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First synthesis of two deoxy Lewis^x pentaosyl glycosphingolipids

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Abstract The Lewis^x–Lewis^x interaction has been increasingly studied, using a variety of techniques including nuclear magnetic resonance spectroscopy, mass spectrometry, vesicle adhesion, atomic force microscopy, and surface plasmon resonance spectroscopy. However, the detailed molecular mechanism of these weak, divalent cation dependent interactions remains unclear, and new models are needed to probe the nature of this phenomenon in term of key roles of the different hydroxyl groups on Lewis^x trisaccharide determinant involved in the Lewis^x–Lewis^x interaction. An interesting solution is to synthesize a series of Lewis^x pentaosyl glycosphingolipid derivatives in which one of the eight hydroxyl groups of Lewis^x trisaccharide is replaced by a hydrogen atom, and to test the adhesion induced by interaction of these derivatives, in order to gain insight into the functions played by the hydroxyl groups of the Lewis^x trisaccharide. This article describes the synthesis of 3d-deoxy and 4d-deoxy Lewis^x pentaosyl glycosphingolipids, to be used for study of the Lewis^x–Lewis^x interaction.

Keywords Carbohydrate · Interaction · Lewis^x · Glycosphingolipid · Synthesis

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Introduction

The major mechanisms of cell adhesion are widely considered to be based on homotypic interaction between protein adhesion receptors (*e.g.*, Ig-like receptors, cadherins), or heterotypic interaction between integrins and specific matrix proteins (*e.g.*, FN, LN). Although these mechanisms are basically protein-to-protein interaction [1, 2], it has been reported that glycosylation of these protein receptors profoundly affects their adhesive function through an unknown mechanism [3–5].

During the last two decades, increasing attention has been paid to carbohydrate-to-protein interaction involving carbohydrate-binding proteins, *i.e.*, endogenous lectins such as galectins [6], selectins [7], and siglecs [8], which play an additional role in mediating cell–cell recognition and adhesion.

The concept of interaction of a specific carbohydrate with its complementary carbohydrate structure was first introduced by Hakomori in 1989–1993 as a new type of molecular interaction involved in cell adhesion [9]. In a seminal work, Hakomori proposed that carbohydrate-to-

carbohydrate interaction is responsible for the initial step of cell adhesion [10]. Embryogenesis, metastasis, and other proliferation processes are, according to this concept, mediated by carbohydrate-to-carbohydrate interactions [10]. One of the structures involved in this novel mechanism is the Lewis^x (Le^x) determinant (Galβ1→4[Fucα1→3]GlcNAcβ1→R). The Le^x antigen—previously defined as Stage-Specific Embryonic Antigen 1 (SSEA-1)—is found in a wide variety of cells and tissues including human cancers, pre-implantation mouse embryos, embryonic carcinoma cells, and human erythrocytes [11].

The interaction between Le^x and Le^x was found to be homotypic, and mediated by the presence of divalent cations such as Ca^{2+} [12, 13]. Recently, the Le^x–Le^x interaction has been studied more extensively, using a variety of techniques including nuclear magnetic resonance (NMR) spectroscopy [14–19], mass spectrometry (MS) [20], vesicle adhesion [21, 22], atomic force microscopy (AFM) [23, 24], and surface plasmon resonance (SPR) spectroscopy [25]. Rat basophilic leukaemia cells pre-incubated with purified Le^x-containing glycosphingolipids have been used as a model [26]. Another model system termed "Glycosylated Foldamer" was demonstrated for study of carbohydrate–carbohydrate interaction in terms of individual carbohydrate motifs [27]. Recently, using a vesicle micromanipulation approach with chemically synthesized natural Le^x pentasaccharidic glycosphingolipid, we demonstrated that in contrast to glyconeolipids [21, 22] which allow strong orientational freedom of the Le^x group, the natural lipid showed a restricted orientation of the Le^x group. The adhesion induced by Le^x–Le^x interaction was thereby considerably enhanced, indicating that relative orientation of the two Le^x groups is a predominant factor in Le^x–Le^x recognition [27]. In another experiment we replaced the Le^x trisaccharide determinant in the headgroup by Le^a trisaccharide, in which the galactose and fucose are permutated relative to Le^x on one vesicle surface. The adhesion energy observed for Le^x–Le^a pair was weak, confirming the homotypic characteristic of this type of the carbohydrate–carbohydrate interaction [28].

The detailed molecular mechanism of these weak, divalent cation dependent interactions remains unclear, and new models are needed to probe the roles of the different hydroxyl groups on Le^x trisaccharide determinant involved in Le^x–Le^x interaction. Our objective is to synthesize a series of Le^x pentaosyl glycosphingolipid derivatives in which one of the eight hydroxyl groups of Le^x trisaccharide is replaced by a hydrogen atom (Scheme 1), and to quantify the adhesion induced by interaction of these derivatives. These studies will provide insight into functions of the hydroxyl groups of the Le^x trisaccharide, which is involved in specific cell adhesion in pre-implantation embryos.

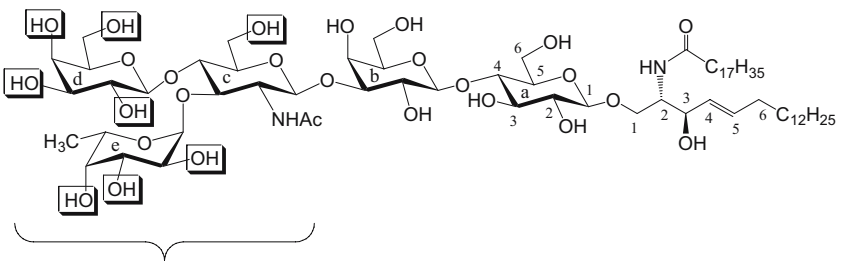
The synthetic work began with chemical modification of galactose residue. Following our successful synthesis of the 3d-deoxy and 4d-deoxy Le^x epitopes [29, 30], we now describe synthesis of 3d-deoxy and 4d-deoxy Le^x pentaosyl glycosphingolipids **2** and **3** (Scheme 2), which are also important bioorganic compounds for study of carbohydrate–carbohydrate interaction.

Results and discussion

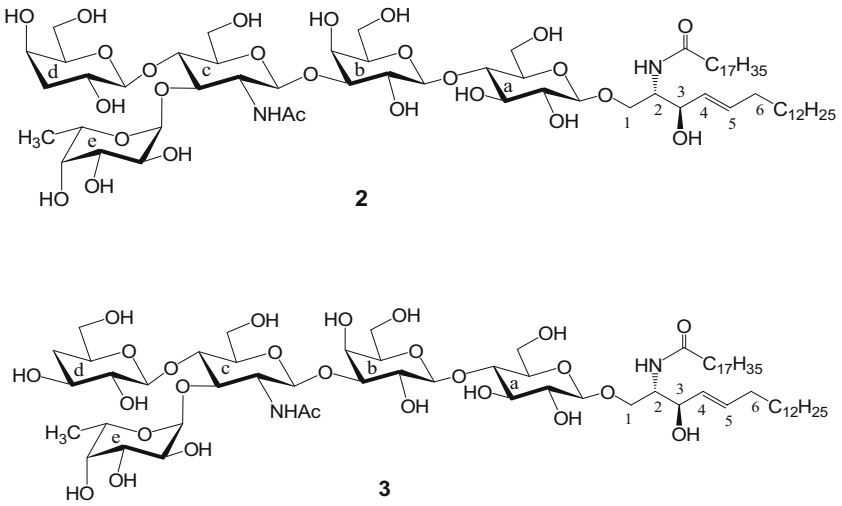
The key intermediates in the synthesis of deoxy Le^x glycosphingolipid **2** and **3** are deoxy Le^x pentasaccharide **8**, **9** and (2S,3R,4E)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol **12**. For the synthesis of **12**, a nine step procedure was chosen [31, 32]. We have previously reported on the preparation of 3d-deoxy Le^x pentasaccharide **8** [29] and 4d-deoxy Le^x pentasaccharide **9** [30] through a convergent synthetic route, using the building block **4**–**7**, as shown in Scheme 3.

After peracetylation of **8**, pentasaccharide **10** was obtained and converted to a trichloroacetimidate donor in order to couple with azidosphigosine derivative **12**. Acid catalyzed cleavage of the 2-(trimethylsilyl)ethyl glycoside was performed in dichloromethane using trifluoroacetic acid to give the hemiacetal as a mixture of α/β isomers which were not separated and further characterized at this

Scheme 1 Le^x pentaosyl glycosphingolipid **1**



Scheme 2 Deoxy Le^x pentaosyl glycosphingolipids **2** (3d-deoxy) and **3** (4d-deoxy)



stage. The hemiacetal was then treated with trichloroacetonitrile in the presence of 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) as a base to provide the trichloroacetimidate **11** (Scheme 4). The ^{1}H NMR spectrum showed that only the α-trichloroacetimidate was formed on the basis of the H-1, H-2 coupling constant ($J_{1a,2a}$=3.7 Hz for **11**). This is because an axial trichloroacetimidate is the thermodynamically more stable isomer [33].

Condensation of trichloroacetimidate **11** with azidosphingosine derivative **12** was performed according to Schmidt's method [34]. $BF_3{\cdot}Et_2O$ was used as promotor of the glycosylation and the desired glycolipid **13** was obtained in 52% yield (Scheme 5). The β configuration of the newly introduced glycosidic linkage was confirmed from the ^{1}H NMR spectrum (J>7 Hz).

The azide group of compound **13** was reduced by triphenylphosphine [35] in a mixture of benzene and water at 45°C for 24 h to give an amino derivative. The reaction temperature needs to be carefully controlled (below 50°C) to avoid the formation of byproduct. Due to its unstability, the amino derivative was not characterized at this stage and condensed directly with stearic acid in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) [36] in dry dichloromethane. ^{1}H NMR spectrum of **14** showed a doublet at δ 5.75 ppm (J=9.3 Hz) corresponding to a proton of NH–CO group. The protecting groups of hydroxyl groups were subsequently removed in basic condition (NaOMe) to provide the target glycosphingolipid **2**.

The compound **3** was synthesized by a same method as for the preparation of compound **2**, described above. The synthesis is outlined in Schemes 6 and 7.

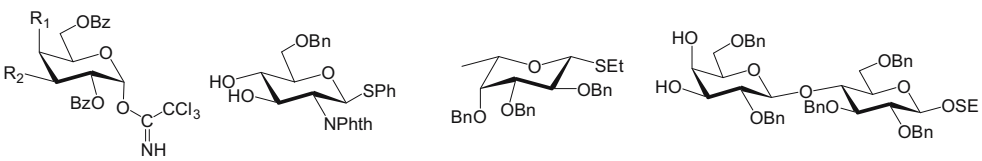
Both compounds **2** and **3** were fully characterized by ^{1}H and ^{13}C NMR, as well as HRMS.

Experimental section

General methods

Optical rotations were measured at 20±2°C with a Perkin-Elmer Model 241 digital polarimeter, using a 10 cm, 1 ml cell. Fast Atom Bombardment (FAB) mass spectra were obtained with a JMS-700 spectrometer. ^{1}H NMR spectra were recorded with a Bruker DRX 400 spectrometer at ambient temperature. Assignments were aided by COSY experiments. ^{13}C NMR spectra were recorded at 100.6 MHz with a Bruker DRX 400 for solutions in $CDCl_3$ or CD_3OD. Reactions were monitored by thin-layer chromatography (TLC) on a precoated plate of silica gel

Scheme 3 Key building blocks for the pentasaccharide synthesis



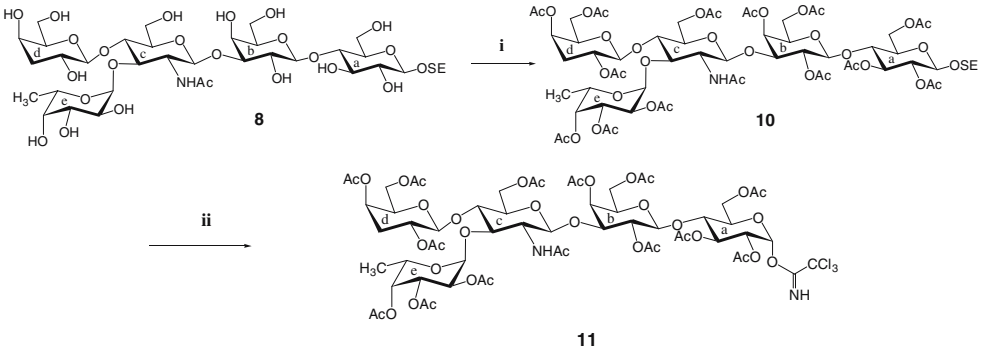
4a R_1= OAc, R_2 = H
4b R_1= H, R_2 = OBz

5

6

7 OSE = $OCH_2CH_2SiMe_3$

Scheme 4 Reagents and conditions: (*i*) Ac_2O, Py, DMAP, 15 h, 94%; (*ii*) TFA, DCM, 0°C for 1 h, then RT for 5 h; and then Cl_3CCN, DBU, DCM, 0°C, 3 h, 60%



60 F_{254} (layer thickness, 0.2 mm; E. Merk, Darmstadt, Germany) and detection by charring with sulfuric acid. Silica gel chromatography was performed on silica gel 60 (230–400 mesh, Merck).

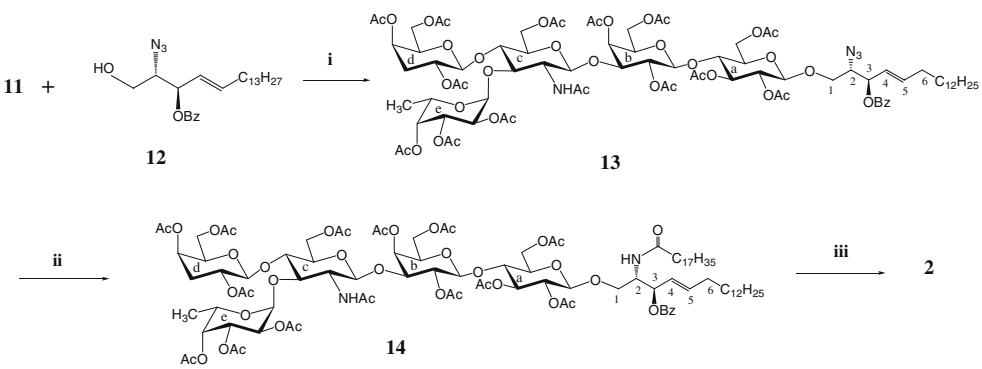
2-(Trimethylsilyl)ethyl(2,4,6-tri-O-acetyl-3-deoxy-β-D-xylo-hexopyranosyl)-(1→4)-[2,3,4-tri-O-acetyl-a-L-fucopyranosyl-(1→3)]-6-O-acetyl-2-deoxy-2-acetamido-β-D-glucopyranoside-(1→3)-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside **10**

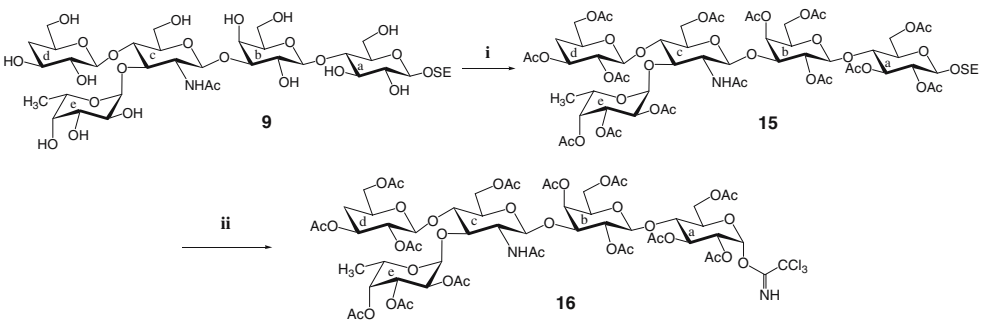
A solution of **8** (20 mg, 0.021 mmol) and DMAP (7 mg) in 3.5 ml of pyridine and 1.8 ml of acetic anhydride was stirred at 30°C for 14 h and then concentrated, co-evaporated with toluene. The residue was purified by flash chromatography (silica gel column, dichloromethane–methanol 30:1) to afford **10** (30 mg, 94%) as a white foam. R_f=0.55 (ethyl acetate–dichloromethane 5:1). $[\alpha]_D$ −27.6 (*c* 1.2, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.43 (d, 1H, *J*=7.4 Hz, NH), 5.35–5.32 (m, 2H), 5.32 (d, 1H, *J*=3.6 Hz, H-1e), 5.21–5.15 (m, 2H), 5.11 (s_{br}, 1H, H-4d), 5.01–4.87 (m, 6H, H-2a, H-2b, H-2d, H-2e, H-5e, H-6), 4.95 (d, 1H, *J*=8.5 Hz, H-1c), 4.58 (d, 1H, *J*=8.4 Hz, H-1b), 4.48 (d, 1H, *J*=8.0 Hz, H-1d), 4.51–4.44 (m, 2H, H-6), 4.35 (d, 1H, *J*=7.8 Hz, H-1a), 4.36–4.33 (m, 2H, 2×H-6), 4.15 (dd, 1H, $J_{5,6}$=5.6 Hz, J_{gem}=12.1 Hz, H-6), 4.07–4.02 (m, 3H, 2×H-6), 3.97–3.91 (m, 1H, O*CH*CH_2Si), 3.85–3.71 (m, 5H), 3.63–3.57 (m, 2H, O*CH*CH_2Si), 3.47 (d, 1H, *J*=9.7 Hz, H-5c), 3.12–3.08 (m, 1H, H-2c), 2.38–2.35 (m, 1H, H-3d), 2.18–1.95 (m, 42H, 14×CH_3CO), 1.73 (ddd, 1H, $J_{3,4}$= 2.5 Hz, $J_{2,3}$=$J_{3,3'}$=14.3 Hz, H-3'd), 1.14 (d, 3H, $J_{5,6}$= 6.4 Hz, H-6e), 0.97–0.89 (m, 2H, CH_2Si), 0.15 (s, 9H, $SiMe_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.65, 171.47, 171.15, 171.07, 171.04, 170.94, 170.92, 170.85, 170.32, 170.10, 170.05, 169.99, 169.78, 169.51 (14×CH_3C=O), 102.08 (C-1b), 101.11 (C-1a), 100.39 (C-1d), 99.59 (C-1c), 95.77 (C-1e), 76.28, 76.20, 74.63, 74.25, 73.55, 73.19, 73.01, 72.72, 72.08, 71.81, 71.56, 71.50, 69.54, 69.22, 68.35, 67.94, 66.82, 64.41 (18×ring C), 67.90 (O*C*H_2CH_2Si), 62.64, 61.99, 61.85, 60.58 (4×C-6), 59.18 (C-2c), 33.39 (C-3d), 23.90–21.03 (14×*C*H_3CO), 18.43 (CH_2Si), 16.21 (C-6e), −1.02 ($SiMe_3$). HRMS (FAB^+) Calcd for $C_{63}H_{93}O_{37}NSiNa$ $[M+Na]^+$: 1506.5093. Found: 1506.5087.

(2,4,6-tri-O-acetyl-3-deoxy-β-D-xylo-hexopyranosyl)-(1→4)-[2,3,4-tri-O-acetyl-a-L-fucopyranosyl-(1→3)]-(6-O-acetyl-2-deoxy-2-acetamido-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-acetyl-a-D-glucopyranosyl trichloroacetimidate **11**

To a solution of compound **10** (50 mg, 0.033 mmol) in 0.84 ml of dichloromethane was added 1.6 ml of trifluoro-

Scheme 5 Reagents and conditions: (*i*) $BF_3{\cdot}Et_2O$, DCM, 4Å powdered molecular sieves, −10°C, 2.5 h, 52%; (*ii*) Ph_3P, H_2O, Benzene, 45°C, 24 h; then stearic acid, EDC, DCM, RT, 24 h, 56%; (*iii*) NaOMe/MeOH, RT, 14 h, 75%



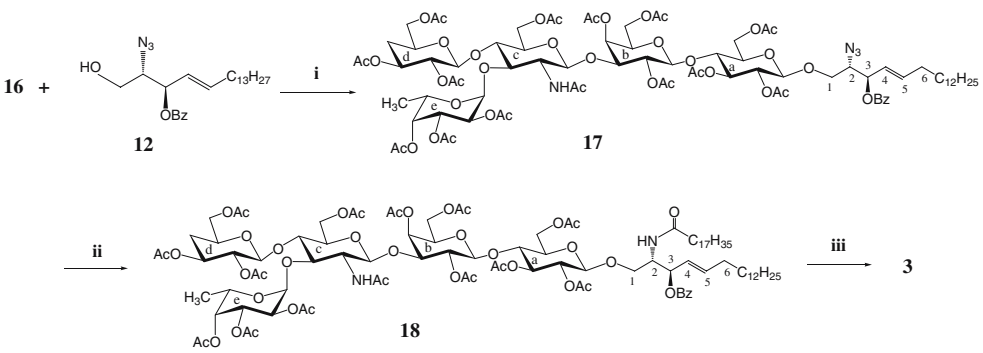


Scheme 6 Reagents and conditions: (*i*) Ac_2O, Py, DMAP, 15 h, 90%; (*ii*) TFA, DCM, 0°C for 1 h, then RT for 5 h; and then Cl_3CCN, DBU, DCM, 0°C, 3 h, 63%

acetic acid dropwise at 0°C. The mixture was stirred at 0°C for 1 h and then at room temperature for 5 h. Then the mixture was diluted with dichloromethane and washed with a saturated aqueous $NaHCO_3$ and then with water, dried over $MgSO_4$. After concentration, the residue was dried in vacuo. (R_f=0.42, dichloromethane–methanol 15:1). The residue was then dissolved in 0.9 ml of dry dichloromethane. 119 μl of trichloroacetonitrile was added to the solution and then 11.9 μl of DBU was added dropwise at −5°C. The mixture was stirred at 0°C for 3 h. After concentration, the residue was purified by flash chromatography (silica gel column, ethyl acetate–dichloromethane–triethylamine 20:10:0.01) to afford **11** as white foam (30 mg, 60%). R_f=0.32 (ethyl acetate–dichloromethane 5:1). $[\alpha]_D$ −10.6 (*c* 1.4, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.67 (s, 1H, HN=C), 6.49 (d, 1H, *J*=3.7 Hz, H-1a), 5.54 (t, 1H, $J_{2,3}=J_{3,4}$=9.8 Hz, H-3a), 5.42 (d, 1H, *J*=7.6 Hz, *NH*Ac), 5.35–5.31 (m, 2H), 5.32 (d, 1H, *J*=3.9 Hz, H-1e), 5.23–5.20 (dd, 1H, *J*= 3.4 Hz, *J*=10.8 Hz), 5.10 ($s_{br,}$ 1H, H-4d), 5.04 (dd, 1H, $J_{1,2}$=3.8 Hz, $J_{2,3}$=10.2 Hz, H-2a), 5.02–4.92 (m, 5H, H-1c, H-2b, H-2e, H-5e, H-6), 4.87–4.81 (m, 1H, H-2d), 4.58 (d, 1H, *J*=8.2 Hz, H-1d), 4.48–4.45 (m, 2H, 2×H-6), 4.38 (d, 1H, *J*=7.9 Hz, H-1b), 4.37–4.31 (m, 3H, H-3c, 2×H-6), 4.19–4.00 (m, 4H, 3×H-6), 3.86–3.74 (m, 5H, H-4a, H-4c), 3.47 (d, 1H, *J*=9.8 Hz, H-5c), 3.11–3.07 (m, 1H, H-2c), 2.36 (ddd, 1H, $J_{2,3}$=5.2 Hz, $J_{3,4}$=2.9 Hz, $J_{3,3'}$= 14.2 Hz, H-3d), 2.18–1.95 (m, 42H, 14×CH_3CO), 1.70 (ddd, 1H, $J_{2,3'}$=11.7 Hz, $J_{3',4}$=2.9 Hz, $J_{3,3'}$=14.2 Hz, H-3'd), 1.13 (d, 3H, $J_{5,6}$=6.5 Hz, H-6e). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.72, 171.47, 171.16, 171.08, 171.02, 170.95, 170.86, 170.80, 170.50, 170.11, 170.03, 169.85, 169.77, 169.48 (14×CH_3CO), 161.41 (C=NH), 102.07 (C-1d), 101.24 (C-1b), 99.60 (C-1c), 95.78 (C-1e), 93.30 (C-1a), 76.39, 75.54, 74.60, 74.21, 73.56, 72.67, 71.80, 71.59, 71.49, 71.40, 70.29, 69.76, 69.53, 69.22, 68.35, 67.94, 66.80, 64.39 (18×ring C), 62.00, 61.95, 61.83, 60.49 (4×C-6), 59.18 (C-2c), 33.34 (C-3d), 23.90–20.89 (14×CH_3CO), 16.21 (C-6e). HRMS (FAB^+) Calcd for $C_{60}H_{81}O_{37}N_2Cl_3Na$ $[M+Na]^+$: 1549.3481. Found: 1549.3446.

(2,4,6-tri-O-acetyl-3-deoxy-β-D-xylo-hexopyranosyl)-(1→4)-[2,3,4-tri-O-acetyl-a-L-fucopyranosyl-(1→3)]-(6-O-acetyl-2-deoxy-2-acetamido-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-O-acetyl-β-D-glucopyranosyl)-(1→1)-(2S, 3R, 4E)-2-azido-3-O-benzoyl-4-octadecene-l,3-diol **13**

A solution of **11** (28 mg, 0.018 mmol, 1.0 equiv.) and 3-*O*-benzyl-azido sphingosine **12** (16.8 mg, 0.036 mmol, 2.0 equiv.) in 0.7 ml of dry dichloromethane was stirred with 4 Å powdered molecular sieves (280 mg) for 20 min at room temperature under an argon atmosphere. $BF_3{\cdot}Et_2O$ (13 μl, 0.103 mmol, 5.7 equiv) was added dropwise at −10°C. The mixture was stirred for 2.5 h at −10°C and then filtered through Celite. The filtrate was washed with a saturated aqueous $NaHCO_3$ and then with water, dried over $MgSO_4$ and concentrated. The residue was applied to a flash chromatography (silica gel column) eluted with cyclohexane–ethyl acetate (1:2) to give the product **13** (17 mg, 52%) as an amorphous solid. R_f=0.55 (ethyl



Scheme 7 Reagents and conditions: (*i*) $BF_3{\cdot}Et_2O$, DCM, 4Å powdered molecular sieves, −10°C, 2.5 h, 58%; (*ii*) Ph_3P, H_2O, Benzene, 45°C, 24 h; then stearic acid, EDC, DCM, RT, 24 h, 50%; (*iii*) NaOMe/MeOH, RT, 16 h, 70%

acetate–dichloromethane 5:1). $[\alpha]_D$ −50.5 (*c* 0.8, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 8.06 (d, 2H, *J*=7.6 Hz, 2× arom H), 7.60 (t, 1H, *J*=7.6 Hz, arom H), 7.47 (t, 2H, *J*= 7.6 Hz, 2×arom H), 5.92 (dt, 1H, $J_{5,6}$=6.8 Hz, $J_{4,5}$=$J_{5,6'}$= 14.8 Hz, H-5cer), 5.63–5.52 (m, 2H, H-3cer, H-4cer), 5.39–5.35 (m, 3H, NH), 5.32 (d, 1H, *J*=3.8 Hz, H-1e), 5.24–5.15 (m, 2H), 5.11 (s_{br}, 1H, H-4d), 5.02–4.92 (m, 6H, H-1c, H-2a, H-2b, H-2e, H-5e, H-6), 4.88–4.82 (m, 1H, H-2d), 4.58 (d, 1H, *J*=8.2 Hz, H-1d), 4.51 (d, 1H, *J*=7.7 Hz, H-1), 4.50–4.43 (m, 2H, 2×H-6), 4.36–4.30 (m, 3H, H-1, H-3c, H-6), 4.10–4.02 (m, 4H, 4×H-6), 3.96–3.93 (m, 1H, H-2cer), 3.89–3.71 (m, 6H, H-1'cer, H-4c), 3.63–3.56 (m, 2H, H-1cer), 3.47 (d, 1H, *J*=9.4 Hz, H-5c), 3.10–3.05 (m, 1H, H-2c), 2.40–2.36 (m, 1H, H-3d), 2.18–1.94 (m, 44H, 14×CH_3CO, CH_2-6cer), 1.73–1.67 (m, 1H, H-3'd), 1.39–1.19 (m, 22H, 11×CH_2), 1.15 (d, 3H, $J_{5,6}$=6.5 Hz, H-6e), 0.89 (t, 3H, *J*=7.0 Hz, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.64, 171.46, 171.15, 171.07, 171.05, 170.92, 170.85, 170.82, 170.27, 170.10, 170.02, 169.92, 169.77, 169.50 (14×CH_3C=O), 165.48 (Ph*C*=O), 139.46 (C-5cer), 133.63 (arom CH), 130.31 (arom C), 130.14 (2×arom CH), 128.87 (2×arom CH), 123.00 (C-4cer), 102.08 (C-1d), 101.21, 100.80 (C-1a, C-1b), 99.56 (C-1c), 95.77 (C-1e), 76.21, 75.96, 74.62, 74.24, 73.55, 73.20, 73.01, 71.81, 71.75, 71.59, 71.46, 69.54, 69.26, 68.35, 64.40 (15× ring C), 75.06 (C-3cer), 72.66 (C-3c), 67.94 (C-2d), 66.81 (C-4d), 63.91 (C-2cer), 59.22 (C-2c), 68.79 (C-1cer), 62.38, 61.84, 60.53, 61.98 (4×C-6), 33.38 (C-3d), 32.78 (C-6cer), 32.32, 30.08, 30.06, 30.05, 30.04, 29.98, 29.80, 29.75, 29.56, 29.13, 23.09 (11×CH_2), 23.88, 21.55–20.94 (14×*C*H_3CO), 1.22 (C-6e), 14.53 (CH_3). HRMS (FAB^+) Calcd for $C_{83}H_{118}O_{39}N_4Na$ $[M+Na]^+$: 1817.7271. Found: 1817.7280.

(2,4,6-tri-O-acetyl-3-deoxy-β-D-xylo-hexopyranosyl)-(1→4)-[2,3,4-tri-O-acetyl-a-L-fucopyranosyl-(1→3)]-(6-O-acetyl-2-deoxy-2-acetamido-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-O-acetyl-β-D-glucopyranosyl)-(1→1)-(2S, 3R, 4E)-2-octadecanamido-3-O-benzoyl-4-octadecene-l,3-diol **14**

To a solution of compound **13** (16 mg, 0.009 mmol) in 2 ml of benzene and 0.08 ml of water was added 10 mg of triphenyl phosphine. The mixture was stirred at 45°C for 24 h. After concentration, the residue was used directly for the next step. R_f=0.46 (dichloromethane–methanol 15:1). The mixture of the residue, stearic acid (9.5 mg, 0.032 mmol, 3.6 equiv), EDC (6.3 mg, 0.032 mmol, 3.6 equiv) in 2 ml dichloromethane was stirred at room temperature for 24 h. Then the mixture was washed with water, dried over $MgSO_4$ and concentrated. The residue was flash chromatographed (silica gel column, ethyl acetate–dichloromethane 2:1) to give **14** as an amorphous solid (10 mg, 56%). R_f=0.50 (ethyl acetate–dichloromethane 5:1). $[\alpha]_D$ +8.1 (*c* 0.8, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 8.04–7.44 (m, 5H, 5×arom H), 5.88 (dt, 1H, $J_{5,6}$=6.7 Hz, $J_{4,5}$=$J_{5,6'}$=15.1 Hz, H-5cer), 5.75 (d, 1H, *J*=9.3 Hz, NH-cer), 5.55 (t, 1H, $J_{3,4}$=$J_{2,3}$=7.4 Hz, H-3cer), 5.47 (dd, 1H, $J_{3,4}$=7.4 Hz, $J_{4,5}$=15.1 Hz, H-4cer), 5.39–5.32 (m, 4H, H-1e, *NH*Ac), 5.22 (dd, 1H, *J*=3.4 Hz, *J*=10.8 Hz), 5.16 (t, 1H, *J*=9.3 Hz), 5.11 (s_{br}, 1H, H-4d), 5.03–4.82 (m, 7H, H-1c, H-2a, H-2b, H-2d, H-2e, H-5e, H-6), 4.58 (d, 1H, *J*=8.2 Hz, H-1d), 4.51–4.47 (m, 2H, H-2cer, H-6), 4.45 (d, 1H, *J*=7.8 Hz, H-1), 4.36–4.29 (m, 3H, H-3c, H-6), 4.32 (d, 1H, *J*=8.1 Hz, H-1), 4.07–4.00 (m, 5H, 4×H-6, H-1cer), 3.87–3.70 (m, 5H, H-4c, H-6), 3.63 (dd, 1H, $J_{1',2}$=4.5 Hz, J_{gem}=10.0 Hz, H-1'cer), 3.57–3.54 (m, 1H, H-5), 3.47 (dt, 1H, $J_{4,5}$=$J_{5,6}$=2.3 Hz, $J_{5,6'}$=9.8 Hz, H-5c), 3.10–3.05 (m, 1H, H-2c), 2.39–2.36 (m, 1H, H-3d), 2.26–1.94 (m, 46H, 14×*CH*$_3$CO, CH_2-6cer, HNCO*CH*$_2$), 1.74–1.65 (m, 1H, H-3'd), 1.65–1.59 (m, 2H, HNCOCH$_2$*CH*$_2$), 1.26 (m, 50H, 25×CH_2), 1.15 (d, 3H, $J_{5,6}$=6.5 Hz, H-6e), 0.89 (t, 6H, *J*=7.0 Hz, 2×CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.10 (HN*C*OCH$_2$), 171.66, 171.46, 171.16, 171.08, 171.06, 170.94, 170.86, 170.80, 170.20, 170.12, 170.10, 170.02, 169.77, 169.51 (14× CH_3C=O), 165.59 (Ph*C*=O), 138.06 (C-5cer), 133.46 (arom CH), 130.64 (arom C), 130.01 (2×arom CH), 128.82 (2× arom CH), 125.01 (C-4cer), 102.07 (C-1d), 101.12, 100.76 (2×C-1), 99.58 (C-1c), 95.77 (C-1e), 76.27, 75.82, 74.61, 74.23, 73.54, 73.17, 72.70, 72.66, 72.04, 71.83, 71.60, 71.43, 69.55, 69.25, 68.35, 67.95, 66.81, 64.40 (18×ring C), 74.44 (C-3cer), 59.23 (C-2c), 67.84 (C-1cer), 62.38, 62.00, 61.84, 60.53 (4×C-6), 51.00 (C-2cer), 37.27 (HNCO*C*H$_2$), 32.75 (CH_2-6cer), 26.15 (HNCOCH$_2$*C*H$_2$), 32.33, 30.12–29.36, 23.10 (25×CH_2), 33.20 (C-3d), 23.89, 21.28–21.04 (14×*C*H_3CO), 16.22 (C-6e), 14.54 (2×CH_3). MS (FAB^+) Calcd for $C_{101}H_{154}N_2O_{40}Na$ $[M+Na]^+$: 2057. Found: 2057.

(3-deoxy-β-D-xylo-hexopyranosyl)-(1→4)-[a-L-fucopyranosyl-(1→3)]-(2-deoxy-2-acetamido-β-D-glucopyranosyl)-(1→3)-(β-D-galactopyranosyl)-(1→4)-(β-D-glucopyranosyl)-(1→1)-(2S, 3R, 4E)-2-octadecanamido-4-octadecene-l,3-diol **2**

A solution of compound **14** (10 mg, 4.9 μmol) in 1.2 ml of NaOMe/MeOH (0.04 M) was stirred at room temperature for 14 h. The mixture was neutralized by Amberlite IR 120/H^+ ion exchange resin. After filtration and concentration, the residue was purified on a Sephadex column (LH20) using dichloromethane–methanol 1/1 as eluant. Compound **2** was obtained as a white amorphous solid (5 mg, 75%). R_f=0.32 (ethyl acetate–isopropanol–water 5:3:2). $[\alpha]_D$ −34.0 (*c* 0.5, MeOH). ^1H NMR (400 MHz, CD_3OD): δ 5.70 (dt, 1H, $J_{5,6}$=6.7 Hz, $J_{4,5}$=$J_{5,6'}$= 15.3 Hz, H-5cer), 5.46 (dd, 1H, $J_{3,4}$=7.7 Hz, $J_{4,5}$= 15.3 Hz, H-4cer), 5.08 (d, 1H, *J*=3.8 Hz, H-1e), 4.72 (d,

1H, *J*=7.7 Hz, H-1), 4.45 (d, 1H, *J*=7.7 Hz, H-1), 4.38 (d, 1H, *J*=7.5 Hz, H-1), 4.32 (d, 1H, *J*=7.8 Hz, H-1), 4.21 (dd, 1H, $J_{5,6}$=4.2 Hz, J_{gem}=10.1 Hz, H-6), 4.10–4.00 (m, 2H, H-3cer), 2.21–2.17 (m, 3H, H-3d, NHCOCH_2), 2.05–2.00 (m, 2H, CH_2-6cer), 2.00 (s, 3H, NHCOCH_3), 1.61–1.58 (m, 3H, H-3'd, NHCOCH$_2CH_2$), 1.31 (m, 50H, 25×CH_2), 1.19 (d, 3H, $J_{5,6}$=6.5 Hz, H-6e), 0.92 (t, 6H, *J*=6.4 Hz, 2×CH_3). ^{13}C NMR (100 MHz, CD_3OD): δ 174.92, 173.55 (2×C=O), 134.12 (C-5cer), 130.37 (C-4cer), 104.68, 104.00, 103.46, 102.82 (4×C-1), 99.31 (C-1e), 82.71, 79.25, 78.77, 76.28, 75.63, 75.53, 75.47, 75.23, 73.81, 73.51, 72.72, 71.96, 70.53, 70.16, 68.96, 68.82, 66.64, 66.01, 65.83 (18×ring C, C-3cer), 68.88 (C-1cer), 62.19, 61.42, 60.50, 60.28 (4×C-6), 56.66 (C-2c), 53.65 (C-2cer), 37.60 (C-3d), 36.36 (HNCOCH_2), 32.47 (C-6cer), 26.17 (NHCOCH$_2CH_2$), 32.10, 29.89–29.43, 22.76 (25×CH_2), 22.16 (NHCOCH_3), 16.60 (C-6e), 13.47 (CH_3), 13.46 (CH_3). HRMS (FAB$^+$) Calcd for $C_{68}H_{124}O_{26}N_2Na$ $[M+Na]^+$: 1407.8340. Found: 1407.8350.

2-(Trimethylsilyl)ethyl(2,3,6-tri-O-acetyl-4-deoxy-β-D-xylo-hexopyranosyl)-(1→4)-[2,3,4-tri-O-acetyl-a-L-fucopyranosyl-(1→3)]-6-O-acetyl-2-deoxy-2-acetamido-β-D-glucopyranoside-(1→3)-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside **15**

The compound was prepared by the same method as **10** and purified by flash chromatography (silica gel column, dichloromethane–methanol 30:1). The compound **15** was obtained as white foam (74 mg, 90%). R_f=0.50 (ethyl acetate–dichloromethane 5:1). $[\alpha]_D$ −10.5 (*c* 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.40 (d, 1H, *J*=7.7 Hz, NH), 5.36 (d, 1H, *J*=3.9 Hz, H-1e), 5.41–5.31 (m, 3H), 5.17 (t, 1H, *J*=9.1 Hz), 4.99–4.88 (m, 7H, H-1c, H-2a, H-2b, H-2e, H-3d, H-5e, H-6), 4.78 (t, 1H, *J*=8.2 Hz, H-2d), 4.60–4.56 (m, 1H, H-6), 4.53 (d, 1H, *J*=8.2 Hz, H-1d), 4.47 (d, 1H, *J*=8.0 Hz, H-1), 4.47–4.43 (m, 1H, H-6), 4.34 (d, 1H, *J*=7.9 Hz, H-1), 4.25 (t, 1H, $J_{2,3}$=$J_{3,4}$=9.2 Hz, H-3c), 4.15–4.02 (m, 5H, 5×H-6), 3.98–3.92 (m, 1H, *CH*CH_2Si), 3.81–3.70 (m, 5H, H-4c, H-5d), 3.62–3.55 (m, 2H, *CH*CH_2Si), 3.45–3.42 (m, 1H, H-5c), 3.14–3.09 (m, 1H, H-2c), 2.18–1.94 (m, 43H, H-4d, 14×CH_3CO), 1.62 (q, 1H, *J*=12.0 Hz, H-4'd), 1.17 (d, 3H, $J_{5,6}$=6.4 Hz, H-6e), 1.00–0.88 (m, 2H, CH_2Si), 0.00 (s, 9H, $SiMe_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.42, 171.41, 171.16, 171.08, 171.04, 170.93, 170.77, 170.68, 170.31, 170.09, 170.06, 169.98, 169.70, 169.38 (14×CH_3C=O), 101.12, 101.01, 100.38 (3×C-1), 99.46 (C-1c), 95.42 (C-1e), 76.21, 76.12, 75.11, 73.51, 73.19, 73.00, 72.08, 72.07, 71.51, 71.48, 70.90, 70.64, 69.51, 69.27, 68.21, 64.71 (16×ring C), 72.76 (C-2d), 72.45 (C-3c), 58.86 (C-2c), 67.88 (O*C*H_2CH_2Si), 65.14, 62.63, 61.90, 60.37 (4×C-6), 33.05 (C-4d), 23.84–21.05 (14×*C*H_3CO), 18.26 (CH_2Si), 16.15 (C-6e), 0.00 ($SiMe_3$). HRMS (FAB$^+$) Calcd for $C_{63}H_{93}NO_{37}SiNa$ $[M+Na]^+$: 1506.5093. Found: 1506.5049.

(2,3,6-tri-O-acetyl-4-deoxy-β-D-xylo-hexopyranosyl)-(1→4)-[2,3,4-tri-O-acetyl-a-L-fucopyranosyl-(1→3)]-(6-O-acetyl-2-deoxy-2-acetamido-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-acetyl-a-D-glucopyranosyl trichloroacetimidate **16**

The compound was prepared by the same method as **11** and purified by flash chromatography (silica gel column, ethyl acetate–dichloromethane–triethylamine 20:10:0.01). Compound **16** was obtained as white foam (17 mg, 63%). R_f=0.33 (ethyl acetate–dichloromethane 5:1). $[\alpha]_D$ −8.3 (*c* 0.7, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.66 (s, 1H, HN=C), 6.47 (d, 1H, *J*=3.7 Hz, H-1a), 5.51 (t, 1H, $J_{2,3}$=$J_{3,4}$=9.9 Hz, H-3a), 5.50 (d, 1H, *J*=9.0 Hz, N*H*Ac), 5.32 (d, 1 H, *J*=3.9 Hz, H-1e), 5.33–5.30 (m, 3H), 5.04 (dd, 1H, $J_{1,2}$=3.7 Hz, $J_{2,3}$=10.0 Hz, H-2a), 5.01–4.88 (m, 5H, H-2e, H-2b, H-3d, H-5e, H-6), 4.91 (d, 1H, *J*=10.2 Hz, H-1c), 4.75 (t, 1H, *J*=9.5 Hz, H-2), 4.55 (dd, 1H, $J_{5,6}$=4.7 Hz, $J_{6',6}$=10.0 Hz, H-6), 4.52 (d, 1H, *J*=8.2 Hz, H-1), 4.42 (dd, 1H, $J_{5,6}$=1.5 Hz, $J_{6',6}$=11.8 Hz, H-6), 4.36 (d, 1H, *J*=7.9 Hz, H-1), 4.21 (t, 1H, $J_{2,3}$=$J_{3,4}$=9.2 Hz, H-3c), 4.17–3.99 (m, 6H, 5×H-6), 3.85–3.68 (m, 5H, H-4a, H-4c, H-5d), 3.42–3.40 (m, 1H, H-5c), 3.14–3.12 (m, 1H, H-2c), 2.17–1.93 (m, 43H, 14×CH_3CO, H-4d), 1.55 (q, 1H, *J*=11.9 Hz, H-4'd), 1.15 (d, 3H, $J_{5,6}$=6.5 Hz, H-6e). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.48, 171.43, 171.15, 171.08, 171.02, 170.79, 170.78, 170.68, 170.47, 170.08, 170.04, 169.83, 169.67, 169.34 (14×CH_3C=O), 161.37 (C=NH), 101.23, 100.99, 99.52 (3×C-1), 95.43 (C-1e), 93.28 (C-1a), 76.22, 75.54, 75.08, 73.51, 72.74, 72.44, 72.06, 71.53, 71.43, 71.38, 70.87, 70.62, 69.48, 69.21, 68.17, 64.68 (16×ring C), 70.28 (C-2a), 69.74 (C-3a), 58.75 (C-2c), 65.12, 61.98, 61.86, 60.26 (4×C-6), 33.02 (C-4d), 23.83–20.87 (14×*C*H_3CO), 16.13 (C-6e). HRMS (FAB$^+$) Calcd for $C_{60}H_{81}N_2O_{37}Cl_3SiNa$ $[M+Na]^+$: 1549.3481. Found: 1549.3483.

(2,3,6-tri-O-acetyl-4-deoxy-β-D-xylo-hexopyranosyl)-(1→4)-[2,3,4-tri-O-acetyl-a-L-fucopyranosyl-(1→3)]-(6-O-acetyl-2-deoxy-2-acetamido-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-O-acetyl-β-D-glucopyranosyl)-(1→1)-(2S, 3R, 4E)-2-azido-3-O-benzoyl-4-octadecene-l,3-diol **17**

The compound was prepared by the same method as **13** and purified by flash chromatography (silica gel column, cyclohexane–ethyl acetate 1:1). Compound **17** was

obtained as an amorphous solid (12 mg, 58%). R_f=0.54 (ethyl acetate–dichloromethane 5:1). $[\alpha]_D$ +1.1 (*c* 1.4, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.06 (d, 2H, *J*= 7.9 Hz, 2×arom H), 7.59 (t, 1H, *J*=7.9 Hz, arom H), 7.46 (t, 2H, *J*=7.9 Hz, 2×arom H), 5.93 (dt, 1H, $J_{5,6}$=6.9 Hz, $J_{4,5}$=$J_{5,6'}$=14.1 Hz, H-5cer), 5.62–5.51 (m, 2H, H-3cer, H-4cer), 5.45 (d, 1H, *J*=7.8 Hz, NH), 5.36 (d, 1H, *J*=3.8 Hz, H-1e), 5.34–5.31 (m, 3H), 5.17 (t, 1H, *J*=9.3 Hz), 5.02–4.88 (m, 7H, H-1c, H-2a, H-2b, H-2e, H-3d, H-5e, H-6), 4.77 (t, 1H, *J*=9.0 Hz, H-2), 4.60–4.50 (m, 3H, H-1, H-1, H-6), 4.43 (d, 1H, J_{gem}=11.1 Hz, H-6), 4.35 (d, 1H, *J*= 7.9 Hz, H-1), 4.25 (t, 1H, $J_{2,3}$=$J_{3,4}$=9.1 Hz, H-3c), 4.19–4.02 (m, 5H, 5×H-6), 3.94 (m, 1H, H-2cer), 3.88–3.69 (m, 6H, H-1cer, H-4c, H-5d), 3.62–3.56 (m, 2H, H-1'cer), 3.43 (d, 1H, *J*=9.7 Hz, H-5c), 3.15–3.10 (m, 1H, H-2c), 2.16–1.93 (m, 45H, 14×CH_3CO, H-4d, CH_2-6cer), 1.62 (q, 1H, *J*=12.0 Hz, H-4'd), 1.93–1.24 (m, 22H, 11×CH_2), 1.17 (d, 3H, $J_{5,6}$=6.3 Hz, H-6e), 0.88 (t, 3H, *J*=5.9 Hz, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.45, 171.18, 171.10, 171.08, 170.84, 170.80, 170.70, 170.29, 170.12, 170.07, 169.93, 169.74, 169.43, 169.40 (14×CH_3*C*=O), 165.48 (Ph*C*=O), 139.46 (C-5cer), 133.63 (arom CH), 130.30 (arom C), 130.14 (2×arom CH), 128.87 (2×arom CH), 122.99 (C-4cer), 101.21, 101.00, 100.78 (3×C-1), 99.45 (C-1c), 95.41 (C-1e), 76.10, 75.97, 75.10, 73.17, 73.01, 72.72, 72.08, 71.74, 71.54, 71.42, 70.89, 70.64, 69.54, 69.31, 68.20, 64.71 (16×ring C), 75.06 (C-3cer), 73.50 (C-5c), 72.42 (C-3c), 63.89 (C-2cer), 58.90 (C-2c), 68.75 (C-1cer), 65.15, 62.36, 60.32, 61.91 (4×C-6), 33.05 (C-4d), 32.78 (C-6cer), 32.31, 30.08–30.04, 29.98, 29.79, 29.75, 29.56, 29.12, 23.09 (11×CH_2), 23.84 (NHCO*C*H_3), 21.42–21.06 (13×*C*H_3CO), 16.15 (C-6e), 14.53 (CH_3). HRMS (FAB^+) Calcd for $C_{83}H_{118}O_{39}N_4Na$ $[M+Na]^+$: 1817.7271. Found: 1817.7334.

(2,3,6-tri-O-acetyl-4-deoxy-β-D-xylo-hexopyranosyl)-(1→4)-[2,3,4-tri-O-acetyl-a-L-fucopyranosyl-(1→3)]-(6-O-acetyl-2-deoxy-2-acetamido-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-O-acetyl-β-D-glucopyranosyl)-(1→1)-(2S, 3R, 4E)-2-octadecanamido-3-O-benzoyl-4-octadecene-l,3-diol **18**

This compound was prepared by the same method as **14** and purified by flash chromatography (silica gel column, ethyl acetate–dichloromethane 2:1). Compound **18** was obtained as an amorphous solid (16 mg, 50%). R_f=0.50 (ethyl acetate–dichloromethane 5:1). $[\alpha]_D$ −24.4 (*c* 0.75, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.03–7.44 (m, 5H, 5×arom H), 5.88 (dt, 1H, $J_{5,6}$=6.9 Hz, $J_{4,5}$=$J_{5,6'}$=14.9 Hz, H-5cer), 5.75 (d, 1H, *J*=9.3 Hz, NH-cer), 5.55 (t, 1H, $J_{3,4}$= $J_{2,3}$=7.4 Hz, H-3cer), 5.47 (dd, 1H, $J_{3,4}$=7.4 Hz, $J_{4,5}$= 14.9 Hz, H-4 cer), 5.37 (d, 1H, *J*=3.7 Hz, H-1e), 5.35–5.32 (m, 5H, *NH*Ac), 5.16 (t, 1H, *J*=9.2 Hz), 5.03–4.88 (m, 7H, H-1c, H-2a, H-2b, H-2e, H-3d, H-5e, H-6), 4.78 (t, 1H, $J_{1,2}$=$J_{2,3}$=8.3 Hz, H-2), 4.59–4.43 (m, 2H, H-2cer, H-6), 4.54 (d, 1H, *J*=8.3 Hz, H-1), 4.44 (d, 1H, *J*=7.8 Hz, H-1), 4.31 (d, 1H, *J*=8.4 Hz, H-1), 4.25 (t, 1H, $J_{3,4}$=$J_{2,3}$=8.9 Hz, H-3c), 4.18 (dd, 1H, $J_{5,6}$=4.4 Hz, $J_{6,6'}$=11.7 Hz, H-6), 4.09–4.00 (m, 5H, H-1cer, 4×H-6), 3.84–3.70 (m, 5H, H-4c, H-5d), 3.63 (dd, 1H, $J_{1',2}$=4.5 Hz, J_{gem}=10.2 Hz, H-1'cer), 3.57–3.54 (m, 1H), 3.44 (d, 1H, *J*=9.4 Hz, H-5c), 3.14–3.08 (m, 1H, H-2c), 2.17–1.94 (m, 47H, 14×CH_3CO, H-4d, CH_2-6cer, HNCOCH_2), 1.69–1.61 (m, 5H, H-4'd, $HNCOCH_2CH_2$, CH_2), 1.27 (m, 48H, 24×CH_2), 1.17 (d, 3H, $J_{5,6}$=6.5 Hz, H-6e), 0.89 (t, 6H, *J*=7.0 Hz, 2×CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.08 (HN*C*OCH_2), 171.42, 171.41, 171.17, 171.09, 171.05, 170.79, 170.78, 170.70, 170.18, 170.10, 170.07, 170.04, 169.68, 169.37 (14×CH_3*C*=O), 165.59 (Ph*C*=O), 138.04 (C-5cer), 133.45 (arom CH), 130.65 (arom C), 130.01 (2×arom CH), 128.81 (2×arom CH), 125.03 (C-4cer), 101.14, 101.02, 100.78 (3×C-1), 99.45 (C-1c), 95.44 (C-1e), 76.12, 75.83, 75.11, 73.51, 73.18, 72.76, 72.72, 72.07, 72.06, 71.56, 71.42, 70.90, 70.65, 69.52, 69.29, 68.21, 64.72 (17×ring C), 74.46 (C-3cer), 72.45 (C-3c), 67.85 (C-1cer), 65.15, 62.36, 61.92, 60.33 (4×C-6), 58.90 (C-2c), 51.01 (C-2cer), 33.07 (C-4d), 37.27 (HNCO*C*H_2), 32.75 (C-6cer), 26.14 ($HNCOCH_2$*C*H_2), 32.33, 30.27–29.35, 23.09 (25×CH_2), 23.85, 21.46–21.04 (14×*C*H_3CO), 16.16 (C-6e), 14.53 (2×CH_3). MS (FAB^+) Calcd for $C_{101}H_{154}N_2O_{40}Na$ $[M+Na]^+$: 2057.9. Found: 2057.9.

(4-deoxy-β-D-xylo-hexopyranosyl)-(1→4)-[a-L-fucopyranosyl-(1→3)]-(2-deoxy-2-acetamido-β-D-glucopyranosyl)-(1→3)-(β-D-galactopyranosyl)-(1→4)-(β-D-glucopyranosyl)-(1→1)-(2S, 3R, 4E)-2-octadecanamido-4-octadecene-l,3-diol **3**

This compound was prepared by the same method as **2** described above and purified on a Sephadex column (LH20) using dichloromethane–methanol 1/1 as eluant. Compound **3** was obtained as a white amorphous solid (5.6 mg, 70%). R_f=0.32 (ethyl acetate–isopropanol–water 5:3:2). $[\alpha]_D$ −19.5 (*c* 0.4, MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ 5.70 (dt, 1H, $J_{5,6}$=6.8 Hz, $J_{4,5}$=$J_{5,6'}$=15.3 Hz, H-5cer), 5.47 (dd, 1H, $J_{3,4}$=7.7 Hz, $J_{4,5}$=15.3 Hz, H-4cer), 5.10 (d, 1H, *J*=3.9 Hz, H-1e), 4.79 (dq, 1H, $J_{5,6}$=6.5 Hz, $J_{4,5}$<1 Hz, H-5e), 4.72 (d, 1H, *J*=7.0 Hz, H-1), 4.46 (d, 1H, *J*=7.7 Hz, H-1), 4.38 (d, 1H, *J*=7.5 Hz, H-1), 4.32 (d, 1H, *J*=7.8 Hz, H-1), 4.21 (dd, 1H, $J_{5,6}$=5.4 Hz, J_{gem}=11.1 Hz, H-6), 4.08–4.07 (m, 2H, H-3cer), 3.08 (dd, 1H, $J_{1,2}$= 7.7 Hz, $J_{2,3}$=9.0 Hz, H-2c), 2.18 (t, 2H, *J*=7.5 Hz, HNCOCH_2), 2.06–1.95 (m, 3H, H-4d, CH_2-6cer), 2.00 (s, 3H, NHCOCH_3), 1.61–1.58 (m, 3H, H-4'd, $HNCOCH_2CH_2$), 1.31 (s, 50H, 25×CH_2), 1.19 (d, 3H, $J_{5,6}$=6.5 Hz, H-6e), 0.92 (t, 6H, *J*=6.8 Hz, 2×CH_3). ^{13}C

NMR (100 MHz, CD_3OD): δ 174.92, 173.51 (2×C=O), 134.12 (C-5cer), 130.37 (C-4cer), 104.00, 103.46 (2×C-1), 102.82 (C-1c), 102.75 (C-1), 99.15 (C-1e), 85.12, 79.01, 76.25, 76.19, 75.62, 75.47, 75.36, 75.23, 74.04, 73.81, 73.09, 72.93, 71.97, 71.07, 70.52, 70.14, 68.89, 68.08, 66.41 (18×ring C, C-3cer), 68.90 (C-1cer), 64.78, 61.41, 60.57, 60.01 (4×C-6), 56.67 (C-2c), 53.65 (C-2cer), 32.47 (C-4d), 36.36 ($HNCOCH_2$), 32.08 (C-6cer), 26.17 ($HNCOCH_2CH_2$), 29.89–29.42, 22.76 (25×CH_2), 22.16 ($NHCOCH_3$), 15.75 (C-6e), 13.46 (CH_3), 13.45 (CH_3). HRMS (FAB^+) Calcd for $C_{68}H_{124}O_{26}N_2Na$ $[M+Na]^+$: 1407.8340. Found: 1407.8331.

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