Synthesis and Molecular Tumbling Properties of Sialyl Lewis X and Derived Neoglycolipids

Christian Gege,[a] Armin Geyer,*[b] and Richard R. Schmidt*[a]

Dedicated to Professor Lutz F. Tietze on the occasion of his 60th birthday

Abstract: The sialyl Lewis X (sLe^X) epitope has become a prominent target for biological studies because of its role in inflammation through binding to selectins. This epitope is located at the terminal end in glycosphingolipids and a lactose unit serves as spacer to the ceramide moiety. This paper focuses on the influence of the spacer structure and spacer length in regard to the mobility of the sLe^X epitope. To this end sLe^X neoglycolipids **1a–c**, with one, two, or three lactose units as spacer between the sLe^X tetrasaccharide epitope and the membrane anchor, were synthesized. The synthetic strategy was also applied to the synthesis of the corresponding Lewis X (Le^X) derivatives. The glycolipids were inserted in model membranes, and the tumbling frequencies of the sLe^X tetrasaccharide epitopes were then analysed by NMR spectroscopy. A nonaethylene glycol spacer decouples the carbohydrate moiety from the membrane mobility while (oligo-)lactoses act as more rigid distance keepers between the Lewis epitope and the surface of the membrane. Quantification of the different degrees of decoupling was possible by analysis of rotational correlation times.

Keywords: glycolipids · molecular dynamics · NMR spectroscopy · sialyl Lewis X · synthesis design

Introduction

Glycosphingolipids, found on the surface of living cells, are functional elements in cell–cell interactions.[1] The amplification of relatively weak monovalent carbohydrate–lectin affinities to strong polyvalent contacts is a prerequisite of many carbohydrate-based recognition processes.[2] High local concentrations of the carbohydrate ligands on membrane surfaces and an adequate presentation of the sugar epitopes result in strong polyvalent receptor affinities.[3] The protein-independent homophilic carbohydrate–carbohydrate interaction is another cell-adhesion process that depends on the pertinent organization of sugar head groups, as has been found by recent studies with the Lewis X (Le^X) antigen family.[4] The Le^X and sialyl Lewis X (sLe^X) sphingolipids occur as oligomeric glycoforms on cellular surfaces (Scheme 1, **Aa**, **Ab**). Fucosylated *N*-acetyllactosamine trisaccharide units and one lactose as a spacer increase the mobility of the terminal sLe^X tetrasaccharide epitope. In this manuscript, the dependence of the epitope dynamics of sLe^X **Aa**,[5] of the membrane-anchored sLe^X neoglycolipids **Bc**,[6] and **1a–c** on spacer structure and spacer length is quantified, because these neoglycolipids were found to be highly effective mimics of the natural sLe^X gangliosides in dynamic cell rolling studies.[6, 7] The synthesis of compounds **1a–c**, that have one, two, or three lactose units as spacer, is also described in this paper.

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Results and Discussion

Synthesis of Compounds 1a–c: For the synthesis of compounds **1a–c**, we employed a modification of our previously reported strategy for the formation of the sLe^X structures.[6] To this end a 3b-*O*-unprotected 2a-*O*-benzoyl-lactose building block was used to which, in three successive steps, the glucosamine residue and then a fucosyl residue was attached. Finally a Neu5Acα(2-3)Gal-disaccharide building block[8] was connected.

3b-*O*-Unprotected 2a-*O*-benzoyl-lactoside **4** (Scheme 2) was readily obtained from known 2a-*O*-unprotected 3b-*O*-allyl-lactoside **2**.[9] 2a-*O*-Benzoylation ($\rightarrow$**3**) and then removal of the 3b-*O*-allyl group under palladium(II) chloride catalysis in aqueous acetic acid[10] afforded acceptor **4** in high yield. Glycosylation with known glucosamine donor **5**[11] under standard conditions, that is, with catalytic amounts of

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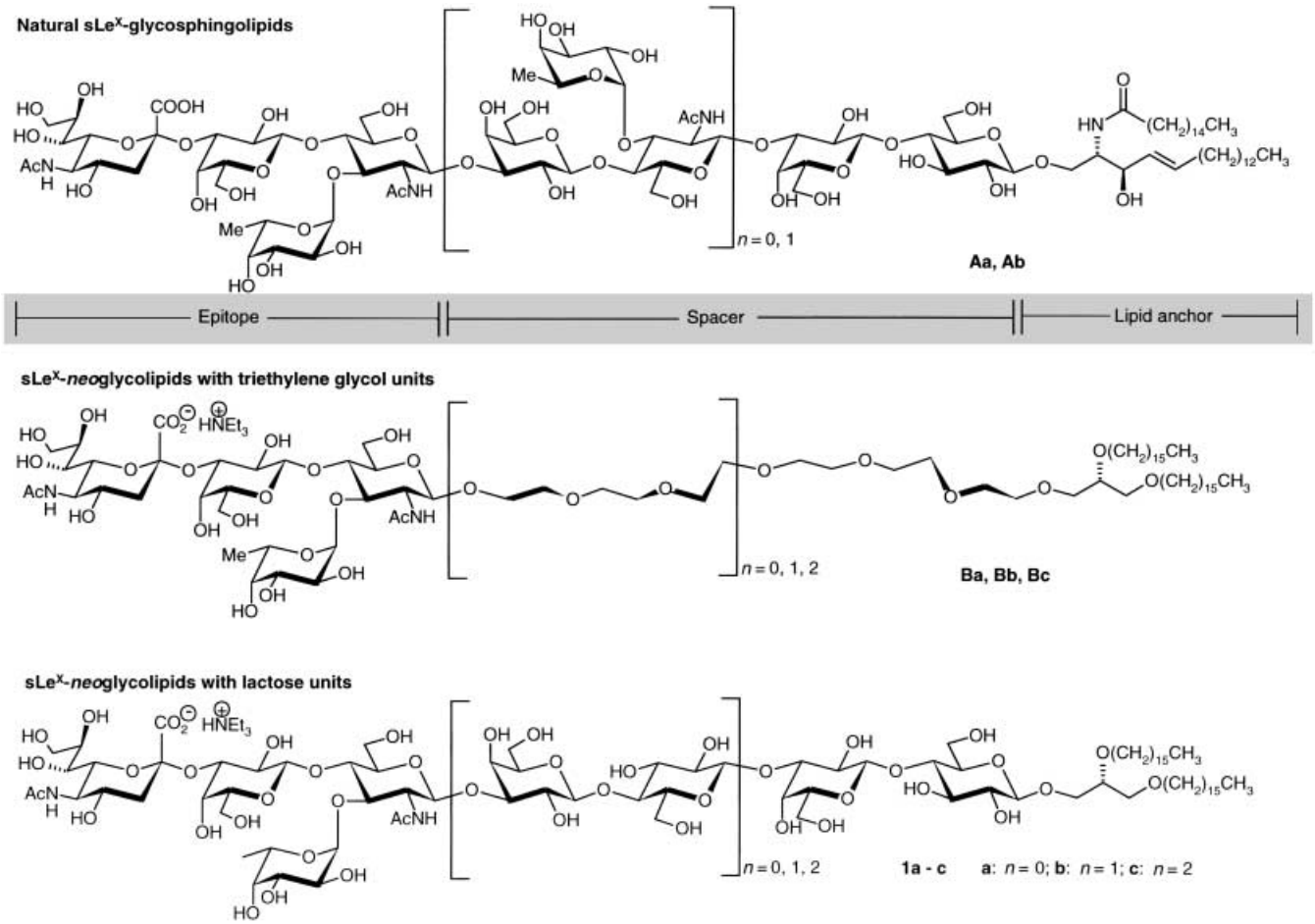

Scheme 1. Natural sialyl Lewis X and derived *neo*glycolipids.

trimethylsilyl trifluoromethanesulfonate (TMSOTf) as activator, led to exclusive β(1-3)-linkage affording trisaccharide **6** in practically quantitative yield (^{1}H NMR: $J_{1c,2c} = 8.3$ Hz; ^{13}C NMR: C-1c, $\delta = 101.51$). Selective removal of the *O*-acetyl groups under Zemplén conditions at 0 °C (→**7**) and then 4c,6c-*O*-benzylidenation with benzaldehyde dimethylacetal in the presence of *p*-toluenesulfonic acid (*p*TsOH) as catalyst gave 3c-*O*-unprotected acceptor **8**. Fucosylation of **8** with known donor **9**[12] under standard conditions furnished exclusively the desired α-linked tetrasaccharide, which was immediately subjected to reductive opening of the 4c,6c-*O*-benzylidene ring with sodium cyanoborohydride in HCl and diethyl ether,[13] thus affording 4c-*O*-unprotected tetrasaccharide **10** as acceptor. It is worth mentioning that the fucosylation of the corresponding *N*-acetyl-protected glucosamine trisaccharide under the same conditions gave only an α/β-mixture (4:1) and moderate yields. This fact demonstrates the good acceptor properties of the trichloroethoxycarbonyl (Troc) residue as amino protecting group.

Glycosylation of **10** with the known Neu5Acα(2-3)Gal donor **11**[6, 14] and TMSOTf as catalyst furnished hexasaccharide **12** in very good yield (Scheme 3). The Troc group in **12** was removed under reductive conditions, and the liberated amino group was acetylated, thus furnishing acetylamino derivative **13**. The new anomeric linkage could be readily assigned in this compound (^{1}H NMR: $J_{1e,2e} = 8.1$ Hz, ^{13}C NMR: C-1e, $\delta = 98.97$). Hydrogenolytic *O*-debenzylation and then *O*-acetylation gave *O*-acyl-protected **14**. The anomeric *O*-acetyl group could be selectively removed with an excess of piperidinium acetate[15] in THF at 50 °C to give the 1-*O*-unprotected derivative **15**. Treatment with trichloroacetonitrile in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as base afforded the desired trichloroacetimidate **16** as hexasaccharide donor.

The synthesis of compounds **1a–c** was completed with the help of dialkylglycerols **17a–c** (Scheme 4); **17a** was obtained as previously described.[6] Its reaction with known donor **18**[16] led, after acid catalyzed removal of the isopropylidene group, to disaccharide neoglycolipid **17b**.[17] Ensuing glycosylation with donor **18** and then de-*O*-isopropylidenation led to tetrasaccharide neoglycolipid **17c**.[17] Glycosylation of acceptors **17a–c** with donor **16** furnished hexa-, octa- and decasaccharides **19a–c**; only β-linkages were formed (^{1}H NMR, **19a**: $J_{1a,2a} = 7.9$ Hz; **19b**: $J_{1c,2c} = 8.0$ Hz, **19c**: $J_{1e,2e} = 7.6$ Hz). Removal of all *O*-acyl groups from **19a–c** under Zemplén conditions and then saponification of the methyl ester of the Neu5Ac moiety furnished target molecules **1a–c** in very good yields. The structural assignments were confirmed by the NMR data.

With building block **10**, the branching to give the Lewis X derivatives is possible at a late stage of the synthetic route.

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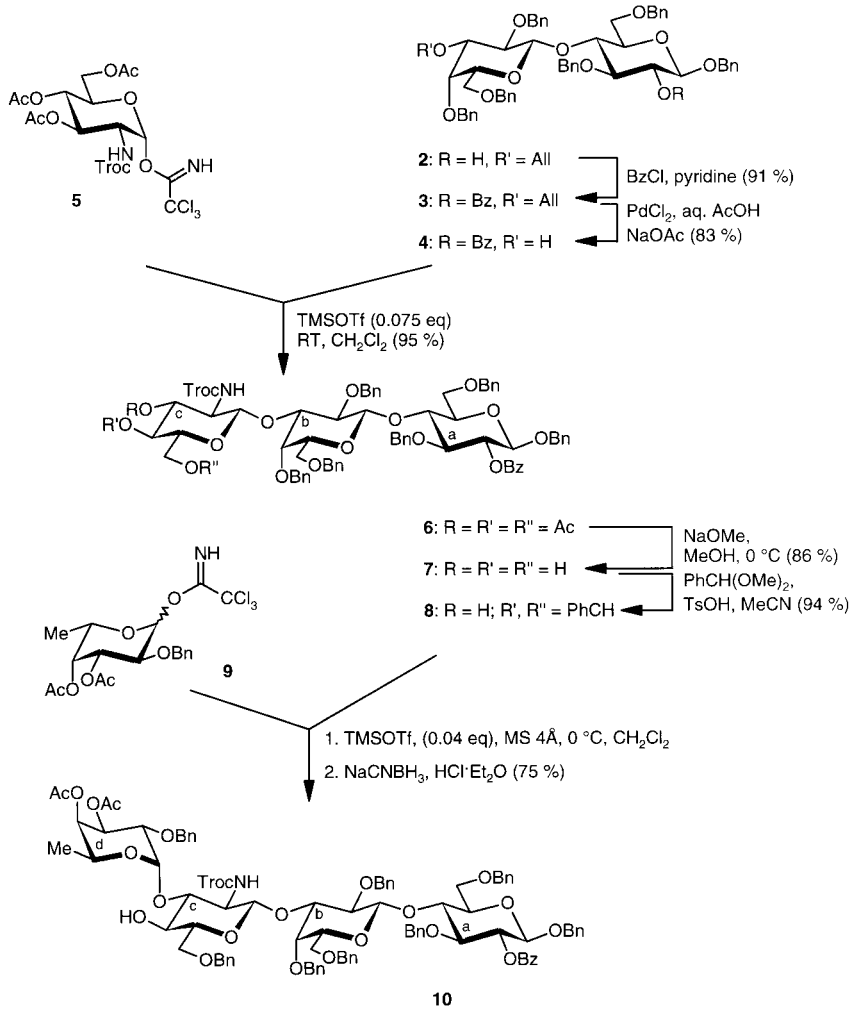
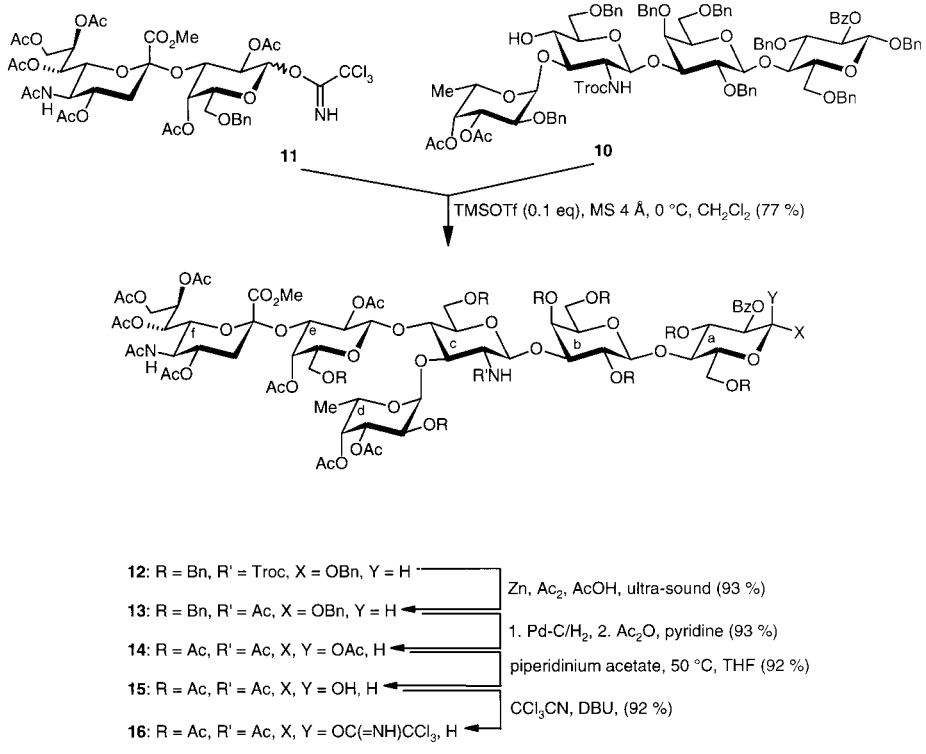


Scheme 2. Synthesis of tetrasaccharide intermediate **10**.



Scheme 3. Synthesis of hexasaccharide donor **16**.

Here with **17a** in hand, the Le^X analogue of **1a** was prepared. To this end, tetrasaccharide **10** was glycosylated with known galactosyl donor **20**[18] under borontrifluoride ether catalysis; the product was treated with zinc in acetic acid/acetic anhydride under sonication, thus furnishing *N*-acetylated pentasaccharide **21** (Scheme 5). The HMQC-spectrum exhibits a large coupling constant for the 1e-H signal at $\delta = 4.59$ ($J_{1e,2e} > 7$ Hz); this indicates its β-linkage. Hydrogenolytic removal of all *O*-benzyl groups and then *O*-acetylation afforded the per-*O*-acylated intermediate **22**. Regioselective 1-*O*-deacetylation with piperidinium acetate ($\rightarrow$**23**) and then treatment with trichloroacetonitrile in the presence of DBU as base furnished trichloroacetimidate **24** as pentaosyl donor. Reaction with **17a** as acceptor afforded the desired β-linked (^{1}H NMR: $J_{1a,2a} = 7.9$ Hz) Le^X neoglycolipid **25**, which on de-*O*-acylation under Zemplén conditions furnished target molecule **26**. The structural assignments were again confirmed by the NMR data collected for compounds **25** and **26**.

Molecular tumbling rates of membrane-anchored sLe^X glycolipids: Deuterated sodium dodecylsulfate ([D_{25}]SDS) micelles form a simple membrane-mimetic environment for the NMR analysis of amphiphilic biomolecules.[19] The tumbling frequency of an SDS micelle is characterized by an average rotational correlation time (τ_c, the average time taken for the molecule to rotate through one radian) of only 5 ns, much shorter than phospholipid-based cell membranes. Yet, this correlation time is long enough to separate the τ_c of the micelle and the τ_c of the carbohydrate head group of a glycolipid which is inserted into the model membrane. Changing the na-

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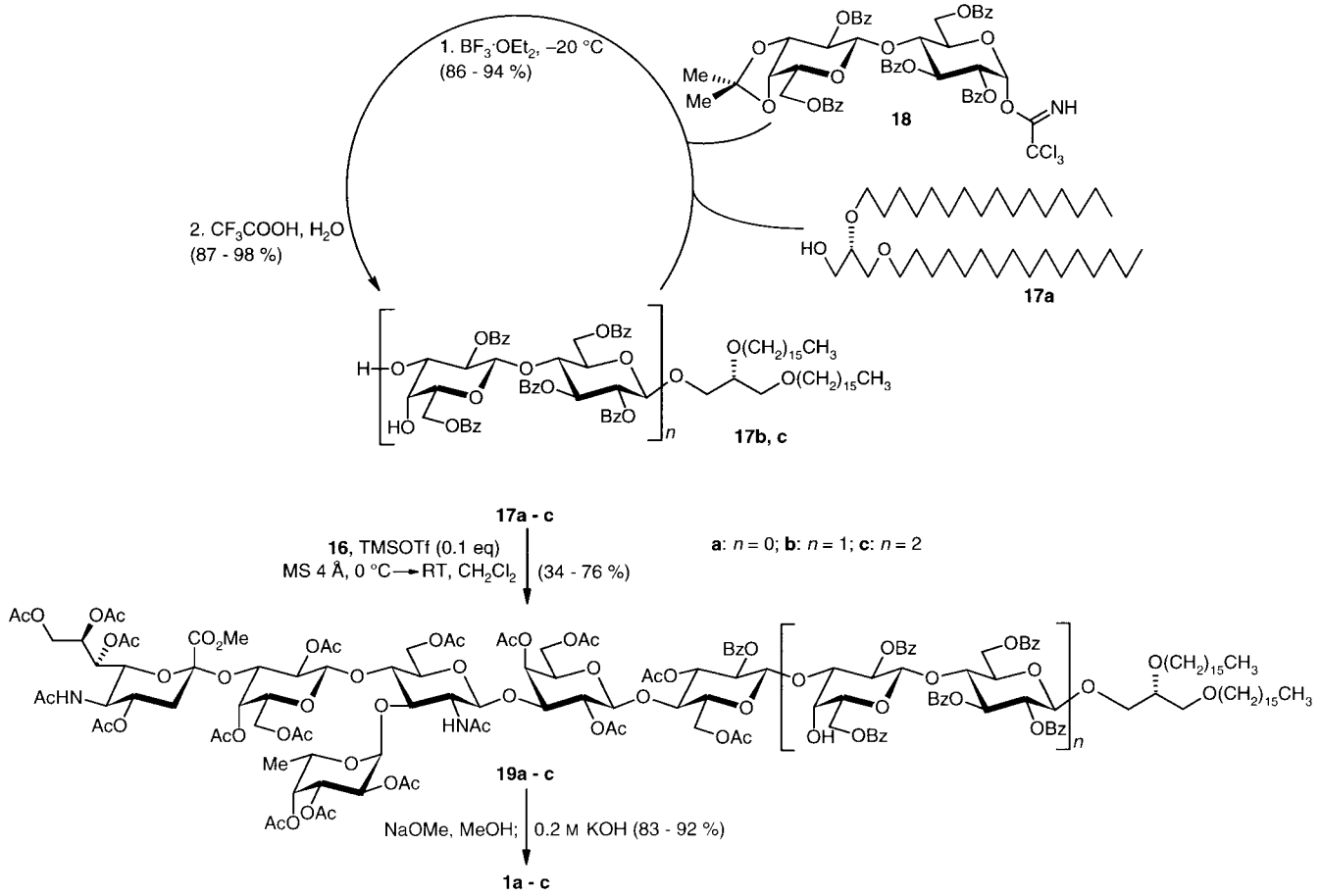

Scheme 4. Synthesis of target molecule **1a–c**.

ture of the spacer between the carbohydrate epitope and the hydrophobic membrane anchor then quantifies the rigidity of the spacer: the flexible spacer decouples the epitope tumbling rate from the membrane mobility while the rigid spacer does not.

Molecular tumbling rates influence the NMR relaxation behaviour. There are several methods for connecting molecular tumbling rates with spin-relaxation properties.[20] Yet, not all of them can be applied to glycolipids that are not isotope enriched and that show line-broadening due to their micelle-anchoring. Dipolar cross relaxation was selected for two reasons, firstly, because of its high sensitivity as a proton-detected method and secondly because of its model-free data analysis. For a fixed interproton distance, the NOE intensity depends only on the reorientation frequency of the corresponding interproton vector relative to the applied magnetic field. Thus, each cross-signal intensity in a NOESY spectrum describes the dynamics of a selected molecular moiety. The strong dependence of the NOE intensity on the molecular tumbling rate in the region of sign inversion of the NOE quantifies the correlation times of individual molecular groups. By this, the nanosecond timescale is analyzed in a measuring window between approximately 0.1 and 10 $\omega_0 \cdot \tau_c$ (ω_0 = spectrometer frequency). For short mixing times (τ_{mix} = 70 ms), each cross-signal intensity in a 2D NOESY spectrum depends on a single interproton distance, and the corresponding cross-relaxation rate σ_{ij} is calculated from the ratio between the cross-signal (I_c) and diagonal-signal (I_d) intensities according to the formula:[21]

$$|\sigma_{ij}| = \frac{1}{2\tau_{mix}} \ln\left[\frac{1+x}{1-x}\right] \qquad x = \frac{I_c}{I_d}$$

Cross-relaxation rates between proton pairs of fixed interatom distances were then analyzed with the formula:

$$\sigma_{ij} = \frac{\gamma^4 h^2}{10 r_{ij}^6}\left(\frac{\mu_0}{(4\pi)^2}\left[\frac{6\tau_c}{1 + (2\omega\tau_c)^2} - \tau_c\right]\right)$$

which contains τ_c as the only variable term. The rigid chair conformations of pyran rings constrain several short interproton distances, which therefore exhibit negligible time averaging. Well-separated ^{1}H NMR resonance signals were observed for the sLe^X tetrasaccharide moieties of **1a–c**, **Aa** and **Bc**. We selected three intraglycosidic NOE contacts of galactose (Gal), fucose (Fuc), and sialic acid (Sia) and one interglycosidic contact to *N*-acetylglucosamine (GlcNAc) Fuc-H1–GlcNAc-H3 for the analysis. The data are listed in Table 1.

A gradation of correlation times was detected within the sLe^X tetrasaccharide units of **1a–c**, **Aa** and **Bc**. The highest mobility (shortest τ_c) was detected for sialic acid which always tumbles at least twice as fast as the Le^X trisaccharide unit. The τ_c's of Fuc are shorter than the τ_c of Gal and describe internal dynamics within the Le^X trisaccharide units. This stepwise decrease of correlation times within the columns of Table 1

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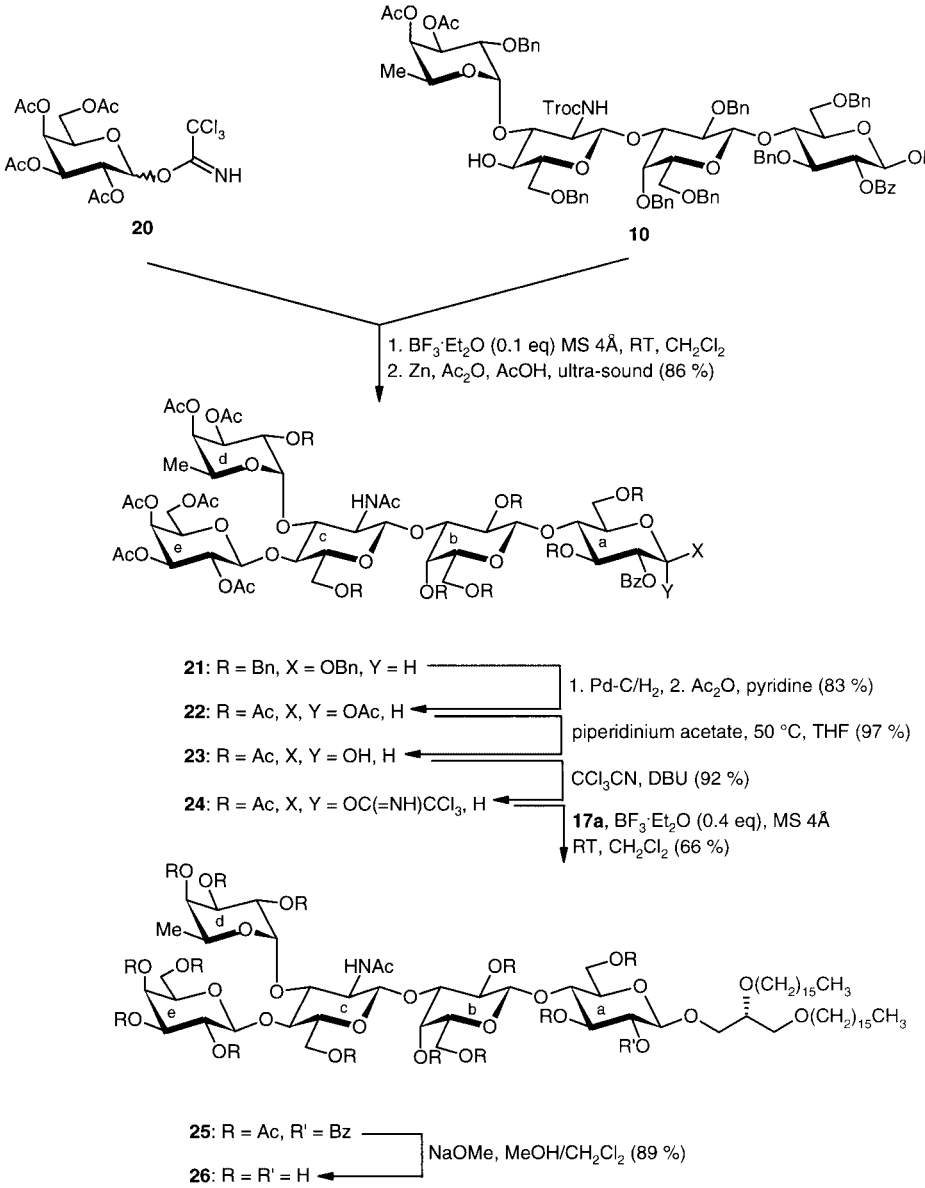

Scheme 5. Synthesis of Le^X *neo*glycolipid **26**.

describes the internal dynamics of each sLe^X tetrasaccharide unit, while the rows in indicate the influence of the spacer unit.

Table 1. The cross-relaxation rates σ_{ij} were determined from NOESY spectra and then transformed into tumbling rates τ_c with the formula given in the text. The structures of naturally occurring sLe^X glycosphingolipid **Aa** and its analogues **1a**–**c** and **Bc** are given in Scheme 1.

	Aa	**1a**	**1b**	**1c**	**Bc**
σ_{ij} [s^{-1}]					
Gal-H1 – Gal-H3	– 0.84	– 0.71	– 0.69	– 0.61	– 0.36
Fuc-H1 – Fuc-H2	– 0.56	– 0.50	– 0.34	– 0.34	n.d.
Fuc-H1 – GlcNAc-H3	– 0.61	– 0.63	– 0.53	– 0.61	– 0.33
Sia-$H3_{ax}$ – $H3_{eq}$	– 2.30	– 2.30	– 1.66	– 1.86	– 1.10
τ_c [10^{-9} s]					
Gal-H1 – Gal-H3	3.6	3.0	3.0	2.6	1.6
Fuc-H1 – Fuc-H2	1.8	1.8	1.3	1.3	n.d.
Fuc-H1 – GlcNAc-H3	2.1	2.2	1.8	2.1	1.2
Sia-$H3_{ax}$ – $H3_{eq}$	1.45	1.45	1.1	1.2	0.8

Neoglycolipid **1a** and the natural sLe^X ganglioside **Aa** have the same hexasaccharide moiety, the structural differences are restricted to the hydrophobic membrane anchors. Their similar correlation times prove that the tumbling behaviour of the sugar head group in the micellar environment is hardly influenced by the membrane anchor. Probably, the aglyconic methylene group acts as a flexible joint between the spacer and the hydrophobic lipid anchor. Two or three lactose units double or triple the distance between the membrane surface and the sLe^X moiety, respectively. Yet the average τ_c's of the sLe^X tetrasaccharide moieties decrease only gradually by approximately 30% between **1a** and **1c**. Although the molecular weight increases from **1a** to **1c**, the average correlation time of the terminal Le^X tetrasaccharide decreases in the same direction. Thus, the oligolactose spacer partly decouples the tumbling of the terminal epitope from the micelle dynamics. The nonaethylene glycol spacer in **Bc**[6] doubles the tumbling frequency of the sLe^X head group—it divides the correlation times by a factor of two—and thus increases the overall mobility of the terminal tetrasaccharide. Thus, the different mobilities of the sLe^X tetrasaccharide units of **1c** and **Bc** are due to the effective decoupling of the carbohydrate tumbling from the micelle tumbling by the nonaethylene spacer. A quantitative description of the degree of decoupling by an oligoethylene spacer is given here for the first time. The tumbling rates are the basis for the interpretation of receptor binding data of **1a**–**1c** and **Bc** described in refs. [6, 7]. The data were measured with isolated glycolipids, which were inserted into model membranes. For the design of multivalent ligands,[22] it has to be taken into account that the membrane anchor also causes differences in the supramolecular arrangement (microdomains) of the glycolipids in the membrane.[7]

Conclusion

In conclusion, an efficient route for the synthesis of Le^X and sLe^X trichloroacetimidate donors **16** and **24** has been devel-

0947-6539/02/0811-2458 $ 20.00+.50/0 *Chem. Eur. J.* **2002**, *8*, No. 11

oped. Glycosylation of dialkylglycerols **17a–c** furnished the target molecules **1a–c** with one to three lactose units as spacer after deprotection. We could separate the influence of the spacer on the presentation of the carbohydrate epitope by quantifying molecular tumbling rates of the sLe^X tetrasaccharide head groups. These results underline the dominating influence of the spacer in the presentation of carbohydrate epitopes and thus on molecular recognition phenomena.

Experimental Section

General techniques: Solvents were purified according to standard procedures. Flash chromatography was performed on Baker silica gel 60 (0.040–0.063 mm) at a pressure of 0.4 bar. Thin layer chromatography was performed on Merck silica gel plastic plates, $60F_{254}$ or Merck silica gel glass plates, HPTLC $60F_{254}$; compounds were visualized by treatment with a solution of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (20 g) and $Ce(SO_4)_2$ (0.4 g) in 10% sulfuric acid (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 at 22 °C on a Bruker AC250 Cryospec or a Bruker DRX600. TMS or the resonance of the deuterated solvent was used as internal standard; solvents: $CDCl_3$, $\delta = 7.26$; D_2O, $\delta = 4.63$. Target molecules (max. 6 mg) were measured in a 320 mmol solution of [D_{25}]sodiumdodecyl sulfate ([D_{25}]SDS) in 0.5 mL D_2O. At this concentration, each micelle bears approximately one or two glycolipids. NOESY spectra were acquired with different mixing times, of which only the shortest τ_{mix} (70 ms) were used for the data analysis. MALDI-mass spectra were recorded on a Kratos Kompact MALDII instrument using a 2,5-dihydroxybenzoic acid matrix.

Benzyl *O*-(3-*O*-allyl-2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranoside (3): A solution of compound **2**[9] (13.3 g, 14.4 mmol) and benzoyl chloride (2.5 mL, 22 mmol) in dry pyridine (50 mL) was heated at 45 °C for 18 h. After addition of some ethanol the mixture was concentrated in vacuo. After addition of dichloromethane (300 mL), the solution was washed with 1M hydrochloric acid and saturated $NaHCO_3$ solution. The organic layer was dried over sodium sulfate and concentrated in vacuo. Purification was accomplished by flash chromatography (petroleum ether/ethyl acetate 5:1 to 4:1) to furnish **3** (13.5 g, 91%) as a colourless oil. $R_f = 0.32$ (petroleum ether/ethyl acetate 4:1); $[\alpha]_D = -5.7$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 3.33$ (dd, $J(2,3) = 9.7$, $J(3,4) = 3.0$ Hz, 1H; 3b-H), 3.36–3.53 [m, 4H; HMQC: 3.37 (6b-H), 3.39 (5b-H), 3.46 (5a-H), 3.51 (6′b-H)], 3.69–3.73 [m, 2H; HMQC: 3.71 (3a-H), 3.72 (2b-H)], 3.78 (dd, $J(5,6) = 1.7$, $^2J = 10.9$ Hz, 1H; 6a-H), 3.85 (dd, $J(5,6') = 4.5$, $^2J = 10.9$ Hz, 1H; 6′a-H), 3.87 (d, $J(3,4) = 3.1$ Hz, 1H; 4b-H), 4.09 (dd, $J(3,4) = J(4,5) = 9.2$ Hz, 1H; 4a-H), 4.17 (m, 2H; OCH_2-CH=CH_2), 4.24–4.96 [m, 14H; HMQC: 4.25, 4.35 (2d, $^2J = 11.8$ Hz, CH_2Ph), 4.45 (1b-H), 4.55 (1a-H), 5 CH_2Ph], 5.19 (dd, $^2J = 1.3$, $^3J = 10.5$ Hz, 1H; OCH_2-CH=CHH), 5.32–5.35 [m, 2H; HMQC: 5.33 (OCH_2-CH=CHH), 5.33 (2a-H)], 5.93 (m, 1H; OCH_2-CH=CH_2), 6.98–7.99 (m, 35H; 7 C_6H_5); ^{13}C NMR (151 MHz, $CDCl_3$, excerpt): $\delta = 68.18$ (6a-C, 6b-C), 73.04 (5b-C), 73.15 (2a-C), 73.44 (4b-C), 75.59 (5a-C), 76.79 (4a-C), 79.94 (2b-C), 80.56 (3a-C), 82.24 (3b-C), 99.43 (1a-C), 102.84 (1b-C), 165.15 (COPh); elemental analysis calcd (%) for $C_{64}H_{66}O_{12}$ (1027.21): C 74.83, H 6.48; found: C 74.91, H 6.52.

Benzyl *O*-(3-*O*-allyl-2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranoside (4): Compound **3** (13.5 g, 13.1 mmol) was dissolved in acetic acid (130 mL) and water (13 mL), then sodium acetate (3.2 g) and palladium chloride (2.7 g) were added. After 24 h, the solution was filtered over celite and washed with diethyl ether. After evaporation in vacuo, the residue was dissolved in diethyl ether (400 mL), and the solution was washed with saturated $NaHCO_3$ solution. The organic layer was dried over sodium sulfate and concentrated in vacuo. Flash chromatography (petroleum ether/ethyl acetate 3:1 to 2:1, added in toluene) furnished **4** (10.7 g, 83%) as a colourless foam. $R_f = 0.42$ (petroleum ether/ethyl acetate 2:1); $[\alpha]_D = -1.3$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 3.47–5.01$ (m, 23H; 21-H, 2b-H, 23-H, 24-H, 25-H, 46-H, 6 CH_2Ph), 5.43 (dd, $J(1,2) = 8.0$, $J(2,3) = 9.3$ Hz, 1H; 2a-H), 7.05–8.08 (m, 35H; 7 C_6H_5); elemental analysis calcd (%) for $C_{61}H_{62}O_{12}\cdot \frac{1}{4}H_2O$ (991.65): C 73.88, H 6.35; found: C 73.73, H 6.32.

Benzyl *O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranoside (6): A solution of **4** (4.10 g, 4.15 mmol) and **5**[11] (2.81 g, 4.49 mmol) in dry dichloromethane (25 mL) with molecular sieves (AW-300) was treated at room temperature with trimethylsilyl trifluoromethanesulfonate (46 μL, 259 μmol). After being stirred for 15 min, the mixture was neutralized with triethylamine and concentrated in vacuo. The residue was purified by flash chromatography (petroleum ether/ethyl acetate 2:1 to 3:2) to give **6** (5.78 g, 95%) as a colourless foam. $R_f = 0.24$ (petroleum ether/ethyl acetate 2:1); $[\alpha]_D = -13.8$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): $\delta =$ 2.00, 2.07, 2.09 (3s, 9H; 3 $COCH_3$), 3.42–3.55 [m, 4H; HMQC: 3.43 (5a-H), 3.44 (6b-H), 3.51 (5b-H), 3.53 (6′b-H)], 3.67 (ddd, $J(4,5) = 10.1$, $J(5,6) = 2.5$, $J(5,6') = 5.1$ Hz, 1H; 5c-H), 3.70–3.81 [m, 5H; HMQC: 3.72 (3b-H), 3.74 (3a-H), 3.77 (2b-H), 3.78 (6a-H), 3.78 (2c-H)], 3.89 (dd, $J(5,6) = 4.0$, $^2J =$ 11.1 Hz, 1H; 6′a-H), 3.92 (d, $J(3,4) = 3.1$ Hz, 1H; 4b-H), 4.18–4.21 [m, 2H; HMQC: 4.19 (4a-H), 4.19 (6c-H)], 4.27 (d, $^2J = 11.7$ Hz, 1H; C*H*HPh), 4.32 (dd, $J(5,6') = 5.1$, $^2J = 12.3$ Hz, 1H; 6′c-H), 4.36 (br d, 1H; NH), 4.39 (d, $^2J = 11.7$ Hz, 1H; C*H*HPh), 4.50–4.65 [m, 9H; HMQC: 4.51 (1b-H), 3.55 (1a-H), CH_2CCl_3, 5 C*H*HPh], 4.75 (d, $^2J = 12.1$ Hz, 1H; C*H*HPh), 4.78 (d, $J(1,2) = 8.3$ Hz, 1H; 1c-H), 4.81 (dd, $J(2,3) = J(3,4) \approx 10.0$ Hz, 1H; 3c-H), 4.87–4.99 (m, 4H; 4 C*H*HPh), 5.05 (dd, $J(3,4) = J(4,5) = 9.7$ Hz, 1H; 4c-H), 5.36 (dd, $J(1,2) = J(2,3) \approx 8.7$ Hz, 1H; 2a-H), 7.01–8.01 (m, 35H; 7 C_6H_5); ^{13}C NMR (151 MHz, $CDCl_3$, excerpt): $\delta = 56.18$ (2c-C), 62.17 (6c-C), 68.04 (6a-C), 68.19 (6b-C), 68.75 (4c-C), 71.74 (5c-C), 72.04 (3c-C), 73.09 (2a-C), 73.36 (5b-C), 75.53 (5a-C), 76.00 (4b-C), 76.24 (4a-C), 80.21 (3b-C), 80.41 (3a-C), 80.94 (2b-C), 99.36 (1a-C), 101.51 (1c-C), 102.56 (1b-C); elemental analysis calcd (%) for $C_{76}H_{80}Cl_3NO_{21}\cdot \frac{1}{2}H_2O$ (1458.83): C 62.57, H 5.60, N 0.93; found: C 62.40, H 5.43, N 0.67.

Benzyl *O*-(2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranoside (7): Compound **6** (8.81 g, 6.04 mmol) was dissolved in dry methanol (100 mL), and sodium methoxide (100 mg, 1.85 mmol) was added at 0 °C. After the mixture had been stirred for 1.5 h at 0 °C, TLC showed that there was no more starting material. The solution was neutralized with Amberlite IR120 (H^+), filtered and evaporated. Flash chromatography (toluene/acetone/methanol 66:33:1) yielded **7** (7.00 g, 86%) as a colourless foam. $R_f = 0.21$ (toluene/acetone/methanol 66:33:1); $[\alpha]_D = -9.3$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 3.14–4.97$ (m, 35H; 31-H, 2b-H, 2c-H, 33-H, 34-H, 35-H, 66-H, NH, CH_2CCl_3, 6 CH_2Ph), 5.31 (dd, $J(1,2) = 8.1$, $J(2,3) = 9.3$ Hz, 1H; 2a-H), 6.93–7.98 (m, 35H; 7 C_6H_5); elemental analysis calcd (%) for $C_{70}H_{74}Cl_3NO_{18}\cdot H_2O$ (1341.73): C 62.66, H 5.71, N 1.04; found: C 62.52, H 5.75, N 0.77.

Benzyl *O*-(4,6-di-*O*-benzylidene-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranoside (8): Compound **7** (3.74 g, 2.79 mmol) was dissolved in dry acetonitrile (30 mL) with molecular sieves (AW-300), then benzaldehyde dimethylacetal (510 μL, 3.39 mmol) and *p*-toluenesulfonic acid (108 mg, 560 μmol) was added at room temperature. After 3 h, the solution was neutralized with triethylamine, filtrated and concentrated in vacuo. The residue was purified by flash chromatography (petroleum ether/ethyl acetate 3:1 to 2:1) to give **8** (3.70 g, 94%) as a colourless foam. $R_f = 0.24$ (petroleum ether/ethyl acetate 2:1); $[\alpha]_D = -25.4$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta =$ 3.37–5.01 (m, 35H; 31-H, 2b-H, 2c-H, 33-H, 34-H, 35-H, 66-H, NH, CH_2CCl_3, 6 CH_2Ph), 5.31 (dd, $J(1,2) = 8.1$, $J(2,3) = 9.3$ Hz, 1H; 2a-H), 5.55 (s, 1H; CHPh), 7.04–7.98 (m, 40H; 8 C_6H_5); elemental analysis calcd (%) for $C_{77}H_{78}Cl_3NO_{18}$ (1411.82): C 65.51, H 5.57, N 0.99; found: C 62.53, H 5.69, N 0.81.

Benzyl *O*-(3,4-di-*O*-acetyl-2-*O*-benzyl-α-L-fucopyranosyl)-(1→3)-(6-*O*-benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranoside (10): A solution of acceptor **8** (3.63 g, 2.57 mmol) and donor **9**[12] (1.86 g, 3.86 mmol) in dry dichloromethane (40 mL) with molecular sieves (AW-300) was treated at 0 °C with trimethylsilyl trifluoromethanesulfonate (19 μL, 106 μmol). After 30 min, the mixture was neutralized with triethylamine and concentrated in vacuo. The residue was purified by flash chromatography (petroleum ether/ethyl

acetate 2:1 to 3:2 to 1:1) to give the tetrasaccharide as a colourless foam. This compound was dissolved in dry tetrahydrofuran (70 mL) with molecular sieves (AW-300), and sodium cyanoborohydride (1.6 g, 80 mmol) was added. The mixture was treated dropwise under cooling with a saturated solution of hydrochloride in diethyl ether until the solution remained acidic. After 30 min, sodium hydrogen carbonate was added, the mixture was filtrated and diluted with a saturated $NaHCO_3$ solution. After extraction with dichloromethane (3×100 mL), the combined organic layers were dried over sodium sulfate and concentrated in vacuo. Flash chromatography (toluene/acetone 9:1 to 8:1 to 7:1) yielded **10** (3.33 g, 75%) as a colourless foam. $R_f = 0.31$ (toluene/acetone 7:1); $[\alpha]_D = -34.0$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 1.08$ (d, $J(5,6) = 6.5$ Hz, 3H; 6d-CH_3), 1.92, 2.09 (2s, 6H; $COCH_3$), 3.22 (br s, 1H; 3c-H), 3.32 (m, 1H; 5a-H), 3.39 (dd, $J(5,6) = 4.6$, $^2J = 8.4$ Hz, 1H; 6b-H), 3.42–3.80 [m, 12H; HMQC: 3.43 (5c-H), 3.46 (5b-H), 3.49 (6′b-H), 3.52 (4c-H), 3.56 (2c-H), 3.65 (3a-H), 3.68 (6a-H), 3.68 (3b-H), 3.70 (2b-H), 3.72 (6c-H), 3.79 (6′a-H), 3.79 (2d-H)], 3.82 (dd, $J(5,6) = 2.9$, $^2J = 10.6$ Hz, 1H; 6′c-H), 3.91 (s, 1H; 4b-H), 4.10 (dd, $J(3,4) = J(4,5) = 9.2$ Hz, 1H; 4a-H), 4.22 (d, $^2J = 11.8$ Hz, 1H; C*H*HPh), 4.33–4.89 [m, 23H; HMQC: 4.34 (5d-H), 4.41 (1b-H), 4.47 (1a-H), 4.50 (NH), 4.75 (1c-H), 4.89 (1d-H), 15 C*H*HPh, CH_2CCl_3], 5.22–5.25 [m, 2H; HMQC: 5.23 (3d-H), 5.24 (4d-H)], 5.30 (dd, $J(1,2) = J(2,3) \approx 8.7$ Hz, 1H; 2a-H), 6.93–8.01 (m, 45H; $9C_6H_5$); ^{13}C NMR (151 MHz, $CDCl_3$, excerpt): $\delta = 15.88$ (6d-C), 56.30 (2c-C), 65.75 (5d-C), 67.90 (6a-C), 68.35 (6b-C), 69.84 (6c-C, 3d-C), 70.41 (4c-C), 71.28 (4d-C), 73.24 (2a-C), 73.41 (2d-C), 73.64 (5b-C), 74.77 (5c-C), 75.42 (5a-C), 76.23 (4a-C, 4b-C), 80.16 (3b-C), 80.40 (3a-C), 80.90 (2b-C), 83.59 (3c-C), 97.79 (1d-C), 99.32 (1a-C), 101.60 (1c-C), 102.49 (1b-C); elemental analysis calcd (%) for $C_{94}H_{100}Cl_3NO_{24}$ (1734.18): C 65.10, H 5.88, N 0.81; found: C 64.87, H 5.91, N 0.73.

Hexasaccharide (12): A solution of acceptor **10** (1.73 g, 1.00 mmol) and donor **11**[6] (1.52 g, 1.56 mmol) in dry dichloromethane (15 mL) with molecular sieves AW-300 was treated at 0 °C with trimethylsilyl trifluoromethanesulfonate (18 μL, 100 μmol). After being stirred for 2 h, the mixture was neutralized with triethylamine, filtrated and concentrated in vacuo. The residue was purified by flash chromatography (toluene/acetone 3:1) to give **12** (1.95 g, 77%) as colourless foam. Remaining acceptor **10** can be recovered. $R_f = 0.33$ (toluene/acetone 2:1); $[\alpha]_D = -30.2$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (600 Hz, $CDCl_3$): $\delta = 1.20$ (d, $J(5,6) = 6.5$ Hz, 3H; 6d-CH_3), 1.73 (dd, $J(3_{ax},4) = {}^2J = 12.4$ Hz, 1H; 3f-H_{ax}), 1.85, 1.86, 1.95, 2.00, 2.07, 2.07, 2.08, 2.08, 2.22 (9s, 27H; $9COCH_3$), 2.56 (dd, $^2J = 12.6$, $J(3_{eq},4) = 4.7$ Hz, 1H; 3f-H_{eq}), 3.30–5.30 (m, 63H; 5 1-H, 5 2-H, 5 3-H, 6 4-H, 6 5-H, 9 6-H, 2 9-H, CO_2CH_3, 2NH, CH_2CCl_3, $9CH_2Ph$), 5.38 (dd, $J(6,7) = 2.8$, $J(7,8) = 9.4$ Hz, 1H; 7f-H), 5.56 (m, 1H; 8f-H), 6.94–7.98 (m, 50H; $10C_6H_5$); elemental analysis calcd (%) for $C_{131}H_{147}Cl_3N_2O_{43}$ (2543.95): C 61.85, H 5.82, N 1.10; found: C 61.83 H 5.73, N 0.71.

Acetylamino hexasaccharide (13): A solution of **12** (1.95 g, 767 μmol) in tetrahydrofuran/acetic anhydride/acetic acid (6:2:1, 24 mL) was treated with activated zinc powder (1.5 g, activation with 2% $CuSO_4$ in water for 5 min, then subsequent washing with water, methanol and dry diethyl ether). The mixture was stirred for 4 h at room temperature with sonication (5×30 min) and then filtered and washed with diethyl ether (400 mL). After careful addition of a saturated sodium bisulfate solution, the mixture was stirred vigorously for 2 h. The organic layer was separated, dried over sodium sulfate, and evaporated under reduced pressure. Purification by flash chromatography (toluene/acetone 5:2 to 2:1) gave **13** (1.81 g, 93%) as a colourless foam. $R_f = 0.52$ (toluene/acetone 1:1); $[\alpha]_D = -35.2$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$, main conformer): $\delta = 1.10$ (d, $J(5,6) = 6.5$ Hz, 3H; 6d-CH_3), 1.50 (s, 3H; $COCH_3$), 1.72 (dd, $^2J = J(3_{ax},4) = 12.4$ Hz, 1H; 3f-H_{ax}), 1.85, 1.86, 1.87, 2.00, 2.04, 2.07, 2.07, 2.09, 2.21 (9s, 27H; $9COCH_3$), 2.57 (dd, $^2J = 12.6$, $J(3_{eq},4) = 4.7$ Hz, 1H; 3f-H_{eq}), 3.32–3.44 [m, 4H; HMQC: 3.32 (6b-H), 3.33 (5a-H), 3.36 (6′b-H), 3.38 (5b-H)], 3.57–3.76 [m, 55H; HMQC: 3.57 (6e-H), 3.57 (6′e-H), 3.61 (6f-H), 3.63 (3b-H), 3.64 (3a-H), 3.66 (6a-H), 3.69 (2b-H), 3.69 (5c-H), 3.72 (5e-H), 3.73 (6′a-H), 3.73 (2c-H), 3.81 (2d-H), 3.82 (6c-H), 3.82 (6′c-H), 3.84 ($COOCH_3$), 3.89 (9f-H), 3.96 (4b-H), 4.06 (4a-H), 4.05 (5f-H), 4.19 (3c-H), 4.19 (4c-H), 4.22 (9′f-H), 4.41 (1b-H), 4.48 (1a-H), 4.59 (3e-H), 4.65 (5d-H), 4.81 (1e-H, $J(1,2) \approx 8.1$ Hz), 4.90 (4f-H), 4.93 (2e-H), 4.99 (1c-H), 5.03 (N_fH), 5.08 (4e-H), 5.18 (3d-H), 5.22 (1d-H), 5.23 (4d-H), $9CH_2Ph$], 5.28 (d, $J(1,2) = J(2,3) \approx 8.7$ Hz, 1H; 2a-H), 5.33 (dd, $J(6,7) = 2.8$, $J(7,8) = 9.5$ Hz, 1H; 7f-H), 5.54 (d, $J(2,NH) = 8.3$ Hz, 1H; N_eH), 5.61 (m, 1H; 8f-H), 6.93–7.98 (m, 50H; $10C_6H_5$); ^{13}C NMR (151 MHz, $CDCl_3$, excerpt): $\delta = 15.86$ (6d-C), 37.57 (3f-C), 49.08 (5f-C), 53.16 (OCH_3), 57.37 (2c-C), 62.40 (9f-C), 64.33 (5d-C), 67.14 (7f-C), 67.22 (8f-C), 67.38 (6e-C), 67.81 (4e-C), 68.08 (6a-C), 68.53 (6b-C), 69.02 (6c-C), 69.41 (4f-C), 70.22 (3d-C), 70.52 (2e-C), 71.65 (3e-C), 71.89 (6f-C), 71.94 (4d-C), 72.09 (5e-C), 72.9 (2d-C, 3c-C), 73.11 (2a-C), 73.34 (5b-C), 74.88 (5c-C), 75.5 (4c-C, 5a-C), 76.2 (4a-C, 4b-C), 79.59 (2b-C), 80.52 (3a-C), 82.36 (3b-C), 96.49 (1d-C), 96.99 (2f-C), 98.97 (1e-C), 99.39 (1a-C), 101.40 (1c-C), 102.54 (1b-C), 167.72 (1f-C); elemental analysis calcd (%) for $C_{130}H_{148}N_2O_{42}$ (2410.59): C 64.77, H 6.19, N 1.16; found: C 64.56 H 5.89, N 0.95.

***O*-acyl-protected hexasaccharide (14)**: Compound **13** (575 mg, 239 μmol) was dissolved in methanol (20 mL) and acetic acid (3 drops). Palladium on charcoal (100 mg, 10% Pd) was added, and the solution was stirred vigorously under a hydrogen atmosphere overnight. The catalyst was filtered off and washed with methanol. After evaporation in vacuo the residue was dissolved in pyridine (15 mL), and acetic anhydride (15 mL) was added. After 16 h, the solvent was evaporated, and the residue was coevaporated with toluene ($3 \times$) and purified by flash chromatography (toluene/acetone 1:1) to give **14** (422 mg, 93%) as a colourless foam. $R_f = 0.30$ (toluene/acetone 1:1); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 1.18$ (d, $J(5,6) = 6.5$ Hz, 3H; 6d-CH_3), 1.64–2.20 (m, 58H; 3f-H_{ax}, 19 $COCH_3$), 2.59 (dd, $^2J = 12.6$, $J(3_{eq},4) = 4.6$ Hz, 1H; 3f-H_{eq}), 3.11–6.43 (m, 45H; 5 1-H, 5 2-H, 5 3-H, 6 4-H, 6 5-H, 9 6-H, 7f-H, 8f-H, 2 9f-H, CO_2CH_3, 2NH), 7.41–7.99 (m, 5H; C_6H_5); elemental analysis calcd (%) for $C_{85}H_{112}N_2O_{51}$ (1977.80): C 51.62, H 5.71, N 1.42; found: C 51.31 H 5.75, N 1.07.

Unprotected hexasaccharide (15): Piperidinium acetate (580 mg) was added to a solution of compound **14** (1.06 g, 535 μmol) in dry tetrahydrofuran (40 mL), and the mixture was stirred for 4 h at 50 °C. After evaporation of the solvent, the residue was purified by flash chromatography (toluene/acetone 1:1) to give **15** (954 mg, 92%) as a colourless foam. $R_f = 0.42$ (toluene/acetone 2:3); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 1.18$ (d, $J(5,6) = 6.5$ Hz, 3H; 6d-CH_3), 1.53–2.20 (m, 55H; 3f-H_{ax}, 18$COCH_3$), 2.59 (dd, 1H; 3f-H_{eq}), 3.15–5.57 (m, 45H; 5 1-H, 4 2-H, 5 3-H, 6 4-H, 6 5-H, 9 6-H, 7f-H, 8f-H, 2 9f-H, CO_2CH_3, 2NH, OH), 5.73 (dd, $J(1,2) = J(2,3) = 9.7$ Hz, 1H; 2a-H), 7.42–8.03 (m, 5H; C_6H_5); elemental analysis calcd (%) for $C_{83}H_{110}N_2O_{50}$ (1935.76): C 51.50, H 5.73, N 1.45; found: C 51.95 H 6.02, N 1.74.

Trichloroacetimidate (16): Trichloroacetonitrile (320 μL, 3.2 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (4 μL) were added to a solution of **15** (614 mg, 317 μmol) in dry dichloromethane (20 mL). After 3 h, the mixture was concentrated in vacuo. Flash chromatography (toluene/acetone 1:1+1% triethylamine) furnished **16** (607 mg, 92%) in a α/β ratio of >19:1 as a pale yellow foam. $R_f = 0.53$ (toluene/acetone 2:3); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 1.18$ (d, $J(5,6) = 6.5$ Hz, 3H; 6d-CH_3), 1.64–2.19 (m, 55H; 3f-H_{ax}, 18$COCH_3$), 2.58 (dd, 1H; 3f-H_{eq}), 3.10–5.78 (m, 45H; 4 1-H, 5 2-H, 5 3-H, 6 4-H, 6 5-H, 9 6-H, 7f-H, 8f-H, 2 9f-H, CO_2CH_3, 2NH), 6.62 (d, $J(1,2) = 3.7$ Hz, 1H; 1a-H), 7.38–7.99 (m, 5H; C_6H_5), 8.56 (s, 1H; =NH); elemental analysis calcd (%) for $C_{85}H_{110}N_3Cl_3O_{50} \cdot H_2O$ (2098.17): C 48.66, H 5.38, N 2.00; found: C 48.97 H 5.90, N 2.02.

Hexasaccharide (19a): A solution of donor **16** (96 mg, 46 μmol) and lipid **17a**[6] (50 mg, 92 μmol) in dry dichloromethane (3 mL) with molecular sieves (AW-300) was treated at 0 °C with trimethylsilyl trifluoromethanesulfonate (3.3 μL, 18 μmol). After being stirred for 3 h, the mixture was neutralized with triethylamine, filtrated and concentrated in vacuo. The residue was purified by flash chromatography (toluene/acetone 2:1 to 3:2) to give **19a** (87 mg, 76%) as a colourless foam. $R_f = 0.56$ (toluene/acetone 1:1, HPTLC); $[\alpha]_D = -22.1$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 0.$ 88 (t, 6H; $2CH_3$), 1.18–1.31 (m, 55H; $26CH_2$, 6d-CH_3), 1.36–1.43 (q, 4H; $2OCH_2CH_2$), 1.68 (dd, $^2J = J(3_{ax},4) = 12.4$ Hz, 1H; 3f-H_{ax}), 1.85, 1.92, 1.94, 1.96, 2.00, 2.05, 2.07, 2.07, 2.08, 2.09, 2.09, 2.10, 2.11, 2.12, 2.13, 2.14, 2.15, 2.20 (18s, 54H; 18$COCH_3$), 2.58 (dd, $^2J = 12.5$, $J(3_{eq},4) = 4.4$ Hz, 1H; 3f-H_{eq}), 3.08–3.17 [m, 3H; HMQC: 3.11, 3.15 (OCH_2), 3.17 (2c-H)], 3.24 (dd, $^2J = 10.2$, $J(2',3') = 5.3$ Hz, 1H; 3′-H), 3.32 (dd, $^2J = 10.2$, $J(2',3') = 4.2$ Hz, 1H; 3′-H), 3.38–3.67 [m, 7H; HMQC: 3.40 (OCH_2), 3.44 (2′-H), 3.46 (5c-H), 3.57 (1′-H), 3.63 (6f-H), 3.65 (5a-H)], 3.71 (dd, $J(2,3) = 9.9$, $J(3,4) = 3.5$ Hz, 1H; 3b-H), 3.75 (dd, $J(4,5) = J(5,6) \approx 6.5$ Hz, 1H; 5b-H), 3.80–3.89 [m, 7H; HMQC: 3.81 (4a-H), 3.83 (1′-H), 3.84 (5e-H), 3.86 ($COOCH_3$), 3.87 (4c-H)], 3.99–4.06 [m, 4H; HMQC: 4.01 (6b-H, 6′b-H), 4.03 (6c-H), 4.03 (5f-H)], 4.08 (dd, $^2J = 12.9$, $J(8,9) = 3.4$ Hz, 1H; 9f-H), 4.16 (dd, $^2J = 11.8$, $J(8,9) = 5.2$ Hz, 1H; 6a-H), 4.21–4.39 [m, 5H; HMQC: 4.22 (3c-H), 4.22 (6e-H), 4.25 (9′f-H), 4.36 (1b-H), 4.37 (6′e-H)], 4.47 (d, $^2J = 10.6$, 1H; 6′a-H), 4.52 (dd, $J(2,3) = 10.1$,

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$J(3,4) = 3.3$ Hz, 1H; 3e-H), 4.64 (d, $J(1,2) = 7.9$ Hz, 1H; 1a-H), 4.77 (d, $J(1,2) = 8.2$ Hz, 1H; 1e-H), 4.80 (d, $^2J = 11.0$ Hz, 1H; 6′c-H), 4.86–5.51 [m, 17H; HMQC: 4.88 (1c-H), 4.88 (4f-H), 4.89 (2e-H), 4.95 (2d-H), 4.95 (4e-H), 4.97 (2b-H), 4.99 (5d-H), 5.12 (N_fH), 5.15 (2a-H), 5.19 (3d-H), 5.31 (4d-H), 5.32 (1d-H), 5.33 (4b-H), 5.35 (3a-H), 5.43 (7f-H), 5.47 (N_cH), 5.50 (8f-H)], 7.41–8.00 (m, 5H; C_6H_5); ^{13}C NMR (151 MHz, $CDCl_3$, excerpt): $\delta =$ 15.80 (6d-C), 37.61 (3f-C), 49.07 (5f-C), 53.15 (OCH_3), 58.4 (2c-C), 60.92 (6c-C), 61.37 (6e-C), 61.52 (6b-C), 61.66 (9f-C), 62.17 (6a-C), 64.10 (5d-C), 66.58 (7f-C), 67.35 (4e-C), 67.57 (8f-C), 67.95 (3d-C), 68.80 (2d-C), 69.01 (4b-C, 1′-C), 69.41 (4f-C), 69.87 (2e-C), 70.04 (3′-C), 70.49 (OCH_2), 70.93 (5e-C), 71.19 (5b-C), 71.23 (2b-C), 71.36 (3e-C), 71.51 (4d-C, OCH_2), 71.86, 71.91 (2a-C, 6f-C), 72.29 (3a-C), 72.4 (3c-C), 72.87 (5a-C), 73.11 (5c-C), 74.23 (4c-C), 75.66 (4a-C), 75.96 (3b-C), 77.31 (2′-C), 95.26 (1d-C), 96.82 (2f-C), 99.39 (1c-C), 99.88 (1e-C), 100.69 (1b-C), 101.11 (1a-C), 165.11 (COPh), 167.84 (1f-C); elemental analysis calcd (%) for $C_{118}H_{180}N_2O_{52}\cdot 2H_2O$ (2494.73): C 56.81, H 7.43, N 1.12; found: C 56.57 H 7.32, N 0.91.

Octasaccharide (19b): A solution of donor **16** (337 mg, 161 μmol) and lipid **17b**[17] (445 mg, 321 μmol) in dry dichloromethane (5 mL) with molecular sieves (AW-300) was treated at room temperature with trimethylsilyl trifluoromethanesulfonate (12 μL, 64 μmol). After being stirred for 3 h, the mixture was neutralized with triethylamine, filtrated and concentrated in vacuo. The residue was purified by flash chromatography (toluene/acetone 8:5 to 1:1) to give **19b** (254 mg, 47%) and unreacted **17b** (301 mg) as colourless foams. $R_f = 0.45$ (toluene/acetone 1:1, HPTLC); $[\alpha]_D = +2.0$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 0.88$ (t, 6H; $2CH_3$), 1.16 (d, 3H; 6f-CH_3), 1.25–1.30 (brs, 52H; $26CH_2$), 1.55 (q, 4H; $2OCH_2CH_2$), 1.65–1.69 (m, 1H; 3h-H_{ax}), 1.78–2.19 (m, 54H; $18COCH_3$), 2.58 (dd, 1H; 3h-H_{eq}), 3.03–3.16 [m, 5H; HMQC: 3.05, 3.08 (OCH_2CH_2), 3.15 (5e-H)], 3.19 (dd, $J(2',3') = 5.4$, $^2J = 10.3$ Hz, 1H; 3′-H), 3.26 (dd, $J(2',3') = 4.2$, $^2J = 10.3$ Hz, 1H; 3′-H), 3.31–3.50 [m, 6H; HMQC: 3.32 (OCH_2), 3.39 (2′-H), 3.43 (5e-H), 3.47 (1′-H), 3.48 (5b-H)], 3.59–3.72 [m, 8H; HMQC: 3.59 (6b-H), 3.63 (5a-H), 3.62 (5c-H), 3.63 (6 h-H), 3.68 (3b-H), 3.68 (3d-H), 3.70 (4c-H), 3.70 (5d-H)], 3.76 (dd, $J(1',2') = 4.8$, $^2J =$ 10.5 Hz, 1H; 1′-H), 3.82–4.44 [m, 22H; HMQC: 3.83 (5g-H), 3.85 (4e-H), 3.85 ($COOCH_3$), 3.93 (4b-H), 3.93 (6d-H), 3.97 (6′d-H), 4.01 (6e-H), 4.02 (5h-H), 4.04 (6c-H), 4.06 (4a-H), 4.07 (9h-H), 4.19 (6′b-H), 4.21 (3e-H), 4.21 (6g-H), 4.23 (9h-H), 4.30 (6a-H), 4.32 (1d-H), 4.37 (6′g-H), 4.39 (6′a-H), 4.42 (6′c-H)], 4.49 (d, $J(1,2) = 8.1$ Hz, 1H; 1b-H), 4.51 (dd, $J(2,3) = 10.1$, $J(3,4) = 3.5$ Hz, 1H; 3g-H), 4.64 (d, $J(1,2) = 8.0$ Hz, 2H; 1a-H, 1c-H), 4.75–5.36 [m, 19H; HMQC: 4.75 (1g-H), 4.78 (6′e-H), 4.88 (1e-H), 4.88 (2g-H), 4.88 (4h-H), 4.92 (2d-H), 4.94 (2f-H), 4.95 (4g-H), 4.99 (5f-H), 5.05 (N_hH), 5.07 (2c-H), 5.18 (3f-H), 5.19 (3c-H), 5.30 (4d-H), 5.30 (4f-H), 5.31 (1f-H), 5.33 (2b-H), 5.34 (2a-H), 5.35 (N_eH)], 5.43 (dd, $J(6,7) = 2.9$, $J(7,8) = 9.6$ Hz, 1H; 7h-H), 5.50 (ddd, $J(7,8) = 9.5$, $J(8,9) = J(8,9') \approx 3.3$ Hz, 1H; 8f-H), 5.62 (dd, $J(2,3) = J(3,4) = 9.5$ Hz, 1H; 3a-H), 7.08–7.97 (m, 30H; $6C_6H_5$); ^{13}C NMR (151 MHz, $CDCl_3$, excerpt): $\delta = 16.22$ (6f-C), 37.75 (3h-C), 49.45 (5h-C), 53.61 (OCH_3), 58.80 (2e-C), 61.25 (6e-C), 61.64 (6g-C), 61.79 (6d-C), 62.09 (6c-C), 62.09 (9h-C), 62.87 (6a-C), 63.16 (6b-C), 64.49 (5f-C), 66.91 (7h-C), 67.68 (4g-C), 67.89 (8h-C), 68.31 (4b-C), 69.20 (1′-C), 69.22 (2f-C), 69.33 (4f-C), 69.80 (4h-C), 70.45 (3′-C), 70.12 (2g-C), 70.68 (2b-C), 71.30 (5g-C), 71.43 (2d-C), 71.59 (5d-C), 71.68 (2c-C), 71.70 (3g-C), 71.86 (4d-C), 72.18 (2a-C), 72.25 (6h-C), 72.39 (3c-C), 72.67 (3e-C), 72.74 (5b-C), 72.97 (3a-C), 73.29 (5a-C), 73.29 (5c-C), 73.48 (5e-C), 74.54 (4e-C), 75.89 (4c-C), 75.78 (4a-C), 76.27 (3d-C), 77.56 (2′-C), 81.31 (3b-C), 95.61 (1f-C), 99.76 (1e-C), 100.21 (1g-C), 100.94 (1b-C), 101.04 (1d-C), 101.44 (1a-C, 1c-C); elemental analysis calcd (%) for $C_{165}H_{220}N_2O_{67}\cdot H_2O$ (3321.55): C 59.67, H 6.74, N 0.84; found: C 59.51 H 6.71, N 0.80.

Decasaccharide 19c: A solution of donor **16** (282 mg, 134 μmol) and lipid **17c**[17] (609 mg, 271 μmol) in dry dichloromethane (6 mL) with molecular sieves (AW-300) was treated at room temperature with trimethylsilyl trifluoromethanesulfonate (10 μL, 54 μmol). After being stirred for 3½ h, the mixture was neutralized with triethylamine, filtrated and concentrated in vacuo. The residue was purified by flash chromatography (toluene/acetone 3:1 to 3:2) to give **19c** (194 mg, 34%) and unreacted **17c** (380 mg) as colourless foams. $R_f = 0.46$ (toluene/acetone 1:1, HPTLC); $[\alpha]_D = +14.2$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 0.88$ (t, 6H; $2CH_3$), 1.17 (d, 3H; 6h-CH_3), 1.25–1.33 (m, 52H; $26CH_2$), 1.53–1.69 (m, 5H; $2OCH_2CH_2$, 3k-H_{ax}), 1.77–2.19 (m, 54H; $18COCH_3$), 2.58 (dd, 1H; 3k-H_{eq}), 3.02–4.52 [m, 54H; HMQC: 3.04, 3.08 (OCH_2), 3.15 (2g-H), 3.17 (3′-H), 3.25 (3′-H), 3.31 (OCH_2), 3.32 (5b-H), 3.37 (2′-H), 3.43 (5g-H), 3.46 (1′-H), 3.47 (6b-H), 3.52 (5d-H), 3.55 (3b-H), 3.58 (6d-H), 3.60 (5a-H), 3.60 (5c-H), 3.60 (5e-H), 3.63 (6k-H), 3.68 (3f-H), 3.69 (3d-H), 3.70 (4e-H), 3.71 (5f-H), 3.73 (1′-H), 3.78 (4b-H), 3.83 (5i-H), 3.85 (4g-H), 3.85 ($COOCH_3$), 3.92 (6′b-H), 3.92 (4c-H), 3.95 (4d-H), 3.96 (6f-H), 3.96 (6′f-H), 4.01 (6g-H), 4.03 (4a-H), 4.03 (5k-H), 4.05 (9k-H), 4.16 (6′d-H), 4.20 (3g-H), 4.23 (9k-H), 4.20–4.27 (6a-H, 6c-H, 6e-H, 6i-H), 4.31 (1f-H), 4.34–4.45 (6′a-H, 6′c-H, 6′e-H, 6′i-H), 4.41 (1b-H), 4.48 (1d-H), 4.51 (3i-H)], 4.61 (d, $J(1,2) = 7.7$ Hz, 2H; 1a-H, 1c-H), 4.64 (d, $J(1,2) = 7.6$ Hz, 1H; 1e-H), 4.68–5.51 [m, 24H; HMQC: 4.76 (1i-H), 4.78 (6′g-H), 4.87 (2i-H), 4.88 (1g-H), 4.88 (4k-H), 4.92 (2f-H), 4.94 (2h-H), 4.95 (4i-H), 5.00 (5h-H), 5.04 (N_kH), 5.07 (2e-H), 5.19 (3h-H), 5.20 (3e-H), 5.24 (2c-H), 5.30 (2b-H), 5.30 (2d-H), 5.30 (4f-H), 5.30 (4h-H), 5.31 (2a-H), 5.31 (1h-H), 5.37 (N_gH), 5.43 (7k-H), 5.48 (3c-H), 5.50 (8k-H)], 5.59 (dd, $J(2,3) = J(3,4) = 9.4$ Hz, 1H; 3a-H), 7.02–8.08 (m, 55H; $11C_6H_5$); ^{13}C NMR (151 MHz, $CDCl_3$, excerpt): $\delta = 15.8$ (6h-C), 37.4 (3k-C), 49.1 (5k-C), 53.1 (OCH_3), 58.4 (2g-C), 60.8 (6g-C), 61.4 (6f-C), 61.6 (9k-C), 62.7 (6b-C), 62.9 (6d-C), 64.1 (5h-C), 66.5 (7k-C), 67.3 (4i-C), 67.6 (8k-C), 67.8 (4b-C), 67.9 (3h-C), 67.9 (4d-C), 68.8 (1′-C), 68.8 (2h-C), 68.9 (4f-C), 69.3 (2i-C), 69.8 (4k-C), 70.1 (3′-C), 70.3 (2b-C), 70.3 (2d-C), 70.4 (OCH_2), 70.9 (5i-C), 71.1 (2f-C), 71.2 (5f-C), 71.3 (2e-C), 71.3 (3i-C), 71.4 (2c-C), 71.4 (OCH_2), 71.5 (4h-C), 71.6 (2a-C), 71.9 (3e-C), 71.9 (6k-C), 72.1 (3c-C), 72.2 (5b-C), 72.3 (3g-H), 72.4 (5d-C), 72.5 (3a-C), 73.0 (5a-C), 73.0 (5c-C), 73.0 (5e-C), 73.1 (5g-C), 74.2 (4g-C), 75.2 (4c-C), 75.3 (4a-C), 75.9 (4e-C), 75.9 (3f-C), 77.2 (2′-C), 80.9 (3b-C), 80.9 (3d-C), 95.2 (1h-C), 99.3 (1g-C), 99.8 (1i-C), 100.4 (1b-C), 100.4 (1d-C), 100.7 (1f-C), 101.0 (1e-C), 101.1 (1a-C), 101.1 (1c-C); elemental analysis calcd (%) for $C_{212}H_{260}N_2O_{82}\cdot 3H_2O$ (4202.40): C 60.59, H 6.38, N 0.67; found: C 60.52 H 6.29, N 0.74.

Neoglycolipid 1a: Compound **19a** (249 mg, 97.7 μmol) was dissolved in dry methanol (50 mL), and sodium methoxide (126 mg, 3.3 mmol) was added. After being stirred for 3 d at room temperature, the solution was neutralized with Amberlite IR120 (H^+), filtered and evaporated. The residue was dissolved in dioxane/water (1:1, 30 mL), and an aqueous solution of potassium hydroxide (0.2 M, 1 mL) was added. After overnight stirring, carbon dioxide was added, and the solution was evaporated in vacuo. Flash chromatography ($CHCl_3$/methanol/water/triethylamine 70:30:5:1) yielded **1a** (153 mg, 83%) as a colourless powder after lyophilization from water. $R_f = 0.23$ ($CHCl_3$/methanol/0.2% aqueous $CaCl_2$ 65:35:8, HPTLC); ^{1}H NMR (600 MHz, [D_{25}]SDS/D_2O): $\delta = 0.79$ (m, 6H; $2CH_3$), 1.16 (d, $J(5,6) = 6.4$ Hz, 3H; 6d-CH_3), 1.21–1.31 (m, 61H; $26CH_2$, $N(CH_2CH_3)_3$), 1.57 (brs, 4H; $2OCH_2CH_2$), 1.79 (t, 1H; 3f-H_{ax}), 2.02, 2.03 (2s, 6H; $2COCH_3$), 2.75 (dd, 1H; 3f-H_{eq}), 3.20 (q, 6H; $N(CH_2Me)_3$), 3.34 (dd, $J(1,2) = J(2,3) = 8.4$ Hz, 1H; 2a-H), 3.48–4.14 (m, 42H; 3a-H, 4a-H, 5a-H, 2 6a-H, 2b-H, 3b-H, 4b-H, 5b-H, 2 6b-H, 2c-H, 3c-H, 4c-H, 5c-H, 2 6c-H, 2d-H, 3d-H, 4d-H, 2e-H, 3e-H, 4e-H, 5e-H, 2 6e-H, 4f-H, 5f-H, 6f-H, 7f-H, 8f-H, 2 9f-H, 5H glycerol, $2O(CH_2)(CH_2)_{14}$), 4.43 (d, $J(1,2) = 7.8$ Hz, 1H; 1a-H), 4.45 (d, $J(1,2) = 7.8$ Hz, 1H; 1b-H), 4.51 (d, $J(1,2) = 7.8$ Hz, 1H; 1e-H), 4.71 (1c-H (in HDO-signal)), 4.82 (q, $J(5,6) = 6.9$ Hz, 1H; 5d-H), 5.12 (d, $J(1,2) = 3.9$ Hz, 1H; 1d-H); FAB-MS (positive Mode, NBA/glycerol/MeOH 1:1:1 + NaI): 1690 [$M^+ - NEt_3$+Na], 1712 [$M^+ -$ $HNEt_3$+2Na]; $C_{84}H_{157}N_3O_{35}$ (1769.16).8888

Neoglycolipid 1b: Compound **19b** (72 mg, 21.6 mmol) was treated as described above to furnish **1b** (39 mg, 86%) as a colourless powder after flash chromatography ($CHCl_3$/methanol/water/triethylamine 70:30:4:1 → 65:35:8:1). $R_f = 0.18$ ($CHCl_3$/methanol/0.2% aqueous $CaCl_2$ 65:35:8, HPTLC); ^{1}H NMR (600 MHz, [D_{25}]SDS/D_2O): $\delta = 0.82$ (m, 6H; $2CH_3$), 1.17 (d, $J(5,6) = 6.4$ Hz, 3H; 6d-CH_3), 1.22–1.32 (m, 61H; $26CH_2$, $N(CH_2CH_3)_3$), 1.58 (brs, 4H; $2OCH_2CH_2$), 1.80 (t, 1H; 3h-H_a), 2.03 (s, 6H; $2COCH_3$), 2.76 (d, 1H; 3h-H_e), 3.18 (q, 6H; $N(CH_2Me)_3$), 3.34–4.21 (m, 56H; 2a-H, 3a-H, 4a-H, 5a-H, 2 6a-H, 2b-H, 3b-H, 4b-H, 5b-H, 2 6b-H, 2c-H, 3c-H, 4c-H, 5c-H, 2 6c-H, 2d-H, 3d-H, 4d-H, 5d-H, 2 6d-H, 2e-H, 3e-H, 4e-H, 5e-H, 2 6e-H, 2f-H, 3f-H, 4f-H, 2g-H, 3g-H, 4g-H, 5g-H, 2 6g-H, 3h-H, 4h-H, 5h-H, 6h-H, 7h-H, 8h-H, 2 9h-H, 5H glycerol, $2O(CH_2)(CH_2)_{14}$), 4.44 (d, 2H; 1a-H, 1d-H), 4.54 (m, 2H; 1b-H, 1f-H), 4.73 (brs, 2H; 1c-H, 1e-H (in HDO-signal)), 4.82 (q, 1H; 5f-H), 5.13 (brs, 1H; 1f-H); FAB-MS (positive mode, NBA/glycerol/MeOH 1:1:1 + NaI): 1993 [$M^+ - NEt_3$+H], 2014 [$M^+ - NEt_3$+Na]; $C_{96}H_{177}N_3O_{45}$ (2093.45).

Neoglycolipid 1c: Compound **19c** (151 mg, 35.9 mmol) was treated as described above to furnish **1c** (80 mg, 92%) as a colourless powder after flash chromatography ($CHCl_3$/methanol/water/triethylamine 70:30:4:1 → 65:35:8:1). $R_f = 0.13$ ($CHCl_3$/methanol/0.2% aqueous $CaCl_2$ 65:35:8, HPTLC); ^{1}H NMR (600 MHz, [D_{25}]SDS/D_2O, excerpt): $\delta = 0.75$ (m, 6H; $2CH_3$), 1.12 (d, $J(5,6) = 6.4$ Hz, 3H; 6d-CH_3), 1.17–1.27 (m, 61H; $26CH_2$, $N(CH_2CH_3)_3$), 1.53 (brs, 4H; $2OCH_2CH_2$), 1.75 (t, 1H; 3j-H_a), 1.97, 1.98 (2

s, 6H; 2 $COCH_3$), 2.72 (dd, 1H; 3j-H_e), 3.16 (q, 6H; $N(CH_2Me)_3$), 3.25–4.15 (m, 68H; 2a-H, 3a-H, 4a-H, 5a-H, 2 6a-H, 2b-H, 3b-H, 4b-H, 5b-H, 2 6b-H, 2c-H, 3c-H, 4c-H, 5c-H, 2 6c-H, 2d-H, 3d-H, 4d-H, 5d-H, 2 6d-H, 2e-H, 3e-H, 4e-H, 5e-H, 2 6e-H, 2f-H, 3f-H, 4f-H, 5f-H, 2 6f-H, 2g-H, 3g-H, 4g-H, 5g-H, 2 6g-H, 2h-H, 3h-H, 4h-H, 2i-H, 3i-H, 4i-H, 5i-H, 2 6i-H, 3j-H, 4j-H, 5j-H, 6j-H, 7j-H, 8j-H, 2 9j-H, 5H glycerol, 2 $O(CH_2)(CH_2)_{14}$), 4.39 (d, 2H; 1a-H, 1f-H), 4.45–4.49 (m, 1H; 1b-H, 1d-H, 1i-H), 4.68 (br s, 3H; 1c-H, 1e-H, 1g-H (in HDO-signal)), 4.77 (q, 1H; 5h-H), 5.12 (d, 1H; 1h-H); MALDI-MS (negative mode, 4-nitroaniline, MeOH): 2314 [M^- – $HNEt_3$], 2412 [M^- – H]; $C_{108}H_{197}N_3O_{55}$ (2417.73).

Benzyl *O*-(2,3,4,6-Tetra-*O*-acetyl-*β*-D-galactopyranosyl)-(1→4)-[(3,4-di-*O*-acetyl-2-*O*-benzyl-*α*-L-fucopyranosyl)-(1→3)]-(2-acetamido-6-*O*-benzyl-2-deoxy-*β*-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-*β*-D-galactopyranosyl)-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-*β*-D-glucopyranoside (21): A solution of acceptor **10** (1.89 g, 1.09 mmol) and donor **20**[18] (1.07 g, 2.18 mmol) in dry dichloromethane (30 mL) with molecular sieves AW-300 was treated at room temperature with borontrifluoride diethyl etherate (27 μL, 0.2 equiv). After being stirred for 1 h, the mixture was neutralized with triethylamine, filtrated and concentrated in vacuo. The residue was purified by flash chromatography (petroleum ether/ethyl acetate 2:1→3:2→1:1) to afford an impure derivative, which was dissolved in tetrahydrofuran/acetic anhydride/acetic acid (6:2:1, 27 mL) and treated with activated zinc powder (1.2 g). The mixture was stirred for 4 h at room temperature with sonication (5 × 30 min) and then filtered and washed with diethyl ether (400 mL). After careful addition of a saturated sodium bisulfate solution, the mixture was stirred vigorously for 1 h. The organic layer was separated, dried over sodium sulfate, and evaporated under reduced pressure. Purification by flash chromatography (petroleum ether/ethyl acetate 1:1→4:5) gave **21** (1.82 g, 86%) as a colourless foam. R_f = 0.30 (petroleum ether/ethyl acetate 1:1); $[\alpha]_D = -39.5$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$, main conformer): $\delta = 1.14$ (d, $J(5,6) = 6.4$ Hz, 3H; 6d-CH_3), 1.39, 1.90, 1.97, 1.98, 2.01, 2.08, 2.15 (7 s, 21H; 7 $COCH_3$), 3.30–3.80 [m, 14H; HMQC: 3.30 (5a-H), 3.36 (6b-H), 3.42 (5b-H), 3.44 (6′b-H), 3.45 (2c-H), 3.47 (5c-H), 3.55 (3b-H), 3.56 (5e-H), 3.65 (3a-H), 3.66 (6a-H), 3.68 (6c-H), 3.70 (2b-H), 3.75 (6′a-H), 3.78 (6′c-H)], 3.84 (dd, $J(1,2) = 3.6$, $J(2,3) = 10.5$ Hz, 1H; 2d-H), 3.99 (d, $J(3,4) = 2.3$ Hz, 1H; 4b-H), 4.03–5.11 [m, 29H; HMQC: 4.06 (4c-H), 4.07 (4a-H), 4.23 (6e-H), 4.28 (3c-H), 4.30 (6′e-H), 4.42 (1b-H), 4.48 (1a-H), 4.59 (1e-H), 4.78 (5d-H), 4.81 (3e-H), 5.01 (2e-H), 5.09 (1c-H), 5.10 (1d-H), 8 CH_2Ph], 5.18 (dd, $J(2,3) = 10.6$, $J(3,4) = 3.2$ Hz, 1H; 3d-H), 5.27–5.31 [m, 3H; HMQC: 5.28 (4d-H), 5.29 (2a-H), 5.31 (4e-H)], 5.34 (d, $J(2,N) = 7.7$ Hz, 1H; NH), 6.95–7.97 (m, 40H; 8 C_6H_5); ^{13}C NMR (151 MHz, $CDCl_3$, excerpt): $\delta = 15.83$ (6d-C), 58.58 (2c-C), 60.77 (6e-C), 64.28 (5d-C), 66.86 (4e-C), 68.06, 68.15 (6a-C, 6c-C), 68.38 (6b-C), 69.19 (2e-C), 70.27 (3d-C), 70.87 (3e-C, 5e-C), 71.98 (4d-C), 73.23 (2a-C), 73.30 (5b-C), 73.36 (2d-C), 73.79 (4c-C), 74.77 (5c-C), 75.51 (5a-C), 76.27 (4b-C), 76.39 (4a-C), 79.53 (2b-C), 80.47 (3a-C), 82.34 (3b-C), 96.83 (1d-C), 99.42 (1a-C), 99.52 (1e-C), 100.93 (1c-C), 102.67 (1b-C); elemental analysis calcd (%) for $C_{107}H_{119}NO_{32}$ (1931.10): C 66.55 H 6.21 N 0.73; found: C 66.26 H 6.20 N 0.59.

Acetyl *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-*α*/*β*-D-gluco-pyranoside (22): Compound **20** (1.82 g, 942 μmol) was treated as described above for compound **14** to furnish **22** (1.20 g, 83%) as a colourless foam after flash chromatography (toluene/acetone 5:2→2:1+1% methanol). R_f = 0.55 (toluene/acetone 1:1); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 1.21$ (d, $J(5,6) = 6.4$ Hz, 3H; 6d-CH_3), 1.94–2.20 (m, 45H; 15 $COCH_3$), 3.11 (m, 1H; 2c-H), 3.38–5.69 (m, 3H; 4 1-H, 4 2-H, 5 3-H, 5 4-H, 5 5-H, 8 6-H, CO_2CH_3, NH), 5.82 (d, $J(1,2) = 8.0$ Hz, ½H; 1a-H_β), 6.42 (d, $J(1,2) = 3.6$ Hz, ½H; 1a-H_α), 7.41–7.99 (m, 5H; C_6H_5); elemental analysis calcd (%) for $C_{67}H_{87}NO_{40}$ (1546.41): C 52.04, H 5.67, N 0.91; found: C 52.25 H 5.94, N 1.16.

***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-*α*/*β*-D-glucopyranose (23)**: A solution of compound **22** (1.20 g, 780 μmol) in dry tetrahydrofuran (10 mL) was treated with piperidinium acetate (1.1 g) and then stirred for 4 h at 50 °C. After evaporation of the solvent, the residue was purified by flash chromatography (toluene/acetone 9:7) to give **23** (1.15 g, 97%) as a colourless foam. R_f = 0.38 (toluene/acetone 1:1); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.19$ (d, $J(5,6) = 6.2$ Hz, 3H; 6d-CH_3), 1.94–2.18 (m, 42H; 14 $COCH_3$), 3.24–5.74 (m, 37H; 5 1-H, 5 2-H, 5 3-H, 5 4-H, 5 5-H, 8 6-H, CO_2CH_3, NH), 7.42–8.02 (m, 5H; C_6H_5); elemental analysis calcd (%) for $C_{65}H_{85}NO_{39} \cdot H_2O$ (1522.39): C 51.28, H 5.76, N 0.92; found: C 51.25 H 5.80, N 0.85.

***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-*α*/*β*-D-glucopyranosyl Trichloracetimidate (24)**: Trichloroacetonitrile (760 μL, 7.6 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (2 drops) were added to a solution of **23** (1.15 g, 756 μmol) in dry dichloromethane (30 mL). After 2 h, the mixture was concentrated in vacuo. Flash chromatography (toluene/acetone 3:2+1% triethylamine) furnished **24** (1.22 g, 95%) in a α/β ratio of > 19:1 as a colourless foam. R_f = 0.20 (toluene/acetone 2:1); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 1.20$ (d, $J(5,6) = 6.6$ Hz, 3H; 6d-CH_3), 1.58–2.19 (m, 42H; 14 $COCH_3$), 3.06 (m, 1H; 2c-H), 3.42–5.77 (m, 35H; 4 1-H, 4 2-H, 5 3-H, 5 4-H, 5 5-H, 8 6-H, CO_2CH_3, NH), 6.63 (d, $J(1,2) = 3.7$ Hz, 1H; 1a-H), 7.27–7.98 (m, 5H; C_6H_5), 8.56 (s, 1H; =NH); elemental analysis calcd (%) for $C_{67}H_{85}N_2Cl_3O_{39} \cdot 2H_2O$ (1684.79): C 47.76, H 5.32, N 1.66; found: C 47.83 H 5.29, N 1.65.

(1,2-Di-*O*-hexadecyl-*sn*-3-glyceryl) *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-glucopyranoside (25): A solution of donor **24** (154 mg, 91.4 μmol) and lipid **17a**[6] (101 mg, 187 μmol) in dry dichloromethane (8 mL) with molecular sieves (AW-300) was treated at room temperature with $BF_3 \cdot Et_2O$ (4.8 μL, 36 μmol). After being stirred for 90 min, the mixture was neutralized with triethylamine, filtrated and concentrated in vacuo. The residue was purified by flash chromatography (toluene/acetone 3:1) to give **25** (125 mg, 66%) as a colourless foam. R_f = 0.44 (toluene/acetone 2:1); $[\alpha]_D = -19.5$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 0.88$ (t, 6H; 2 CH_3), 1.20–1.31 (m, 55H; 26 CH_2, 6d-CH_3), 1.36–1.43 (m, 4H; 2 OCH_2CH_2), 1.93, 1.94, 1.97, 1.98, 2.00, 2.01, 2.04, 2.08, 2.08, 2.10, 2.11, 2.14, 2.16, 2.19 (14 s, 42H; 14 $COCH_3$), 3.05–3.17 [m, 3H; HMQC: 3.04 (2c-H), 3.10, 3.14 (OCH_2)], 3.23, 3.31 (2 dd, $J(2',3') = 5.4$, $^2J = 10.3$ Hz, 2H; 2 3′-H), 3.38–3.46 [m, 4H; HMQC: 3.40 (OCH_2), 3.42 (5c-H), 3.44 (2′-H)], 3.57 (dd, $J(1',2') = 5.9$, $^2J = 10.5$ Hz, 1H; 1′-H), 3.65 (m, 1H; 5a-H), 3.71 (dd, $J(2,3) = 9.9$, $J(3,4) = 3.6$ Hz, 1H; 3b-H), 3.76–3.89 [m, 5H; HMQC: 3.77 (5b-H), 3.79 (4c-H), 3.82 (4a-H), 3.84 (1′-H), 3.87 (5e-H)], 3.93 (dd, $J(5,6) \approx 1.9$, $^2J \approx 12.0$ Hz, 1H; 6c-H), 4.02, 4.06 (2 dd, 2H; 6b-H, 6′b-H), 4.16 (dd, $J(5,6) = 5.2$, $^2J = 11.8$ Hz, 1H; 6a-H), 4.28–4.34 [m, 2H; HMQC: 4.29 (3c-H), 4.32 (6e-H)], 4.36 (d, $J(1,2) = 7.9$ Hz, 1H; 1b-H), 4.46 (d, $^2J = 10.4$ Hz, 1H; 6′a-H), 4.52 (dd, $J(5,6) = 5.7$, $^2J = 11.4$ Hz, 1H; 6′e-H), 4.61 (d, $J(1,2) = 8.1$ Hz, 1H; 1e-H), 4.65 (d, $J(1,2) = 7.9$ Hz, 1H; 1a-H), 4.94–4.97 [m, 5H; HMQC: 4.94 (5d-H), 4.95 (2b-H), 4.95 (1c-H), 4.95 (2d-H), 4.96 (6′c-H)], 5.01 (dd, $J(2,3) = 10.4$, $J(3,4) = 3.4$ Hz, 1H; 3e-H), 5.09 (dd, 1H; 2e-H), 5.14–5.19 [m, 2H; HMQC: 5.15 (2a-H), 5.18 (3d-H)], 5.30 (d, $J(3,4) = 3.2$ Hz, 1H; 4b-H), 5.32–5.37 [m, 4H; HMQC: 5.32 (1d-H), 5.34 (NH), 5.35 (3a-H), 5.36 (4d-H)], 5.41 (d, $J(3,4) = 3.1$ Hz, 1H; 4e-H), 7.41–8.00 (m, 5H; C_6H_5); ^{13}C NMR (151 MHz, $CDCl_3$, excerpt): $\delta = 16.14$ (6d-C), 59.19 (2c-C), 60.11 (6c-C), 60.86 (6e-C), 61.87 (6b-C), 62.58 (6a-C), 64.45 (5d-C), 67.05 (4e-C), 68.35 (3d-C), 69.20 (2d-C), 69.41 (2e-C), 69.42 (1′-C), 69.52 (4b-C), 70.45 (3′-C), 70.87 (OCH_2), 71.33 (3e-C), 71.42 (5e-C), 71.49 (2b-C), 71.54 (5b-C), 71.78 (4d-C), 71.91 (OCH_2), 72.27 (2a-C), 72.34 (3c-C), 72.67 (3a-C), 73.24 (5a-C), 73.42 (5c-C), 74.75 (4c-C), 76.06 (4a-C), 76.34 (3b-C), 77.74 (2′-C), 95.65 (1d-C), 99.47 (1c-C), 100.93 (1e-C), 101.07 (1b-C), 101.54 (1a-C); elemental analysis calcd (%) for $C_{100}H_{155}NO_{41} \cdot 2H_2O$ (2063.34): C 58.21, H 7.77, N 0.68; found: C 58.19 H 7.57, N 0.84.

(1,2-Di-*O*-hexadecyl-*sn*-3-glyceryl) *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-glucopyranoside (26): Compound **25** (106 mg, 51.3 μmol) was dissolved in dry methanol/dichloromethane (2:1, 30 mL), and sodium methoxide (54 mg, 1 mmol) was added. After being stirred overnight at room temperature, the solution was neutralized with Amberlite IR120 (H^+), filtered and evaporated at low temperature to avoid premature foaming. Flash chromatography ($CHCl_3$/methanol/water 70:30:4) yielded **26** (64.0 mg, 89%) as a colourless powder after lyophilization from water/dioxane. R_f = 0.29 ($CHCl_3$/methanol/water 65:35:8, HPTLC); $[\alpha]_D = -29.7$ ($c = 1.0$ in $CHCl_3$/MeOH 3:1); ^{1}H NMR (600 MHz, [D_{25}]SDS/D_2O): $\delta = 0.76$ (br s, 6H; 2 CH_3), 1.12 (d, $J(5,6) =$

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6.4 Hz, 3H; 6d-CH_3), 1.17–1.27 (m, 52H; 26CH_2), 1.53 (br s, 4H; 2OCH_2CH_2), 2.00 (s, 3H; $COCH_3$), 3.30 (dd, $J(1,2) = J(2,3) \approx 8.3$ Hz, 1H; 2a-H), 3.44–4.09 (m, 35H; 3a-H, 4a-H, 5a-H, 26a-H, 2b-H, 3b-H, 4b-H, 5b-H, 26b-H, 2c-H, 3c-H, 4c-H, 5c-H, 26c-H, 2d-H, 3d-H, 4d-H, 2e-H, 3e-H, 4e-H, 5e-H, 26e-H, 5H glycerol, 2OCH_2CH_2), 4.37–4.42 (m, 3H; 1a-H, 1b-H, 1e-H), 4.69 (d, $J(1,2) = 8.3$ Hz, 1H; 1c-H), 4.76 (q, $J(5,6) \approx 6.6$ Hz, 1H; 5d-H), 5.09 (d, $J(1,2) = 3.3$ Hz, 1H; 1d-H); MALDI-MS (positive mode, DHB, $CHCl_3$/methanol): 1398.7 [M^++Na], 1415.2 [M^++K]; $C_{67}H_{125}NO_{27}$ (1376.72).

Acknowledgement

This work was supported by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie. We are grateful to Anke Friemel (University Konstanz) for her help in the structural assignments by NMR experiments.

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Received: November 2, 2001 [F3651]

  0947-6539/02/0811-2463 $ 20.00+.50/0