** (1.01 g,

Table 1
Inhibition constants (IC_{50} values) for thapsigargin analogues towards SERCA1a

Compd			IC_{50} (nM)
1a	R^1 = –$COCH_3$	R^2 = –$CO(CH_2)_2CH_3$	0.2
11a	R^1 = –H	R^2 = –$CO(CH_2)_2CH_3$	20
11b	R^1 = –H	R^2 = –$CO(CH_2)_6CH_3$	20 ± 8.5
9a	R^1 = –$CO(CH_2)_2CH_3$	R^2 = –$CO(CH_2)_2CH_3$	2.5 ± 0.3
9c	R^1 = –$CO(CH_2)_6CH_3$	R^2 = –$CO(CH_2)_2CH_3$	16 ± 2.7
12a	R^1 = –COC_6H_5	R^2 = –$CO(CH_2)_2CH_3$	15.6 ± 0.1
12b	R^1 = –COC_6H_4–C_6H_5	R^2 = –$CO(CH_2)_2CH_3$	>70
9d	R^1 = –$COCH_2C_6H_4C_6H_5$	R^2 = –$CO(CH_2)_2CH_3$	27 ± 1.6
9e	R^1 = –$COCH_3$	R^2 = –H	10
9b			1.6
13			>70
7a			19 ± 1.1
14			11.1 ± 1.9

(*continued on next page*)

Table 1 (*continued*)

Compd		IC_{50} (nM)
		
15	R = H	>70
16a	R = 	0.8 ± 0.7
16b	R = 	9 ± 4.5
16c	R = 	18 ± 2.5
16d	R = 	3 ± 2.0
16e	R = 	44 ± 8.6
16f	R = 	>70

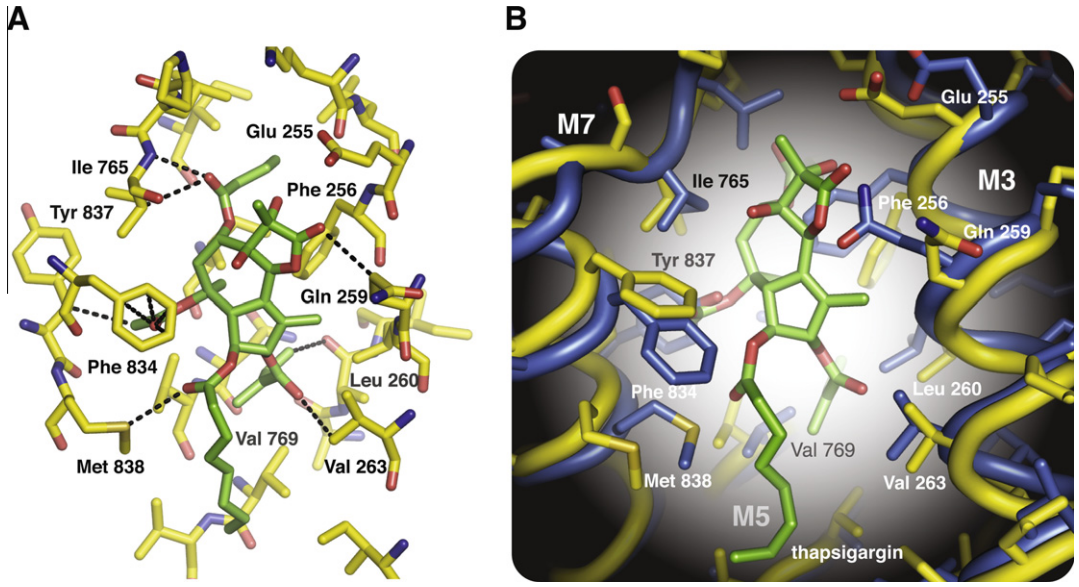


Figure 1. Thapsigargin (carbons as green sticks and oxygen as red sticks) in the binding cavity of SERCA1a in the E2 conformation. A: The binding cavity with adjacent amino acid residues accentuated. B: Superimpositions of structures of SERCA1a in the E2 conformation in the absence (PDB code 3B9R, blue) and in the presence of bound thapsigargin (PDB code 1XP5, yellow).

87%) was purified by column chromatography using heptanes–ethyl acetate (3:1) as an eluent.

^{1}H NMR ($CDCl_3$) δ: 5.78 m, 1H, H-6); 5.73 (m, 1H, H-3); 5.49 (dd, J = 4.2 Hz and 5.4 Hz, 1H, H-2); 4.30 (dd, J = 3.0 Hz and 4.2 Hz, 1H, H-8); 4.06 (s, 1H, OH); 3.99 (br s, 1H, H-1); 2.75 (dd, J = 4.8 and 14.7 Hz, 1H, H-9a); 2.64 (dd, J = 2.7 and 14.7 Hz, 1H, H-9b), 1.88 (s, 3H, H-15), 1.56 (s, 3H, H-13); 1.47 (3H, s, H-14). Acetate 1.88 (s, 3H, H-2). Angeloate 6.10 (qq, J = 1.5 Hz; 7.2 Hz, 1H, H-3), 1.99

(dq, J = 1.5 and 7.2 Hz, 3H, H-4), 1.91 (m, 3H, Me-2). Hexanoate 2.30 (m, 2H, H-2), 1.60 (m, 2H, H-3), 1.30 (m, 4H, H-4 and H-5); 0.89 (t, J = 6.9 Hz, 3H, H-6). Isopropylidene, 1.54 (s, 3H, H-1), 1.42 (s, 3H, H-3), (H, H-3).

5.1.3. Isopropylidene 5

Compound **3** (1.00 g, 1.7 mmol) was stirred in 10% KOH in MeOH (15 mL) for 5 min. The mixture was neutralized with Dowex 50WX8-100 ion exchange resin, filtered and concentrated in vacuo. Compound **5** (0.61 g, 79%) was purified by column chromatography using heptanes–ethyl acetate (1:3→1:6) as eluents.

^{1}H NMR ($CDCl_3$) δ (ppm): 6.21 (1H, qq, J = 1.5 Hz; 7.2 Hz, CH_3CH = $C(CH_3)COO$–); 5.77–5.80 (1H, m, H-6); 5.33–5.35 (1H, m, H-3); 4.53 (1H, s, –OH); 4.22–4.26 (2H, m, H-2 + H-8); 3.65 (s, 1H, OH); 3.25–3.28 (1H, m, H-1); 3.09 (1H, s, –OH); 2.29 (1H, dd, J = 4.2 Hz; 15.0 Hz, H-9); 2.09 (1H, dd, J = 2.7 Hz; 15.0 Hz, H-9); 2.03 (3H, dq, J = 1.5 Hz; 7.5 Hz, CH_3CH = $C(CH_3)COO$–); 1.93 (6H, m, CH_3CH = $C(CH_3)COO$– + H-15); 1.54 (3H, s, H-13); 1.53 (3H, s, –$OC(CH_3)(CH_3)O$–); 1.41 (3H, s, –$OC(CH_3)(CH_3)O$–); 1.23 (3H, s, H-14).[20]

5.1.4. Isopropylidene 6a

Compound **5** (385 mg, 0.85 mmol) was dissolved in dichloromethane (55 mL). 4-Dimethylaminopyridine (DMAP, 10 mg, 0.085 mmol) and a solution of octanoic anhydride (368 mg, 1.36 mmol) in CH_2Cl_2 (15 mL) was added. The mixture was stirred at room temperature for 1 h, and further octanoic anhydride (368 mg, 1.36 mmol) in CH_2Cl_2 (2 mL) and DMAP (10 mg, 0.085 mmol) was added. The reaction mixture was stirred over night at room temperature. Saturated NH_4Cl-solution (150 mL) and EtOAc (150 mL) was added, and the layers were separated. The aqueous layer was extracted three times with ethyl acetate (75 mL). The combined organic layer was dried over Na_2SO_4, filtered and concentrated in vacuo. Compound **6a** (463 mg, 94%) was purified by column chromatography using heptane–ethyl acetate (4:1) as an eluent.

^{1}H NMR ($CDCl_3$) δ (ppm): 5.81 (s, 2H, H-3 and H-6); 5.38 (dd, J = 4.2 and 5.4 Hz, 1H, H-2); 4.28 (dd, J = 3.0 and 4.5 Hz, 1H, H-8); 3.44 (br s, 1H, H-1); 2.27 (1H, dd, J = 4.2 and 15.3 Hz, H-9a), 2.05 (dd, J = 3.0 and 15.3 Hz, 1H, H-9b); 1.87 (3H, d, J = 1.8 Hz, H-15), 1.54 (3H, s, H-13), 1.25 (3H, s, H-14). Angeloate 6.11 (qq, J = 1.5 and 7.5 Hz, 1H, H-3), 1.99 (dq, J = 1.5 and 6.9 Hz, 3H, H-4), 1.91 (dq, J = 1.5 Hz, 3H, Me-2), Isopropylidene:; 1.55 (s, 3H, H-3) 1.42 (s, 3H, H-1). Octanoate 2.32 (dt, J = 3.0 and 7.5 Hz, 2H, H-2), 1.60 (m, 2H, H-3), 1.28 (m, 8H, H-4–H-7), 0.87 (t, J = 6.9 Hz, 3H, H-8).[27]

5.1.5. Butanoate (7a) and triol (8a) (general procedure A)

Compound **6a** (50 mg, 0.085 mmol) and butanoic anhydride (0.35 mL, 2.1 mmol) was dissolved in CH_2Cl_2 (6 mL). p-TsOH acid monohydrate (49 mg, 0.26 mmol) was added and the mixture was stirred at room temperature for 45 min. A saturated aqueous $NaHCO_3$-solution (20 mL) and EtOAc (20 mL) was added and the layers were separated. The aqueous layer was extracted three times with EtOAc (15 mL) and the combined organic layer was washed with brine (20 mL), dried over Na_2SO_4, filtered and concentrated in vacuo. The product was purified by column chromatography using heptanes–ethyl acetate (9:1) as an eluent to give compound **7a** (18.2 mg, 33%) and compound **8a** (33 mg, 63%).

Compound (**7a**): colorless syrup (18.2 mg, 33%).

^{1}H NMR ($CDCl_3$) δ: 5.78 (s, 1H, H-6); 5.74 (s, 1H, H-3); 5.46 (dd, J = 3.9 Hz, 1H, H-2); 4.27 (br s, 1H, H-8); 3.96 (br s, 1H, H-1); 3.29 (s, 1H, OH); 2.73 (dd, J = 3.9 and 14.7 Hz, 1H, H-9a); 2.66 (dd, J = 3.0 and 14.7 Hz, 1H, H-9b), 1.87 (s, 3H, H-15) 1.56 (s, 3H, H-13), 1.47 (s, 3H, H-14). Angeloate 6.10 (qq, J = 1.5 and 7.5 Hz, 1H, H-3), 1.98 (dq, J = 1.5 and 7.2 Hz, 3H, H-4), 1.91 (dq, J = 1.5 Hz, 3H, Me-2), butyrate 2.10 (m, 2H, H-2), 1.55 (m, 2H, H-3), 0.91 (t, J = 7.2 Hz, 3H, H-4). Isopropylidene 1.53 (s, 3H, H-3) 1.42 (s, 3H, H-1). Octanoate 2.32 (dt, J = 3.0 and 7.5 Hz, 2H, H-2), 1.60 (m, 2H, H-3), 1.28 (m, 8H, H-4–H-7), 0.87 (t, J = 6.9 Hz, 3H, H-8).

For the data of **8a**, see below.

5.1.6. Triol (8a) (general procedure B)

Butanoate (**7a**) (18.2 mg, 0.028 mmol) was dissolved in MeOH (2 mL). Three molar of HCl (39 μL) was added dropwise under stirring and the mixture was heated to 45 °C for 45 min. The reaction mixture was cooled to room temperature and a saturated aqueous solution of $NaHCO_3$ (10 mL) and EtOAc (10 ml) was added. The layers were separated and the aqueous layer was extracted three times with EtOAc (10 mL). The combined organic layer was dried over Na_2SO_4, filtered and concentrated in vacuo. Compound **8a** (15.5 mg, 87%) was purified by column chromatography using heptanes–ethyl acetate (5:2) as an eluent.

^{1}H NMR ($CDCl_3$) δ (ppm) Guaianolide: 5.81 (br s, 1H, H-6); 5.69 (br s, 1H, H-3); 5.42 (dd, J = 3.3 and 3.6 Hz, 1H, H-2); 4.86 (s, 1H, OH); 4.36 (br s, 1H, H-8); 4.21 (br s, 1H, H-1); 4.05 (s, 1H, OH); 3.34 (s, 1H, OH); 2.80 (dd, J = 3.0 and 14.1 Hz, 1H, H-9a); 2.50 (dd, J = 3.3 and 14.4 Hz, 1H, H-9b), 1.84 (3H, m, H-15), 1.50 (3H, s, H-13); 1.45 (3H, s, H-14). Angeloate 6.11 (qq, J = 1.2 and 7.2 Hz, 1H, H-3), 1.98 (dq, J = 1.5 and 7.2 Hz, 3H, H-4), 1.91 (dq, J = 1.5 Hz, 3H, Me-2), butyrate 2.10 (m, 2H, H-2), 1.55 (m, 2H, H-3), 0.90 (t, J = 7.5 Hz, 3H, H-4). Octanoate 2.32 (m, 2H, H-2), 1.60 (m, 2H, H-3), 1.28 (m, 8H, H-4–H-7), 0.87 (t, J = 6.9 Hz, 3H, H-8).[27]

5.1.7. Dibutyrate (9a) (general procedure C)

Compound **8a** (44.6 mg, 0.073 mmol) was dissolved in dichoromethane (0.5 mL) and DMAP (1.8 mg, 0.015 mmol) and 800 μL of a solution of butanoic anhydride (150 μL) in dichloromethane (5.0 mL) was added. The mixture was stirred at room temperature for 1 h. The reaction was quenched by addition of a saturated aqueous ammonium chloride solution (30 mL) and ethyl acetate (30 mL). The layers were separated and the aqueous layer was extracted three times with ethyl acetate (15 mL). The combined organic layer was dried over Na_2SO_4, filtered and concentrated in vacuo. Compound **9a** (44.7 mg, 90%) was purified by column chromatography using heptane–ethyl acetate (9:2) as an eluent.

^{1}H NMR ($CDCl_3$) δ: Guaianloide 5.68 (m, 1H, H-3); 5.66 (m, 1H, H-6); 5.63 (dd, J = 3.6 Hz, 1H, H-8); 5.45 (t, J = 3.3 Hz, 1H, H-2); 4.29 (br s, 1H, H-1); 3.38 (s, 1H, OH); 3.02 (dd, J = 3.0 and 14.7 Hz, 1H, H-9a); 2.94 (s, 1H, OH); 2.20 (overlapped, H-9b), 1.87 (s, 3H, H-15); 1.48 (s, 3H, H-13); 1.40 (s, 3H, H-14). Angeloate 6.11 (qq, J = 1.5 and 6.9 Hz, 1H, H-3), 1.98 (dq, J = 1.5 and 7.5 Hz, 3H, H-4), 1.92 (dq, J = 1.5 Hz, 3H, Me-2), butyrate 2.10 (m, 4H, H-2), 1.55 (m, 4H, H-3), 0.94 and 0.89 (each t, J = 7.5 Hz, 3H, H-4). Octanoate 2.32 (m, 2H, H-2), 1.60 (m, 2H, H-3), 1.28 (m, 8H, H-4–H-7), 0.87 (t, J = 6.9 Hz, 3H, H-8). ^{13}C NMR ($CDCl_3$) δ Guaianolide: 175.5 (C-12), 141.3 (C-4), 130.3 (C-5), 84.5 (C-3); 84.1 (C-10); 78.4 (C-7 or C-11); 78.3 (C-7 or C-11); 77.8 (C-2); 76.8 (C-6); 66.0 (C-8); 57.2 (C-1); 38.2 (C-9), 23.0 (C-14), 15.8 (C-13), 12.9 (C-15). Angeloate 166.8 (C-1) 138.5 (C-3), 127.2 (C-2), 20.6 (Me-2), 15.9 (C-4). Butyrate 172.4 and 172.4 (C-1), 36.5 and 34.1 (C-2), 18.1 and 17.9 (C-3), 13.7 and 13.5 (C-4). Octanoate 173.4 (C-1), 37.3 (C-2) 31.6 (C-6), 29.0 (C-4), 29.0 (C-5), 24.7 (C-3), 22.6 (C-7), 14.1 (C-8). $[\alpha]_D^{25.2}$ = −31.2 (*c* 0.36, acetone). HRMS calcd for $C_{36}H_{54}NaO_{12}$ 701.3507, found 701.3496.

5.1.8. Dioctanoate (7c)

Synthesized according to the general procedure **A** using isopropylidene (**6a**) (50 mg, 0.085 mmol) and octanoic anhydride (27 mg, 0.100 mmol) as starting materials. Compound **7c** was purified by

column chromatography using heptane–ethyl acetate (12:1) as an eluent. The product was slightly contaminated with octanoic anhydride.

^{1}H NMR ($CDCl_3$) δ Guaianolide: 5.79 (1H, br s, H-6), 5.75 (br s, 1H, H-3), 5.46 (dd, J = and 5.4 Hz, 1H, H-2), 4.27 (dd, J = 3.6 Hz, 1H, H-8), 3.94 (br s, 1H, H-1), 2.70 (d, J = 3.6 Hz, 2H, H-9a), 1.87 (s, 3H, H-15, 1.56 (s, 3H, H-13) 1.47 (3H, s, H-14). Angeloate 6.10 (qq, J = 1.5 and 7.2 Hz, 1H, H-3), 1.99 (dq, J = 1.5 and 7.2 Hz, 3H, H-4), 1.92 (m, 3H, Me-2. Isopropylidene 1.53 (s, 3H, H-1), 1.42 (s, 3H, H-3). Octanoate 2.32 and 2.12 (m, each 2H, H-2), 1.60 (m, 4H, H-3), 1.28 (m, 16H, H-4–H-7), 0.88 (t, J = 6.9 Hz, 6H, H-8).

5.1.9. Triol (8c)

Synthesized according to the general procedure **B** using dioctanoate (**7c**) as starting material. Compound **8c** (40.4 mg, 70%) was purified by column chromatography using heptanes–ethyl acetate (3:1) as an eluent.

^{1}H NMR ($CDCl_3$) δ Guaianolide: 5.81 (br s, 1H, H-6); 5.68 (br s, 1H, H-3); 5.42 (dd, J = 3.3 Hz, 1H, H-2); 4.81 (s, 1H, OH); 4.35 (br s, 1H, H-8); 4.20 (br s, 1H, H-1); 4.02 (s, 1H, OH); 3.18 (s, 1H, OH); 2.81 (dd, J = 3.0 and 14.4 Hz, 1H, H-9a), 2.48 (dd, J = 3.3 and 14.4 Hz, 1H, H-9b), 1.84 (s, 3H, H-15), 1.50 (s, 3H, H-13); 1.44 (s, 3H, H-14). Angeloate 6.10 (qq, J = 1.5 and 7.5 Hz, 1H, H-3), 1.98 (dq, J = 1.5 and 7.2 Hz, 3H, H-4), 1.91 (t, J = 1.5 Hz, 3H, Me-2). Octanoate 2.32 and 2.12 (m, each 2H, H-2), 1.60 (m, 4H, H-3), 1.28 (m, 16H, H-4–H-7), 0.87 (t, J = 6.9 Hz, 6H, H-8).

5.1.10. Octanoate (9c)

Synthesized according to the general procedure **C** using triol **8c** (40.4 mg, 0.061 mmol) as starting material. Compound **9c** (44.0 mg, 98%) was purified by column chromatography using heptane–ethyl acetate (5:1) as an eluent.

^{1}H NMR ($CDCl_3$) δ: Guaianloide 5.68 (br s, 1H, H-3); 5.66 (br s, 1H, H-6); 5.63 (t, J = 3.9 Hz, 1H, H-8); 5.45 (t, J = 3.3 Hz, 1H, H-2); 4.28 (br s, 1H, H-1); 3.28 (s, 1H, OH); 3.00 (t, J = 3.3 and 14.7 Hz, 1H, H-9a), 2.91 (s, 1H, OH); 2.20 (overlapped, H-9), 1.87 (s, 3H, H-15); 1.57–1.71 (4H, m, –OCOCH$_2$C*H*$_2$-2-octanoyl and butanoyl); 1.49 (3H, s, H-13); 1.45–1.49 (2H, m, –OCOCH$_2$C*H*$_2$-10-octanoyl); 1.40 (3H, s, H-14). Angeloate 6.11 (qq, J = 1.2 and 7.2 Hz, 1H, H-3), 1.99 (dq, J = 1.5 and 7.5 Hz, 3H, H-4), 1.92 (dq, J = 1.5 Hz, 3H, Me-2), Butyrate 2.10 (m, 4H, H-2), 1.55 (m, 4H, H-3), 0.95 (t, J = 7.5 Hz, 3H, H-4). Octanoate 2.32 and 2.10 (each m, 2H, H-2), 1.60 (m, 4H, H-3), 1.28 (m, 8H, H-4–H-7), 0.87 (t, J = 6.9 Hz, 6H, H-8).

^{13}C NMR ($CDCl_3$) δ: Guaianloide 175.4 (C-12), 141.3 (C-5), 130.3 (C-4), 84.3 (C-10), 84.1 (C-3), 78.5 (C-7 or C-11), 78.4 (C-7 or C-11), 77.8 (C-2), 76.7 (C-6), 66.0 (C-8), 57.4 (C-1), 38.2 (C-9), 22.9 (C-14), 16.0 (C-13), 12.9 (C-15). Angeloate 166.9 (C-1) 138.5 (C-3), 127.2 (C-2), 20.6 (Me-2), 15.8 (*C*-4). Butyrate 172.4 (C-1), 36.5 (C-2), 18.0 (C-3), (C-4). Octanoate 173.4 and 172.4 (C-1), 35.4 and 34.1 (C-2) 31.7 (C-6), 29.1 and 29.0 (C-4), 29.0 and 28.9 (C-5), 24.7 (C-3), 22.6 and 22.6 (C-7), 14.1 (C-8). $[\alpha]_D^{26.2}$ = −32.2 (*c* 0.35, acetone). HRMS calcd for $C_{40}H_{62}NaO_{12}$ 757.4133, found 757.4126.

5.1.11. Biphenylacetate (7d)

Synthesized according to the general procedure **A** using isopropylidene **6a** (65.2 mg, 0.113 mmol) and 4-biphenylacetic anhydride as starting materials. The reaction mixture was stirred at room temperature over night. The crude reaction product was used in the next step.

5.1.12. Triol (8d)

Synthesized according to the general procedure **B** using 4-biphenylacetate (**7d**) as starting material. Compound **8d** (26.9 mg, 33%) was purified by column chromatography using heptane–ethyl acetate (7:1→5:1) as an eluent.

^{1}H NMR ($CDCl_3$) δ Guaianolide: 5.75 (br s, 1H, H-6); 5.67 (br s, 1H, H-3); 5.49 (t, J = 3.6 Hz, 1H, H-2); 4.71 (s, 1H, OH); 4.30 (br s, 1H, H-1); 4.21 (t, J = 3.0 Hz, 1H, H-8); 3.90 (s, 1H, OH); 3.00 (s, 1H, OH); 2.82 (dd, J = 2.4 and 13.5 Hz, 1H, H-9a), 2.20 (overlapped, H-9), 1.82 (s, 3H, H-15), 1.42 (s, 3H, H-13); 1.35 (s, 3H, H-14) l). Angeloate 6.11 (qq, J = 1.2 and 7.5 Hz, 1H, H-3), 1.99 (dq, J = 1.5 and 7.5 Hz, 3H, H-4), 1.91 (q, J = 1.5 Hz, 3H, Me-2). Biphenylacetate: 7.48–7.59 (m, 4H, H-8, H-9, H-11 and H-12); 7.38–7.43 (m, 2H, H-3 and H-5); 7.34 (m, 1H, H-10); 7.27 (m, 2H, H-2 and H-6), 3.55 (d, J = 15.6 Hz, 1H, H-αa); 3.47 (d, J = 15.6 Hz, 1H, H-αb). Octanoate 2.32 (m, 2H, H-2), 1.60 (m, 2H, H-3), 1.28 (m, 8H, H-4–H-7), 0.83 (t, J = 6.9 Hz, 6H, H-8).

5.1.13. Biphenylacetate (9d)

Synthesized according to the general procedure **C** using triol **8d** (26.9 mg, 0.037 mmol) as a starting material. Compound **9d** (18.5 mg, 63%) was purified by column chromatography using heptane–ethyl acetate (7:1) as an eluent.

^{1}H NMR ($CDCl_3$) δ: 5.68 (s, 1H, H-3), 5.63 (s, 1H, H-6), 5.57 (t, J = 3.6 Hz, 1H, H-8), 5.53 (dd, J = 3.0 Hz, 1H, H-2), 4.32 (br s, 1H, H-1), 3.07 (s, 1H, OH); 2.97 (dd, J = 3.0 and 15.0 Hz, 1H, H-9a), 2.73 (s, 1H, OH), 1.86 (s, 3H, H-15), 1.42 (s, 3H, H-13); 1.41 (s, 3H, H-14). Angeloate 6.11 (qq, J = 1.2 and 7.5 Hz, 1H, H-3), 2.01 (dq, J = 1.5 and 7.5 Hz, 3H, H-4), 1.92 (q, J = 1.5 Hz, 3H, Me-2). Biphenylacetate: 7.51–7.58 (m, 4H, H-8, H-9, H-11 and H-12); 7.39–7.45 (m, 2H, H-3 and H-5); 7.33 (m, 1H, H-10), 3.54 (d, J = 15.9 Hz, 1H, H-αa); 3.46 (d, J = 15.9 Hz, 1H, H-αb). Butyrate 2.20 (m, 2H, H-2), 1.55 (m, 2H, H-3), 0.91 (t, J = 7.5 Hz, 3H, H-4). Octanoate 2.32 (m, 2H, H-2), 1.60 (m, 2H, H-3), 1.28 (m, 8H, H-4–H-7), 0.84 (t, J = 6.9 Hz, 6H, H-8). ^{13}C NMR ($CDCl_3$) δ: 175.0 (C-12), 142.0 (C-4), 129.6 (C-5), 85.0 (C-10), 84.2 (C-3), 78.5 (C-7 and C-11), 77.7 (C-2), 77.4 (C-6), 65.9 (C-8), 58.0 (C-1), 38.2 (C-9), 23.0 (C-13), 16.1 (C-14), 13.1 (C-15). Angeloate 166.9 (C-1), 139.8 (C-3), 127.3 (C-2), 20.7 (Me-2), 15.9 (*C*-4). Biphenylacetate 171.2 (C=O), 140.6 (C-4), 138.8 (C-7), 132.7 (C-1), 130.2 (C-2 and C-6), 128.6 (C-3 and C-5), 127.2 and 126.9 (C-8–C12), 41.9 (C-α). Butyrate 172.5 (C-1), 36.5 (C-2), 18.0 (C-3), 13.8 (C-4). Octanoate 172.3 (C-1), 34.3 (C-2) 31.7 (C-6), 29.1 (C-4), 29.1 (C-5), 24.9 (C-3), 22.7 (C-7), 14.2 (C-8). HRMS calcd for $[C_{46}H_{58}NaO_{12}]^+$ 825.3820, found 825.3806.

5.1.14. Tetraol (10)

Synthesized according to the general procedure **B** using isopropylidene **6a** (50 mg, 0.086 mmol) as starting material. Compound **10** (41.4 mg, 89%) was purified by column chromatography using heptane–ethyl acetate (1:2) as an eluent.

^{1}H NMR ($CDCl_3$) δ: 5.80 (br s, 1H, H-6); 5.73 (br s, 1H, H-3); 5.37 (s, 1H, OH); 5.23 (t, J = 3.0 Hz, 1H, H-2), 4.28 (s, 2H, H-8 and OH), 3.79 (s, 1H, OH); 3.51 (br s, 1H, H-1); 3.43 (s, 1H, OH); 2.19 (dd, J = 1.2 and 13.5 Hz, 1H, H-9a); 2.04 (dd, J = 1.2 and 13.5 Hz, 1H, H-9b), 1.86 (s, 3H, H-15); 1.47 (s, 3H, H-13), 1.20 (s, 3H, H-14). Angeloate 6.14 (qq, J = 1.2 and 7.2 Hz, 1H, H-3), 1.98 (dq, J = 1.5 and 7.5 Hz, 3H, H-4), 1.89 (q, J = 1.5 Hz, 3H, Me-2). Octanoate 2.32 (m, 2H, H-2), 1.60 (m, 2H, H-3), 1.28 (m, 8H, H-4–H-7), 0.86 (t, J = 6.9 Hz, 6H, H-8). ^{13}C NMR ($CDCl_3$) δ Guaianolide: 61.9 (C-1), 78.8 (C-2), 83.5 (C-3), 141.1 (C-4), 131.3 (C-5), 76.7 (C-6), 78.6 (C-7), 66.3 (C-8), 44.9 (C-9), 72.8 (C-10), 78.6 (C-11), 174.9 (C-12), 16.4 (C-13), 24.8 (C-14), 13.1 (C-15). Angeloate: 166.9 (C-1), 127.1 (C-2), 139.4 (C-3), 16.0 (C-4), 20.0 (Me-2). Butyrate: 172.4 (C-1), 36.6 (C-2), 18.1 (C-3), 13.9 (C-4). Octanoate: 173.8 (C-1), 34.5 (C-2), 25.0 (C-3), 29.0 (C-4), 29.0 (C-5), 31.7 (C-6), 22.7 (C-7), 14.2 (C-8). HRMS calcd for $[C_{32}H_{48}NaO_{11}]^+$: 631.3089, found 631.3073.

5.1.15. Triol (11a)

Synthesized according to the general procedure **C** using tetraol **10** (41.4 mg, 0.077 mmol) as starting material. Compound **11a**

(38.2 mg, 82%) was purified by column chromatography using heptane–ethyl acetate (3:1) as an eluent.

^{1}H NMR ($CDCl_3$) δ Guaianolide: 5.73 (s, 1H, H-6); 5.68 (s, 1H, H-3); 5.55 (dd, J = 3.3 Hz, 1H, H-8); 5.24 (dd, J = 2.4 Hz, 1H, H-2), 3.76 (s, 1H, OH), 3.57 (br s, 1H, H-1), 3.40 (s, 1H, OH), 3.05 (s, 1H, OH), 2.35 (overlapped, H-9a), 1.97 (dd, J = 3.9 and 11.4 Hz, 1H, H-9b), 1.88 (s, 3H, H-15), 1.47 (s, 3H, H-13), 1.16 (s, 3H, H-14). Angeloate 6.14 (qq, J = 1.5 and 7.2 Hz, 1H, H-3), 2.00 (dq, J = 1.8 and 6.9 Hz, 3H, H-4), 1.90 (q, J = 1.5 Hz, 3H, Me-2). Butyrate 2.26 (t, J = 7.5 Hz, 2H, H-2), 1.60 (m, 2H, H-3), 0.93 (t, J = 7.5 Hz, 3H, H-4). Octanoate 2.32 (m, 2H, H-2), 1.60 (m, 2H, H-3), 1.28 (m, 8H, H-4–H-7), 0.87 (t, J = 6.9 Hz, 6H, H-8). ^{13}C NMR ($CDCl_3$) δ: Guaianolide: 61.9 (C-1), 78.8 (C-2), 83.5 (C-3), 141.1 (C-4), 131.3 (C-5), 76.7 (C-6), 78.6 (C-7), 66.3 (C-8), 44.9 (C-9), 72.8 (C-10), 78.6 (C-11), 174.9 (C-12), 16.4 (C-13), 24.8 (C-14), 13.1 (C-15). Angeloate: 166.9 (C-1), 127.1 (C-2), 139.4 (C-3), 16.0 (C-4), 20.0 (Me-2). Butyrate: 172.4 (C-1), 36.6 (C-2), 18.1 (C-3), 13.9 (C-4). Octanoate: 173.8 (C-1), 34.5 (C-2), 25.0 (C-3), 29.0 (C-4), 29.0 (C-5), 31.7 (C-6), 22.7 (C-7), 14.2 (C-8). HRMS calcd for $C_{32}H_{48}NaO_{11}^+$: 631.3089, found 631.3073.

5.1.16. Dioctanoate (11b)

Synthesized according to the general procedure **C** using tetraol **10** (41.4 mg, 0.077 mmol) as starting material. Compound **11b** (40 mg, 80%) was purified by column chromatography using heptane–ethyl acetate (3:1) as an eluent. ^{1}H NMR data ($CDCl_3$): δ Guaianolide: 3.56 (br s, 1H, H-1), 5.24 (br s, 1H, H-2), 5.74 (br s, 1H, H-3), 5.68 (s, 1H, H-6), 5.55 (bf s, 1H, H-8), 2.30 (overlapped, 1H, H-9a), 1.96 (overlapped, 1H, H-9b), 1.48 (s, 3H, H-13), 1.17 (s, 3H, H-14), 1.90 (br s, 1H, H-15), 3.77, 3.24, 2.89 (each s, 1H, OH). Angeloate: 6.16 (dq, J = 7.0 and 1.5 Hz, 1H, H-3), 2.02 (overlapped, 3H, H-4), 1.99 (br s, 1H, 3H, Me-2). 2 times octanoate: 2.35 and 2.28 (overlapped, 4H, H-2), 1.61 and 1.59 (overlapped, 4H, H-3), 1.28 (br s, 16H, H-4–H-7), 0.88 (t, J = 7.5 Hz, 6H, H-8). ^{13}C NMR ($CDCl_3$) δ Guaianolide: 61.6 (C-1), 79.1 (C-2), 83.5 (C-3), 140.5 (C-4), 131.5 (C-5), 76.9 (C-6), 78.6 (C-7), 66.3 (C-8), 44.6 (C-9), 72.9 (C-10), 78.6 (C-11), 175.3 (C-12), 16.3 (C-13), 24.9 (C-14), 13.0 (C-15). Angeloate: 167.0 (C-1), 127.1 (C-2), 139.5 (C-3), 16.0 (C-4), 20.7 (Me-2). Two times octanoate: 175.0 and172.8 (C-1), 35.0 and 34.9 (C-2), 24.8 and 24.6 (C-3), 29.1 and 29.0 (C-4), 29.2 (C-5), 31.7 (C-6), 22.7 (C-7), 14.2 (C-8), HRMS calcd for $C_{36}H_{56}NaO_{11}^+$: 687.3715, found 687.3693.

5.1.17. Benzoate (12a)

Synthesized according to the general procedure **A** using triol (**11a**) (38.2 mg, 0.063 mmol) as starting material and stirring for 10 days. Compound **12** (17.3 mg, 39%) was purified by column chromatography over silica gel using heptane–ethyl acetate (4:1) as an eluent followed by column chromatography over LiChrosorp RP-18 using methanol–water (6:1) as an eluent.

^{1}H NMR ($CDCl_3$) δ: Guaianloide 5.74–5.77 (overlapped, 3H, H-3, H-6 and H-8), 5.52 (dd, J = 3.9 and 4.8 Hz, 1H, H-2), 4.68 (br s, 1H, H-1), 3.63 (dd, J = 3.0 and 15.0 Hz, 1H, H-9a); 3.38 (s, 1H, OH), 3.15 (s, 1H, OH), 2.18 (dd, J = 3.6 and 14.7 Hz, 1H, H-9b), 1.87 (s, 3H, H-15), 1.56 (s, 3H, H-14); 1.52 (s, 3H, H-13). Angeloate 6.07 (qq, J = 1.2 and 7.2 Hz, 1H, H-3), 1.97 (dq, J = 1.5 and 7.2 Hz, 3H, H-4), 1.92 (q, J = 1.5 Hz, 3H, Me-2). Benzoate 7.87 (m, 2H, H-2 and H-6), 7.52 (m, 1H, H-4); 7.39 (m, 2H, H-3 and H-5). Butyrate 2.32 (t, J = 7.2 Hz, 2H, H-2), 1.60 (m, 2H, H-3), 0.95 (t, J = 7.2 Hz, 3H, H-4). Octanoate 2.00 (m, 2H, H-2), 1.60 (m, 2H, H-3), 1.20 (m, 8H, H-4–H-7), 0.85 (t, J = 7.2 Hz, 6H, H-8).

^{13}C NMR ($CDCl_3$) δ Guaianolide: 175.2 (C-12), 141.4 (C-4), 129.0 (C-5), 85.3 (C-10); 83.3 (C-3); 78.5 (C-7 and C-11); 78.3 (C-2); 77.1 (C-6); 66.1 (C-8); 54.6 (C-1); 38.4 (C-9), 23.7 (C-13), 16.1 (C-14), 13.0 (C-15). Angeloate 166.7 (C-1), 138.2 (C-3), 127.3 (C-2), 20.6 (Me-2), 15.7 (C-4). Benzoate 165.6 (C=O), 137.9 (C-4), 129.3 (C-2 and C-6), 128.2 (C-3 and C-5). Butyrate 172.3 (C-1), 36.6 (C-2), 18.0 (C-3), 13.7 (C-4). Octanoate 172.5 (C-1), 33.6 (C-2) 31.5 (C-6), 28.8 (C-4), 28.2 (C-5), 24.2 (C-3), 22.6 (C-7), 14.1 (C-8). HRMS calcd for $[C_{39}H_{52}NaO_{12}]^+$ 735.3351, found 735.3330.

5.1.18. 4-Phenylbenzoate (12b)

To a solution of **11a** (20 mg, 33 μmol) and DMAP (10 mg, 82 μmol) in dichloromethane (1 mL) was added 4-phenylbenzoic anhydride (110 mg, 290 μmol). The solution was stirred at room temperature overnight, filtered and concentrated. Compound **12b** (6 mg, 25%) was isolated from the residue by column chromatography over silica gel using toluene–ethyl acetate (3:1) added acetic acid (0.5%) as an eluent. ^{1}H NMR data ($CDCl_3$): δ Guaianolide: 4.70 (br s, 1H, H-1), 5.56 (dd, J = 4.3 and 3.8 Hz, 1H, H-2), 5.76 (br s, 1H, H-3), 5.76 (s, 1H, H-6), 5.79 (overlapped, 1H, H-8), 3.65 (dd, J = 13.9 and 2.06 Hz, 1H, H-9a), 2.22 (dd, J = 13.9 and 4.1 Hz, 1H, H-9b), 1.55 (s, 3H, H-13), 1.59 (s, 3H, H-14), 1.93 (br s, 1H, H-15). Angeloate: 6.10 (dq, J = 7.3 and 1.5 Hz, 1H, H-3), 1.99 (dd, J = 7.4 and 2.3 Hz, 3H, H-4), 1.93 (br s, 1H, 3H, Me-2). Butanoate: 2.32 (t, 7.3 Hz,, 2H, H-2), 1.66 (overlapped, 2H, H-3), 0.97 (t, J = 7.2 Hz, 3H, H-4). Octanoate: 2.32 (overlapped, 2H, H-2), 1.61 (overlapped, 2H, H-3), 1.28 (br s, 8H, H-4–H-7), 0.78 (t, J = 7.0 Hz, 3H, H-8). 4-Phenylbenzoate 8.10 (m, 2H, H2 and H6), 7.61 (m, 2H, H-3 and H-5), 7.61 (m, 2H, H-8 and H-12), 7.46 (m, 3H, H-9, H-10 and H-11). ^{13}C NMR ($CDCl_3$) δ Guaianolide: 54.7 (C-1), 78.4 (C-2), 83.9 (C-3), 141.6 (C-4), 138.4 (C-5), 77.2 (C-6), 78.6 (C-7), 66.3 (C-8), 38.5 (C-9), 85.4 (C-10), 78.6 (C-11), 175.4 (C-12), 15.8 (C-13), 23.7 (C-14), 13.0 (C-15). Angeloate: 165.7 (C-1), 129.3 (C-2), 139.7 (C-3), 16.2 (C-4), 20.6 (Me-2). Butyrate: 172.3 (C-1), 33.8 (C-2), 18.1 (C-3), 13.8 (C-4). Octanoate: 172.8 (C-1), 36.6 (C-2), 24.3 (C-3), 29.0 (C-4), 29.7 (C-5), 31.6 (C-6), 22.5 (C-7), 13.7 (C-8). 4-Phenylbenzoate 167.0 (C=O), 129.1 (C-1), 128.9 (C-2 and C-6), 127.1 (C-3 and C-5), 145.8 (C-4), 129.3 (C-7), 127.2 (C-8 and H-12), 127.5 (H-9 and H-11), 130.0 (H-10). HRMS calcd for $C_{45}H_{56}NaO_{12}^+$: 811.3664, found 811.3638.

5.1.19. Compound (6b)

To a solution of **5** (53 mg, 0.12 mmol) and DMAP (23 mg, 0.19 mmol) in DCM (9 mL) was added a solution of benzoic anhydride (81.5 mg, 0.36 mmol) in dichloromethane (1 mL). After stirring for 20 h a saturated aqueous solution of ammonium chloride (15 mL) and ethyl acetate (15 mL) was added. The aqueous phase was extracted two times with ethyl acetate (15 mL) and the combined organic phases were dried (sodium sulfate) and concentrated. Compound **6b** (31 mg, 48%) was isolated by column chromatography over LiChroprep RP-18 using methanol–water (4:1) as an eluent. ^{1}H NMR data ($CDCl_3$): δ Guaianolide: 3.56 (br s, 1H, H-1), 5.58 (br t, J = 4.5 Hz, 1H, H-2), 5.98 (br s, 1H, H-3), 5.83 (s, 1H, H-6), 4.19 (br t, J = 3 Hz, 1H, H-8), 2.20 (dd, J = 14 and 2 Hz, H-9a), 2.03 (dd, J = 14 and 2 Hz, 1H, H-9b), 1.28 (s, 3H, H-13), 1.52 (s, 3H, H-14), 1.93 (br s, 1H, H-15), 3.22 (1H, OH). Angeloate: 6.11 (q, J = 7.1 Hz, 1H, H-3), 2.00 (d, J = 7 Hz, 3H, H-4), 1.96 (br s, 1H, 3H, Me-2). Benzoate: 8.02 (m, 2H, H-2 and H-6), 7.54 (m, 1H, H-4), 7.40 (m, 2H, H-3 and H-5), Isopropanylen: 1.51 (s, 3H, Me-β), 1.42 (s, 3H, Me-α).

5.1.20. Compound (7b)

To a solution of **6b** (29 mg, 52 mmol) in isopropenyl acetate (2 mL) was added *p*-toluenesulfornic acid (30 mg). After 2 h a solution of sodium hydrogen carbonate (M, 15 mL) and ethyl acetate (15 mL) was added. The aqueous phase was extracted two times with ethyl acetate (15 mL) and the combined organic phases dried and concentrated. Compound **7b** (25 mg, 79%) was isolated from the residue by column chromatography over Sigel using toluene–ethyl acetate (3:1) as an eluent. ^{1}H NMR data ($CDCl_3$): δ Guaianolide: 4.17 (br s, 1H, H-1), 5.74 (br t, J = 6 Hz, 1H, H-2), 5.91 (br s,

1H, H-3), 5.83 (s, 1H, H-6), 4.25 (br t, J = 3 Hz, 1H, H-8), 2.81 (dd, J = 14 and 2 Hz, H-9a), 2.57 (dd, J = 14 and 2 Hz, 1H, H-9b), 1.53 (s, 3H, H-13), 1.43 (s, 3H, H-14), 1.91 (br s, 1H, H-15), 3.51 (1H, OH). Acetate: 1.61 (s, 3H, H-2). Angeloate: 6.1 (qq, J = 6.1 and 1.5 Hz, 1H, H-3), 2.00 (d, J = 7 Hz, 3H, H-4), 1.96 (br s, 1H, 3H, Me-2). Benzoate: 8.03 (m, 2H, H-2 and H-6), 7.56 (m, 1H, H-4), 7.42 (m, 2H, H-3 and H-5), Isopropanylen: 1.51 (s, 3H, Me-β), 1.43 (s, 3H, Me-α).

5.1.21. Compound (8b)

To a solution of **7b** (25 mg, 0.41 mmol) in methanol (1.5 mL) was added 75 μmol hydrogen chloride and the solution was left for 2 h at 45 °C. The reaction mixture was added a saturated aqueous solution of sodium acetate (10 mL) and ethyl acetate (10 mL). The aqueous phase was three times extracted with ethyl acetate (10 mL) and the combined organic phases concentrated and dried (sodium sulfate). Compound **8b** (17 mg, 74%) was isolated by column chromatography over LiChroprep RP-18 using methanol–water (3:1) as an eluent. ^{1}H NMR data ($CDCl_3$): δ Guaianolide: 4.40 (br s, 1H, H-1), 5.72 (br t, J = 4.5 Hz, 1H, H-2), 5.90 (br s, 1H, H-3), 5.84 (s, 1H, H-6), 4.34 (br t, J = 3 Hz, 1H, H-8), 2.90 (dd, J = 15 and 3 Hz, H-9a), 2.47 (dd, J = 15 and 3 Hz, 1H, H-9b), 1.48 (s, 3H, H-13), 1.51 (s, 3H, H-14), 1.88 (br s, 1H, H-15), 4.07 and 3.33 (each 1H, OH). Acetate: 1.70 (s, 3H, H-2). Angeloate: 6.12 (qq, J = 6.1 and 1.5 Hz, 1H, H-3), 1.99 (d, J = 7 Hz, 3H, H-4), 1.95 (br s, 1H, 3H, Me-2). Benzoate: 8.01 (m, 2H, H-2 and H-6), 7.55 (m, 1H, H-4), 7.42 (m, 2H, H-3 and H-5).

5.1.22. Compound (9b)

To a solution of **8b** (17 mg, 0.30 mmol) and DMAP (9 mg, 7 mmol) in DCM (5 mL) was added butanoic anhydride (10 mg, 0.70 mmol) dissolved in dichloromethane (1 mL). After stirring for 75 min a saturated solution of ammonium chloride (15 mL) and ethyl acetate (15 mL) was added. The aqueous phase was extracted two times with ethyl acetate (15 mL) and the combined organic phases were dried (sodium sulfate) and concentrated. Compound **9b (**13 mg, 66%) was isolated by column chromatography over LiChroprep RP-18 using methanol–water (5:1) as an eluent: ^{1}H NMR data ($CDCl_3$): δ Guaianolide: 4.48 (br s, 1H, H-1), 5.76 (br t, J = 4 Hz, 1H, H-2), 5.90 (br s, 1H, H-3), 5.71 (s, 1H, H-6), 5.66 (br t, J = 2 Hz, 1H, H-8), 3.14 (dd, J = 14 and 2 Hz, H-9a), 2.31 (dd, J = 14 and 2 Hz, 1H, H-9b), 1.47 (s, 3H, H-13), 1.48 (s, 3H, H-14), 1.86 (br s, 1H, H-15), 2.80 and 3.51 (each 1H, OH). Angeloate: 6.12 (q, J = 7.1 Hz, 1H, H-3), 2.00 (d, J = 7 Hz, 3H, H-4), 1.96 (br s, 1H, 3H, Me-2). Acetate: 1.90 (s, 3H, H-2), Benzoate: 8.02 (m, 2H, H-2 and H-6), 7.42 (m, 2H, H-3 and H-5), 7.51 (m, 1H, H-4). Butyrate: 2.28 (t, J = 7.5 Hz, 2H, H-2), 1.63 (sex, J = 7.5 Hz, 2H, H-3), 0.95 (t, J = 7.5 Hz, 3H, H-4). ^{13}C NMR ($CDCl_3$) δ Guaianolide: 56.7 (C-1), 78.9 (C-2), 83.9 (C-3), 141.8 (C-4), 129.8 (C-5), 77.4 (C-6), 78.9 (C-7), 66.4 (C-8), 38.6 (C-9), 84.9 (C-10), 79.1 (C-11), 175.5 (C-12), 16.6 (C-13), 23.3 (C-14), 13.4 (C-15). Angeloate: 167.2 (C-1), 133.3 (C-2), 138.7 (C-3), 16.2 (C-4), 20.9 (Me-2). Acetate: 171.1 (C.-1), 22.8 (C-2). Butyrate: 172.8 (C-1), 36.9 (C-2), 18.3 (C-3), 14.1 (C-4). Benzoate: 165.7 (C-1), 127.7 (C-2), 129.9 (C-3 and C-7), 128.6 (C-4 and C-6), 133.3 (C-5). HRMS calcd for $C_{33}H_{40}NaO_{12}^+$: 651.2412, found 651.2408.

5.1.23. Compounds (8a, 13a, and 14)

To a solution of **7a** (78 mg, 135 nmol) in isopropenyl acetate (5 mL) is added *p*-TsOH acid (83 mg, 83 nmol) and the mixture is left for 105 min. The reaction is quenched by addition of a saturated aqueous solution of sodium hydrogen carbonate (20 mL). The mixture is extracted three times with ethyl acetate (10 mL) and the combined organic phases dried (sodium sulfate) and concentrated. The residue was fractionated by column chromatography over LiChroprep RP-18 using methanol–water (6:1) as an eluent to give compound **8a** (14 mg, 16%), compound **14** (16 mg, 17%), and compound **13a** (14 mg, 16%).

Compound **14**: ^{1}H NMR data ($CDCl_3$): δ Guaianolide: 4.25 (br s, 1H, H-1), 5.43 (dd, J = 3.5 and 2.1 Hz, 1H, H-2), 5.66 (br s, 1H, H-3), 5.66 (s, 1H, H-6), 5.48 (t, J = 3.6 Hz, 1H, H-8), 2.87 (dd, J = 14.9 and 3.5 Hz, 1H, H-9a), 2.49 (dd, J = 14.9 and 3.5 Hz, 1H, H-9b), 1.67 (s, 3H, H-13), 1.36 (s, 3H, H-14), 1.88 (br s, 1H, H-15), 3.50 (s, 1H, OH). Angeloate: 6.11 (dq, J = 7.2 and 1.3 Hz, 1H, H-3), 2.00 (dd, J = 7.2 and 1.3 Hz, 3H, H-4), 1.91 (br s, 1H, 3H, Me-2). Three times butyrate: 2.16–2.35 (overlapped, 6H, H-2), 1.75–1.45 (overlapped, 6H, H-3), 1.03–0.90 (t, overlapped, 9H, H-4). Octanoate: 2.10 (overlapped, 2H, H-2), 1.61 (overlapped, 2H, H-3), 1.28 (br s, 8H, H-4–H-7), 0.90 (t, J = 7.5 Hz, 3H, H-8). ^{13}C NMR ($CDCl_3$) δ Guaianolide: 57.9 (C-1), 77.2 (C-2), 83.8 (C-3), 142.0 (C-4), 129.5 (C-5), 77.8 (C-6), 78.9 (C-7), 67.6 (C-8), 37.4 (C-9), 84.0 (C-10), 82.0 (C-11), 173.0 (C-12), 18.4 (C-13), 20.7 (C-14), 13.1 (C-15). Angeloate: 166.9 (C-1), 129.5 (C-2), 138.7 (C-3), 16.0 (C-4), 22.7 (Me-2). Three times butyrate: 172.3, 172.2, 170.9 (C-1), 36.1, 36.0, 34.3 (C-2), 18.4, 18.4, 18.1, (C-3), 13.8, 13.7, 13.5 (C-4). Octanoate: 172.3 (C-1), 37.4 (C-2), 24.8 (C-3), 29.2 (C-4), 29.2 (C-5), 31.7 (C-6), 22.7 (C-7), 14.2 (C-8), HRMS calcd for $C_{40}H_{60}NaO_{13}^+$: 771.3926, found 771.3912.

Compound **13a**: ^{1}H NMR data ($CDCl_3$): δ Guaianolide: 3.70 (br s, 1H, H-1), 5.42 (br t, J = 4.3 Hz, 1H, H-2), 5.77 (br s, 1H, H-3), 5.94 (s, 1H, H-6), 5.04 (br t, J = 3.5 Hz, 1H, H-8), 2.66 (d, J = 3.5 Hz, 2H, H-9), 1.56 (s, 3H, H-13), 1.49 (s, 3H, H-14), 1.90 (br s, 1H, H-15). Angeloate: 6.10 (dq, J = 7.2 and 1.4 Hz, 1H, H-3), 2.00 (dd, J = 7.4 and 1.2 Hz, 3H, H-4), 1.92 (br s, 1H, 3H, Me-2). Two times butyrate: 2.39 and 2.08 (overlapped, 4H, H-2), 1.8–1.5 (overlapped, 4H, H-3), 0.95 (t, J = 7.2 Hz, 6H, H-4). Octanoate: 2.28 (overlapped, 2H, H-2), 1.61 (overlapped, 2H, H-3), 1.28 (br s, 8H, H-4–H-7), 0.88 (t, J = 7.5 Hz, 3H, H-8). Isopropanylen: 1.58 (s, 3H, Me-β), 1.43 (s, 3H, Me-α). ^{13}C NMR ($CDCl_3$) δ Guaianolide: 56.8 (C-1), 78.5 (C-2), 83.7 (C-3), 138.5 (C-4), 127.4 (C-5), 76.4 (C-6), 83.6 (C-7), 63.6 (C-8), 38.9 (C-9), 84.0 (C-10), 78.0 (C-11), 172.6 (C-12), 17.2 (C-13), 22.6 (C-14), 12.5 (C-15). Angeloate: 167.2 (C-1), 127.4 (C-2), 138.5 (C-3), 15.8 (C-4), 20.6 (Me-2). Two times butyrate: 171.1 and 171.1 (C-1), 37.3 and 36.4 (C-2), 18.3 and 18.1 (C-3), 13.6 (C-4). Octanoate: 172.4 (C-1), 34.3 (C-2), 24.7 (C-3), 29.0 (C-4), 29.1 (C-5), 31.7 (C-6), 20.6 (C-7), 14.2 (C-8). Isopropanylen: 101.2 (C-2) 30.3 (C-1), 22.0 (C-3), HRMS calcd for $C_{39}H_{58}NaO_{12}^+$: 741.3820, found 741.3797.

5.1.24. Compound (13b)

To a solution of **5** (42 mg, 73 μmol) in isopropenyl octanoate (2 mL) is added *p*-TsOH acid (39 mg, 0.22 mmol) and the solution is left for 70 h. The reaction was quenched with a saturated aqueous solution of sodium hydrogen carbonate (15 mL). The mixture was extracted three times with ethyl acetate (15 mL) and the organic phases were combined, dried (sodium sulfate) and concentrated. Compound **13b** (27 mg, 445 μmol) was isolated from the residue by column chromatography over LiChroprep RP-18 using methanol–water (25:1) as an eluent.

^{1}H NMR data ($CDCl_3$): δ Guaianolide: 3.71 (br s, 1H, H-1), 5.42 (br s, 1H, H-2), 5.78 (br s, 1H, H-3), 5.94 (s, 1H, H-6), 5.06 (br s, 1H, H-8), 2.66 (m, 1H, H-9a), 2.46 (dd, J = 14.9 and 3.6 Hz, 1H, H-9b), 1.58 (s, 3H, H-13), 1.43 (s, 3H, H-14), 1.89 (br s, 1H, H-15). Angeloate: 6.09 (dq, J = 7.0 and 1.5 Hz, 1H, H-3), 1.99 (qd, J = 7.0 and 1.5 Hz, 3H, H-4), 1.92 (br s, 1H, 3H, Me-2). Isopropylidene 1.56 (s, 1H, H-1), 1.48 (s, 3H, H-3). Three times octanoate: 2.4–2.1 (overlapped, 6H, H-2), 1.6 (overlapped, 6H, H-3), 1.26 (br s, 24H, H-4–H-7), 0.88 (t, J = 7.5 Hz, 9H, H-8).

5.1.25. Compound (15)

To a solution of thapsigargin (**1a**, Tg, 500 mg, 0.786 mmol) and 4-methylmorpholino N-oxide (106 mg, 0.905 mmol) in acetone–

water (3:1, 4 mL) was added osmium tetraoxide (0.153 mL, 15 μmol) and the solution was stirred for 4 h at room temperature. To the bright orange solution was added sodium periodate (496 mg, 2.3 mmol) and the solution was left under stirring for the night. The reaction mixture was quenched by adding an aqueous solution of sodium thiosulfate (1 M, 2 mL) and water (1 mL). The greenish mixture was filtered and the filtrate acidified with M hydrochloric acid and extracted with ethyl acetate until a colorless aqueous phase was obtained. The combined organic phases were dried (sodium sulfate) and concentrated in vacuo. The residue was dissolved in methanol (7.5 mL), pyridine (6 mL), and water (3.7 mL), and the solution was refluxed over night. Compound **15** (191 mg, 44%) was isolated from the residue of the reaction mixture by column chromatography over silica gel using toluene–ethyl acetate (4:1) added 1% acetic acid as an eluent.

^{1}H NMR data ($CDCl_3$): δ Guaianolide: 4.35 (br s, 1H, H-1), 4.88 (dd, J = 3.3 and 4.4 Hz, 1H, H-2), 4.41 (br s, 1H, H-3), 5.66 (s, 1H, H-6), 5.64 (t, J = 3.30 Hz, 1H, H-8), 3.24 (dd, J = 14.7 and 3.3 Hz, 1H, H-9a), 2.16 (overlapped, H-9b), 1.47 (s, 3H, H-13), 1.37 (s, 3H, H-14), 1.94 (br s, 1H, H-15). Acetate 1.98 (s, 3H, H-2). Butanoate: 2.4 (overlapped, H-2), 1.6 (overlapped, H-3), 0.94 (t, J = 7.2 Hz, 3H, H-4). Octanoate: 2.3 (overlapped, 2H, H-2), 1.61 (overlapped, 2H, H-3), 1.28 (br s, 8H, H-4–H-7), 0.87 (t, J = 7.0 Hz, 3H, H-8). ^{13}C NMR ($CDCl_3$) δ Guaianolide: 55.6 (C-1), 85.0 (C-2), 84.0 (C-3), 144.5 (C-4), 126.6 (C-5), 77.4 (C-6), 78.9 (C-7), 66.4 (C-8), 38.5 (C-9), 85.0 (C-10), 78.9 (C-11), 175.4 (C-12), 16.6 (C-13), 23.7 (C-14), 13.3 (C-15). Acetate 170.5 (C-1), 23.0 (C-2). Butyrate: 172.8 (C-1), 34.5 (C-2), 18.3 (C-3), 14.1 (C-4). Octanoate: 176.0 (C-1), 36.8 (C-2), 25.2 (C-3), 29.4 (C-4), 29.3 (C-5), 32.0 (C-6), 22.9 (C-7), 14.1 (C-8). HRMS calcd for $C_{29}H_{44}NaO_{12}^+$: 591.2776, found 591.2774.

5.1.26. Compound (16a)

To a solution of compound **15** (25 mg, 43 μmol) and DMAP (14 mg, 115 μmol) in dichloromethane (3 mL) was added benzoic anhydride (19.8 mg, 0.88 mmol). The solution was stirred for 2 days at room temperature, concentrated and the residue dissolved in ethyl acetate (3 mL). The solution was washed with M hydrochloric acid (2 mL), the organic phase dried over sodium sulfate and concentrated. Compound **16a** (21.4 mg, 74%) was isolated from the residue by column chromatography over silica gel using toluene–ethyl acetate (9:1) as an eluent. ^{1}H NMR data ($CDCl_3$): δ Guaianolide: 4.39 (br s, 1H, H-1), 5.58 (t, J = 2.9, 1H, H-2), 5.83 (br s, 1H, H-3), 5.69 (s, 1H, H-6), 5.67 (t, J = 3.30 Hz, 1H, H-8), 3.12 (dd, J = 14.3 and 2.2 Hz, 1H, H-9a), 2.39 (overlapped, 1H, H-9b), 1.49 (s, 3H, H-13), 1.46 (s, 3H, H-14), 1.90 (br s, 1H, H-15). Acetate 1.90 (s, 3H, H-2). Benzoate 8.05 (m, 2H, H-2 and H-6), 7.58 (m, 1H, H-4), 7.45 (m, 2H, H-3 and H-5). Butanoate: 2.3 (overlapped, H-2), 1.6 (overlapped, H-3), 0.95 (t, J = 7.3 Hz, 3H, H-4). Octanoate: 2.3 (overlapped, 2H, H-2), 1.61 (overlapped, 2H, H-3), 1.28 (br s, 8H, H-4–H-7), 0.86 (t, J = 7.0 Hz, 3H, H-8). ^{13}C NMR ($CDCl_3$) δ Guaianolide: 57.8 (C-1), 77.1 (C-2), 85.0 (C-3), 141.6 (C-4), 129.5 (C-5), 77.8 (C-6), 78.9 (C-7), 66.4 (C-8), 38.6 (C-9), 85.4 (C-10), 78.9 (C-11), 175.8 (C-12), 16.4 (C-13), 23.7 (C-14), 13.3 (C-15). Acetate 171.7 (C-1), 23.0 (C-2). Benzoate 166.0 (C═O), 131.0 (C-1), 130.0 (C-2 and C-6), 128.6 (C-3 and C-5), 133.4 (C-4). Butyrate: 172.8 (C-1), 34.5 (C-2), 18.4 (C-3), 14.1 (C-4). Octanoate: 172.7 (C-1), 36.9 (C-2), 25.2 (C-3), 29.3 (C-4), 29.5 (C-5), 32.0 (C-6), 23.1 (C-7), 14.5 (C-8). HRMS calcd for $C_{36}H_{48}NaO_{12}^+$: 695.3038, found 695.3021.

5.1.27. Compound (16b)

Compound **16b** (24 mg, 53%) was prepared as described above for compound **16a** using 3-methylbenzoic anhydride (30 mg, 0.12 mmol), DMAP (13 mg, 0.1 mmol) and **15** (38.3 mg, 66 μmol) as starting materials.

^{1}H NMR data ($CDCl_3$): δ Guaianolide: 4.37 (br s, 1H, H-1), 5.58 (t, J = 3.3, 1H, H-2), 5.84 (br s, 1H, H-3), 5.70 (br s, 1H, H-6), 5.67 (overlapped, 1H, H-8), 3.10 (br d, J = 14.7, 1H, H-9a), 2.3 (overlapped, 1H, H-9b), 1.50 (s, 3H, H-13), 1.48 (s, 3H, H-14), 1.91 (br s, 1H, H-15). Acetate 1.91 (s, 3H, H-2). Butanoate: 2.3 (overlapped, 2H, H-2), 1.6 (overlapped, H-3), 0.96 (t, J = 7.5 Hz, 3H, H-4). 3-Methylbenzoate: 7.84 (m, 1H, H-2 and H-6), 7.40 (m, 3H, H-4, and H-5), 2.41 (s, 3H, Me). Octanoate: 2.3 (overlapped, 2H, H-2), 1.6 (overlapped, 2H, H-3), 1.3 (br s, 8H, H-4–H-7), 0.86 (t, J = 6.6 Hz, 3H, H-8). ^{13}C NMR ($CDCl_3$) δ Guaianolide: 57.9 (C-1), 77.9 (C-2), 85.3 (C-3), 141.6 (C-4), 130.5 (C-5), 77.1 (C-6), 78.8 (C-7), 66.4 (C-8), 38.6 (C-9), 84.9 (C-10), 78.9 (C-11), 175.8 (C-12), 16.5 (C-13), 23.5 (C-14), 13.4 (C-15). Acetate 171.1 (C-1), 23.0 (C-2). Butyrate: 172.8 (C-1), 36.9 (C-2), 18.4 (C-3), 14.1 (C-4). 3-Methylbenzoate 166.2 (C═O), 129.8 (C-1), 130.5 (C-2), 138.9 (C-3), 134.2 (C-4), 128.5 (C-5), 127.1 (C-6), 21.7 (Me). Octanoate: 172.7 (C-1), 34.6 (C-2), 25.2 (C-3), 29.3 (C-4), 29.4 (C-5), 32.0 (C-6), 23.0 (C-7), 14.7 (C-8). HRMS calcd for $C_{37}H_{50}NaO_{12}^+$: 709.3194, found 709.3172.

5.1.28. Compound (16c)

Compound **16c** (45 mg, 85%) was prepared as described above for compound **16a** using 4-biphenylacetic anhydride (56.3 mg, 0.139 mmol), DMAP (11.6 mg, 94 μmol) and **15** (40.5 mg, 70 μmol) as starting materials.

^{1}H NMR data ($CDCl_3$): δ Guaianolide: 4.28 (br s, 1H, H-1), 5.44 (t, J = 3.4, 1H, H-2), 5.61 (overlapped, 1H, H-3), 5.61 (overlapped, 1H, H-6), 5.61 (overlapped, 1H, H-8), 3.04 (dd, J = 14.3 and 2.7 Hz, 1H, H-9a), 2.32 (overlapped, 1H, H-9b), 1.43 (s, 3H, H-13), 1.32 (s, 3H, H-14), 1.77 (br s, 1H, H-15). Acetate 1.89 (s, 3H, H-2). Biphenylacetate 7.6 (m, 4H, H-4, H-8, H-10 and H-14), 7.4 (m, 2H, H-5 and H-7), 7.3 (m, 3H, H-11, H-12 and H-13), 3.74 (s, 2H, H-2). Butanoate: 2.3 (overlapped, 2H, H-2), 1.6 (overlapped, H-3), 0.92 (t, J = 7.3 Hz, 3H, H-4). Octanoate: 2.3 (overlapped, 2H, H-2), 1.61 (overlapped, 2H, H-3), 1.28 (br s, 8H, H-4–H-7), 0.86 (t, J = 7.0 Hz, 3H, H-8). ^{13}C NMR ($CDCl_3$) δ Guaianolide: 57.5 (C-1), 77.1 (C-2), 85.0 (C-3), 141.2 (C-4), 130.5 (C-5), 77.8 (C-6), 78.9 (C-7), 66.4 (C-8), 38.6 (C-9), 85.2 (C-10), 78.8 (C-11), 175.8 (C-12), 16.5 (C-13), 23.7 (C-14), 13.3 (C-15). Acetate 171.1 (C-1), 23.0 (C-2). Biphenylacetate 172.9 (C═O), 41.1 (C-2), 133.0 (C-3), 130.0 (C-4 and C-8), 129.0 (C-5 and C-7), 140.2 (C-6), 140.9 (C-9), 127.2 (C-10 and C-14), 127.4 (C-11 and C-13), 129.9 (C-12). Butyrate: 172.7 (C-1), 34.5 (C-2), 18.4 (C-3), 14.1 (C-4). Octanoate: 172.7 (C-1), 36.9 (C-2), 25.2 (C-3), 29.3 (C-4), 29.5 (C-5), 32.0 (C-6), 23.1 (C-7), 14.5 (C-8). HRMS calcd for $C_{43}H_{54}NaO_{12}^+$: 785.3507, found 785.3435.

5.1.29. Compound (16d)

Compound **16d** (50 mg, 76%) was prepared as described above for **16a** using 2-phenylbenzoic acid (19 mg, 96 μmol) and compound **15** (50 mg, 66 μmol) as starting materials. ^{1}H NMR data ($CDCl_3$): δ Guaianolide: 4.17 (br s, 1H, H-1), 5.28 (t, J = 3.2, 1H, H-2), 5.70 (br s, 1H, H-3), 5.54 (br s, 1H, H-6), 5.58 (t, J = 3.5 Hz, 1H, H-8), 2.94 (br d, J = 14.7, 1H, H-9a), 2.3 (overlapped, 1H, H-9b), 1.45 (s, 3H, H-13), 1.15 (s, 3H, H-14), 1.60 (br s, 1H, H-15). Acetate 1.91 (s, 3H, H-2). Butanoate: 2.3 (overlapped, 2H, H-2), 1.6 (overlapped, H-3), 0.96 (t, J = 7.3 Hz, 3H, H-4). Octanoate: 2.3 (overlapped, 2H, H-2), 1.6 (overlapped, 2H, H-3), 1.3 (br s, 8H, H-4–H-7), 0.88 (t, J = 6.6 Hz, 3H, H-8); 2-Phenylbenzoate 7.87 (dd, J = 7.3 and 1.2 Hz, 1H, H-3), 7.54 (td, J = 6.1 and 1.5 Hz, H-5), 7.43 (td, J = 6.1 and 1.5 Hz, H-4), 7.4 (overlapped, 6H, H-6, H-8–H-12). ^{13}C NMR ($CDCl_3$) δ Guaianolide: 57.6 (C-1), 77.0 (C-2), 84.8 (C-3), 141.2 (C-4), 130.8 (C-5), 72.9 (C-6), 78.8 (C-7), 66.3 (C-8), 38.5 (C-9), 85.2 (C-10), 78.9 (C-11), 175.5 (C-12), 16.5 (C-13), 22.3 (C-14), 13.0 (C-15). Acetate 170.9 (C-1), 23.0 (C-2). Butyrate: 172.7 (C-1), 36.9 (C-2), 18.3 (C-3), 14.1 (C-4). Octanoate: 172.7 (C-1), 34.6 (C-2), 25.2 (C-3), 29.3 (C-4), 29.3 (C-5), 32.0 (C-6), 23.2 (C-7), 14.5 (C-8). 2-Phenylbenzoate 168.4 (C═O), 130.4 (C-1), 142.2

(C-2), 127.4 (C-3), 131.1 (C-4), 130.3 (C-6), 141.6 (C-7), 128.9 (C-8 and C-12), 128.5 (C-9 and C-11), 127.5 (C-10). HRMS calcd for $C_{42}H_{52}NaO_{12}^{+}$: 771.3351, found 771.3325.

5.1.30. Compound (16e)

Compound **16e** (25 mg, 71%) was prepared as described below for compound **16f** using 4-methylbenzoic anhydride (30 mg, 0.12 mmol), DMAP (10 mg, 80 μmol) and **15** (30.5 mg, 53 μmol) as starting materials.

^{1}H NMR data ($CDCl_3$): δ Guaianolide: 4.30 (br s, 1H, H-1), 5.53 (t, J = 3.1, 1H, H-2), 5.78 (br s, 1H, H-3), 5.65 (br s, 1H, H-6), 5.61 (t, J = 3.7 Hz, 1H, H-8), 3.03 (dd, J = 14.7 and 3.2 Hz, 1H, H-9a), 2.3 (overlapped, 1H, H-9b), 1.46 (s, 3H, H-13), 1.43 (s, 3H, H-14), 1.86 (br s, 1H, H-15). Acetate 1.86 (s, 3H, H-2). Butanoate: 2.3 (overlapped, 2H, H-2), 1.6 (overlapped, H-3), 0.93 (t, J = 7.5 Hz, 3H, H-4). 4-Methylbenzoate: 7.89 (m, 2H, H-2 and H-6), 7.40 (m, 2H, H-3 and H-5). 4-Methylbenzoate 7.89 (m, 2H, H-2 and H-6), 7.20 (m, 2H, H-3 and H-5), 2.38 (s, 3H, Me). Octanoate: 2.3 (overlapped, 2H, H-2), 1.61 (overlapped, 2H, H-3), 1.3 (br s, 8H, H-4–H-7), 0.82 (t, J = 6.7 Hz, 3H, H-8). ^{13}C NMR ($CDCl_3$) δ Guaianolide: 57.9 (C-1), 77.8 (C-2), 85.2 (C-3), 142.0 (C-4), 130.8 (C-5), 77.1 (C-6), 78.8 (C-7), 66.4 (C-8), 38.6 (C-9), 84.8 (C-10), 78.9 (C-11), 175.6 (C-12), 16.6 (C-13), 23.5 (C-14), 13.4 (C-15). Acetate 171.1 (C-1), 23.0 (C-2). Butyrate: 172.6 (C-1), 36.9 (C-2), 18.4 (C-3), 14.1 (C-4). 4-Methylbenzoate 166.0 (C═O), 144.1 (C-1), 130.0 (C-2 and C-6), 129.3 (C-3 and C-5), 127.2 (C-4), 22.1 (Me). Octanoate: 172.8 (C-1), 34.6 (C-2), 25.2 (C-3), 29.3 (C-4), 29.4 (C-5), 32.0 (C-6), 23.0 (C-7), 14.5 (C-8). HRMS calcd for $C_{37}H_{50}NaO_{12}^{+}$: 709.3194, found 709.3170.

5.1.31. Compound (16f)

To a solution of **15** (50 mg, 66 μmol) in dichloromethane (2 mL) was added 4-phenylbenzoic acid (19 mg, 96 μmol), DMAP (20 mg, 160 μmol), and DCC (20 mg, 97 μmol). The reaction mixture was stirred at room temperature overnight, filtered and concentrated. Compound **16f** (35 mg, 53%) was isolated from the residue by column chromatography over silica gel using toluene–ethyl acetate (5:1) added acetic acid (1%) as an eluent. ^{1}H NMR data ($CDCl_3$): δ Guaianolide: 4.39 (br s, 1H, H-1), 5.62 (t, J = 3.3, 1H, H-2), 5.88 (br s, 1H, H-3), 5.72 (br s, 1H, H-6), 5.68 (t, J = 3.5 Hz, 1H, H-8), 3.11 (br d, J = 14.7, 1H, H-9a), 2.3 (overlapped, 1H, H-9b), 1.51 (s, 3H, H-13), 1.52 (s, 3H, H-14), 1.95 (br s, 1H, H-15). Acetate 1.92 (s, 3H, H-2). Butanoate: 2.3 (overlapped, 2H, H-2), 1.6 (overlapped, H-3), 0.96 (t, J = 7.5 Hz, 3H, H-4). Octanoate: 2.3 (overlapped, 2H, H-2), 1.6 (overlapped, 2H, H-3), 1.3 (br s, 8H, H-4–H-7), 0.86 (t, J = 6.6 Hz, 3H, H-8); 4-phenylbenzoate 8.13 (m, 2H, H-2 and H-6, 7.68 (m, 2H, H-3 and H-5), 7.63 (m, 2H, H8 and H-12), 7.50 (m, 2H, H-9 and H-11), 7.41 (m, 1H, H-10). ^{13}C NMR ($CDCl_3$) δ Guaianolide: 57.6 (C-1), 76.6 (C-2), 84.6 (C-3), 141.5 (C-4), 130.7 (C-5), 77.6 (C-6), 78.7 (C-7), 66.2 (C-8), 38.3 (C-9), 85.1 (C-10), 78.6 (C-11), 175.3 (C-12), 16.3 (C-13), 22.6 (C-14), 13.2 (C-15). Acetate 170.8 (C-1), 23.2 (C-2). Butyrate: 172.4 (C-1), 36.6 (C-2), 18.1 (C-3), 13.8 (C-4). Octanoate: 172.4 (C-1), 34.3 (C-2), 24.9 (C-3), 29.0 (C-4), 29.1 (C-5), 31.7 (C-6), 22.7 (C-7), 14.2 (C-8). 4-Phenylbenzoate 165.5 (C═O), 128.3 (C-1), 127.2 (C-2 and C-6), 128.8 (C-3 and C-5), 145.8 (C-4), 139.9 (C-7), 128.0 (C-8 and C-12), 130.2 (C-7 and C-11), 128.1 (C-9). HRMS calcd for $C_{42}H_{52}NaO_{12}^{+}$: 771.3351, found 771.3322.

5.2. Assay for SERCA inhibition

Sarcoplasmic reticulum from rabbit skeletal muscle was prepared as previously described.[32] The incubation medium contained phosphoenolpyruvate (1 mM), MgATP (concd) NADH (0.15 mM), lactate dehydrogenase (60 μg/mL), and Ca^{2+} (concd). The reaction, which took place at 20 °C, was initiated by addition of SERCA to the medium at concentration of 4 nM and a concentration of 5–10 μg protein/mL. The reaction was followed by measuring the optical density at 340 nm. The experiments were repeated two or three times.

Acknowledgments

This work has been supported by the Danish Cancer Society and the Carlsbergfondet.

Supplementary data

Supplementary data (^{1}H NMR spectra of the compounds **9a–9d**, **11b**, **12a**, **12b**, **13**, **14**, and **16a**–**16f**) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.06.032.

References and notes

1. Bobe, R.; Bredoux, R.; Corvazier, E.; Martin, V.; Gelebart, P.; Lacabaratz-Porret, C.; Launay, S.; Fanchaouy, M.; Dally, S.; Chaabane, C.; Kovacs, T.; Enouf, J. *Curr. Top. Biochem. Res.* **2005**, *7*, 1.
2. Thastrup, O.; Cullen, P. J.; Drøbak, B. K.; Hanley, M. R.; Dawson, A. P. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 2466.
3. Papp, B.; Enyedi, A.; Kovacs, T.; Sarkadi, B.; Wuytack, F.; Thastrup, O.; Gardos, G.; Bredoux, R.; Levy-Toledano, S.; Enouf, J. *J. Biol. Chem.* **1991**, *266*, 14593.
4. Lytton, J.; Westlin, M.; Hanley, M. R. *J. Biol. Chem.* **1991**, *266*, 17067.
5. Xu, C.; Ma, H. L.; Inesi, G.; Al-Shawi, M. K.; Toyoshima, C. *J. Biol. Chem.* **2004**, *279*, 17973.
6. Sagara, Y.; Inesi, G. *J. Biol. Chem.* **1991**, *266*, 13503.
7. Wootton, L. L.; Michelangeli, F. *J. Biol. Chem.* **2006**, *281*, 6970.
8. Furuya, Y.; Isaacs, J. T. *Prostate* **1994**, *25*, 301.
9. Furuya, Y.; Lundmo, P.; Short, A. D.; Gill, D. L.; Isaacs, J. T. *Cancer Res.* **1994**, *54*, 6167.
10. Lin, X. S.; Denmeade, S. R.; Cisek, L.; Isaacs, J. T. *Prostate* **1997**, *33*, 201.
11. Christensen, S. B.; Skytte, D. M.; Denmeade, S. R.; Dionne, C.; Moller, J. V.; Nissen, P.; Isaacs, J. T. *Anticancer Agents Med. Chem.* **2009**, *9*, 276.
12. Janssen, S.; Jakobsen, C. M.; Rosen, D. M.; Ricklis, R. M.; Reinecke, U.; Christensen, S. B.; Lilja, H.; Denmeade, S. R. *Mol. Cancer Ther.* **2004**, *3*, 1439.
13. Denmeade, S. R.; Lou, W.; Lovgren, J.; Malm, J.; Lilja, H.; Isaacs, J. T. *Cancer Res.* **1997**, *57*, 4924.
14. Jakobsen, C. M.; Denmeade, S. R.; Isaacs, J. T.; Gady, A.; Olsen, C. E.; Christensen, S. B. *J. Med. Chem.* **2001**, *44*, 4696.
[15]. Denmeade, S. R.; Isaacs, J. T.; Lilja, H. Patent US 7,053,042 B1, 2006.
16. Denmeade, S. R.; Isaacs, J. T. *Cancer Biol. Ther.* **2005**, *4*, e69.
17. Denmeade, S. R.; Jakobsen, C. M.; Janssen, S.; Khan, S. R.; Garrett, E. S.; Lilja, H.; Christensen, S. B.; Isaacs, J. T. *J. Natl. Cancer Inst.* **2003**, *95*, 990.
18. Andersen, A.; Treiman, M.; Poulsen, J. C. J.; Cornett, C.; Moldt, P.; Olsen, C. E.; Christensen, S. B. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 657.
19. Christensen, S. B.; Andersen, A.; Poulsen, J. C.; Treiman, M. *FEBS Lett.* **1993**, *335*, 345.
20. Nielsen, S. F.; Thastrup, O.; Pedersen, R.; Olsen, C. E.; Christensen, S. B. *J. Med. Chem.* **1995**, *38*, 272.
21. Sohoel, H.; Jensen, A. M.; Moller, J. V.; Nissen, P.; Denmeade, S. R.; Isaacs, J. T.; Olsen, C. E.; Christensen, S. B. *Bioorg. Med. Chem.* **2006**, *14*, 2810.
22. Christensen, S. B.; Andersen, A.; Smitt, U. W. *Prog. Chem. Natl. Prod.* **1997**, *71*, 131.
23. Toyoshima, C.; Nakasako, M.; Nomura, H.; Ogawa, H. *Nature* **2000**, *405*, 647.
24. Sohoel, H.; Liljefors, T.; Ley, S. V.; Oliver, S. F.; Antonello, A.; Smith, M. D.; Olsen, C. E.; Isaacs, J. T.; Christensen, S. B. *J. Med. Chem.* **2005**, *48*, 7005.
25. Toyoshima, C.; Nomura, H. *Nature* **2002**, *418*, 605.
26. Rasmussen, U.; Christensen, S. B.; Sandberg, F. *Acta Pharm. Suec.* **1978**, *15*, 133.
27. Andrews, S. P.; Ball, M.; Wierschem, F.; Cleator, E.; Oliver, S.; Högenauer, K.; Simic, O.; Antonello, A.; Hüniger, U.; Smith, M. D.; Ley, S. V. *Chem.-A Eur. J.* **2007**, *13*, 5688.
28. Ball, M.; Andrews, S. P.; Wierschem, F.; Cleator, E.; Smith, M. D.; Ley, S. V. *Org. Lett.* **2007**, *9*, 663.
29. Andersen, A.; Cornett, C.; Lauridsen, A.; Olsen, C. E.; Christensen, S. B. *Acta Chem. Scand.* **1994**, *48*, 340.
30. Christensen, S. B. *Acta Chem. Scand.* **1988**, *B 42*, 623.
31. Rothman, E. S.; Swern, D.; Perlstein, T.; Serota, S. *J. Org. Chem.* **1962**, *27*, 3123.
32. Moller, J. V.; Lind, K. E.; Andersen, J. P. *J. Biol. Chem.* **1980**, *255*, 1912.
33. Sorensen, T. L.; Moller, J. V.; Nissen, P. *Science* **2004**, *304*, 1672.