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Synthesis of dimeric lactose and dimeric (sialyl) Lewis^X glycolipids

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Abstract

To investigate structural requirements for the homophilic interaction between carbohydrates on planar model membranes, divalent derivatives with enforced proximity between the two carbohydrate epitopes (lactose, Lewis^X, and sialyl Lewis^X) were synthesized by use of a dimeric membrane anchor as scaffold.

Keywords: Carbohydrate–carbohydrate interaction; Lewis antigen; Neoglycolipids; Molecular recognition

1. Introduction

Glycolipids, found on the surface of living cells, constitute an important class of biomolecules that are involved in biomolecular recognition, for example in specific cell–cell binding events.[1] The amplification of relatively weak monovalent carbohydrate–lectin affinities to strong polyvalent contacts is a prerequisite of many carbohydrate-based recognition processes.[2] The sialyl Lewis^X (sLe^X) epitope Neu5Acα(2→3)Galβ(1→4)[Fucα(1→3)]GlcNAc is a prominent example because of its implication in inflammation through binding to selectins.[3] The proper orientation of the sLe^X glycolipid on planar membrane surfaces leads to high local concentrations (lateral clusters) resulting in enhanced selectin affinities.[4]

The polyvalent and protein-independent homophilic carbohydrate–carbohydrate interaction is another cell-adhesion process depending on a suitable organization of sugar head groups, as has been recently found for the Lewis^X (Le^X) antigen family.[5] With two covalently tethered Le^X trisaccharide epitopes, the calcium mediated self-assembly of the carbohydrate moieties within the complex could be characterized by NMR spectroscopy.[6]

In continuation of these investigations on homophilic carbohydrate interactions, dimeric glycolipid derivatives were synthesized, which represent the smallest possible carbohydrate cluster (Fig. 1, **1**–**3**). Thus, the tethering of the membrane anchor causes an enforced proximity between the carbohydrate moieties, and the flexibility of the carbohydrate residue is reduced to a rotational mobility when these molecules are inserted in planar membranes.

Considerations of the preferred conformation and van-der-Waals radii of glycolipids reveal, that for each hydrophilic carbohydrate head group two lipophilic alkyl chains are required in order to be suitable constituents in a physiologically relevant planar membrane environment.[7] In our case, a 2-branched fatty alkyl residue was used to mimic the ceramide moiety of naturally occurring glycosphingolipids.[8] For the attachment of two carbohydrate moieties and two 2-branched fatty alkyl residues, a D-threitol based scaffold was used (Fig. 2, **4**). The inherent C_2 symmetry simplifies the NMR-spectroscopic interpretation; in addition, the carbohydrate moieties are separated by six single bonds that allow for considerable conformational flexibility. Furthermore, the primary hydroxy groups enable glycosylation with high yield.

2. Results and discussion

The synthesis of the 2-branched alkylalcohol was accomplished as described for homologous compounds.[9] Dimethyl malonate was alkylated twice with 1-decyl bromide, followed by saponification of the methyl ester and decarboxylation (Scheme 1). The re-

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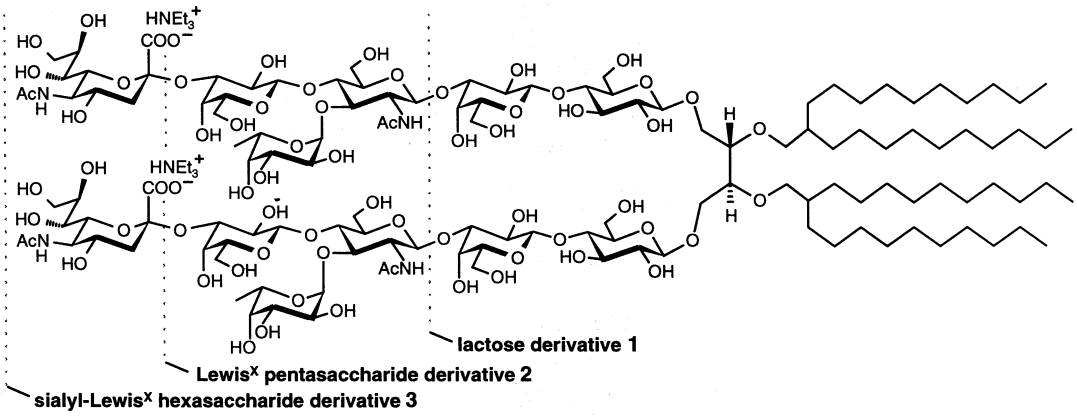


Fig. 1. Dimeric glycolipids **1**–**3** with lactose, Lewis^X, and sialyl Lewis^X head groups.

sulting acid was reduced with $LiAlH_4$ and the formed alcohol **5**[8] was activated with tosyl chloride in pyridine to afford tosylate **6**.

Reaction of 1,4-*O*-benzyl protected D-threitol derivative **7**[10] with an excess of tosylate **6** and sodium hydride as base in DMF–THF furnished the protected membrane anchor **8** in 93% yield (Scheme 2). After hydrogenolytic debenzylation, the membrane anchor **4** was obtained, which served as an acceptor for the following glycosylations.

The assembly of the target compounds is shown in Scheme 3. Glycosylation of **4** with a threefold excess of perbenzoylated lactosyl trichloroacetimidate **9**[11] was performed in the presence of TMSOTf as a catalyst affording the dimeric glycolipid **10** in 83% yield. Exclusively, the desired β-glycoside was isolated ($J_{1a,2a}$ 7.9 Hz). Removal of all *O*-benzoyl groups under Zemplén conditions gave the target compound **1** in 85% yield.

For the synthesis of the Le^X derivative, trichloroacetimidate **11**[12] was used with a threefold excess. Its glycosidation with acceptor **4** was performed in CH_2Cl_2 at room temperature. To avoid the isolation of the corresponding orthoester,[13] 0.4 equiv of TMSOTf and a further 0.2 equiv of TMSOTf after 30 min were used and the reaction time was prolonged to 4.5 h to give **12**. After removal of all *O*-acyl groups under Zemplén conditions, the target compound **2** with the desired β-glycosidic linkage (^{1}H NMR: $J_{1a,2a}$ 7.8 Hz) was isolated in 69% after two steps.

With sLe^X trichloroacetimidate **13**,[12,13] as hexaosyl donor, the corresponding sLe^X intermediate **14** was synthesized under the same conditions as described above. Finally, removal of all *O*-acyl groups and saponification of the methyl ester the target compound **3** was isolated as triethylammonium salt after chromatography on reversed-phase silica gel with MeOH–water–NEt_3 as eluent in 54% after two steps.

In conclusion, an efficient method for the construction of dimeric glycolipids with lactose, Le^X and sLe^X, having up to 12 sugar residues as head group has been investigated. These compounds should prove useful for the investigation of homophilic carbohydrate interactions when inserted in bicelles[†] as planar model membranes.[15] Also other cluster types are currently generated to explore systematically the effect of multivalent ligand representations on biological activity.[‡17]

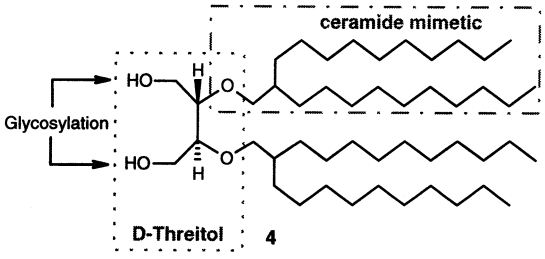
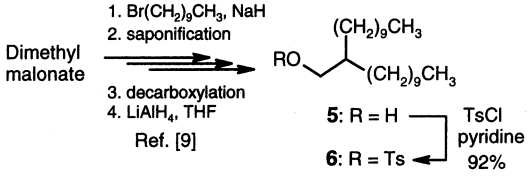
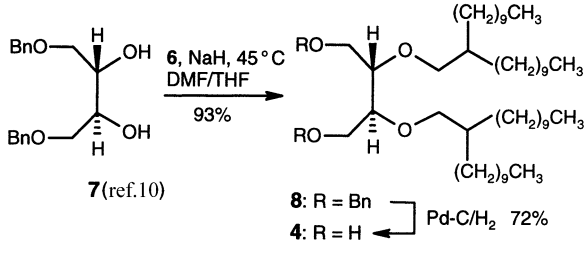


Fig. 2. Structure of the required lipid anchor **4**.



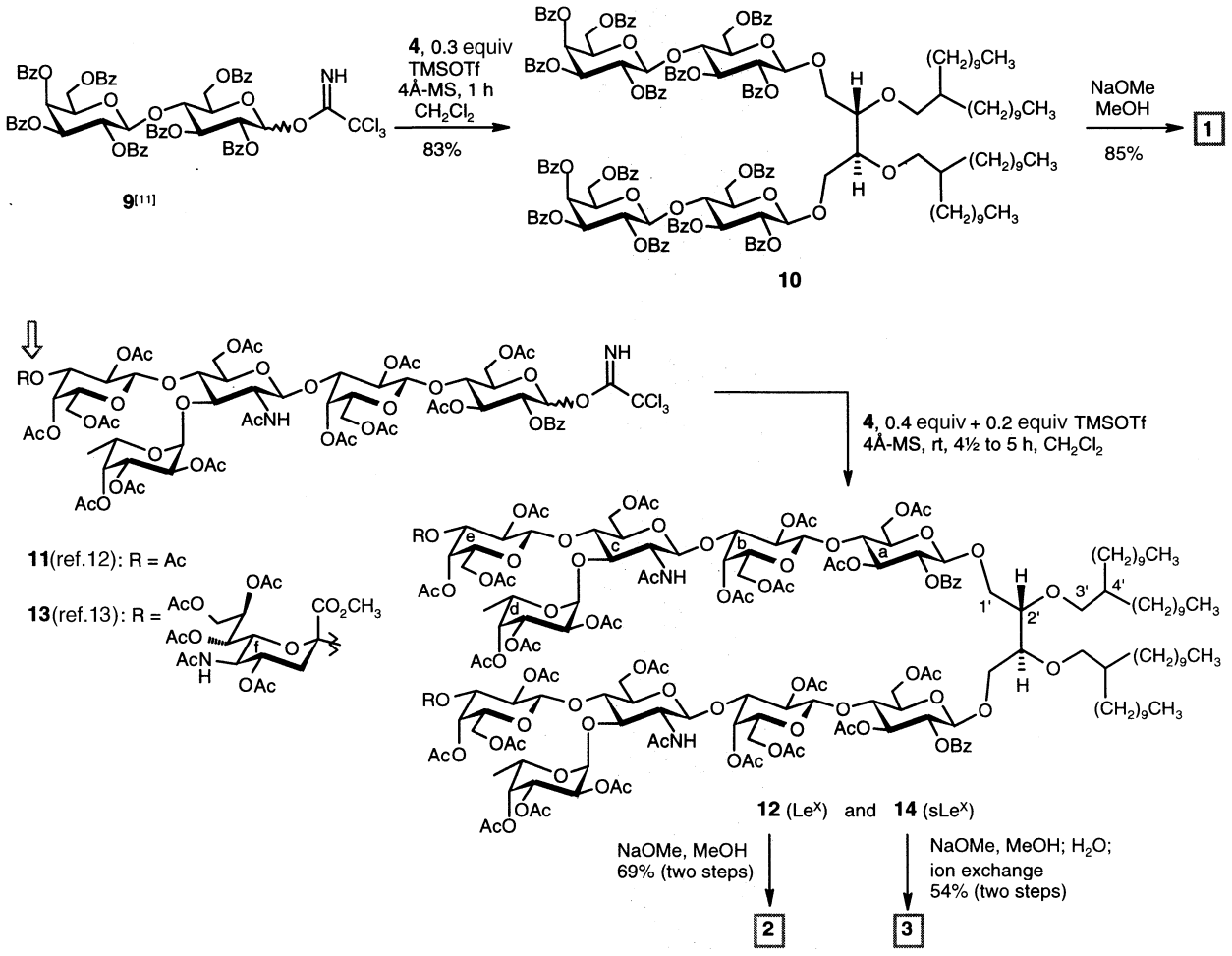
Scheme 1. Synthesis of 2-(decyl) dodecyl tosylate (**6**).



Scheme 2. Generation of lipid anchor **4**.

[†] For bicelle experiments, see Ref. 14.

[‡] For a review on multivalent ligand design, see Ref. 16.



Scheme 3. Synthesis of the target molecules **1**–**3**.

3. Experimental

General methods.—Solvents were purified according to the standard procedures. Flash chromatography was performed on J.T. Baker Silica Gel 60 (40–63 μm) or RP-18 Silica Gel (40 μm) at a pressure of 0.4 bar. TLC was performed on E. Merck Silica Gel plastic plates $60F_{254}$ or E. Merck Silica Gel glass plates HPTLC $60F_{254}$, or E. Merck Silica Gel glass plates RP-18 $60F_{254S}$; compounds were visualized by treatment with a solution of $(NH_4)_6Mo_7O_{24}{\cdot}4\ H_2O$ (20 g) and $Ce(SO_4)_2$ (0.4 g) in 10% H_2SO_4 (400 mL) and heating at 150 °C. Optical rotations were measured on a Perkin–Elmer polarimeter 241 in a 1 dm cell at 22 °C. NMR measurements were recorded on a Bruker AC250 Cryospec or a Bruker DRX600 spectrometer with TMS as internal standard. The carbohydrate monomers were assigned in alphabetical order, beginning with the aglycon, based in part on carbon–proton shift-correlation heteronuclear multiple quantum coherence (HMQC). MALDI-mass spectra were recorded on a Kratos Kompact MALDI I instrument using a 2,5-dihydroxybenzoic acid matrix unless otherwise stated.

2-Decyl-dodecyl-1-tosylate (**6**).—To a solution of 2-decyl-dodecan-1-ol **5**[8] (2.77 g, 8.48 mmol) in dry pyridine (10 mL) was added TsCl (1.94 g, 10.2 mmol) at rt. The mixture was stirred for 5 h, concentrated and diluted with water and extracted with $CHCl_3$ (3 × 50 mL). The organic layers were combined, dried (Na_2SO_4), filtered, and concentrated. The residue, purified by flash chromatography (25:1–19:1 petroleum ether–EtOAc), led to **6** (3.77 g, 92%) as a colorless oil. R_f 0.67 (9:1 petroleum ether–EtOAc); ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.79, 7.34 (2 d, 4 H, C_6H_4), 3.91 (d, J_{vic} 5.3 Hz, 2 H, OCH_2), 2.45 (s, 3 H, CH_3), 1.59–1.54 (m, 1 H, CH), 1.31–1.16 (m, 36 H, 18 CH_2), 0.88 (t, 6 H, 2 CH_3). MALDI-MS (positive mode, THF): 503.1 $[M + Na]^+$.

*1,4-Di-*O*-benzyl-2,3-di-*O*-(2-decyl-dodecyl)-*D*-threitol* (**8**).—To a suspension of **6** (2.16 g, 4.50 mmol, 3 equiv) and 1,4-di-*O*-benzyl-D-threitol **7**[10] (454 mg, 1.50 mmol) in dry 3:2 DMF–THF (25 mL) was added NaH (144 mg, 6.0 mmol). The mixture was stirred overnight under argon at 50–55 °C. Then additional **6** (750 mg, 1.56 mmol) and NaH (36 mg, 1.5 mmol) was added and

stirred again overnight. After addition of MeOH, the mixture was concentrated, diluted with brine and extracted with $CHCl_3$ (3 × 100 mL). The organic layers were combined, dried (Na_2SO_4), filtered, and concentrated. Flash chromatography (20:1 petroleum ether–EtOAc) of the residue furnished **8** (1.28 g, 93%) as a colorless oil. $[\alpha]_D$ +3.2° (*c* 1, $CHCl_3$); R_f 0.54 (20:1 petroleum ether–EtOAc); ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.32–7.27 (m, 10 H, 2 C_6H_5), 4.51 (s, 4 H, 2 CH_2Ph), 3.68–3.48 (m, 8 H, 4 CH_2), 3.36 (dd, 2 H, CHO), 1.54 (m, 2 H, 2 CH), 1.30–1.25 (m, 72 H, 36 CH_2), 0.88 (t, 12 H, 4 CH_3). MALDI-MS (positive mode, THF): 941.0 $[M + Na]^+$, 957.0 $[M + K]^+$.

2,3-Di-O-*(2-decyl-dodecyl)*-D-*threitol* (**4**).—To a solution of **8** (1.21 g, 1.32 mmol) in EtOAc (40 mL) was added AcOH (3 drops) and 10% Pd–C (180 mg). Hydrogenolysis was performed at atmospheric pressure overnight and then the mixture was filtered through Celite, washed and concentrated. Flash chromatography (9:1–7:1 petroleum ether–EtOAc) of the residue led to **4** (763 mg, 72%) as a colorless oil. $[\alpha]_D$ +0.8° (*c* 1, $CHCl_3$); R_f 0.16 (9:1 petroleum ether–EtOAc); ^{1}H NMR (250 MHz, $CDCl_3$): δ 3.82–3.44 (m, 10 H, 2 CHO, 4 CH_2), 2.36 (s, 2 H, 2 OH), 1.54 (m, 2 H, 2 CH), 1.34–1.26 (m, 72 H, 36 CH_2), 0.88 (t, 12 H, 4 CH_3). MALDI-MS (positive mode, THF): 761.4 $[M + Na]^+$, 777.3 $[M + K]^+$.

1,4-Di-O-*[(2,3,4,6-tetra*-O-*benzoyl-β*-D-*galactopyranosyl)-(1→4)-2,3,6-tri*-O-*benzoyl-β*-D-*glucopyranosyl]-2,3-di*-O-*(2-decyl-dodecyl)*-D-*threitol* (**10**).—A solution of *O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-α/β-D-glucopyranosyl trichloroacetimidate (**9**)[11] (234 mg, 193 μmol, 3 equiv) and **4** (47.6 mg, 64.3 μmol) in dry CH_2Cl_2 (3 mL) was stirred with molecular sieves AW-300 for 30 min. Then TMSOTf (3.5 μL, 19 μmol, 0.3 equiv) was added under argon at rt. After 1 h the solution was neutralized with NEt_3, filtrated, and concentrated. Flash chromatography (14:1 toluene–EtOAc) of the residue gave **10** (153 mg, 83%) as a colorless foam. $[\alpha]_D$ +32.3° (*c* 1, $CHCl_3$); R_f 0.37 (12:1 toluene–EtOAc); ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.00–7.12 (m, 70 H, 14 C_6H_5), 5.73–5.70 (m, 4 H, 2b-H, 4b-H), 5.64 (d, $J_{2,3} = J_{3,4}$ 9.5 Hz, 2 H, 3a-H), 5.38–5.34 (m, 4 H, 2a-H, 3b-H), 4.81 (d, $J_{1,2}$ 7.9 Hz, 2 H, 1b-H), 4.49–4.42 (m, 4 H, 6a-H, 6′a-H), 4.31 (d, $J_{1,2}$ 7.9 Hz, 2 H, 1a-H), 4.15 (dd, $J_{3,4} = J_{4,5}$ 9.4 Hz, 2 H, 4a-H), 3.85–3.83 (m, 4 H, 2′-H, 5b-H), 3.67 (m, 4 H, 6b-H, 6′b-H), 3.54 (m, 2 H, 5a-H), 3.31 (m, 4 H, 1′-CH_2), 3.12–3.03 (m, 4 H, 3′-CH_2), 1.58 (s, 2 H, 4′-H), 1.30–0.96 (m, 72 H, 36 CH_2), 0.88 (t, 12 H, 4 CH_3). Anal. Calcd for $C_{170}H_{194}O_{38}\cdot H_2O$ (2863.40): C, 71.31; H, 6.90. Found: C, 71.35; H, 6.92.

1,4-Di-O-*[(β*-D-*galactopyranosyl)-(1→4)-β*-D-*glucopyranosyl]-2,3-di*-O-*(2-decyl-dodecyl)*-D-*threitol* (**1**).—To a solution of **10** (145 mg, 50.6 μmol) in dry MeOH (10 mL) was added NaOMe (10 mg). The mixture was stirred for 2 days at rt, then neutralized with Amberlite IR-120 (H^+), filtered, and concentrated. Flash chromatography (75:25:4–70:30:5 $CHCl_3$–MeOH–water) of the residue furnished **1** (60 mg, 85%) as a colorless amorphous solid after lyophilization from water–dioxane. R_f 0.47 (14:6:1 $CHCl_3$–MeOH–water, HPTLC); ^{1}H NMR (600 MHz, 65:25:4 $CDCl_3$–CD_3OD–D_2O): δ 4.29 (d, $J_{1,2}$ 6.8 Hz, 2 H, 1b-H), 4.25 (d, $J_{1,2}$ 7.8 Hz, 2 H, 1a-H), 3.95 (br d, J_{gem} 9.0 Hz, 2 H, 1′-H), 3.83–3.33 [m, 30 H, HMQC: 3.78 (6′a-H), 3.78 (6a-H), 3.76 (4b-H), 3.71 (6′b-H), 3.61 (6b-H), 3.59 (1′-H), 3.53 (5b-H), 3.52 (4a-H), 3.48 (3a-H), 3.48 (2′-H), 3.45 (3b-H), 3.45 (2b-H), 3.42 (3′-H), 3.34 (3′-H), 3.34 (5a-H)], 3.24 (dd, 2 H, 2a-H), 1.48 (s, 2 H, 4′-H), 1.25–1.08 (m, 72 H, 36 CH_2), 0.80 (t, 12 H, 4 CH_3). ^{13}C NMR (151 MHz, 65:25:4 $CDCl_3$–CD_3OD–D_2O, excerpt): 103.99 (1b-C), 103.56 (1a-C), 79.95 (2′-C), 79.76 (4a-C), 76.09 (5b-C), 75.48, 75.25, 75.21 (3a-C, 5a-C, 3′-C), 73.77 (3b-C), 73.55 (2a-C), 71.66 (2b-C), 69.43 (4b-C), 68.91 (1′-C), 61.78 (6b-C), 61.30 (6a-C). MALDI-MS (positive mode, $CHCl_3$–MeOH): 1410.6 $[M + Na]^+$, 1427.6 $[M + K]^+$.

1,4-Di-O-*[(2,4,6-tri*-O-*acetyl-β*-D-*galactopyranosyl)-(1→4)-[(2,3,4-tri*-O-*acetyl-α*-L-*fucopyranosyl)-(1→3)]-(2-acetamido-6*-O-*acetyl-2-deoxy-β*-D-*glucopyranosyl)-(1→3)-(2,4,6-tri*-O-*acetyl-β*-D-*galactopyranosyl)-(1→4)-3,6-di*-O-*acetyl-2*-O-*benzoyl-β*-D-*glucopyranosyl]-2,3-di*O-*(2-decyl-dodecyl)*-D-*threitol* (**12**).—A solution of Le^X trichloroacetimidate **11**[12] (209 mg, 124 μmol, 3 equiv) and **4** (31.0 mg, 41.3 μmol) in dry CH_2Cl_2 (5 mL) was stirred with molecular sieves AW-300 for 30 min. Then TMSOTf (3.0 μL, 17 μmol, 0.4 equiv) was added under argon at rt and after 30 min again TMSOTf (1.5 μL, 0.2 equiv). After further 4 h, the solution was neutralized with NEt_3, filtrated, and concentrated. Flash chromatography (2:1 toluene–acetone) of the residue gave **12** (135 mg, 88%) as a pale yellow foam. R_f 0.33 (3:2 toluene–acetone, HPTLC); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.95–7.43 (m, 10 H, 2 C_6H_5), 5.41–3.06 [m, 78 H, HMQC: 5.40 (4e-H), 5.36 (4d-H), 5.32 (1d-H), 5.32 (3a-H), 5.30 (4b-H), 5.18 (3d-H), 5.10 (2a-H), 5.09 (2e-H), 5.01 (3e-H), 4.96 (6′c-H), 4.96 (2b-H), 4.95 (5d-H), 4.95 (2d-H), 4.95 (1c-H), 4.60 (1e-H), 4.41 (6′a-H), 4.39 (6′e-H), 4.39 (1b-H), 4.32 (1a-H), 4.30 (3c-H), 4.20 (6e-H), 4.12 (6a-H), 4.10 (6′b-H), 4.03 (6b-H), 3.93 (6c-H), 3.89 (1′-CHH), 3.87 (5e-H), 3.80 (4c-H), 3.77 (4a-H), 3.75 (5b-H), 3.70 (3b-H), 3.52 (5a-H), 3.42 (5c-H), 3.35 (1′-CHH), 3.35 (2′-H), 3.14, 3.07 (3′-CH_2), 3.05 (2c-H), N_cH], 2.19–1.90 (m, 84 H, 28 $COCH_3$), 1.63 (s, 2 H, 4′-H), 1.32–0.92 (m, 78 H, 36 CH_2, 6d-CH_3), 0.89 (t, 12 H, 4 CH_3). MALDI-MS (positive mode, THF): 3734.9 $[M + Na]^+$, 3750.2 $[M + K]^+$.

1,4-Di-O-*[(β*-D-*galactopyranosyl)-(1→4)-[(α*-L-*fucopyranosyl)-(1→3)]-(2-acetamido-2-deoxy-β*-D-*glucopyranosyl)-(1→3)-(β*-D-*galactopyranosyl)-(1→4)-β*-D-

*glucopyranosyl]-2,3-di-*O*-(2-decyl-dodecyl)-*D*-threitol* (**2**).—To a solution of **12** (130 mg, 35.0 μmol) in dry MeOH (40 mL) was added NaOMe (40 mg). The mixture was stirred for 3 days at rt, then neutralized with Amberlite IR-120 (H^+), filtered, and adsorbed on RP-18 Silica Gel. Flash chromatography with RP-18 Silica Gel (2:1–5:1–1:0 MeOH–water) furnished **2** (57.9 mg, 78%) as a colorless amorphous solid after lyophilization from water. R_f 0.36 (MeOH, RP-18); ^{1}H NMR (600 MHz, CD_3OD): δ 5.05 (d, $J_{1,2}$ 3.8 Hz, 2 H, 1d-H), 4.82 (5d-H in solvent signal), 4.70 (d, $J_{1,2}$ 7.9 Hz, 2 H, 1c-H), 4.43 (d, $J_{1,2}$ 7.5 Hz, 2 H, 1e-H), 4.37 (d, $J_{1,2}$ 7.7 Hz, 2 H, 1b-H), 4.32 (d, $J_{1,2}$ 7.8 Hz, 2 H, 1a-H), 4.05–3.23 [m, 54 H, HMQC: 4.05 (4b-H), 3.95 (6′c-H), 3.91 (3c-H), 3.90 (2c-H), 3.90 (6c-H), 3.89 (6′a-H), 3.87 (4c-H), 3.86 (3d-H), 3.84 (6a-H), 3.79 (4e-H), 3.78 (6′b-H), 3.76 (6′e-H), 3.71 (4d-H), 3.67 (6b-H), 3.66 (6e-H), 3.64 (2d-H), 3.62 (2b-H), 3.58 (5b-H), 3.57 (4a-H), 3.53 (3b-H), 3.52 (3a-H), 3.50 (2e-H), 3.45 (5e-H), 3.45 (3e-H), 3.43 (5c-H), 3.40 (5a-H), 3.26 (2a-H)], 1.97 (s, 6 H, $COCH_3$), 1.55 (m, 2 H, 4′-H), 1.38–1.28 (m, 72 H, 36 CH_2), 1.17 (d, $J_{5,6}$ 6.5 Hz, 6 H, 6d-CH_3), 0.90 (t, 12 H, 4 CH_3). MALDI-MS (positive mode, MeOH): 2436.4 $[M + Na]^+$.

*1,4-Di-*O*-[(methyl-5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonulopyranosylonate)-(2→3)-(2,4,6-tri-*O*-acetyl-β-*D*-galactopyranosyl)-(1→4)-[(2,3,4-tri-*O*-acetyl-α-*L*-fucopyranosyl)-(1→3)]-(2-acetamido-6-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-acetyl-β-*D*-galactopyranosyl)-(1→4)-3,6-di-*O*-acetyl-2-*O*-benzoyl-β-*D*-glucopyranosyl]-2,3-di-*O*-(2-decyl-dodecyl)-*D*-threitol* (**14**).—A solution of sLe^X trichloroacetimidate **13**[13] (346 mg, 165 μmol, 3 equiv) and **4** (40.7 mg, 55.0 μmol) in dry CH_2Cl_2 (5 mL) was stirred with molecular sieves AW-300 for 30 min. Then TMSOTf (4.0 μL, 22 μmol, 0.4 equiv) was added under argon at rt and after 30 min again TMSOTf (2.0 μL, 0.2 equiv). After further 4.5 h, the solution was neutralized with NEt_3, filtrated, and concentrated. Flash chromatography (20:1–15:1 $CHCl_3$–MeOH) of the residue gave **14** (166 mg, 66%) as a colorless foam. $[\alpha]_D$ −19.5° (*c* 1, $CHCl_3$); R_f 0.26 (3:2 toluene–acetone, HPTLC); ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.00–7.42 (m, 10 H, 2 C_6H_5), 5.33–5.31 [m, 12 H, HMQC: 5.51 (8f-H), 5.44 (7f-H), 5.33 (1d-H), 5.33 (4b-H), 5.32 (N_cH), 5.31 (4d-H)], 5.23 (dd, $J_{2,3} = J_{3,4}$ 9.5 Hz, 2 H, 3a-H), 5.20 (dd, $J_{2,3}$ 10.9, $J_{3,4}$ 3.1 Hz, 2 H, 3d-H), 5.10 (dd, $J_{1,2}$ 8.2, $J_{2,3}$ 9.5 Hz, 2 H, 2a-H), 5.02–4.77 [m, 20 H, HMQC: 5.00 (5d-H), 4.98 (2b-H), 4.95 (4e-H), 4.95 (2d-H), 4.90 (1c-H), 4.89 (2e-H), 4.88 (4f-H), 4.81 (6′c-H), 4.77 (1e-H), N_fH], 4.52 (dd, $J_{2,3}$ 10.1, $J_{3,4}$ 3.2 Hz, 2 H, 3e-H), 4.43–4.37 [m, 4 H, HMQC: 4.42 (6′a-H), 4.38 (6′e-H)], 4.33 (d, $J_{1,2}$ 7.9 Hz, 2 H, 1a-H), 4.31 (d, $J_{1,2}$ 8.0 Hz, 2 H, 1b-H), 4.43–3.07 [m, 50 H, HMQC: 4.25 (9′f-H) 4.22 (3c-H), 4.22 (6e-H), 4.13 (6a-H), 4.08 (9f-H), 4.03 (5f-H), 4.00 (6b-H, 6′b-H), 3.88 (1′-CHH), 3.87 (4c-H), 3.86 ($COOCH_3$), 3.84 (5e-H), 3.77 (4a-H), 3.73 (5b-H), 3.69 (3b-H), 3.63 (6f-H), 3.51 (5a-H), 3.45 (5c-H), 3.35 (2′-H), 3.34 (1′-CHH), 3.18 (2c-H), 3.14, 3.07 (3′-CH_2)], 2.58 (dd, $J_{3,4}$ 4.2, J_{gem} 12.4 Hz, 2 H, 3f-H_e), 2.20–1.85 (m, 108 H, 36 $COCH_3$), 1.68 (t, $J_{3,4} = J_{gem}$ 12.3 Hz, 2 H, 3f-H_a), 1.62 (s, 2 H, 4′-H), 1.32–0.94 (m, 78 H, 36 CH_2, 6d-CH_3), 0.89 (t, 12 H, 4 CH_3). ^{13}C NMR (151 MHz, $CDCl_3$, excerpt): δ 100.8 (1a-C), 100.7 (1b-C), 99.9 (1e-C), 99.4, (1c-C), 95.3 (1d-C), 78.6 (2′-C), 76.0 (3b-C), 75.6 (4a-C), 74.4 (3′-C), 74.3 (4c-C), 73.1 (5c-C), 72.8 (5a-C), 72.5 (3a-C), 72.3 (3c-C), 71.9 (6f-C), 71.8 (2a-C), 71.6 (4d-C), 71.4 (3e-C), 71.2 (2b-C), 71.2 (5b-C), 70.9 (5e-C), 70.1 (1′-C), 69.8 (2e-C), 69.4 (4f-C), 69.0 (4b-C), 68.9 (2d-C), 68.0 (3d-C), 67.6 (8f-C), 67.4 (4e-C), 66.6 (7f-C), 64.1 (5d-C), 62.1 (6a-C), 61.7 (9f-C), 61.5 (6b-C), 61.4 (6e-C), 60.9 (6b-C), 58.5 (2cC), 53.2 (OCH_3), 49.1 (5f-C), 38.3 (4′-C), 37.4 (3f-C), 15.8 (6d-C). MALDI-MS (positive mode, THF): 4598.7 $[M + Na]^+$, 4613.1 $[M + K]^+$. Anal. Calcd for $C_{214}H_{314}N_4O_{102}$ (4574.70): C, 56.19; H, 6.92; N, 1.22. Found: C, 56.20; H, 6.84; N, 0.85.

*1,4-Di-*O*-[(triethylammonium-5-acetamido-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonulopyranosylonate)-(2→3)-(β-*D*-galactopyranosyl)-(1→4)-[(α-*L*-fucopyranosyl)-(1→3)]-(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-(1→3)-(β-*D*-galactopyranosyl)-(1→4)-β-*D*-glucopyranosyl]-2,3-di-*O*-(2-decyl-dodecyl)-*D*-threitol* (**3**).—To a solution of **14** (159 mg, 34.8 μmol) in dry MeOH (40 mL) was added NaOMe (80 mg). The mixture was stirred for 2 days at rt, then water (0.5 mL) was added and stirred for 3 h. After neutralization with Amberlite IR-120 (H^+), the mixture was filtered and adsorbed on RP-18 Silica Gel. Flash chromatography with RP-18 Silica Gel (5:1–8:1–1:0 MeOH–water + 0.5% NEt_3) afforded **1** (91.4 mg, 82%) as a colorless amorphous solid after lyophilization from water. R_f 0.71 (MeOH, RP-18); ^{1}H NMR (600 MHz, CD_3OD): δ 5.04 (d, $J_{1,2}$ 3.7 Hz, 2 H, 1d-H), 4.82 (5d-H in solvent signal), 4.68 (d, $J_{1,2}$ 7.7 Hz, 2 H, 1c-H), 4.50 (d, $J_{1,2}$ 7.7 Hz, 2 H, 1e-H), 4.37 (d, $J_{1,2}$ 7.7 Hz, 2 H, 1b-H), 4.32 (d, $J_{1,2}$ 7.8 Hz, 2 H, 1a-H), 4.05–3.25 [m, 68 H, HMQC: 4.05 (4b-H), 4.03 (3e-H), 4.02 (6′c-H), 3.93 (2c-H), 3.91 (3c-H), 3.89 (6a-H), 3.87 (4e-H), 3.88 (6c-H), 3.85 (4c-H), 3.84 (9′f-H), 3.84 (6a-H), 3.77 (6′b-H), 3.75 (6′e-H), 3.71 (4d-H), 3.70 (5f-H), 3.70 (4f-H), 3.69 (6f-H), 3.69 (6b-H), 3.64 (6e-H), 3.63 (7f-H), 3.62 (2d-H), 3.61 (2b-H), 3.59 (9f-H), 3.59 (5b-H), 3.57 (3d-H), 3.56 (4a-H), 3.54 (3b-H), 3.53 (2e-H), 3.51 (3a-H), 3.45 (8f-H), 3.45 (5e-H), 3.43 (5c-H), 3.39 (5a-H), 3.25 (2a-H)], 3.20 (q, 12 H, $N(CH_2Me)_3$), 2.87 (dd, 2 H, 3f-H_e), 2.00, 1.97 (2 s, 12 H, 2 $COCH_3$), 1.71 (t, 2 H, 3f-H_a), 1.55 (m, 2 H, 4′-H), 1.37–1.28 (m, 90 H, 18 CH_2, $N(CH_2CH_3)_3$), 1.15 (d, $J_{5,6}$ 6.5 Hz, 6 H, 6d-CH_3), 0.90 (t, 12 H, 2 CH_3). MALDI-MS (negative mode, matrix 4-nitroaniline, MeOH): 2990 $[M - NEt_3 - HNEt_3]^-$, 3012 $[M - 2HNEt_3 + Na]^-$.

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