New Highly Hydrophobic Lewis X Glycolipids: Synthesis and Monolayer Behaviour

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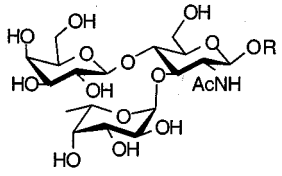
Two highly hydrophobic Lewis X glycolipids **2** and **3** were prepared. The glycoconjugate **2** was constructed in the following way: pentaerythrytol was used as a distributor on which three racemic phytol hydrophobic chains and a triethyleneglycol spacer β-glycosylated with the pentasaccharide Gal (β 1–4)[Fuc (α 1–3)] GlcNAc (β 1–3) Gal (β 1–4) Glc were anchored. The glycoconjugate **3** was constructed in a similar way, the sugar moiety being the trisaccharide Gal (β 1–4)[Fuc (α 1–3)] GlcNAc, the so-called Lewis X determinant. A triethyleneglycol spacer was used in order to introduce the mobility required for the study of single carbohydrate–carbohydrate interactions. Three phytyl chains increase the hydrophobicity of the lipid moiety compared to the natural ceramide glycolipid **1**. These glycolipids display a liquid-expanded behaviour with a high compressibility in monolayer studies. These properties associated with a low solubility in water make them good candidates for the study of the interaction between two Lewis X functionalized vesicles.

Introduction

Although polysaccharide−polysaccharide interactions have clearly been demonstrated, it was only in the nineties that Hakomori opened a new trend in cellular biology in suggesting that initial cell-cell recognition may be based on the interaction of well-defined cell surface carbohydrates with matching carbohydrate structures on the surface of another cell. This new concept in biological science − inherently logical when realising that the carbohydrate chains of glycoconjugates are the most exposed structures at the cell surface − was launched with the Lewis X system. The carbohydrate chains of glycosphingolipids and glycoproteins at the murine cell surface undergo a complex sequence of rapid changes during embryogenesis. In a mouse model of pre-implantation embryo, the expression of stage-specific embryonic antigen 1 (SSEA-1) was first observed at the eight-cell stage, maximal at the 16- or 32-cell stage, and rapidly declined after compaction.[1] The SSEA-1 epitope was identified as Lewis X,[2,3] which is *N*-acetyl lactosamine α-L-fucosylated at the 3-position of *N*-acetyl-D-glucosamine.

Specific homotypic and Ca^{2+}-mediated interactions between Lewis X and Lewis X determinants of glycosphingolipid[4] and glycoproteins[5] have been investigated and provide a possible basis for initial cell recognition in pre-implantation embryos and in embryonal carcinoma cells. This



Lewis X

demonstration was based on several experiments, especially the observed selective self-aggregation of liposomes containing cholesterol and purified Lewis X natural glycolipids. This was recently confirmed[6] using as a model rat basophilic leukaemia cells, which do not express Lewis X and do not exhibit autoaggregation; aggregation was observed after the incorporation of natural Lewis X glycosphingolipids. This aggregation was dependent on the presence of Ca^{2+}. MS[7] and NMR[8,9] spectroscopic studies have also shown that Lewis X−Lewis X interactions exist in the presence of Ca^{2+}.

In order to study and quantify the putative Ca^{2+}-mediated Lewis X−Lewis X homotypic interaction, we decided to study the adhesion between electrically neutral, giant vesicles that include tailored synthetic lipids bearing an Lewis X group at the surface of the vesicle. This article discusses the chemical synthesis of these tailored glycolipids and their monolayer behaviour.

Results and Discussion

Selection of Tailored Glycolipids

We first used the extracted natural glycolipid **1** in this study. Although pure according to the ^1H NMR spectrum, it is difficult to estimate the exact composition of the long-chain saturated fatty acid. The lipid moiety of this natural

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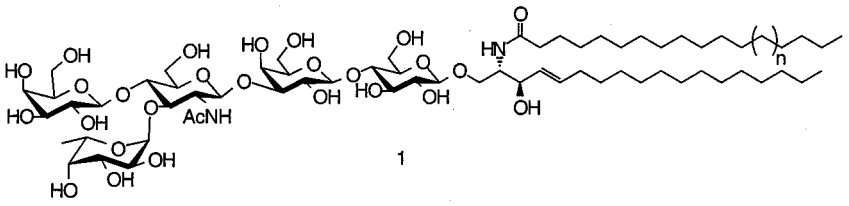

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product is thus not perfectly defined. We checked the monolayer behaviour of **1**. The compression isotherms were obtained with an automatic Langmuir balance[10] film and are shown in Figure 1.

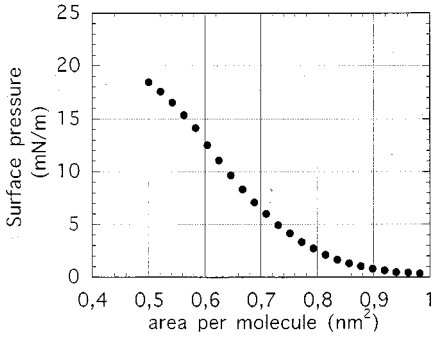


Figure 1. Compression isotherm of **1** (t = 20 °C); a chloroform/methanol (4:1) solution of the lipid was spread on pure water, and 15 minutes were allowed for the solvent to evaporate before the pressure area measurements were made; ultrapure water was obtained from an Elgastat maxima unit HPLC model (TOC less than 3ppb); the compression rate was 0.01 nm^2/minute; the maximum pressure was 16 mN/m because higher pressures caused hysteresis in the surface pressure versus molecular area curve, due to lipid departure from the monolayer

Compound **1** displays a "liquid-expanded" behaviour characterised by a high compressibility. A "liquid-expanded" state indicates an enhanced mobility of the molecules. This mobility is necessary to make the molecules accessible for interaction with other molecules.

The stability of the monolayers is a prerequisite for surface interaction and adhesion measurements. The solubility of the lipid was probed by measuring the stability of the monolayer under a constant surface pressure. Any desorption of lipid decreases the pressure, which can be readjusted to its initial value by moving the compression barrier. In the film balance used, the pressure can be kept constant by moving the compression barrier until the pressure reaches the required value. It was not possible to study the desorption of compound **1** at higher surface pressures than 16 mN/m because of the strong monolayer instability above this value.

The percentage of lipid loss can be monitored by the movement of the compression barrier, and can be plotted as a function of time, as shown in Figure 2.

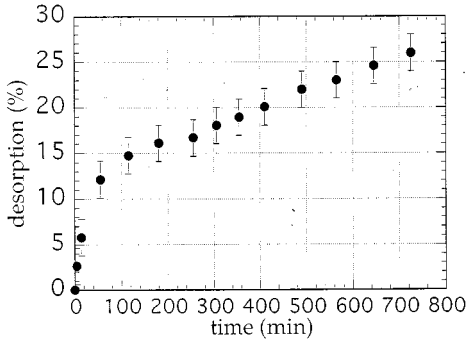
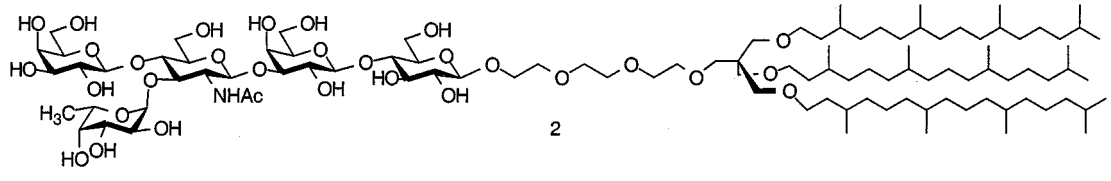


Figure 2. Desorption of **1** at a pressure of 16 mN/m and at t = 20 °C, from the air/water interface; the surface pressure of the monolayer is kept constant; any lipid desorbed from the monolayer reduces the surface pressure, which is then readjusted to the required value by moving the compression barrier

Lewis X sphingolipid **1** with its large hydrophilic pentasaccharide headgroup and its relatively small hydrophobic chains is too soluble in water (15% loss in 2 h, 25% loss in 12 h compared to ca 3% in 12 h for standard phospholipid) for accurate adhesion studies. We therefore synthesized a less soluble lipid with a Lewis X headgroup. Specific



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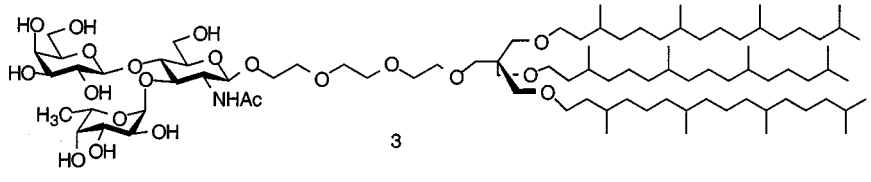

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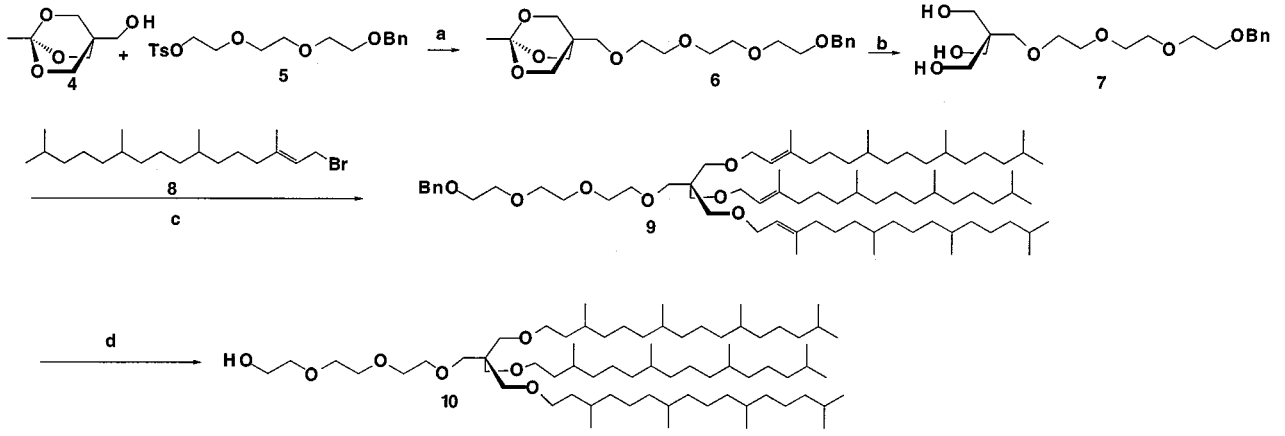
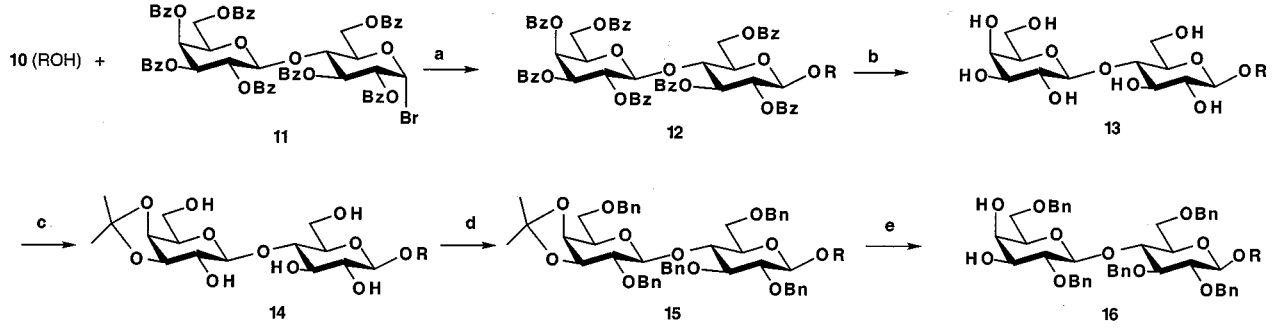
binding requires, in general, that the molecules have sufficient mobilities so that they can take the exact position and orientation for which the interaction will occur. This is even more so in a designed molecule that nature has not selected, 2in contrast to the natural glycolipid **1**. To give the Lewis X an adequate orientational mobility, it was attached to the hydrophobic part by a flexible spacer.

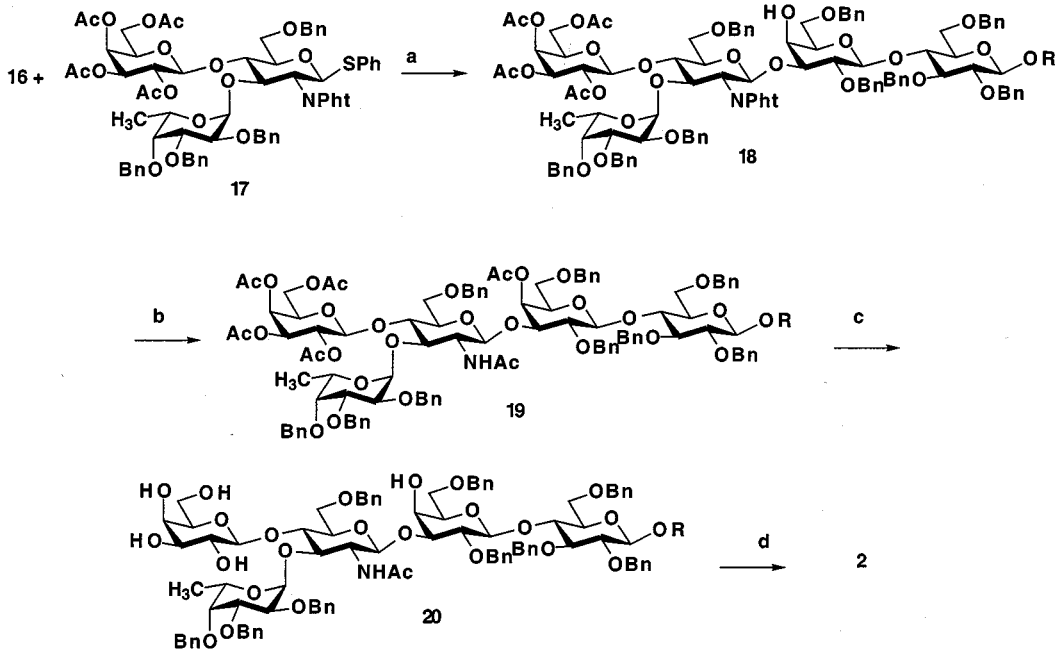
Three racemic phytyl chains increase the hydrophobicity of the lipid moiety. The methyl chain ramifications ensure translational mobility of the hydrophobic part by pre-



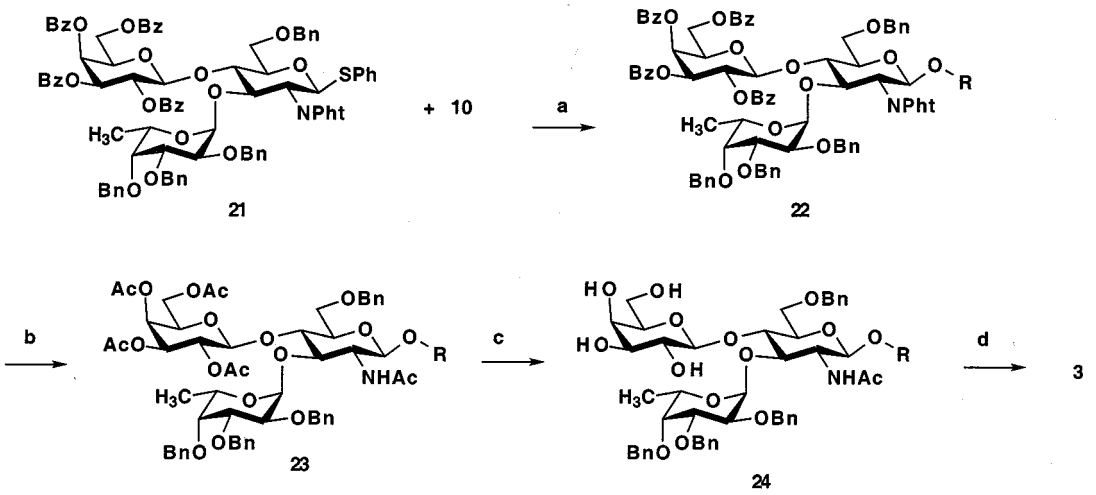
Scheme 1. Reagents: a) NaH, DMF (75%); b) HCl, H_2O/MeOH (97%); c) NaH, DMF (85%); d) H_2, PtO_2 then Pd/C, MeOH, AcOEt (82%)



Scheme 2. Reagents: a) AgOTf, CH_2Cl_2 −20 °C (80%); b) NaOMe, CH_2Cl_2, MeOH TA (90%); c) dimethoxypropane, CSA, DMF TA, (55%); d) BnBr, NaH, DMF, 80 °C, (65%); e) CF_3COOH, CH_2Cl_2, TA (70%)



Scheme 3. Reagents: a) NIS, TfOH, CH_2Cl_2, −30 °C (66%); b) N_2H_4, aq. EtOH, reflux then Ac_2O, Pyr. (65%); c) MeONa, CH_2Cl_2, MeOH (85%); d) H_2, Pd/C, MeOH, AcOEt (73%)



Scheme 4. Reagents: a) NIS, TfOH, CH_2Cl_2 (92%); b) N_2H_4, EtOH, then Ac_2O/pyr; c) NaOMe, MeOH (83%, three steps); d) H_2: Pd/C, MeOH/AcOEt (85%)

venting crystallisation of the chains under surface pressure of lipid monolayers spread on water.

Chemical Synthesis of the Tailored Glycolipids

Glycolipids **2** and **3** were synthesised from three blocks: a lipid unit **10**, a lactose unit **11**,[11] and a Lewis X unit **17**[12] or **21**.[13] The synthesis of the lipid part **10** is presented in Scheme 1. The known selectively protected pentaerythrytol **4**[14] was condensed with the tosylate **5**[15] in DMF, in the presence of NaH. The orthoester protection was removed with hydrochloric acid, and the resulting triol **7** was treated with phytyl bromide **8**, prepared from the Poulter procedure.[16]

A simultaneous catalytic hydrogenation of the double bonds and hydrogenolysis of the benzyl group was first attempted in the presence of palladium on charcoal. It failed to give the expected compound **10** as cleavage of phytyl chains occurred (probably via isomerisation of the allylic ether to enol ether). When the catalyst was changed to PtO_2, a clean hydrogenation of the double bonds first took place. Further treatment with palladium on charcoal cleaved the benzyl ether to give **10**. The platinum-catalysed hydrogenation has to be carefully controlled in order to avoid saturation of the phenyl group.

Compound **10** was then condensed with the bromide **11** in the presence of silver triflate to give the benzoylated β-lactoside **12** (Scheme 2). Benzoates were removed and a 3′,4′-*O*-isopropylidene group was introduced to give **14** (55%), together with isomeric isopropylidenes (4′,6′ and 2,3′). Benzylation of **14**, followed by removal of the isopropylidene group, gave the protected glycolipid **16**.

The final steps are shown in Scheme 3. Glycosylation of the diol **16** with the thioglycoside **17** selectively gave the pentasaccharide **18** in 66% yield. The newly created glycosidic bond was β as indicated by ^{1}H NMR spectroscopy: for H-1 $GlcN^{III}$ $\delta = 5.35$ and $J_{1,2} = 8.5$ Hz. Uneventful deprotection steps gave the target molecule **2**.

The synthesis of the second Lewis X glycolipid **3** is shown in Scheme 4. The alcohol **10** was glycosylated with the benzoylated Lewis X trisaccharidic donor **21** to give the protected glycolipid **22**. Removal of all the protecting groups afforded compound **3** in a 70% overall yield.

Monolayer Behaviour of the Synthetic Tailored Glycolipids

The compression isotherms of the Lewis X glycolipids **2** and **3** are shown in Figure 3.

These glycolipids display a "liquid expanded" behaviour with a high compressibility. These properties, associated with a low solubility in water (Figure 4 and Table 1), make

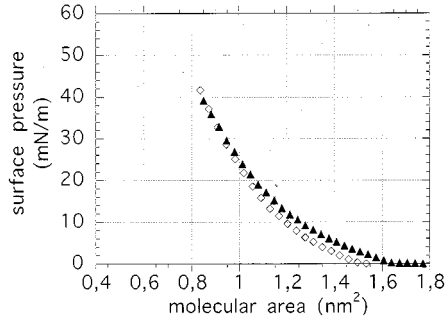


Figure 3. Compression isotherms of monolayers of compound **2** (triangle) and compound **3** (diamond); both compounds have almost the same compression isotherms; however, compound **2**, with its pentasaccharide has a higher molecular area than compound **3**, which has a trisaccharide

Table 1. Desorption of glycolipids **1, 2** and **3**

Compound	2 h	10 h	Pressure
1[a]	15%	25%	16 mN/m
2	1%	5%	32 mN/m
3	1%	2%	30 mN/m

[a] The desorption of compound **1** could not be studied at higher surface pressures than 16 mN/m because of the strong monolayer instability above this value and also because higher surface pressures induce higher desorptions. 32 and 30 mN/m are sufficiently close values to allow comparison of the compounds. This illustrates the much better stability to desorption of compounds **2** and **3** relative to **1**.

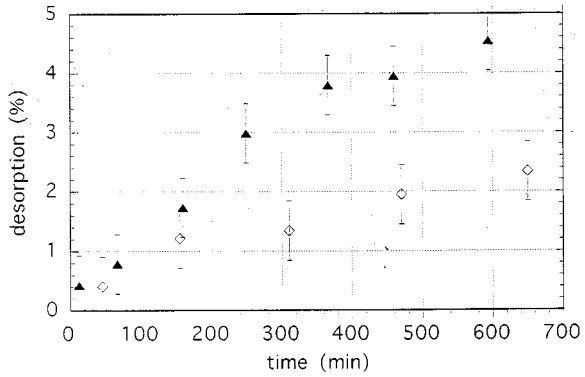


Figure 4. Desorption of **2** (triangle, pressure 32 mN/m) and **3** (square, pressure 30 mN/m), t = 20 °C from the air/water interface; almost the same pressure was used to compare both compounds; although the pressure influences the desorption, a difference of 2 mN/m is not significant and still allows for the comparison; adding two sugar units noticeably increase the solubility, which is directly related to desorption

them good candidates for the study of the interaction of Lewis X functionalized vesicles, which will be reported elsewhere.

Experimental Section

General: Melting points: Büchi 510 apparatus, uncorrected. − Optical rotations: Perkin-Elmer 241 digital polarimeter. − Mass spectra: Nermag R10-10 spectrometer, C.I. (ammonia) or FAB (NBA)$^+$ as indicated. − Elemental analyses: performed by Service d′Analyse de l′Université Pierre et Marie Curie, 75252 Paris Cedex 05, France. − NMR: Bruker AM-400 (400 MHz and 100.6 MHz, for ^{1}H and ^{13}C, respectively), TMS as internal standard. −TLC: silica gel 60 F_{254} (Merck) and detection by charring with conc. H_2SO_4. − Flash column chromatography: silica gel 60 (230−400 mesh, Merck).

12-Benzyloxy-2-bis(hydroxymethyl)-4,7,10-trioxadodecan-1-ol Orthoacetate (6): NaH (1.0 g, 60% in oil, 2 equiv.) was added portionwise to a solution of **4** (1.9 g, 11.9 mmol) in DMF (50 mL) at 0 °C. A solution of **5** (5.6 g, 1.2 equiv.) in DMF (40 mL) was added dropwise. The resulting solution was stirred for 15 h at room temp. Methanol was added and then the solution was concentrated. A solution of the residue in ether was washed with water, sat. aq. NaCl, dried ($MgSO_4$) and concentrated. Column chromatography of the residue (hexane/AcOEt 45:55) gave **6** (3.95 g, 87%) − ^{1}H NMR (250 MHz, $CDCl_3$): δ = 7.32−7.19 (m, 5 H, arom), 4.52 (s, 2 H, Bn), 3.93 (s, 6 H, CH_2O), 3.65−3.42 (m, 12 H, CH_2O), 3.18 (s, 2 H, CH_2O), 1.40 (s, 3 H, CH_3). − ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 138.05, 128.13, 127.51, 127.37 (C arom), 108.25 (C orthoester), 73.00, 71.05, 70.52, 70.46, 70.44, 70.29, 69.49, 69.26 (CH_2−O), 34.76 (C quat), 23.24 (Me). − $C_{20}H_{30}O_7$ (382.46): C 62.81, H 7.91; found C 62.99, H 8.00.

12-Benzyloxy-2-bis(hydroxymethyl)-4,7,10-trioxadodecan-1-ol (7): A mixture of **6** (3.5 g, 9.2 mmol), methanol (8 mL) and 10^{-2} M aq. HCl (32 mL) was stirred for 2 h at room temp., neutralised with solid Na_2CO_3 and concentrated. A solution of the residue in methanol was filtered and concentrated to give **7** (3.2 g, 97%) which was used as such in the following step. − ^{1}H NMR (250 MHz, $CDCl_3$): δ = 7.30−7.18 (m, 5 H, arom), 4.49 (s, 2 H, CH_2Ph), 3.62−3.48 (m, 20 H, CH_2O), 3.10 (br s, 3 H, OH). − ^{13}C NMR (62.9 MHz, $CDCl_3$/[D$_4$]MeOH): δ = 137.72, 128.10, 127.53, 127.42 (C arom), 72.98, 71.37, 70.39−68.92, 62.98 (CH_2O), 45.07 (C quat).

1-Bromo-3,7,11,15-tetramethylhexadec-2-ene (*Z*, *E*) (8): Phosphorus tribromide (0.72 mL, 0.5 equiv.) was added to a solution of phytol (*Z*+*E*) (4.6 g, 15.5 mmol) in dry hexane (50 mL) at 0 °C. The reaction mixture was stirred for 10 min. and then methanol (2 mL) was added. The reaction mixture was washed with aq. $NaHCO_3$, water, dried ($MgSO_4$) and concentrated. The residue **8** (5.6 g, quant) was dried under vacuum (room temp., 1 Torr) for 2 h and used immediately in the following step. − ^{1}H NMR (250 MHz, $CDCl_3$): δ = 5.45 (br t, J = 8.4 Hz, 1H, CH=), 3.96 and 3.95 [2* d, J = 8.4 Hz, CH−Br (*Z*) and (*E*)], 2.23 and 1.96 [2*t, J = 7.5 Hz, 2 H, CH_2−C= (*Z*) and (*E*)], 1.70 and 1.65 [2*s, 3H, Me−C= (*Z*) and (*E*)], 1.50−0.90 (m, 18 H, CH, CH_2), 0.80−0.70 (m, 12 H, Me).

11-Tris(3,7,11,15-tetramethylhexadec-2-enyloxymethyl)-3,6,9-trioxa-1-benzyloxyundecane (9): NaH (1.08 g, 60% in oil, 2 equiv./OH) was added to a solution of triol **7** (1.64 g,4.58 mmol), and **8** (3.4 equiv. prepared from 4.6 g of phytol) in DMF (30 mL) at 0 °C. The solution was stirred for 15 h at room temp., and concentrated after addition of methanol. A solution of the residue in cyclohexane was washed with water, dried ($MgSO_4$) and concentrated. The residue was chromatographed (cyclohexane/AcOEt 95:5→90:10) to give **9** (4.7 g, 85%). − ^{1}H NMR (250 MHz, $CDCl_3$): δ = 7.30−7.21 (m, 5 H, arom), 5.22 (br t, J = 6.4 Hz, 3 H, H−C=), 4.49 (s, 2 H, Bn), 3.91−3.81 (m, 6 H, O−CH_2−C=), 3.64−3.46 (m, 2 H, O−CH_2−CH_2O), 3.33 (s, 3 H, CH_2−C), 3.26 (s, 6 H, CH_2−C), 2.00−1.85 (m, 6 H, −CH_2−C=), 1.70−0.90 (m, 66 H, CH_2), 0.82−0.72 (m, 36 H, CH_3). − ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 139.3, 139.0, 135.15 (C arom, C=, *cis trans*), 128.1, 127.49, 127.33 (C arom), 122.26, 121.36 (C=, *cis trans*), 73.05, 70.85, 70.59, 70.51, 70.23, 69.31, 69.07, 67.85, 67.58 (CH_2O), 45.18 (C quat), 39.78, 39.21, 37.28, 37.22, 37.18, 37.13, 36.86, 36.77, 36.68, 36.59, 32.59, 32.51, 32.25, 27.78, 25.41, 25.03, 24.61, 24.30, 23.25, 22.54, 22.45, 19.57, 19.50, 16.16 (chains). − $C_{78}H_{144}O_7$ (1194.01): C 78.46, H 12.36; found C 78.50, H 12.23.

11-Tris(3,7,11,15-tetramethylhexadecyloxymethyl)-3,6,9-trioxa-1-undecanol (10): A mixture of **9** (4.5 g) in AcOEt (50 mL) was stirred under hydrogen (1 atm.) in the presence of a catalytic amount of PtO_2 for 3 h and filtered. 10% Pd/C (400 mg) was added to the filtrate, and the reaction mixture was stirred under hydrogen for 1 h, filtered and concentrated. Column chromatography (hexane/AcOEt 4:1) gave **10** (3.40 g, 82%). − ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.76 (t, J = 5 Hz, 2 H, H-1), 3.71 (s, 4 H, H-3, H-4), 3.67−3.63 (m, 4 H, H-2, H-5), 3.62−3.85 (m, 2 H, H-6), 3.74−3.35 (m, 12 H, CH_2O), 1.55−0.90 (m, 76 H, chains), 0.81, 0.78, 0.76 (3s, 45 H, CH_3). − ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 72.53, 70.99, 70.65, 70.45, 70.39, 69.76, 69.69, 61.72 (CH_2O), 45.53 (C quat), 39.32, 37.48, 37.41, 37.36, 37.34, 37.24, 36.72, 36.64, 32.72, 29.97, 27.89, 26.65, 24.72, 24.42, 24.35, 22.64, 22.55, 19.68, 19.62 (chains). − $C_{71}H_{144}O_7$ (1109.93): C 76.83, H 13.03; found C 76.86, H 13.14.

11-Tris(3,7,11,15-tetramethylhexadecyloxymethyl)-3,6,9-trioxa-1-undecyl-2,3,6-tri-*O*-benzoyl-4-*O*-(2,3,4,6-tetrabenzoyl-β-D-galactopyranosyl) β-D-Glucopyranoside (12): A mixture of **10** (1.6 g), **11** (2.2 g, 1.3 equiv.), 4 Å molecular sieves (3.5 g) and CH_2Cl_2 (40 mL) was stirred for 30 min at room temp. and then cooled to −20 °C. AgOTf (600 mg, 1.2 equiv./bromide) was then added. After 15 min at −20 °C, Et_3N was added, the reaction mixture was filtered through a Celite pad, washed with water, dried ($MgSO_4$) and concentrated. The residue was chromatographed (hexane/AcOEt 75:25) to give **12** (2.62 g, 86%). $[\alpha]_D$ = +26 (c = 1.07, $CHCl_3$). −

^{1}H NMR (250 MHz, $CDCl_3$): δ = 7.98−7.02 (m, 35 H, arom), 5.87−5.67 (m, 3 H, H-2 Glc^I, H-3 Gal^I, H-4 Gal^{II}), 5.47 (dd, $J_{2',3'}$ = 9.8, $J_{1',2'}$ = 8.0 Hz, 1 H, H-2 Gal^{II}), 5.36 (dd, $J_{3',4'}$ = 3.5 Hz, 1 H, H-3 Gal^{II}), 4.87 (d, $J_{1,2}$ = 7.9 Hz, 1 H, H-1 Glc^I or H-1 Gal^{II}), 4.80 (d, $J_{1,2}$ = 7.9 Hz, 1H, H-1 Glc^I or Gal^{II}), 4.60 (dd, $J_{5,6a}$ = 2, $J_{6a,6b}$ = 12.2 Hz, H-6a Gal^{II}), 4.49 (dd, $J_{5,6a}$ = 4.2 Hz, H-6b Gal^{II}), 4.26 (dd, $J_{3,4}$ = 9.5, $J_{4,5}$ = 9.5 Hz, 1 H, H-4 Glc^I), 3.97−3.28 (m, 30 H, H-6a Glc^I, H-6b Glc^I, H-5 Glc^I, H-5 Gal^{II}, CH_2−O), 1.54−0.90 (m, 72 H, chains), 0.83−0.72 (m, 45 H, Me). − ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 165.80−164.75 (CO), 133.35−128.19 (C arom), 101.27, 100.97 (C-1, C-1′), 76.0−67.50, 61.03 (CH−O), 45.34, 43.40, 39.35, 37.51, 37.44, 37.29, 36.74, 36.66, 32.77, 29.98, 27.94, 24.79, 24.47, 24.40, 22.7, 22.61, 19.73, 19.67 (chains). − $C_{132}H_{192}O_{24}$ (2162.98): C 73.30, H 8.95; found C 73.31, H 8.95.

11-Tris(3,7,11,15-tetramethylhexadecyloxymethyl)-3,6,9-trioxa-1-undecyl-4-*O*-(β-D-galactopyranosyl) β-D-Glucopyranoside (13): A solution of NaOMe (0.4 M) in methanol (20 mL) was added to a solution of **12** (2.4 g) in CH_2Cl_2 (20 mL). The reaction mixture was stirred at 50 °C for 2 h then at room temp. for 15 h, neutralised [IR −120 (H^+ form)], filtered and concentrated. Column chromatography of the residue (toluene to remove methyl benzoate then CH_2Cl_2/MeOH 4:1) gave **13** (1.4 g, 90%) as a waxy solid. − $[\alpha]_D$ = −4 (c = 1.02, $CHCl_3$). − ^{1}H NMR (400 MHz, [D_5]pyridine) selected data: δ = 5.09 (d, $J_{1,2}$ = 8 Hz, 1 H, H-1 Gal^{II}), 4.77 (d, $J_{1,2}$ = 8 Hz, 1 H, H-1 Glc^I), 4.55−4.41 (m, 5 H, H-2 Gal^{II}, H-6a Glc^I, H-6b Glc^I, H-6a Gal^{II}, H-4 Gal^{II}), 5.02 (dd, $J_{5,6b}$ = 5, $J_{6a,6b}$ = 11 Hz, 1 H, H-6b Gal^{II}), 4.29 (dd, $J_{3,4}$ = $J_{4,5}$ = 9 Hz, 1 H, H-4 Glc^I), 4.32 (dd, $J_{2,3}$ = 9 Hz, 1 H, H-3 Glc^I), 4.13 (dd, $J_{2,3}$ = 9, $J_{3,4}$ = 3 Hz, 1 H, H-3 Gal^{II}), 3.99 (dd, 1 H, H-2 Glc^I). − $C_{83}H_{164}O_{17}$ (1434.22): C 69.51, H 11.53; found C 69.40, H 11.64.

11-Tris(3,7,11,15-tetramethylhexadecyloxymethyl)-3,6,9-trioxa-1-undecyl-4-*O*(3,4-*O*-isopropylidene-β-D-galactopyranosyl)-β-D-Glucopyranoside (14): A mixture of **13** (300 mg), dimethoxypropane (65 μL, 2.5 equiv.) and camphorsulfonic acid (20 mg) in DMF (10 mL) was stirred at room temp. for 15 h, and then neutralised (Et_3N) and concentrated. A solution of the residue in a 10:1 MeOH/water mixture (20 mL) was stirred at 80 °C for 2 h, and concentrated. The residue was chromatographed (toluene/acetone 3:2) to give **14** (169 mg, 55%) which was used as such in the following step.

11-Tris(3,7,11,15-tetramethylhexadecyloxymethyl)-3,6,9-trioxa-1-undecyl-2,3,6-tri-*O*-benzyl-4-*O*-(2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl) β-D-Glucopyranoside (15): Benzyl bromide (0.1 mL, 1.5 equiv./OH) was added to a mixture of **14** (169 mg) and NaH (50 mg, 60% in oil, 2 equiv./OH) in DMF (100 mL). The reaction mixture was stirred at 80 °C for 30 min and then at room temp. for 15 h. Methanol was then added. The reaction mixture was stirred for 1 h and concentrated. A solution of the residue in CH_2Cl_2 was washed with water, dried ($MgSO_4$) and concentrated. Flash chromatography of the residue (toluene/AcOEt 85:15) gave **15** (143 mg, 65%) which was used as such in the following step. − ^{13}C NMR (62 MHz, $CDCl_3$): δ = 138.94, 138.67, 138.45, 138.33, 138.18 (C arom quat), 128.45−127.17 (C arom), 109.64 (OCO, isopropylidene), 103.61 (C-1 Glc^I or Gal^{II}), 101.75 (C-1 Glc^I or Gal^{II}), 82.82, 81.59, 80.53, 79.27, 77.43, 76.19−68.18 (C−O), 45.30, 44.85, 39.29, 37.49−36.62, 32.75, 32.71, 29.95, 29.61, 27.89, 26.32, 24.72, 24.42, 24.36, 22.64, 22.55, 19.67, 19.61 (chains, methyl).

11-Tris(3,7,11,15-tetramethylhexadecyloxymethyl)-3,6,9-trioxa-1-undecyl-2,3,6-tri-*O*-benzyl-4-*O*-(2,6-di-*O*-benzyl-β-D-galactopyranosyl) β-D-Glucopyranoside (16): A mixture of **15** (143 mg), CF_3COOH (0.4 mL) and CH_2Cl_2 (4 mL) was stirred at room temp. for 1.5 h, neutralised (solid K_2CO_3) filtered, and concentrated. Flash chromatography (hexane/AcOEt 65:35) of the residue gave **16** (100 mg, 70%). − $[\alpha]_D$ = +7 (c = 1.2, $CHCl_3$). − ^{1}H NMR (400 MHz, $CDCl_3$ + CCl_3−CO−NCO): δ = 7.40−7.10 (m, 25 H, arom), 5.52 (d, $J_{3,4}$ = 3.5 Hz, 1 H, H-4 Gal^{II}), 4.93 (dd, $J_{2,3}$ = 9 Hz, 1 H, H-3 Gal^{II}), 4.96 (d, J = 11 Hz, 1 H, CH_2Ph), 4.90 (d, J = 11 Hz, 1 H, CH_2Ph), 4.80 (d, J = 10 Hz, 1 H, CH_2Ph), 4.76 (d, J = 12 Hz, 1 H, CH_2Ph), 4.70 (d, J = 11 Hz, 1 H, CH_2Ph), 4.69 (d, J = 12 Hz, 1 H, CH_2Ph), 4.64 (d, J = 12 Hz, 1 H, CH_2Ph), 4.55 (d, J = 11 Hz, 1 H, CH_2Ph), 4.52 (d, J = 8 Hz, 1 H, H-1 Glc^I or Gal^{II}), 4.46 (d, J = 8 Hz, 1 H, H-1 Glc^I or Gal^{II}), 4.41 (d, J = 12 Hz, 1 H, CH_2Ph), 4.23 (d, J = 12 Hz, 1 H, CH_2Ph), 4.07 (m, 1 H, H-5 Gal^{II}), 4.05 (dd, $J_{2,3}$ = 9 Hz, H-2 Gal^{II}), 3.90−3.30 (m, 36 H, CHO), 1.40−1.05 (m, 72 H, chains), 0.90−0.80 (m, 45 H, methyl). − $C_{118}H_{194}O_{17}$ (1884.84): C 75.19, H 10.37; found C 75.11, H 10.38.

11-Tris(3,7,11,15-tetramethylhexadecyloxymethyl)-3,6,9-trioxa-1-undecyl-2,3,4,5-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl β-D-Glucopyranoside (18): A mixture of glycosyl donor **17** (30 mg, 1.5 equiv.), diol **16** (30 mg), 4 Å molecular sieves (60 mg) and CH_2Cl_2 (1.5 mL) was stirred for 30 min at room temp. NIS (13.8 mg, 2.5 equiv./**17**) was added and the reaction mixture was cooled to −30 °C. A solution of TfOH (0.1 equiv./NIS) [58 μL of a solution of TfOH (34 μL) in CH_2Cl_2 (4 mL)] was then added. The reaction mixture was stirred for 30 min at −30 °C, neutralised (Et_3N), filtered, washed with 10% aq. sodium thiosulfate, aq. $NaHCO_3$, water, dried ($MgSO_4$) and concentrated. Column chromatography of the residue (hexane/AcOEt 7:3) and (toluene acetone 91:9) gave **18** (32 mg 66%) as a white amorphous powder. $[\alpha]_D$ = + 6 (c = 1.0 $CHCl_3$). − ^{1}H NMR (400 MHz, $CDCl_3$) selected data: δ = 5.35 (d, $J_{1,2}$ = 8.5 Hz, 1H, H-1 GlcN ^{III}), 5.29 (d, $J_{3,4}$ = 3.5 Hz, 1 H, H-4 Gal^{IV}), 5.06 (dd, $J_{1,2}$ = 8, $J_{2,3}$ = 10 Hz, 1 H, H-2 Gal^{IV}), 4.84 (dd, H-3 Gal^{IV}), 4.73 (d, 1 H, H-1 Gal^{IV}), 4.60 (m, 1 H, H-5 Fuc^V). − ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.03, 169.98, 169.81, 168.66 (CO, Ac), 138.97−137.52 (C arom quat), 128.65−128.22 (C arom), 103.68 (C-1), 101.89 (C-1), 99.51 (C-1), 98.89 (C-1), 97.49 (C-1 Fuc), 83.37, 82.87, 81.48, 79.61, 78.00, 75.68, 75.68, 75.17, 74.97, 74.59, 72.46, 70.89, 70.57, 70.50, 70.40, 70.37, 70.25, 70.20, 69.75, 69.69, 68.95, 66.83, 68.32, 67.84, 67.67, 67.41, 66.65, 66.45 (CHO rings), 75.37, 74.89, 74.09, 73.68, 73.30, 72.94, 72.81, 72.27 (CH_2Ph), 60.20, 56.13, 45.30, 39.32, 37.49−37.24, 36.70, 36.71, 32.78−32.72, 29.94, 27.92, 24.76, 24.44, 24.39, 24.39, 22.69, 22.60, 20.67, 20.56, 20.50, 20.47, 19.74−19.61, 16.68. − $C_{180}H_{259}NO_{36}$ (3013.05): C 71.75, H 8.66; found C 71.81, H 8.68.

11-Tris(3,7,11,15-tetramethylhexadecyloxymethyl)-3,6,9-trioxa-1-undecyl-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-6-*O*-benzyl-2-deoxy-2-acetamido-β-D-glucopyranosyl-(1→3)-4-*O*-acetyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl β-D-Glucopyranoside (19): A mixture of **18** (25 mg), hydrazine monohydrate (0.7 mL) and 90% aq. ethanol (7 mL) was stirred at 80 °C for 15 h, and then concentrated. A solution of the residue in a mixture of Ac_2O (1 mL) and pyridine (2 mL) was stirred for 15 h and concentrated. Flash chromatography of the residue (toluene/acetone 85:15) gave **19** (16 mg, 65%) which was used as such in the following step. − ^{1}H NMR (400 MHz, $CDCl_3$) selected data: δ = 5.56 (d, $J_{2,NH}$ = 9.3 Hz, 1 H, H-1 GlcN ^{III}), 5.55 (d, $J_{1,2}$ = 3.8 Hz, 1 H, H-1 Fuc^V), 5.32 (d, $J_{3,4}$ =

3.6 Hz, 1 H, H-4 Gal^{IV}), 5.23 (d, $J_{3,4}$ = 3.7 Hz, 1 H, H-4 Gal^{II}), 5.18 (d, $J_{2,NH}$ = 5.4 Hz, 1 H, NH), 4.97 (dd, $J_{1,2}$ = 8.0, $J_{2,3}$ = 10.5 Hz, 1 H, H-2 Gal^{IV}), 4.77 (dd, 1 H, H-3 Gal^{IV}), 4.59 (m, 1 H, H-5 Fuc^{V}), 4.53 (d, $J_{1,2}$ = 8.5 Hz, 1 H, H-1 Gal^{IV}), 4.49 (d, $J_{1,2}$ = 8.45 Hz, 1 H, H-1 Gal^{II} or H-1 Glc^{I}), 4.43 (d, $J_{1,2}$ = 8.6 Hz, 1 H, H-1 Gal^{II} or H-1 Glc^{I}).

11-Tris(3,7,11,15-tetramethylhexadecyloxymethyl)-3,6,9-trioxa-1-undecyl-β-D-galactopyranosyl-(1→4)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-6-*O*-benzyl-2-deoxy-2-acetamido-β-D-glucopyranosyl-(1→3)-2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-Glucopyranoside (20): A 0.2 M solution of NaOMe in methanol (5 mL) was added to a solution of **19** (16 mg) in CH_2Cl_2 (5 mL). The reaction mixture was stirred for 15 h at room temp., neutralised [IR-120 (H^+ form)], filtered and concentrated. Flash chromatography (CH_2Cl_2/MeOH 95:5) gave **20** (12.5 mg, 85%), which was used as such in the following step.

11-Tris(3,7,11,15-tetramethylhexadecyloxymethyl)-3,6,9-trioxa-1-undecyl-β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl)-(1→3)]-2-deoxy-2-acetamido-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4) β-D-Glucopyranoside (2): A solution of **20** (12.5 mg) in MeOH/AcOEt 50:50 (5 mL) was stirred under hydrogen (1 atm.) in the presence of a catalytic amount of 10% Pd/C for 3 h. The suspension was filtered through celite. The celite pad was washed with CH_2Cl_2/MeOH 2:1. The organic solutions were pooled and concentrated. The residue was purified by flash chromatography (CH_2Cl_2/MeOH/H_2O 13:6:1) to give **2** (7 mg, 73%). − $[\alpha]_D$ = + 18 (*c* = 0.8, $CHCl_3$/MeOH 2:1 v/v). − ^{1}H NMR (400 MHz, [D_5]pyridine): δ = 9.27 (d, *J* = 8.5 Hz, 1 H, NH), 5.89 (d, $J_{1,2}$ = 8.5 Hz, 1 H, H-1 Fuc^{V}), 5.55 (q, $J_{1,2}$ = 6.5 Hz, 1 H, H-5 Fuc^{V}), 5.40 (d, $J_{1,2}$ = 7.95 Hz, 1 H, H-1), 5.27 (d, $J_{1,2}$ = 7.5 Hz, 1 H, H-1), 4.98 (d, $J_{1,2}$ = 7.8 Hz, 1 H, H-1). − ^{13}C NMR (100 MHz, [D_5]pyridine): δ = 171.77 (CO), 104.98, 104.22, 103.80, 103.19, 100.15 (C-1), 83.32, 80.69, 77.23, 76.53, 76.37, 76.26, 76.18, 74.95, 74.82, 74.27, 73.30, 72.57, 71.27, 70.94, 70.79, 70.63, 70.51, 70.42, 70.02, 69.77, 69.30, 69.14, 68.81, 67.24, 61.73−61.68, 61.43−60.72, 57.18, 45.75, 39.30, 37.56, 37.52, 37.48, 37.34, 36.95, 36.86, 32.90−32.83, 30.10, 28.01, 24.92, 24.62, 24.59, 23.17, 22.68, 22.58, 19.84, 19.78, 19.71, 16.99. − MS (FAB): *m*/*z* = 1966.8 [M + Na^+].

11-Tris(3,7,11,15-tetramethylhexadecyloxymethyl)-3,6,9-trioxa-1-undecyl-2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-Glucopyranoside (22): A mixture of glycosyl donor **21** (180 mg, 0.121 mmol), **10** (161 mg, 0.145 mmol), 4 Å molecular sieves (400 mg) and CH_2Cl_2 (4 mL) was stirred for 30 min at room temp. NIS (81 mg, 0.36 mmol) was added and the reaction mixture was cooled to −30 °C. TfOH (5 μL) was added to the solution. The reaction mixture was stirred for 30 min at −30 °C, neutralised (Et_3N), filtered, washed with 10% aq. sodium thiosulfate, aq. $NaHCO_3$, water, dried ($MgSO_4$) and concentrated. Column chromatography of the residue (toluene/AcOEt 9:1) and (hexane/AcOEt 75:25) gave **22** (275 mg, 92%). − $[\alpha]_D$ = 0; $[\alpha]_{436}$ = +10 (*c* = 1.1, $CHCl_3$). − ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.20−7.10 (m, 44 H, arom), 5.82 (d, $J_{3,4}$ = 3.6 Hz, 1 H, H-4 Gal^{IV}), 5.74 (dd, $J_{2,3}$ = 10.3, $J_{1,2}$ = 8.3 Hz, 1 H, H-2 Gal^{IV}), 5.36 (dd, 1 H, H-3 Gal^{IV}), 5.09 (d, $J_{1,2}$ = 8.9 Hz, 1 H, H-1 GlcN^{III}), 50.7 (d, $J_{1,2}$ = 4.5, 1 H, H-1 Fuc^{V}), 5.01 (d, 1 H, H-1 Gal), 4.93 (d, *J* = 12 Hz, 1 H, Bn), 4.84 (dd, $J_{3,4}$ = 9.0, $J_{2,3}$ = 10.4 Hz, 1 H, H-3 GlcN ^{III}), 4.79 (br q, *J* = 6.0 Hz, 1 H, H-5 Fuc^{V}), 4.71 (d, *J* = 11 Hz, 1 H, Bn), 4.51−4.42 (m, 33 H, CH−O, Bn, H-6 Gal), 4.40 (dd, 1 H, H-2 GlcN ^{III}), 4.34 (dd, $J_{5,6a}$ = 7.1, $J_{6a,6b}$ = 11.2 Hz, 1 H, H-6b Gal), 4.30 (d, *J* = 12.5 Hz, 1 H, Bn), 4.27 (dd, $J_{4,5}$ = 10.0 Hz, 1 H, H-4 GlcN ^{III}), 4.23 (d, *J* = 11.3 Hz, 1 H, Bn), 3.97 (dd, $J_{3,4}$ = 2.6, $J_{2,3}$ = 10.2 Hz, 1 H, H-3 Fuc^{V}), 3.94 (br d, *J* = 11.0 Hz, 1 H), 3.90−3.80 (m, 3 H, H-5 Gal, H-2 Fuc^{V}), 3.67 (br d, *J* = 11.0 Hz, 1 H), 3.60 (br d, 1 H, H-4 Fuc^{V}), 3.58−3.20 (m, 26 H, CH_2O), 1.65−1.05 (m, 72 H, chains), 0.92−0.85 (m, 45 H, Me). − $C_{153}H_{217}NO_{26}$ (2486.42): C 73.90, H 8.79; found C 73.86, H 8.73.

11-Tris(3,7,11,15-tetramethylhexadecyloxymethyl)-3,6,9-trioxa-1-undecyl-β-D-galactopyranosyl-(1→4)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-6-*O*-benzyl-2-deoxy-2-acetamido β-D-Glucopyranoside (24): A mixture of **22** (195 mg) hydrazine monohydrate (0.1 mL) and 90% aq. ethanol (7 mL) was stirred at 80 °C for 15 h, and concentrated. A solution of the residue in a mixture of Ac_2O (10 mL) and pyridine (20 mL) was stirred for 15 h and concentrated. Flash chromatography of the residue (toluene/acetone 85:15) gave **23** (156 mg, 92%).

A 0.2 M solution of NaOMe in methanol (6 mL) was added to a solution of **23** (156 mg) in CH_2Cl_2 (2 mL). The reaction mixture was stirred 15 h at room temp., neutralised (IR-120, H^+ form) filtered and concentrated. Flash chromatography (toluene/acetone 60:40→55:45) gave **24** (99 mg, 90%), which was used as such in the following step. − ^{1}H NMR (400 MHz, $CDCl_3$) selected data: δ = 7.50−7.25 (m, 20 H, arom), 6.00 (d, $J_{2,NH}$ = 7.8 Hz, 1 H, NH), 5.29 (d, $J_{3,4}$ = 3.3 Hz, 1 H, H-4 Gal^{IV}), 5.16 (d, $J_{1,2}$ = 3.7 Hz, 1 H, H-1 Fuc^{V}), 5.03 (dd, $J_{1,2}$ = 8.2, $J_{2,3}$ = 10.5 Hz, 1 H, H-2 Gal^{IV}), 5.00 (d, *J* = 12 Hz, 1 H, Bn), 4.96 (d, $J_{1,2}$ = 7.2 Hz, 1 H, H-1 GlcN^{III}), 4.60 (d, $J_{1,2}$ = 8.1 Hz, 1 H, H-2 Gal^{II}), 4.51 (q, $J_{5,6}$ = 6.5 Hz, 1 H, H-5 Fuc^{V}), 2.04, 2.03, 1.99, 1.91 (4 s, 12 H, OAc), 1.79 (s, 3 H, NHAc).

11-Tris(3,7,11,15-tetramethylhexadecyloxymethyl)-3,6,9-trioxa-1-undecyl-β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl)-(1→3)]-2-deoxy-2-acetamido β-D-Glucopyranoside (3): A solution of **24** (90 mg) in MeOH/AcOEt 3:1 (13 mL) was stirred under hydrogen (1 atm.) in the presence of 10% Pd/C (10 mg) for 3 h. The suspension was filtered through celite. The celite pad was washed with CH_2Cl_2/MeOH 9:1. The organic solutions were pooled and concentrated. The residue was purified by flash chromatography (CH_2Cl_2/MeOH/H_2O 75:25:0.5) to give **2** (62 mg, 85%). − $[\alpha]_D$ = + 29 (*c* = 1.2, $CHCl_3$/MeOH 4:1). − ^{1}H NMR (400 MHz, [D_5]pyridine): δ = 5.86 (d, $J_{1,2}$ = 3.7 Hz, 1 H, H-1 Fuc^{V}), 5.61 (br q, $J_{5,6}$ = 6.5 Hz, 1 H, H-5 Fuc^{V}), 5.32 (d, $J_{1,2}$ = 7.7 Hz, 1 H, H-1), 5.01 (d, $J_{1,2}$ = 8.3 Hz, 1 H, H-1), 4.82 (dd, *J* = 3 Hz, *J* = 10 Hz, 1 H, H-3 Fuc^{V}), 4.72 (dd, *J* = 9.4 Hz, 1 H), 4.65 (dd, *J* = 3, *J* = 12 Hz, 1 H, H-6), 4.59−4.51 (m, 4 H), 4.48−4.41 (m, 2 H), 4.37−4.25 (m, 3 H), 3.75−3.580 (m, 26 H, OCH_2CH_2O), 4.11 (dd, *J* = 3.1, *J* = 9.5 Hz, 1 H), 4.02−3.95 (m, 2 H), 2.10 (s, 3 H, Ac), 1.78−0.85 (m, 118 H, chain + H-5 fuc). − MS (FAB, NBA): *m*/*z* = 1643.4 [M + Na].

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