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Glycosylation of stannyl ceramides promoted by modified montmorillonite in supercritical carbon dioxide

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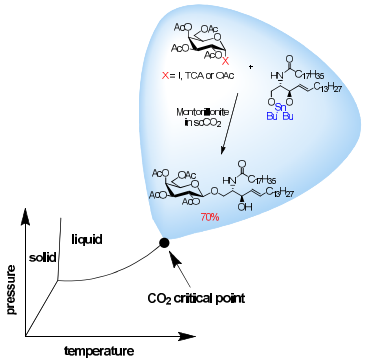

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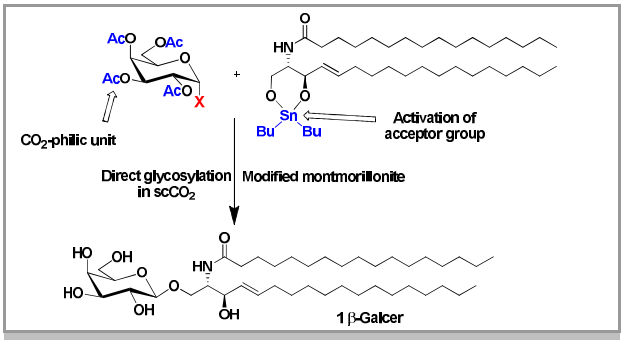
Abstract The direct glycosylation of ceramides in supercritical carbon dioxide ($scCO_2$) successfully proceeded to produce β-glycolipids with a high yield and full stereoselectivity. The reaction is promoted by montmorillonite modified with a super-acid (CF_3SO_3H). The value of this protocol was demonstrated in the efficient synthesis of isoglobotrihexosylceramide (iGB3).

Key words Glycosylation, β-glycolipids, iGB3, supercritical carbon dioxide, montmorillonite

In recent years, supercritical fluid extraction (SFE) has been successfully introduced as an interesting alternative to conventional solid–liquid extraction in the analysis and isolation of natural products.[1] One such group of natural products is glycolipids,[2] which together with neutral lipids,[3] phospholipids[4] and fatty acids[5] have been isolated from different cellular matrices by employing SFE. Although a number of gases could be used for SFE, $scCO_2$ (Tc=31.1 °C, Pc=73.8 bar) is the most commonly used because it is non-toxic, non-flammable, inexpensive and readily available in high purity from a variety of sources. Historically, $scCO_2$ has been considered a nonpolar solvent because of its low dielectric constant and dipole moment; however, recent studies suggest that $scCO_2$ may exhibit a certain polar behaviour, and this behaviour[6] helps explain the high solubility of sugar acetates in $scCO_2$ that allowed the design of new nonfluorous CO_2-philic agents.[7]

Against this background, we envisioned going beyond the simple extraction of glycolipids and considered the idea of synthesizing these types of molecules in $scCO_2$ as the reaction medium. The synthetic strategy was designed to take advantage of the presence of the CO_2-philic unit[7] in the donor group and to use a stannyl ceramide as an active acceptor group,[8] aiming to directly glycosylate the ceramide[9] (Scheme 1). As a reaction medium, $scCO_2$ has been used in diverse synthetic processes with varying results.[10] In the specific case of carbohydrate chemistry, glycosidation reaction[11] has been described under metal free conditions[12] and in the presence of sulfated zirconia[13] with $scCO_2$ as the solvent. To the best of our knowledge, the direct glycosylation of ceramides to obtain glycosphingolipids (GSLs) in $scCO_2$ has not been previously explored



Scheme 1 Synthetic strategy.

GSLs constitute a heterogeneous group of biomolecules involved in cellular trafficking and signalling functions.[14] These compounds are sites for host–pathogen/toxin interactions and for the generation of pathological/infectious forms of proteins associated with Alzheimer's and prion diseases[15] and HIV.[16] These observations, in conjunction with our interest in providing a homogeneous material for use in bioorganic studies, encouraged us to develop alternative glycosylation protocols in the presence of heterogeneous promoters to replace the typical acids such as $BF_3{\cdot}Et_2O$ or TBDMSOTf.[17] Thus, we herein report a direct, selective protocol for the glycosylation of ceramides based on the high reactivity of stannyl ceramides and the solubility of sugar acetates in $scCO_2$ in the presence of montmorillonite modified with a super-acid (CF_3SO_3H), which offers a range of advantages over liquid acids, as it is noncorrosive, inexpensive, stable, and readily reusable (Scheme 1).

Natural montmorillonite is a clay composed of an octahedral alumina sheet and two tetrahedral silica sheets, with isomorphous substitution, which produces an excess negative charge. That charge is compensated by the adsorption of exchangeable cations on the surface layer.[18] In this case, treating montmorillonite with CF_3SO_3H incremented the number of Brønsted catalytic sites by replacing exchangeable cations with protons.[19] The acid treatment also changed the structure of the material by damaging the original lamellar structure, allowing free aluminium ions to migrate from the octahedral sheets into the interlayer region, where they displaced sodium ions and increased the number of Lewis catalytic sites, which affected the montmorillonite surface area, pore volume and diameter (Table 1).[20] The chemical compositions of the natural and modified montmorillonite (Table 2), as determined by energy-dispersive X-ray spectroscopy (EDS), corroborate the migration of cations from the montmorillonite interstitial spaces into the interlayer region.

Table 1 Properties of montmorillonite clays

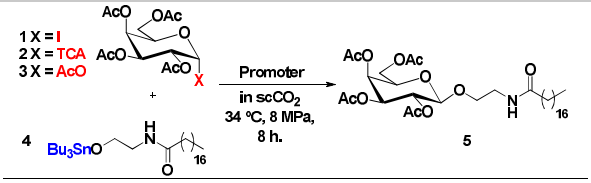
Parameters	Montmorillonite	
	Natural	Modified with CF_3SO_3H
Surface area (m^2/g)	23	185
Pore volume (cc/g)	0.140	0.603
Pore diameter (Å)	23.00	107.79

Table 2 Elements present in the clay samples as determined by EDS

Element	Elemental concentration weight (%)	
	Natural montmorillonite	Modified montmorillonite
Na_2O	2.60	0.13
K_2O	0.33	0.30
MgO	3.52	2.06
Al_2O_3	21.30	13.36
SiO_2	65.48	74.60
CaO	1.05	1.65
SO_3	-	2.30
TiO_2	0.33	0.27
MnO	0.31	0.03
FeO	5.08	5.30

After the montmorillonite was modified with CF_3SO_3H, the reaction of stannyl derivative **4** as an acceptor group and galactosyl donors **1**-**3** was chosen as the model reaction to test the potential of $scCO_2$ as a reaction medium (Table 3). The reaction was carried out under 8 MPa of CO_2 at 34 °C, and the results of these studies are summarized in table 3. Initially, glycosyl iodide[21] **1** was used as the donor group, and the results showed an excellent yield when the reaction was performed in the presence of TBAI (tetrabutylammonium iodide) and modified montmorillonite. The yield decreased when only modified montmorillonite was used as the promoter in the reaction medium (Table 3, entry 2). With no promoter, this reaction did not occur after 8 h (Table 3, entry 3). Alternatively, we also investigated the reactivity of stannyl ether **4** with others donors with different leaving groups such as trichloroacetimidate[22] (TCA) and acetate[23] in the appropriate reaction conditions. The trichloroacetimidate was expected to behave similarly to the iodo derivative when used as a donor. Effectively, when **2** was reacted with the stannyl ether **4**, β-glycilioid **5** was obtained in a similar yield (Table 3, entry 4). The reaction of penta-*O*-acetyl-β-D-galactopyranose **3**, a readily accessible glycosyl donor, was probed with the stannylated acceptor **4** in the presence of modified montmorillonite, led to a decrease in the yield of the β-Galcer analogue **5** (Table 3, entry 5). The effect of the reaction pressure was examined, and when the pressure was increased or reduced, the yield of **5** notably decreased. Notably the modified montmorillonite was recovered from the reaction medium and reused three additional times with similar reactivity before the structural base of montmorillonite was lost.

Table 3 Glycosylation of stannyl ether 4 in $scCO_2$[a]



Entry	Donor	Promoter	Yield (%)[b]	Ratio (α/β)
1	**1**	Modified montmorillonite and TBAI	80	0/1
2	**1**	Modified montmorillonite	20	0/1
3	**1**	-	0	-
4	**2**	Modified montmorillonite and TBAI	75	0/1
5	**3**	Modified montmorillonite and TBAI	60	0/1
1	**1**	Modified montmorillonite and TBAI	80	0/1

[a] Reaction conditions: donor (1.2 mmol), acceptor (1 mmol), TBAI (1.2 mmol) and montmorillonite (50 mg) in $scCO_2$ at 34 °C and 8 MPa for 8 h.
[b] Yields of isolated product after chromatographic purification.

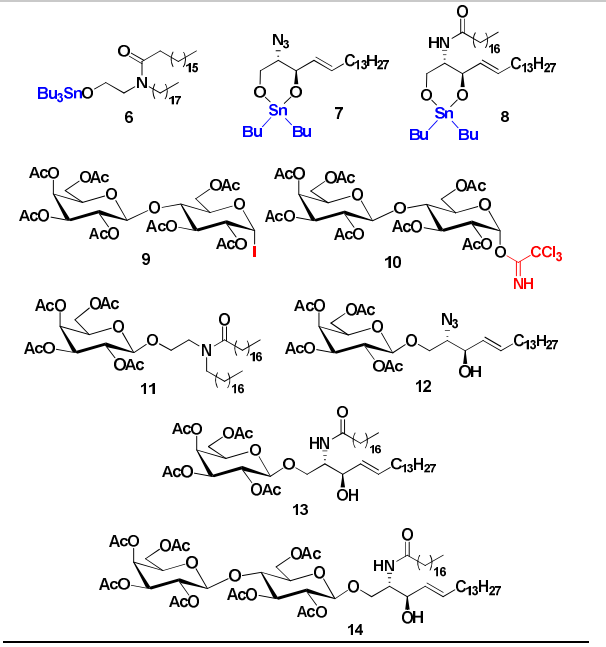
It is noteworthy that, our approach uses stannyl ether with the purpose of increasing the nucleophilicity of oxygen in **4**. TBAI is used to activate both the glycosyl idodide **1**[21] and stannyl ether **4**.[8] Meanwhile, modified montmorillonite gives acid conditions to activatee glycosyl donor **2** and **3** and to promote the rearrangement of the orthoester, which is the principal intermediate in the glycosylation reaction.[8]

To examine the scope of these glycosylation protocols in the synthesis of biologically relevant glycolipids, the glycosylation of azidosphingosine, ceramide and analogues was tested (Table 4). Thus, when the ceramide analogue **6** was reacted with glycosyl iodide **1** following the general protocol of glycosylation, the glycolipid **11** was exclusively obtained in an 80 % yield. Similar yields were obtained when **2** was used as the donor group (Table 4, entries 1 and 2). The classical protocol for the selective glycosylation of diols requires a series of programmed protection–deprotection steps to ensure that only one of the hydroxyls reacts.[17] Azido-sphingosine has two hydroxyl functional groups in positions 1 and 3 that can be simultaneously protected as stannyl acetal. When stannyl acetal **7**, which is obtained from the reaction of azidosphingosine with Bu_2SnO with the exclusion of water, was reacted with **1** in the standard conditions, the β-*O*-glycoside **12** was obtained in a 73 % yield. Additionally, the use of **2** as a donor group in this case afforded similar yields (Table 4, entries 3 and 4). The reaction was fully chemo- and stereoselective by exclusively involving the primary hydroxyl group.

Similarly, stannyl acetal **8** was also reacted with glycosyl donors **1** and **2** in the presence of modified montmorillonite, and the

galactosyl ceramide **13** was obtained in 70 and 65 % yields, respectively. Additionally, the reaction was fully chemo- and stereoselective in this case (Table 4, entries 5 and 6). Finally, glycosylation of **8** with the corresponding hepta-*O*-acetyllactosyl iodide **9** or hepta-*O*-acetyllactosyl trichloroacetimidate **10** also afforded the corresponding glycolipid **14** in good yields (Table 4, entries 7 and 8). In all cases, when the crude reaction products were analysed by ^{1}H NMR, no α-anomer or the corresponding elimination product (glycal) was observed.

Table 4 Synthesis of β-glycosphingolipids in $scCO_2$[a]



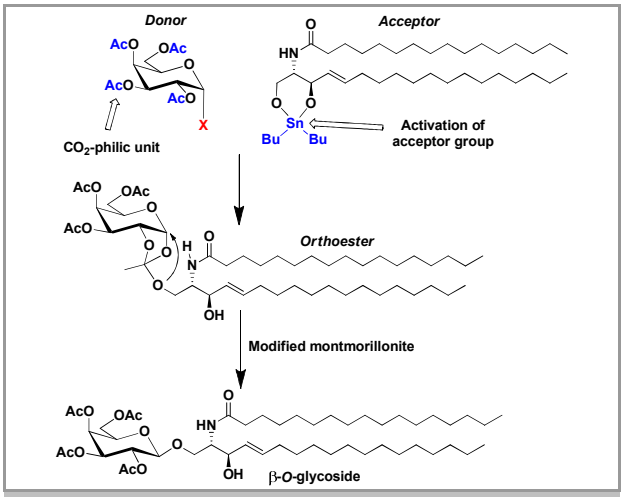
Entry	Donor	Acceptor	Glycolipid	Yield (%)[b]	Ratio (α/β)
1	**1**	**6**	**11**	80	0/1
2	**2**	**6**	**11**	77	0/1
3	**1**	**7**	**12**	73	0/1
4	**2**	**7**	**12**	70	0/1
5	**1**	**8**	**13**	70	0/1
6	**2**	**8**	**13**	65	0/1
7	**9**	**8**	**14**	68	0/1
8	**10**	**8**	**14**	65	0/1

[a] Donor (1.2 mmol), acceptor (1 mmol), TBAI (1.2 mmol) and montmorillonite (50 mg) in $scCO_2$ at 34 °C and 8 MPa for 8 h.

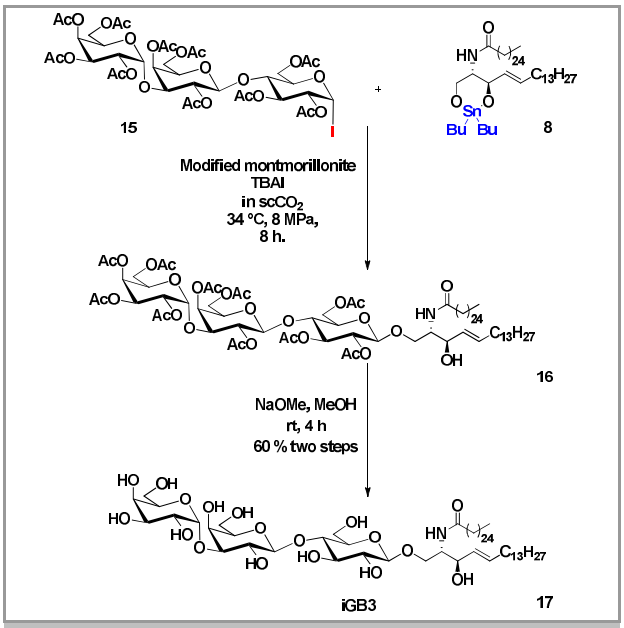
[b] Yields of isolated product after chromatographic purification.

To explain the results described above, it is important to consider that in the diverse synthetic strategies for glycosphingolipids, one of the principal drawbacks is the low yields obtained by the direct glycosylation of ceramides (> 60 % using CH_2Cl_2, THF, benzene or toluene as solvent), which have been attributed to the poor nucleophilicity of sphingosines and ceramides.[17] In this case, we considered that the use of stannyl ceramides increases the nucleophilic character of ceramides and therefore also increases the yield of the reaction.[8] On the other hand, modified montmorillonite promotes the rearrangement of the orthoester[24] intermediate to obtain the desired β-*O*-glycoside, which is formed in the first step of the general reaction (Scheme 2).[8]

Finally, the synthetic utility of this new approach was further demonstrated by rapidly synthesizing isoglobotrihexosylceramide **17** (iGB3). The donor **15** and stannyl ceramide **8** would serve as building blocks for glycosylation. Thus, treating the iodide donor **15** with **8** in the presence of TBAI and modified montmorillonite under 8 MPa of CO_2 at 34 °C followed by eliminating the acetate groups furnished iGb3 **17** in a 60 % yield as a unique anomer. The ^{1}H and ^{13}C NMR spectroscopic data for **17** were consistent with the data reported for the synthetic product[25] (Scheme 3).



Scheme 2 Proposed mechanism for glycosylation reaction.



Scheme 3 Synthesis of iGB3 in $scCO_2$.

In summary, the highly stereoselective glycosylation protocol described above offers an efficient strategy for directly synthesizing β-glycolipids. The synthetic strategy was designed to exploit the presence of the CO_2-philic unit in the donor group and used a stannyl ceramide as an active acceptor group. The overall process occurs with complete chemoselectivity (differentiation of the primary and secondary OH on the lipid moiety) and stereoselectivity (β-anomer only). To further demonstrate its synthetic value, we showed that the method can

be used to rapidly prepare the biologically active compound iGB3.

All reactions were conducted under a dried argon stream. Solvents were purchased in capped Pure Solv System-4® bottles and used without further purification and stored under argon. Yields refer to the chromatographically and spectroscopically (^{1}H and ^{13}C) homogeneous materials, unless otherwise stated. TMSI was stored at -15 °C under dry atmosphere. All other solvents and reagents were purchase from Sigma-Aldrich Co. LLC., and used without further purification. The sphingosine[26] and azidosphingosine[27] were synthesized using literature procedures. All glassware utilized was flame-dried before use. The reactions were monitored by tin layer chromatographic (TLC) carried out on 0.25 mm E. Merck silica gel plates. The developed TLC plates were visualized under a short-wave UV lamp and by heating plates that were dipped in $Ce(SO_4)_2$ Flash column chromatography (FCC) was performed using flash silica gel (230-400) and employed a solvent polarity correlated with TLC mobility. Optical rotations were measured on a Perkin Elmer 343 polarimeter. NMR experiments were conducted on a Bruker 300 MHz instrument using $CDCl_3$ (99.9% D) as the solvent, with chemical shifts (δ) reference to internal standards $CDCl_3$ (7.26 ppm ^{1}H, 77.23 ppm ^{13}C) or Me_4Si as an internal reference (0.00 ppm) Chemical shifts are relative to the deuterated solvent peak and are in parts per million (ppm).

Preparation of acceptors 4 and 6

A mixture of corresponding amide (1 mmol) and bis-(tributyltin) oxide (0.5 mmol) in 20 ml of dry toluene, was heated to reflux overnight and was subjected to azeotropic dehydration using a Dean-Stark or 4 Å molecular sieves. Removal of solvent under reduced pressure afforded the stannyl ethers (acceptors) **4** and **6**, which was used for the glycosyl-coupling reaction without further purification.

Preparation of acceptors 7 and 8

A mixture of azidosphingosine or sphingosine (1 mmol) and dibutyltin oxide (1 mmol) in dry toluene (20 mL), was heated to reflux overnight and was subjected to azeotropic dehydration using a Dean-Stark or 4 Å molecular sieves. Removal of solvent under reduced pressure afforded the stannyl ethers (acceptors) **7** and **8**, which was used for the glycosyl-coupling reaction without further purification.

General procedure of glycosylation

Glycosyl donor (1.2 mmol), acceptor (1 mmol), TBAI (1.2 mmol) and modified montmorillonite (50 mg) were added to a reactor (50 mL). Then, the reactor was heated at to 34 °C and CO_2 was introduced with high pressure pump. The reaction mixture was stirred at 34˚C and 8.0 MPa for 8 hours. Afterwards, the modified motmorillonite was removed by filtration using methanol. The resulting residue was purified by flash column chromatography on silica gel using hexane-AcOEt-MeOH as mobile phase to give the desired product.

β-*O*-glycoside 5:[8] **(2*R*,3*S*,4*S*,5*R*,6*R*)-2-(acetoxymethyl)-6-(2-stearamidoethoxy)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate.**

White solid (80 %). TLC (hexane/AcOEt/MeOH 60:30:10) R_f 0.40; m.p. 137–139˚C; $[\alpha]_D^{25}$ – 6 (c = 1.0, $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$): δ 5.85 (1H, t, *J* = 5.2 Hz), 5.39 (1H, d, *J* = 3.2 Hz), 5.19 (1H, dd, *J* = 10.8, 8.4 Hz), 5.01 (1H, dd, *J* = 10.8, 3.6 Hz), 4.46 (1H, d, *J* = 8.4 Hz), 4.16-4.13 (2H, m), 3.91 (1H, t, *J* = 6.4 Hz), 3.87 (1H, ddd, *J* = 10.4, 6.4, 4.0 Hz), 3.67 (1H, ddd, *J* = 10.4, 6.8, 3.6 Hz), 3.51 (2H, q, *J* = 5.2 Hz), 2.17 (2H, t, *J* = 7.2 Hz), 2.07 (3H, s), 2.06 (3H, s), 2.04 (3H, s), 1.98 (3H, s), 1.61 (2H, quint, *J* = 7.2, Hz), 1.33 (2H, sex, *J* = 7.6, Hz); 1.25-1.24 (26H, m), 0.87 (3H, t, *J* = 7.2 Hz).

^{13}C NMR (75 MHz, $CDCl_3$): δ 172.0, 170.2, 170.0, 169.9, 168.8, 100.0, 71.8, 70.9, 68.2, 67.0, 61.2, 61.0, 41.6, 36.5, 31.8, 29.5, 28.6, 25.6, 22.7, 20.7, 20.5 14.1.

Anal. Calcd. for $C_{34}H_{59}NO_{11}$: C, 62.08; H, 9.04; N, 2.11. Found: C, 62.03; H, 9.01; N, 2.05.

β-*O*-glycoside 11:[8] **(2*R*,3*R*,4*R*,5*R*,6*R*)-2-(acetoxymethyl)-6-(2-(*N*-octadecylstearamido)ethoxy)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate.**

White solid (80 %). TLC (Hexane-AcOEt-MeOH 60:30:10) R_f 0.50; m.p. 136-138 ˚C; $[\alpha]_D^{25}$ – 10 (c = 1.0, $CHCl_3$).

^{1}H NMR (300 MHz $CDCl_3$): δ 5.37 (1H, d, *J* = 3.2 Hz), 5.17 (1H, dd, *J* = 10.4, 8.0 Hz), 5.00 (1H, dd, *J* = 10.4, 3.2 Hz), 4.47 (1H, d, *J* = 8.0 Hz), 4.17-4.01 (2H, m), 3.96 (1H, t, *J* = 6.4 Hz), 3.90 (1H, ddd, *J* = 10.0, 6.0, 4.0 Hz), 3.77 (1H, ddd, *J* = 10.0, 6.4, 3.6 Hz), 3.52 (2H, q, *J* = 5.0 Hz), 3.27 (2H, t, *J* = 7.2 Hz), 2.32 (2H, t, *J* = 7.2 Hz), 2.09 (3H, s), 2.04 (3H, s), 2.02 (3H, s), 1.01 (3H, s), 1.62-1.54 (4H, m), 1.25-1.24 (58H, m), 0.87 (6H, t, *J* = 7.2 Hz).

^{13}C NMR (75 MHz, $CDCl_3$): δ 173.0, 172.9, 170.8, 170.5, 170.0, 169.0, 100.1, 72.0, 71.0, 68.8, 67.1, 61.5, 58.9, 50.2, 47.3, 34.3, 31.8, 30.4, 29.6, 29.3, 28.9, 28.6, 27.7, 22.7, 20.8, 20.4, 14.0.

Anal. Calcd. for $C_{52}H_{95}NO_{11}$: C, 68.61; H, 10.52; N, 1.54. Found: C, 68.58; H, 10.47, N, 1.50.

β-*O*-glycoside 12:[8] **(2*R*,3*R*,4*R*,5*R*,6*R*)-2-(acetoxymethyl)-6-(((2*S*,3*R*,*Z*)-2-azido-3-hydroxyoctadec-4-en-1-yl)oxy)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate.**

White solid (73 %). TLC (Hexane-AcOEt-MeOH 60:30:10) R_f 0.60; m.p. 50-52 ˚C; $[\alpha]_D^{25}$ – 20 (c = 1.0, $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$): δ 5.8.0 (1H, dt, *J* = 15.6, 6.4 Hz), 5.48 (1H, dd, *J* = 15.6, 7.2 Hz), 5.37 (1H, d, *J* = 3.2 Hz), 5.19 (1H, dd, *J* = 10.5, 7.5 Hz), 5.00 (1H, dd, *J* = 10.5, 3.2 Hz), 4.50 (1H, d, *J* = 8.0 Hz), 4.25 (1H, dd, *J* = 6.8, 6.0 Hz), 4.2-4.06 (2H, m), 3.96 (1H, t, *J* = 6.4 Hz), 3.93 (1H, dd, *J* = 12.8, 6.0 Hz), 3.69 (1H, dd, *J* = 10.4, 4.4 Hz), 3.45 (1H, m), 2.06 (2H, q, *J* = 7.2 Hz), 2.05 (3H, s), 2.04 (3H, s), 2.03 (3H, s), 1.96 (3H, s), 1.37-135 (2H, m), 1.25-1.24 (20H, m), 0.87 (3H, t, *J* = 7.2 Hz).

^{13}C NMR (75 MHz, $CDCl_3$): δ 170.2, 169.3, 169.2, 165.0, 139.0, 122.7, 100.5, 72.8, 72.5, 72.0, 71.0, 68.3, 68.0, 63.5, 61.8, 32.4, 31.9, 29.6-28.7, 22.7, 20.6, 20.4, 14.0.

Anal. Calcd. for $C_{32}H_{53}N_3O_{11}$: C, 58.61; H, 8.15; N, 6.41. Found: C, 58.57; H, 8.12; N, 6.38.

β-*O*-glycoside 13:[8] **(2*R*,3*R*,4*R*,5*R*,6*R*)-2-(acetoxymethyl)-6-(((2*S*,3*R*,*Z*)-3-hydroxy-2-stearamidooctadec-4-en-1-yl)oxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate.**

White solid (70 %). TLC (Hexane-AcOEt-MeOH 60:30:10) R_f 0.60; m.p. 50-52 ˚C; $[\alpha]_D^{25}$ – 14.5 (c = 1.0, $CHCl_3$).

^{1}H NMR (300, MHz $CDCl_3$): δ 5.79 (1H, t, *J* = 7.0 Hz), 5.75 (1H, dt, *J* = 15.3, 6.7 Hz), 5.49 (1H, dd, *J* = 15.3, 7.5 Hz), 5.38 (1H, d, *J* = 3.2 Hz), 5.20 (1H, dd, *J* = 10.6, 8 Hz), 5.00 (1H, dd, *J* = 10.6, 3.6 Hz), 4.47 (1H, d, *J* = 8 Hz), 4.25 (1H, dd, *J* = 7.5, 3.4 Hz), 4.16 (1H, dd, *J* = 10.0, 4.6 Hz), 4.15-3.99 (3H, m), 3.92 (1H, t, *J* = 6.4 Hz), 3.65 (1H, dd, *J* = 10.0, 3.2 Hz), 2.20 (2H, t, *J* = 7.5 Hz), 2.05 (3H, s), 2.04 (3H, s), 2.03 (2H, dt, *J* = 7.2, 6.7, Hz), 2.03 (3H, s), 1.99 (3H, s), 1.61 (2H, quint, *J* = 7.5 Hz), 1.36-134 (2H, m), 1.34-1.20 (48H, m), 0.87 (6H, t, *J*=6.9, Hz).

^{13}C NMR (75 MHz, $CDCl_3$): δ 174.3, 170.2, 170.1, 169.9, 168.8, 134.0, 128.8, 101.0, 71.9, 71.7, 71, 68.2, 68.0, 67.1, 61.3, 53.0, 36.0, 31.9, 31.5, 29.0, 29-28.8, 25.5, 21.2, 20.6, 20.5, 13.5.

Anal. Calcd. for $C_{49}H_{87}NO_{12}$: C, 66.70; H, 9.94; N, 1.59. Found: C, 66.67; H, 9.90, N, 1.55.

β-*O*-glycoside 14:[8] **(2*R*,3*R*,4*R*,5*R*,6*S*)-2-(acetoxymethyl)-6-(((2*R*,3*R*,4*R*,5*R*,6*R*)-4,5-diacetoxy-2-(acetoxymethyl)-6-(((2*S*,3*R*,*Z*)-3-hydroxy-2-stearamidooctadec-4-en-1-yl)oxy)tetrahydro-2*H*-pyran-3-yl)oxy)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate.**

White solid (68 %). TLC (Hexane-AcOEt-MeOH 60:30:10) Rf 0.35; m.p. 50–52 °C; [α]D25 –13.2 (c = 1.0, $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$): δ 5.97 (1H, d, *J* = 7.0 Hz), 5.76 (1H, dt, *J* = 15.3, 6.7 Hz), 5.50 (1H, dd, *J* = 15.3, 7.5 Hz), 5.33 (1H, d, *J* = 2.6 Hz), 5.10 (1H, dd, *J* = 10.4, 9.2 Hz), 5.08 (1H, dd, *J* =1 0.4, 8.0 Hz), 4.94 (1H, dd, *J* = 10.4, 3.4 Hz), 4.87 (1H, dd *J* = 9.6, 8.0 Hz), 4.49 (1H, d, *J* = 8.0 Hz), 4.48 (1H, d, *J* = 8.0 Hz), 4.35 (1H, dd, *J* = 9.6, 1.0 Hz), 4.16-4.01 (2H, m), 4.02-3.93 (3H, m), 3.88 (1H, t, *J* = 6.4 Hz), 3.81 (1H, t, *J* = 9.6 Hz), 3.62 (1H, dd, *J* = 10.0, 3.2

Hz), 3.55 (1H, m) 2.20 (2H, t, *J* = 7.5 Hz), 2.14 (3H, s), 2.07 (3H, s), 2.05 (3H, s), 2.03 (2H, dt, *J* = 7.2, 6.7, Hz), 2.02 (3H, s), 2.01 (3H, s), 1.96 (3H, s), 1.95 (3H, s), 1.62-1.61 (2H, m), 1.36-1.35 (2H, m), 1.34-1.20 (48H, m), 0.87 (6H, t, *J* = 6.9 Hz).

^{13}C NMR (75 MHz, $CDCl_3$): δ 173.0, 170.7, 170.6, 170.5, 170.2, 170.1, 169.9, 169.5, 133.5, 128.8, 101.0, 100.7, 76.5, 74.5, 73.1, 73.0, 72.2, 71.4, 71.1, 69.4, 67.9, 67.0, 62.3, 61.2, 52.2, 36.1, 31.9, 31.6, 21.2-21.0, 14.0.

Anal. Calcd. for $C_{61}H_{103}NO_{20}$: C, 62.59; H, 8.87; N, 1.20. Found: C, 62.56; H, 8.82, N, 1.17.

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White solid (60 %).

^{1}H NMR (Py-*d*5, 300 MHz): δ 8.31 (1H, d, *J* = 8.0 Hz), 7.53 (1H, br s), 7.32 (1H, br s), 6.98 (1H, br s), 6.66 (1H, br s), 6.58 (1H, br s), 6.52 (1H, br s), 6.42 (1H br s), 6.38 (1H, br s), 6.36 (1H, br s), 6.09 (1H, br s), 6.01 (1H dd, *J*=15.5 Hz, 6.0 Hz), 5.91 (1H, dt, *J* = 15.5, 6.5 Hz), 5.76 (1H, br s), 5.64 (1H, d, *J* = 3.8 Hz), 5.05-5.00 (2H, m), 4.87 (1H, d, *J* = 12.0 Hz), 4.81-4.75 (3H, m), 4.72-4.69 (1H, m), 4.66-4.65 (1H, m), 4.57-4.54 (1H, m), 4.54-4.49 (3H, m), 4.47-4.39 (4H, m), 4.32-4.29 (1H, m), 4.24-4.20 (3H, m), 4.15 (1H, dd, *J* = 9.5, 2.5 Hz), 4.05-4.00 (2H, m), 3.86-3.84 (1H, m), 2.42 (2H, t, *J* = 7.0 Hz) 2.06 (2H, q, *J* = 6.5 Hz), 1.83-1.82 (2H, m), 1.39-1.21 (66H, m), 0.87 (3H, t, *J* = 6.5 Hz), 0.86 (3H, t, *J* = 7.0 Hz).

^{13}C NMR (Py-*d*5, 75 MHz): δ 174.2, 133.5, 133.2, 106.3, 98.6, 83.0, 81.0, 77.6, 77.5, 77.4, 75.6, 73.7, 73.6, 72.5, 71.7, 71.4, 71.3, 66.9, 63.1, 62.9, 62.7, 55.8, 37.8, 33.6, 33.0, 31.4, 31.0, 30.9, 30.8, 30.7, 30.6, 30.5, 27.3, 23.3, 15.0.

Anal. Calcd. for $C_{62}H_{117}NO_{18}$: C, 63.94; H, 10.13; N, 1.20. Found: C, 63.91; H, 10.09; N, 1.16.

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Supporting Information

YES (this text will be updated with links prior to publication)

Primary Data

NO (this text will be deleted prior to publication)

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