Synthesis of a Structurally Defined Antigen – Immunostimulant Combination for Use in Cancer Vaccines

Wulf Dullenkopf,[a] Gerd Ritter,[b] Sheila R. Fortunato,[b] Lloyd J. Old,[b] and Richard R. Schmidt[a]

Abstract: Ganglioside GM_2 expressed on the cell surface of human cancers is a promising target for immunotherapy because GM_2 antibodies are cytotoxic in vitro and GM_2 antibody formation can be induced upon vaccination in cancer patients. We recently reported on the efficient chemical synthesis of GM_2; clinical trials with these synthetic GM_2 conjugated to a purified carrier protein (KLH) are currently under way. In our efforts to generate a totally synthetic GM_2 cancer vaccine, we have now synthesized GM_2 neoglycolipid **1**, which consists of the GM_2-tetrasaccharide epitope that is linked through a spacer to the B-cell stimulatory glycolipid **4**. Target compound **1** was constructed from the GM_2 tetrasaccharide donor **2**, the 9-hydroxynonanoate **3** spacer, and the 6-amino-6-deoxy derivative (**5**) of compound **4**. Building block **5** was obtained from Z-protected 6-azido-6-deoxy-*N*-leucyl-glucosamine derivative **12**, which was available from glucosamine by two different approaches; the route with the Z-protected derivative of **4** (**10**) as intermediate gave the best yields. The neoglycolipid **1** reacted with a number of different GM_2-reactive antibodies. Vaccination of rabbits with **1** resulted in induction of antibodies against GM_2, thus confirming the viability of this novel concept for the construction of a totally synthetic vaccine.

Keywords: antigens · gangliosides · glycolipids · immunostimulants · synthesis

Introduction

Ganglioside GM_2 has been considered an attractive target for vaccine-based therapy of GM_2-expressing cancers,[1] because a) GM_2 is expressed in a large number of different cancer types including melanoma, glioma, seminoma, lung cancer, colon cancer, renal cancer, and prostate cancer;[2] b) GM_2-reactive antibodies are cytotoxic for GM_2^+ tumor cells;[3, 4] c) GM_2 is immunogenic in humans as indicated by the presence of naturally occurring serum antibodies to GM_2,[4] the relative ease of isolating GM_2 monoclonal antibodies from humans,[5, 6] and the induction of GM_2 antibodies in melanoma patients following vaccination with GM_2-containing vaccines;[6, 8] d) melanoma patients with GM_2 antibodies, either induced by vaccination or naturally occurring, appear to have more favorable prognosis;[4] and e) no deleterious effects are associated with an immune response to GM_2.[4]

We have recently developed a practical chemical synthesis of ample quantities of the building blocks that consist of GM_2-oligosaccharides and GM_2 itself for use in cancer vaccine studies.[9] A first clinical vaccine trial in melanoma patients with synthetic GM_2 conjugated to the biological carrier protein keyhole limpet hemocyanin (KLH) has been started. In our efforts to develop a totally synthetic GM_2 cancer vaccine, we have designed a novel conjugate molecule in which a carbohydrate cancer epitope is linked through a spacer to an immunostimulant as shown in Scheme 1. We prepared neoglycolipid **1** by ligating GM_2-tetrasaccharide and the artifical B-cell stimulatory glycolipid BAY R 1005 (Scheme 1, **4**) through the ω-hydroxynonanoate spacer. BAY R 1005 has been reported to amplify the proliferation of B lymphocytes in response to stimulation with antigens.[10, 11] We report here on the synthesis of **1** and on its serological characterization.

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Results and Discussion

Synthesis of target molecule 1: The retrosynthesis of target molecule **1** (shown in Scheme 1) disintegrates the molecule into known GM_2-tetrasaccharide donor **2**,[9] the spacer **3**,[12] and the 6-amino-6-deoxy derivative **5** of BAY R 1005 (**4**); the 6-amino group in **5** is introduced in order to provide a

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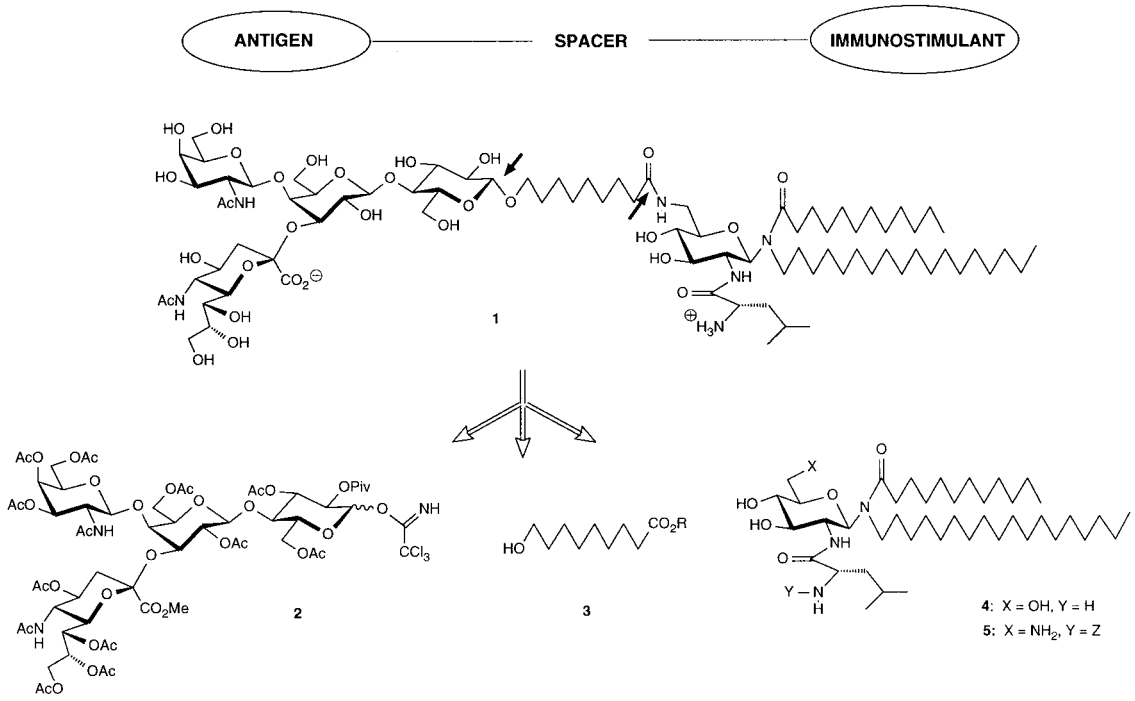
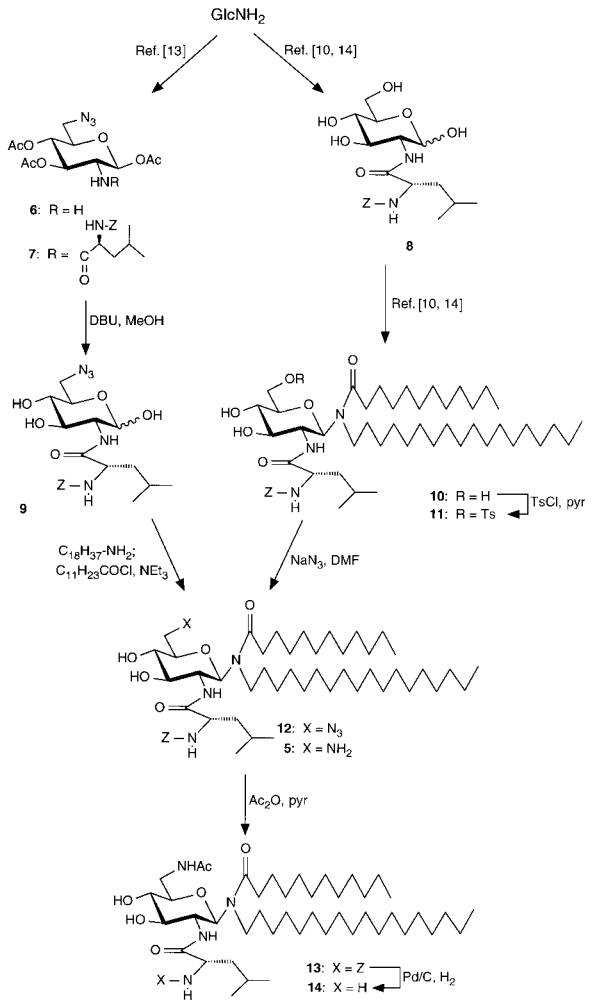


Scheme 1. Retrosynthesis of neoglycolipid **1**.

convenient linkage between the spacer and the adjuvant. For the synthesis of **5**, glucosamine was transformed into the 6-azido derivative **6** by the use of previously published procedures (Scheme 2).[13] Attachment of the *N*-benzyloxycarbonyl (Z)-protected leucyl residue to the 2-amino group was performed with commercially available Z-Leu-OH in the presence of water-soluble carbodiimide (WSC) *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride as condensing agent, this leads to *N*-leucyl-glucosamine derivative **7** in good yield. Removal of all *O*-acetyl groups was carried out in methanol in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as base; after addition of acetic acid the *O*-deprotected compound **9** was isolated. Treatment of **9** with

Abstract in German: *Gangliosid GM_2, das auf menschlichen Krebszellen exprimiert wird, stellt ein interessantes Ziel für die Immunotherapie dar, weil Antikörper gegen GM_2 cytotoxisch sind und somit die Antikörperproduktion durch Impfung von Krebspatienten induziert werden kann. Klinische Studien mit synthetischem GM_2, über dessen effiziente chemische Synthese wir kürzlich berichtet haben, das an ein Carrierprotein (KLH) konjugiert wurde, wurden bereits aufgenommen. In unserem Bemühen um einen totalsynthetischen GM_2-Krebsimpfstoff haben wir jetzt das GM_2-Neoglycolipid **1** aus einem GM_2-Epitop bestehend hergestellt, das über einen Spacer an das B-Zellen stimulierende Glycolipid **4** gebunden ist. Das Neoglycolipid **1** reagiert mit verschiedenen GM_2-reaktiven Antikörpern. Die Immunisierung von Kaninchen mit **1** führte zur Induktion von Antikörpern gegen GM_2. Auf diese Weise konnte der Erfolg dieses neuen Konzeptes zur Darstellung von totalsynthetischen Impfstoffen bestätigt werden.*



Scheme 2. Synthesis of building block **14**.

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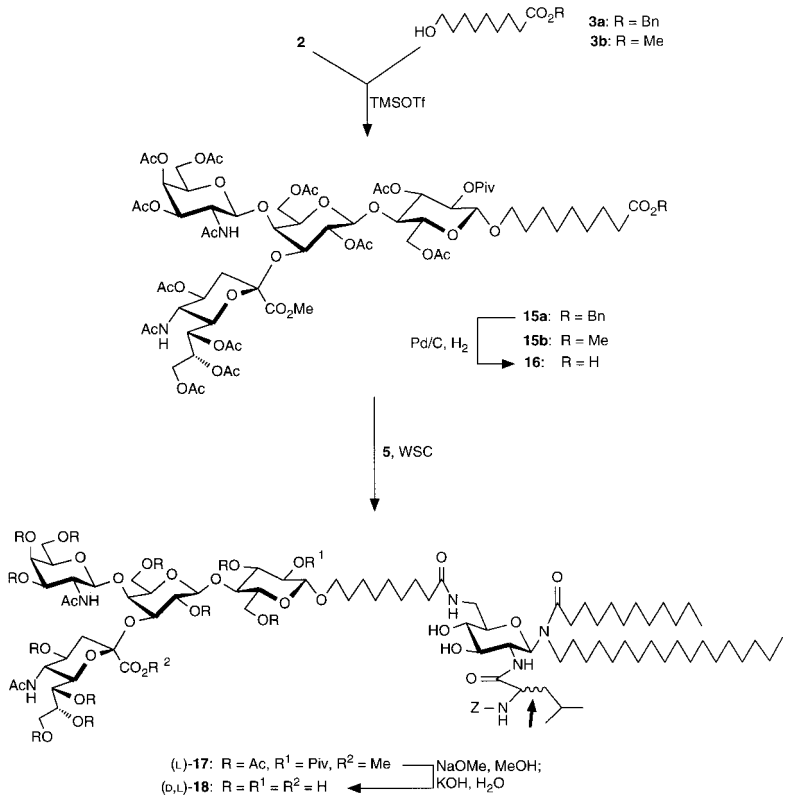
octadecylamine and then with lauryl chloride in the presence of triethylamine afforded the desired neoglycolipid **12**, but only in 37% yield. Therefore, an alternative approach for the synthesis of **10** was investigated. To this end, by the use of literature procedures glucosamine was first transformed into Z-protected *N*-leucyl-glucosamine **8**, which was then converted into Z-protected *N*-leucyl BAY R 1005 (**10**).[10, 14] Regioselective 6-*O*-tosylation of **10** could be carried out with tosyl chloride in pyridine at $-10\,^{\circ}C$ in good yield (→**11**); ensuing treatment with sodium azide in DMF afforded the desired neoglycolipid **12** in very good overall yield. Reduction of the azido group in **12** to the amino group with propanedithiol in pyridine/water[15] afforded building block **5**. For future adjuvant activity studies, **5** was transformed into the deprotected *N*-acetyl derivative **14**; *N*-acetylation of **5** with acetic anhydride provided 6-acetylamino derivative **13** and ensuing hydrogenolysis of the Z group afforded **14**. For both compounds, ^{1}H NMR indicated the presence of two rotamers at room temperature.

For the ligation of building blocks **2**, **3**, and **5**, tetraglycosyl donor **2**[9, 16] was first coupled to the benzyl ester of ω-hydroxynonanoate (**3a**) in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) as catalyst; thus, spacer-linked GM_2 derivative **15a** was obtained in good yield (Scheme 3).

Hydrogenolysis of the carboxylate benzyl group afforded acid **16**, which was fully *O*-acyl protected. Reaction with **5** in the presence of WSC as condensing agent afforded the protected target molecule (L)-**17**, which could be fully assigned by the ^{1}H NMR data. However, removal of the *O*-acyl groups under Zemplén conditions[17] and ensuing hydrolysis of the methylester moiety led to racemisation of the leucyl residue to give (D,L)-**18** as a mixture of diastereomers. Therefore, *O*-deacylation and ester hydrolysis in the spacer-linked GM_2-tetrasaccharide moiety had to be performed prior to attachment of **5**; this leads to the generation of an intermediate, in which for the condensation with the amino group of **5** two different carboxylate groups, the spacer and the neuraminic acid carboxylate group, are available. However, we anticipated that the spacer carboxylate group would be more reactive than the neuraminic acid carboxylate group because of steric hinderance and because of the negative inductive effect of the α-oxygen in the neuraminic acid residue. Reaction of **2** with the methylester of ω-hydroxynonanoate (**3b**) in the presence of TMSOTf as catalyst gave spacer-linked GM_2 derivative **15b** in good yield (Scheme 3). Removal of the *O*-acyl groups under Zemplén conditions and subsequent hydrolysis of the two methylester residues led to totally deprotected **19** as a dipotassium salt (Scheme 4). Condensation of **19** with **5** in the presence of 1-ethyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) as condensing agent gave, as expected, only (L)-**18**, which carried only one Z-protective group at the leucyl residue. Hydrogenolysis in dioxane/water with palladium on carbon as catalyst and then purification of the product in the presence of triethylamine afforded target molecule **1** as its triethylammonium salt. The structural assignments of **1** and of the intermediates were readily performed with NMR spectrocopical data.

Serological characterisation of 1: GM_2 neoglycolipid **1** was tested for its reactivity with several GM_2-reactive antibodies, including mAb 45.66 (human IgM), KM966 (mouse-human chimeric IgG), a polyclonal rabbit immune serum against GM_2, and serum from a melanoma patient immunized with bovine-brain-derived GM_2-KLH/QS21 vaccine, by ELISA (enzyme-linked immunosorbent assay) and immune thin-layer chromatography (ITLC). GM_2 neoglycolipid **1** showed good reactivity with all four GM_2 antibodies in both assay systems. As an example, ELISA reactivity of neoglycolipid **1** with mAb 45.66 and the patient's immune serum is shown in Figure 1. The reactivity with GM_2 neoglycolipid **1** was weaker than with GM_2 ganglioside (Figure 2C, page 2436). In order to test whether the GM_2 neoglycolipid **1** can elicit antibodies against GM_2 ganglioside, rabbits were immunized with GM_2 neoglycolipid **1** in complete Freund's adjuvant (CFA). Immunization resulted



Scheme 3. Synthesis of precursor **15b**; epimerisation upon saponification.

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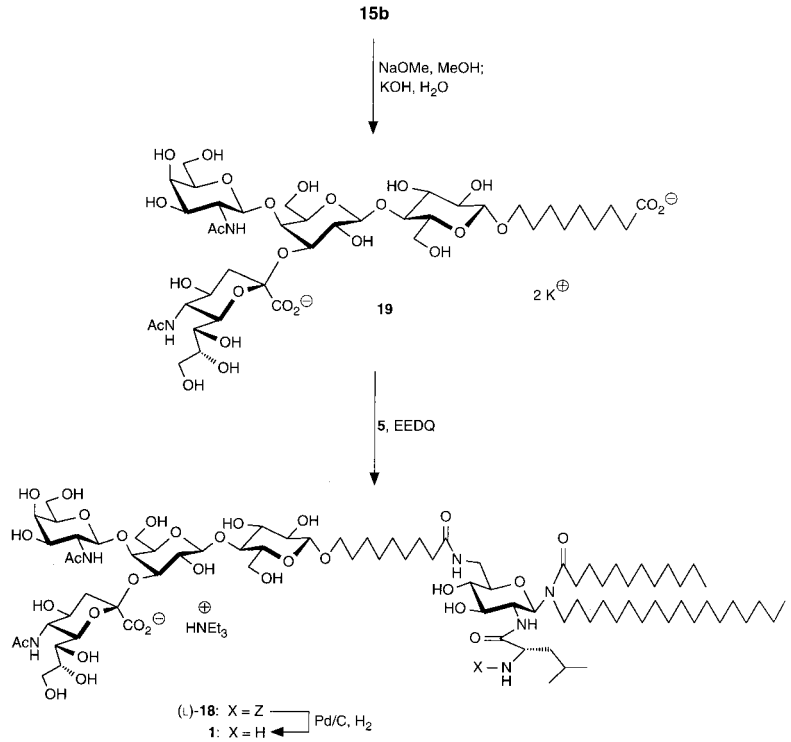

Scheme 4. Synthesis of neoglycolipid **1**.

in induction of IgG antibodies against GM_2 neoglycolipid **1** and synthetic GM_2 (sGM_2) ganglioside as determined by ELISA and ITLC (Figures 2A, and B). IgG ELISA reactivity with the GM_2 neoglycolipid **1** (peak titer 1:9600) was stronger than with sGM_2 ganglioside (peak titer 1:1600); this indicates that IgG antibodies were induced against unique epitopes on the GM_2 neoglycolipid **1** as well as epitopes that are shared with sGM_2 and bovine-brain-derived GM_2 ganglioside.

Conclusion

Our results indicate that novel synthetic glycolipids in which the synthetic carbohydrate antigen is linked to an appropriate synthetic immunostimulatory carrier may lead to useful immunogens for inducing antibodies to ganglioside and other carbohydrate cancer antigens.

Experimental Section

Solvents were purified according to the standard procedures. Melting points are reported in degree Celsius (uncorrected). NMR measurements were performed at 22 °C on a Bruker AC250 Cryospec or Bruker DRX600. TMS or the resonance of the deuterated solvent was used as internal standard; solvents: $CDCl_3$, δ=7.24; CD_3OD, δ=3.315; D_2O, δ=4.63; [D_6]DMSO, δ=2.49, was used as external standard. MALDI-mass spectra were recorded on a Kratos Kompact Maldi 1 and 2,5-dihydroxybenzoic acid (DHB) or 6-aza-2-thiothymine (ATT) were used as matrices. FAB-mass spectra were measured on a Finnigan MAT 312/AMD 5000 (790 eV, 70 °C). Optical rotations were measured on a Perkin–Elmer polarimeter 241/MS in a 1 dm cell at 22 °C. Thin-layer chromatography (TLC) was performed on Merck silica gel 60 F_{254} plastic plates or Merck amino phase glass plates. Compounds were visualized by treatment with a solution of $(NH_4)_6Mo_7O_{24} \times 4H_2O$ (20 g) and $Ce(SO_4)_2$ (0.4 g) in 10% sulfuric acid (400 mL). Flash chromatography was performed on J.T. Baker silica gel 60 (0.040–0.063 mm) at a pressure of 0.3 bar.

Serological assays were performed as described previously.[18] Immunizations: Two rabbits were immunized four times at four different sites with GM_2 neoglycolipid **1** (7 nmol GM_2-tetrasaccharide) in CFA/IFA every two weeks followed by two boost injections four weeks apart, four weeks after

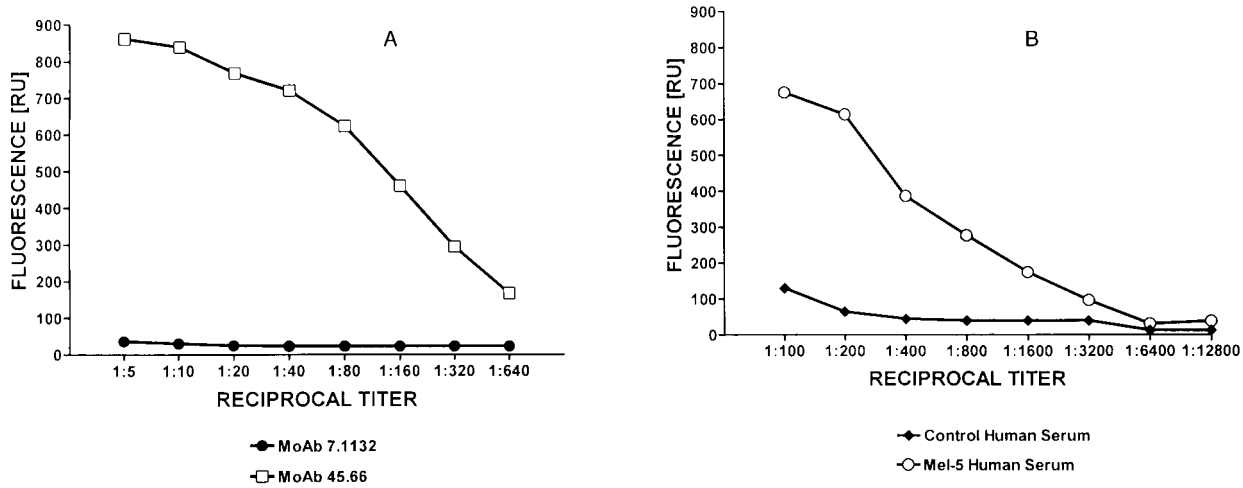

Figure 1. ELISA reactivity of GM_2 antibodies with GM_2 neoglycolipid **1**. A) Human monoclonal antibodies (tissue culture supernantant, IgM) 45.66 (anti-GM_2) and 7.1132 (isotype control); B) Sera from melanoma patient prior and post immunization with bovine brain-derived GM_2-KLH/QS21 vaccine. Method: 200 pmol GM_2 neoglycolipid **1** were incubated with varying amounts of GM_2 antibody; reactivity was quantitated with Fitc-conjugated species, isotype specific secondary antibodies and a microplate fluorospectrometer.

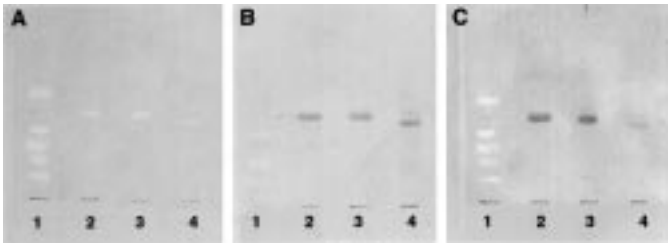

Figure 2. Reactivity of serum of rabbit 322 immunized with GM_2 neoglycolipid **1** plus Freund adjuvant as determined by immune thin-layer chromatography. A) Pre-vaccination serum, B) immune serum obtained after six immunizations, and C) Human GM_2-monoclonal antibody 45.66. Lane 1: gangliosides GM_3, GM_1, GD_3, GD_{1a} and GD_{1b}; lane 2: bovine brain GM_2; lane 3: synthetic GM_2 (C18:0); lane 4: GM_2 neoglycolipid **1**. The plate was developed in chloroform/methanol/water containing 0.2% calcium chloride 55:45:10 (v/v) before overlaying with diluted serum 1:100. Specific reactivity was visualized with HRP-conjugated goat anti-rabbit IgG and diaminobenzidine as chromogen.

the fourth injection. Blood was collected from the ear vein prior to and two weeks after each injection.

1,3,4-Tri-*O*-acetyl-6-azido-2-*N*-(*N*-benzyloxycarbonyl)leucyl-2,6-dideoxy-*β*-D-glucopyranoside (7): Water soluble carbodiimide (WSC; 2.01 g, 10.5 mmol) was added to a solution of 1,3,4-tri-*O*-acetyl-2-amino-6-azido-2,6-dideoxy-*β*-D-glucopyranoside (**6**;[13] 3.15 g, 9.54 mmol) and Z-Leu-OH (2.78 g, 10.5 mmol) in dry dichloromethane (20 mL). The mixture was stirred for 20 min at room temperature, diluted with dichloromethane (20 mL), washed with brine (2×10 mL) and water (10 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was dissolved in hot methanol. After 12 h needles were filtered off, washed with cold ethanol and dried in vacuo to give **7** (4.34 g, 79%). $R_f=0.38$ (toluene/ethyl acetate 1:1); m.p. 193.5 °C; $[\alpha]_D=-17.2$ ($c=1$, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta=0.88$, 0.91 (2s, 6H; CH (CH_3)$_2$), 1.31–1.45 (m, 1H; C*H*(CH_3)$_2$), 1.53–1.66 (m, 2H; CHC*H*$_2$), 2.03–2.05 (m, 9H; 3 $COCH_3$), 3.36–3.38 (m, 2H; 6-H, 6′-H), 3.74–3.84 (m, 1H; 5-H), 3.99–4.18 (m, 1H; C*H*CH$_2$), 4.26 (ddd, J(NH,2) ≈ J(2,3) ≈ 9.2 Hz, 1H; 2-H), 5.02–5.23 (m, 5H; CH_2-phenyl, 3-H, 4-H, CO_2NH), 5.77 (d, J(1,2) = 8.7 Hz, 1H; 1-H), 6.37 (brs, 1H; NH), 7.34–7.36 (m, 5H; phenyl); $C_{26}H_{35}N_5O_{10}$ (577.6): calcd C 54.06, H 6.10, N 12.12; found C 54.16, H 6.10, N 12.06.

6-Azido-2-*N*-(*N*-benzyloxycarbonyl)-L-leucyl-2,6-dideoxy-*α*-D-glucopyranoside (9): A solution of **7** (1.05 g, 1.82 mmol) in dry methanol was treated with DBU (1 mL). After 2 h acetic acid (1 mL) was added, and the solvent was removed in vacuo. The residue was chromatographed over silica gel (toluene/acetone 2:1). Final purification was achieved by crystallization (acetone/petrol ether 1:1) to yield **9** (451 mg, 57%) as a colorless solid. $R_f=0.41$ (toluene/acetone 1:1); m.p. 104 °C; $[\alpha]_D=+33.9$ ($c=1$, methanol); ^{1}H NMR (250 MHz, MeOD): $\delta=0.91-0.95$ (m, 6H; CH(CH_3)$_2$), 1.53–1.73 (m, 3H; CH_2CH(CH_3)$_2$), 3.29–3.54 (m, 3H; 4-H, 6-H, 6′-H), 3.66 (dd, J(2,3) = 9.6 Hz, J(3,4) = 8.7 Hz, 1H; 3-H), 3.83 (dd, J(1,2) = 3.4 Hz; 1H; 2-H), 3.91–3.98 (m, 1H; 5-H), 4.21 (dd, J(vic) ≈ J'(vic) ≈ 6.0 Hz, 1H; C*H*CH$_2$), 5.08–5.11 (m, 3H, 1-H; CH_2-phenyl), 7.27–7.37 (m, 5H; phenyl); $C_{20}H_{29}N_5O_6$ (435.5): calcd C 52.97, H 6.44, N 15.44; found C 52.81, H 6.61, N 15.16.

***N*-[2-*N*-(*N*-Benzyloxycarbonyl)-L-leucyl-2,6-dideoxy-6-*O*-tosyl-*β*-D-glucopyranosyl]-*N*-octadecyldodecanamide (11)**: Tosyl chloride (0.376 g, 1.97 mmol) was added to a solution of *N*-[2-*N*-(*N*-benzyloxycarbonyl)leucyl-2,6-dideoxy-*β*-D-glucopyranosyl]-*N*-octadecyldodecanamide (**10**;[10, 14] 1.11 g, 1.31 mmol) in pyridine (50 mL) at −10 °C. After 3 h the reaction mixture was allowed to warm to room temperature. After another hour methanol (1 mL) was added, and the solvent was removed in vacuo. Chromatography of the residue on silica gel (toluene/ethyl acetate 1:1) gave **11** (930 mg, 70%) as a colorless syrup. $R_f=0.38$ (toluene/acetone 1:1); $[\alpha]_D=+0.5$ ($c=1$, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): $\delta=0.74-0.84$ (brs, 12H; CH(CH_3)$_2$, $CH_2(CH_2)_9CH_3$, $CH_2(CH_2)_{16}CH_3$), 1.03–1.55 (m, 53H; $CH_2(CH_2)_9CH_3$, $CH_2(CH_2)_{16}CH_3$, CH_2CH(CH_3)$_2$), 2.17–2.41 (m, 5H; C$H_2(CH_2)_9CH_3$, $C_6H_4CH_3$), 3.04–3.06 (brs, 2H; C$H_2(CH_2)_{16}CH_3$), 3.37 (brs, 1H; 5-H), 3.80 (brs, 1H; 2-H), 4.00 (brs, 1H; C*H*CH$_2$), 4.12 (brs, 1H; 6-H), 4.26 (brs, 1H; 6′-H), 5.01 (m, 2H; $CH_2C_6H_5$), 5.26 (d, J(NH,CH) = 6 Hz, 1H; $NHCO_2$), 5.5 (d, J(1,2) = 9.2 Hz, 1H; 1-H), 6.84 (d, J(2,NH) = 7.2 Hz, 1H; NH), 7.19–7.70 (m, 9H; C_6H_5, $C_6H_4CH_3$); $C_{57}H_{95}N_3O_{10}S$ (1014.4): calcd C 67.49, H 9.44, N 4.14; found C 67.4, H 9.76, N 4.35.

***N*-[6-Azido-2-*N*-(*N*-benzyloxycarbonyl)-L-leucyl-2,6-dideoxy-*β*-D-glucopyranosyl]-*N*-octadecyldodecanamide (12)**: a) From **11**. A solution of **11** (740 mg, 0.73 mmol) and sodium azide (200 mg, 3.00 mmol) in DMF (10 mL) was stirred for 4 h at 80 °C, then cooled to room temperature and diluted with ethyl acetate (30 mL). The solid was filtered off and the filtrate was concentrated in vacuo. Further purification by chromatography (toluene/acetone 1:1) on silica gel afforded **12** (638 mg, 97%) as a colorless solid.

b) From **9**: A suspension of **9** (700 mg, 1.55 mmol) and octadecylamine (629 mg, 2.33 mmol) in dry methanol (15 mL) was stirred under reflux for 2–3 h. After the mixture had been allowed to reach room temperature, excess of octadecylamine was filtered off, and the filtrate was concentrated in vacuo. The residue was extensively dried in vacuo and then taken up in dry dichloromethane (10 mL). Triethylamine (550 μL, 4.65 mmol) and lauroyl chloride (615 μL, 4.65 mmol) were added, and the mixture was stirred for 3 h. After concentration in vacuo, purification on silica gel and subsequent crystallisation (methanol) afforded **12** (510 mg, 37%) as a colorless needles. $R_f=0.41$ (toluene/acetone 1:1); m.p. 87.9 °C; $[\alpha]_D=+18.3$ ($c=1$, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta=0.84-0.93$ (m, 12H; CH(CH_3)$_2$, $CH_2(CH_2)_9CH_3$, $CH_2(CH_2)_{16}CH_3$), 1.11–1.60 (m, 53H; CH_2CH(CH_3)$_2$, $CH_2(CH_2)_9CH_3$, $CH_2(CH_2)_{16}CH_3$), 2.25–2.31 (m, 2H; C$H_2(CH_2)_9CH_3$), 3.15–3.21 (brs, 2H; C$H_2(CH_2)_{16}CH_3$), 3.35–3.60 (m, 5H; 3-H, 4-H, 5-H, 6-H, 6′-H), 3.75 (brs, 1H; OH), 3.92–4.09 (m, 1H; 2-H), 4.58 (brs, 1H; OH), 5.08–5.15 (m, 2H; $CH_2C_6H_5$), 5.43 (d, J(NH,CH) = 8.1 Hz, 1H; CO_2NH), 5.63 (d, J(1,2) = 9.8 Hz, 1H; 1-H), 6.97 (d, J(2,NH) = 7.5 Hz, 1H; NH), 7.29–7.38 (m, 5H; C_6H_5); rotamers caused a second set of signals of very weak intensity; MS (FAB, positive mode, matrix: 3-nitrobenzylalcohol/NaI): m/z: 908 $[M+Na]^+$; $C_{50}H_{88}N_6O_7$ (885.29): calcd C 67.84, H 10.02, N 9.49; found C 67.68, H 9.95, N 9.40.

***N*-[6-Amino-2-*N*-(*N*-benzyloxycarbonyl)-L-leucyl-2,6-dideoxy-*β*-D-glucopyranosyl]-*N*-octadecyldodecanamide (5)**: A solution of compound **12** (648 mg, 0.73 mmol) and 1,3-propanedithiol (222 mL, 2.19 mmol) in a mixture of pyridine/water (4:1, 10 mL) was stirred at room temperature. After 12 h the solvent was removed in vacuo. The residue was coevaporated twice with toluene and then chromatographed on silica gel ($CHCl_3$/MeOH 20:1 → 10:1; with 1% Et_3N) to give compound **5** (448 mg, 77%) as a colorless oil. $R_f=0.37$ (methanol/$CHCl_3$ 1:10); $[\alpha]_D=+13.5$ ($c=1$, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$/MeOD 95:5): $\delta=0.87-0.91$ (m, 12H; CH(CH_3)$_2$, $CH_2(CH_2)_9CH_3$, $CH_2(CH_2)_{16}CH_3$), 1.01–1.70 (m, 53H; CH_2CH(CH_3)$_2$, $CH_2(CH_2)_9CH_3$, $CH_2(CH_2)_{16}CH_3$), 2.23–2.29 (m, 2H; C$H_2(CH_2)_9CH_3$), 2.88 (dd, J(5,6) = 5.4 Hz, J(6,6′) = 13.1 Hz, 1H; 6-H), 3.05 (dd, J(5,6′) = 3.1 Hz, 1H; 6′-H), 3.15–3.19 (m, 2H; C$H_2(CH_3)_{16}CH_3$), 3.35–3.51 (m, 3H; 3-H, 4-H, 5-H), 3.94 (dd, J(2,3) ≈ J(3,4) ≈ 7.2 Hz, 1H; 2-H), 4.10 (dd, J = 4.0, 9.9 Hz, C*H*CH$_2$), 5.02, 5.10 (2d, J(gem) = 12.8 Hz, 2H; $CH_2C_6H_5$), 5.60 (d, J(1,2) = 9.7 Hz, 1H; 1-H), 7.27–7.35 (m, 5H; C_6H_5); MS (FAB, positive mode, matrix: 3-nitrobenzylalcohol/NaI): m/z: 908 $[M+Na]^+$; $C_{50}H_{90}N_4O_7$ (859.3): calcd C 69.88, H 10.56, N 6.52; found C 69.65, H 10.68, N 6.53.

***N*-[6-Acetamido-2-*N*-(*N*-benzyloxycarbonyl)-L-leucyl-2,6-dideoxy-*β*-D-glucopyranosyl]-*N*-octadecyldodecanamide (13)**: Compound **5** (150 mg, 0.175 mmol) was treated with acetic anhydride (17 mL) in dichloromethane (10 mL) at room temperature. After 30 min the mixture was concentrated in vacuo. The residue was purified on silica gel (toluene/acetone 1:1) to give oily **13** (151 mg, 96%). $R_f=0.25$ (toluene/acetone 1:1); $[\alpha]_D=-18$ ($c=1$, $CHCl_3$). The ^{1}H NMR spectra showed two sets of signals (rotamers). Only significant signals are given: ^{1}H NMR (600 MHz, [D_6]DMSO/D_2O 95:5): $\delta=0.74-0.77$ (brs, 12H; CH(CH_3)$_2$, $CH_2(CH_2)_9CH_3$, $CH_2(CH_2)_{16}CH_3$), 1.01–1.54 (m, 53H; CH_2CH(CH_3)$_2$, $CH_2(CH_2)_9CH_3$, $CH_2(CH_2)_{16}CH_3$), 1.98–2.31 (m, 2H; C$H_2(CH_2)_9CH_3$), 2.86–3.22 (m, 5H; 4-H, 5-H, 6-H, C$H_2(CH_2)_{16}CH_3$), 3.37–3.70 (m, 3H; 2-H, 3-H, 6′-H), 3.88–3.99 (m, 1H; C*H*CH$_2$), 4.93–4.97 (m, 2H; $CH_2C_6H_5$), 5.09, 5.49 (2d, J(1,2) = 8.5 Hz, 1H; 1-H, rotamers), 7.27–7.35 (m, 5H; C_6H_5); $C_{52}H_{92}N_4O_8$ (901.3): calcd C 69.29, H 10.29, N 6.22; found C 69.06, H 10.62, N 6.16.

***N*-[6-Acetamido-2-*N*-L-leucyl-2,6-dideoxy-*β*-D-glucopyranosyl]-*N*-octadecyldodecanamide (14)**: A mixture of **13** (220 mg, 0.244 mmol), Pd/C (10%, 20 mg), and acetic acid (15 μL, 0.244 mmol) in methanol was treated with

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hydrogen (1 atm) under vigorous stirring at room temperature. After 24 h the catalyst was filtered off over Celite, and the filtrate was concentrated in vacuo. The residue was chromatographed on silica gel ($CHCl_3$/MeOH/Et_3N 100:5:1 →50:5:1) to give **14** (160 mg, 86%) as a sirup. $R_f = 0.18$ ($CHCl_3$/MeOH 12:1); $[\alpha]_D = 12.8$ ($c = 1$, $CHCl_3$). The ^1H NMR spectra showed two sets of signals (rotamers). Only significant signals are given: ^1H NMR (600 MHz, $CDCl_3$): $\delta = 0.81 - 0.91$ (m, 12H; $CH_2(CH_2)_9CH_3$, $CH_2(CH_2)_{16}CH_3$, $CH(CH_3)_2$), 1.11 – 1.71 (m, 53H; $CH_2(CH_2)_9CH_3$, $CH_2(CH_2)_{16}CH_3$, $CH_2CH(CH_3)_2$), 1.98 (s, 3H; $COCH_3$), 2.15 – 2.42 (m, 2H; $CH_2(CH_2)_9CH_3$), 3.06 – 4.12 (m, 9H; 2-H, 3-H, 4-H, 5-H, 6-H, 6′-H, NH_2, $CHCH_2$), 5.48, 5.73 (2d, $J(1,2) = 9.9$ Hz, 1H), 6.08 – 6.23 (br s, 1H; NH), 7.63, 8.11 (2br s, 1H; NH); $C_{44}H_{86}N_4O_6$ (767.2); calcd C 68.88, H 11.29, N 7.30; found 68.61, H 11.18, N 7.22.

Benzyl 9-*O*-[(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)-(1→4)-{[methyl-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-D-nonulopyranosyl)onat]-(2→3)}-(2,6-di-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-3,6-di-*O*-acetyl-2-*O*-pivaloyl-β-D-glucopyranosyl]nonanoate (15a): A solution of the known trichloroacetimidate **2** (200 mg, 0.129 mmol) and benzyl 9-hydroxynonanoate (**3a**) (68 mg, 0.259 mmol) in dry dichloromethane (1 mL) was treated under argon with a solution of TMS triflate (130 μL of a 0.1N solution in dichloromethane). After 15 min the mixture was neutralized with Et_3N and concentrated in vacuo. Chromatography of the residue on silica gel (toluene/acetone 3:2) afforded **15a** as an oil (165 mg, 78%), $R_f = 0.37$ (toluene/acetone 1:1); $[\alpha]_D = -15.9$ ($c = 1$, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$): $\delta = 1.10 - 1.12$ (m, 9H; $C(CH_3)_3$), 1.19 – 1.25 (m, 10H; $CH_2(CH_2)_5(CH_2)_2CO_2CH_2C_6H_5$), 1.47 – 2.31 (m, 44H; 13 $COCH_3$, $CH_2(CH_2)_5(CH_2)_2CO_2CH_2C_6H_5$, 3c′-H), 2.77 (dd, $J(3,3') = 13$ Hz, $J(3,4) = 4.3$ Hz, 1H; 3c-H), 3.26 – 3.37 (m, 2H; 2d-H, $CH_2(CH_2)_5(CH_2)_2CO_2CH_2C_6H_5$), 3.47 (dd, $J(3,4) \approx J(4,5) \approx 2.0$ Hz, 1H; 4b-H), 3.53 – 3.56 (m, 2H; 5a-H, 5b-H), 3.76 – 3.83 (m, 7H; $CH_2(CH_2)_5(CH_2)_2CO_2CH_2C_6H_5$, OCH_3, 4a-H, 5d-H, 6c-H), 3.91 – 4.10 (m, 6H; 6b-H, 6′-H, 5c-H, 9′c-H, 6d-H, 6′d-H), 4.16 – 4.19 (m, 2H; 3b-H, 6′a-H), 4.32 (dd, $J(8,9) = 2.6$ Hz, $J(9,9') = 12.7$ Hz, 1H; 9c-H), 4.39 (d, $J(1,2) = 8.0$ Hz, 1H; 1a-H), 4.41 (dd, $J(5,6) < 2$ Hz, $J(6,6') = 10.9$ Hz, 1H; 6a-H), 4.55 (d, $J(1,2) = 7.7$ Hz, 1H; 1b-H), 4.76 (ddd, $J(3',4) = J(4,5) = 11.3$ Hz, $J(3,4) = 4.3$ Hz, 1H; 4c-H), 4.88 (dd, $J(2,3) = 8.2$ Hz, 1H; 2a-H), 4.93 (dd, $J(2,3) = 10.1$ Hz, 1H; 2b-H), 5.06 (s, 2H; $CH_2C_6H_5$), 5.10 – 5.15 (m, 3H; Nc-H, 3a-H, 1d-H), 5.30 – 5.35 (m, 2H; 7c-H, 4d-H), 5.49 – 5.50 (m, 1H; 8c-H), 5.83 (dd, $J(2,3) = 11.2$ Hz, $J(3,4) = 3.4$ Hz, 1H; 3d-H), 6.03 (d, $J(2,NH) = 7.1$ Hz, 1H; Nd-H), 7.27, 7.31 (m, 5H; C_6H_5); MS (MALDI, positive mode, matrix: DHB): m/z: 1665 $[M+Na]^+$; $C_{75}H_{106}N_2O_{38}$ (1643.7): calcd C 54.80, H 6.50, N 1.78; found C 54.93, H 6.74, N 2.07.

Methyl 9-*O*-[(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)-(1→4)-{[methyl-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-D-nonulopyranosyl)onat]-(2→3)}-(2,6-di-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-3,6-di-*O*-acetyl-2-*O*-pivaloyl-β-D-glucopyranosyl]nonanoate (15b): A solution of trichloroacetimidate **2**[9, 16] (1 g, 0.645 mmol) and methyl 9-hydroxynonanoate (**3b**) (365 mg, 1.94 mmol) in dry dichloromethane (2 mL) was treated under argon with a solution of TMS triflate (645 μL of 0.1N solution in dichloromethane). After 15 min the mixture was neutralized with Et_3N and concentrated in vacuo. Chromatography of the residue on silica gel (toluene/acetone 2:1) afforded **15b** (730 mg, 73%) as a colorless foam. $R_f = 0.31$ (toluene/acetone 1:1); $[\alpha]_D = -8.0$ ($c = 0.5$, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$): $\delta = 1.11 - 1.13$ (m, 9H; $C(CH_3)_3$), 1.19 – 1.25 (m, 10H; $CH_2(CH_2)_5(CH_2)_2CO_2CH_3$), 1.55 – 2.25 (m, 44H; 13 $COCH_3$, $CH_2(CH_2)_5(CH_2)_2CO_2CH_3$, 3′c-H), 2.77 (dd, $J(3,3') = 13$ Hz, $J(3,4) = 4.3$ Hz; 3c-H), 3.25 – 3.37 (m, 2H; 2-d, $CH_2(CH_2)_5(CH_2)_2CO_2CH_3$), 3.47 (dd, $J(3,4) \approx J(4,5) \approx 2.0$ Hz, 1H; 4b-H), 3.53 – 3.83 (m, 9H; $CH_2(CH_2)_5(CH_2)_2CO_2CH_3$, OCH_3, 4a-H, 5a-H, 5b-H, 5d-H, 6c-H), 3.90 – 4.10 (m, 6H; 6b-H, 6′b-H, 5c-H, 9′c-H, 6d-H, 6′d-H), 4.16 – 4.19 (m, 2H; 3b-H, 6′a-H), 4.32 (dd, $J(8,9) = 2.5$ Hz, $J(9,9') = 12.6$ Hz, 1H; 9c-H), 4.39 (d, $J(1,2) = 8.1$ Hz, 1H; 1a-H), 4.41 (dd, $J(5,6) < 2$ Hz, $J(6,6') = 10.9$ Hz, 1H; 6a-H), 4.55 (d, $J(1,2) = 7.7$ Hz, 1H; 1b-H), 4.76 (ddd, $J(3',4) = J(4,5) = 11.3$ Hz, $J(3,4) = 4.3$ Hz, 1H; 4c-H), 4.88 (dd, $J(2,3) = 8.2$ Hz, 1H; 2a-H), 4.93 (dd, $J(2,3) = 10.1$ Hz, 1H; 2b-H), 5.10 – 5.15 (m, 3H; Nc-H, 3a-H, 1d-H), 5.30 – 5.35 (m, 2H; 7c-H, 4d-H), 5.49 – 5.50 (m, 1H; 8c-H), 5.83 (dd, $J(2,3) = 11.2$ Hz, $J(3,4) = 3.4$ Hz, 1H; 3d-H), 6.03 (d, $J(2,NH) = 7.1$ Hz, 1H; Nd-H); C 51.53, H 6.61, N 1.76; found C 51.64, H 6.65, N 2.39.

9-*O*-[2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)-(1→4)-{[methyl-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-D-nonulopyranosyl)onat]-(2→3)}-(2,6-di-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-3,6-di-*O*-acetyl-2-*O*-pivaloyl-β-D-glucopyranosyl]nonanoic acid (16): Compound **15a** (100 mg, 0.06 mmol) was dissolved in dioxane/acetic acid (4 mL, 5:1). Then Pd/C (10%) was added, and the mixture was treated with hydrogen (1 atm) for 60 – 90 min at room temperature. The catalyst was filtered off over Celite, and the solvent was removed under reduced pressure. The residue was purified by chromatography on silica gel (toluene/acetone/acetic acid 50:50:1) to yield **16** (89 mg, 94%) as a colorless foam. $R_f = 0.11$ (toluene/acetone 1:1); $[\alpha]_D = -15.8$ ($c = 1$, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$): $\delta = 1.11 - 1.13$ (m, 9H; $C(CH_3)_3$), 1.2 – 1.29 (m, 10H; $CH_2(CH_2)_5(CH_2)_2CO_2H$), 1.47 – 2.31 (m, 44H; 13 $COCH_3$, $CH_2(CH_2)_5(CH_2)_2CO_2H$, 3′c-H), 2.79 (dd, $J(3,3') = 13$ Hz, $J(3,4) = 4.3$ Hz; 3c-H), 3.23 – 3.37 (m, 2H; 2-d, $CH_2(CH_2)_5(CH_2)_2CO_2CH_2$-phenyl), 3.48 (dd, $J(3,4) \approx J(3,4) \approx 2.0$ Hz, 1H; 4b-H), 3.56 – 3.58 (m, 2H; 5a-H, 5b-H), 3.77 – 3.82 (m, 7H; $CH_2(CH_2)_5(CH_2)_2CO_2CH_2$-phenyl, OCH_3, 4a-H, 5d-H, 6c-H), 3.92 – 4.12 (m, 6H; 6b-H, 6′b-H, 5c-H, 9′c-H, 6d-H, 6′d-H), 4.17 – 4.20 (m, 2H; 3b-H, 6′a-H), 4.33 (dd, $J(8,9) = 2.6$ Hz, $J(9,9') = 12.7$ Hz, 1H; 9c-H), 4.41 (d, $J(1,2) = 8.0$ Hz, 1H; 1a-H), 4.45 (dd, $J(5,6) < 2$ Hz, $J(6,6') = 10.9$ Hz, 1H; 6a-H), 4.57 (d, $J(1,2) = 7.7$ Hz, 1H; 1b-H), 4.80 (ddd, $J(3',4) = J(4,5) = 11.3$ Hz, $J(3,4) = 4.3$ Hz, 1H; 4c-H), 4.89 (dd, $J(2,3) = 8.3$ Hz, 1H; 2a-H), 4.95 (dd, $J(2,3) = 10.0$ Hz, 1H; 2b-H), 5.10 (d, $J(4,NH) = 10.3$ Hz, 1H; Nc-H), 5.13 (dd, $J(1,2) = 8.3$ Hz, 1H; 1d-H), 5.18 (dd, $J(2,3) \approx J(3,4) \approx 9.8$ Hz, 1H; 3d-H), 5.32 – 5.37 (m, 2H; 7c-H, 4d-H), 5.50 – 5.53 (m, 1H; 8c-H), 5.84 (dd, $J(2,3) = 11.1$ Hz, $J(3,4) = 3.3$ Hz, 1H; 3d-H), 6.15 (d, $J(2,NH) = 7.3$ Hz, 1H; Nd-H); MS (MALDI, positive mode, matrix: DHB): m/z: 1575 $[M+Na]^+$; $C_{68}H_{100}N_2O_{38}$ (1553.6); calcd C 51.97, H 6.41, N 1.79; found C 51.67, H 6.54, N 2.10.

6-[9-*O*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)-(1→4)-{[methyl-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-D-nonulopyranosyl)onat]-(2→3)}-(2,6-di-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-3,6-di-*O*-acetyl-2-*O*-pivaloyl-β-D-glucopyranosyl]nonylamido-2-*N*-(*N*-benzyloxycarbonyl)-L-leucyl 2,6-dideoxy-β-D-glucopyranosyl]-*N*-octadecyldodecanamide [(L)-17]: Compound **5** (55 mg, 0.064 mmol), compound **16** (99 mg, 0.064 mmol), and WSC were dissolved in dry dichloromethane (5 mL). After 2 h the reaction mixture was diluted with dichloromethane and extracted with water (3 × 10 mL). The organic layer was dried over $MgSO_4$, filtered, and evaporated in vacuo. The residue was purified on silica gel (toluene/acetone 1:1 →2:3) to give (L)-**17** (101 mg, 66%) as a colorless foam. $R_f = 0.63$ ($CHCl_3$/MeOH 8:1); $[\alpha]_D = -21.8$ ($c = 1$, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$): $\delta = 0.83 - 0.89$ (m, 21H; $CH_2(CH_2)_9CH_3$, $CH_2(CH_2)_{16}CH_3$, $CH(CH_3)_2$, $C(CH_3)_3$), 1.13 – 2.17 (m, 107H; $CH_2(CH_2)_9CH_3$, $CH_2(CH_2)_{16}CH$, $CH_2CH(CH_3)_2$), 3′d-H $[CH_2(CH_2)_6CH_2CON$, 13 $COCH_3]$, 2.79 (dd, $J(gem) = 13$ Hz, $J(3,4) = 4.4$ Hz, 1H; 3d-H), 3.0 – 4.12 (m, 25H; 2a-H, 3-H, 4-H, 5a-H, 6a-H, 6′a-H, 4b-H, 5b-H, 6b-H, 4c-H, 5c-H, 6c-H, 3d-H, 5d-H, 9d-H, 2e-H, 5e-H, 6e-H, 6′e-H, $CH_2(CH_2)_6CH_2CON$, $CH_2(CH_2)_{16}CH_3$, $CHCH_2$], 4.79 (m, 1H; 4d-H), 4.18 – 4.24 (m, 2H; 6′b-H, 3c-H), 4.32 (dd, $J(9,9') = 12.2$ Hz, $J(8,9) = 3.5$ Hz, 1H; 9d-H), 4.41 (m, $J(1,2) = 8.0$ Hz, 2H; 1b-H, 6′c-H;), 4.56 (d, $J(1,2) = 7.8$ Hz, 1H; 1c-H), 4.79 (m, 1H; 1c-H), 4.79 (m, 1H; 4d-H), 4.87 (dd, $J(2,3) = 8.5$ Hz, 1H; 2b-H), 4.94 (dd, $J(2,3) = 9.8$ Hz, 1H; 2c-H), 5.08 – 5.,24 (m, 6H; 3b-H, 1e-H, CH_2-phenyl, ND-H, CO_2NH), 5.32 – 6.07 (m, 5H; 1a-H, 7d-H, 8d-H, 4e-H, Ne-H), 6.52 (br s, 1H; NH-a), 7.28 – 7.32 (m, 5H; phenyl); MS (FAB, positive mode, matrix: NaI/3-nitrobenzylalcohol): m/z: 2417 $[M+Na]^+$; $C_{118}H_{188}N_6O_{44}$ (2394.9): C 59.18, H 7.91, N 3.51; found C 59.00, H 8.04, N 3.71.

6-[9-*O*-[(2-Acetamido-2-deoxy-β-D-galactopyranosyl)-(1→4)-[5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-D-nonulopyranosylonat-(2→3)]-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranosyl]noylamido-2-*N*-(*N*-benzyloxycarbonyl)-L-leucyl-2,6-dideoxy-β-D-glucopyranosyl]-*N*-octadecyldodecanamide potassium salt [(D,L)-18]: Compound (L)-**17** (100 mg, 0.041 mmol) was treated at room temperature with a solution of sodium methoxide in methanol (10 mL, 0.05 mol) for 12 h, and was then neutralized with Amberlite (IR120, H^+-form). The solid was filtered off, and the solvent was removed under reduced pressure. The residue was dissolved in a solution of potassium hydroxide (4 mL, 0.2 mol). After stirring for 3 d the solution was again neutralized with Amberlite (IR120, H^+-form), and the solvent was removed by lyophylisation. Purification was carried out as described for compound (L)-**18** to yield compound (D,L)-**18** (48 mg, 61%). $R_f = 0.41$ and 0.42 [$CHCl_3$/MeOH/$CaCl_2$ (2% water) 55:45:10]; ^1H NMR (600 MHz, [D_6]DMSO/D_2O 95:5) and MS (FAB,

positive mode, matrix: DMSO/3-nitrobenzylalcohol) showed no differences to the data for compound (L)-**18**.

9-*O*-[(2-Acetamido-2-deoxy-β-D-galactopyranosyl)-(1→4)-[5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-D-nonulopyranosylonat-(2→3)]-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranosyl]nonanoate dipotassium salt (19): Compound **15b** (400 mg, 0.255 mmol) was dissolved in a solution of sodium methoxide in methanol (10 mL, 0.05 M). After 12 h stirring the solution was neutralized with Amberlite (IR120, H^+-form), filtered, and concentrated in vacuo. The residue was treated with a solution of potassium hydroxide in water (4 mL, 0.2 M). The reaction was monitored by TLC on amino-phase silica gel. After 3 d the mixture was neutralized as described above. The product was purified on a sephadex column (LH-20, $CHCl_3$/MeOH 1:1) to give (251 mg, quant.) of the dipotassium salt **19**. $R_f = 0.5$ (EtOH/water 1:1, amino-phase silica gel); $[\alpha]_D = -4.5$ ($c = 1$, MeOH); ^{1}H NMR (600 MHz, MeOD): $\delta = 0.88-1.55$ (m, 12H; $CH_2(CH_2)_6CH_2CO_2^-$), 1.81 (m, 1H; 3′c-H), 1.91, 1.92 (2s, 6H; $COCH_3$), 2.12 (br s, 2H; $CH_2(CH_2)_6CH_2CO_2^-$), 2.64 (m, 1H; 3c-H), 3.15 (dd, $J(1,2) \approx J(2,3) = 7.8$ Hz, 1H; 2a-H), 3.21–3.39 (m, 5H; 3a-H, 5a-H, 2b-H, 6c-H, 7c-H), 3.44–3.80 (m, 18H; 4a-H, 6a-H, 6′a-H, 5b-H, 6b-H, 6′b-H, 4c-H, 5c-H, 8c-H, 9c-H, 9′c-H, 3d-H, 4d-H, 5d-H, 6d-H, 6′d-H, $CH_2(CH_2)_6CH_2CO_2^-$), 3.85 (dd, $J(1,2) \approx J(2,3) = 8.1$ Hz, 1H; 2d-H), 3.9 (dd, $J(2,3) = 8.5$ Hz, $J(3,4) < 2$ Hz, 1H; 3b-H), 4.05 (d, 1H; 1a-H), 4.19 (d, 1H; 1a-H), 4.39 (d, $J(1,2)$ 7.8 Hz, 1H; 1b-H), 4.73 (d, 1H; 1d-H); MS (FAB, negative mode, matrix: DMSO/glycerol 1:1): m/z: 991 $[M+H]^-$ 990.3 for $C_{40}H_{66}N_2O_{26}$.

6-[9-*O*-[(2-Acetamido-2-deoxy-β-D-galactopyranosyl)-(1→4)-[5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-D-nonulopyranosylonat-(2→3)]-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranosyl]nonylamido-2-*N*-(*N*-benzyloxycarbonyl)leucyl-2,6-dideoxy-β-D-glucopyranosyl]-*N*-octadecyldodecanamide potassium salt [(L)-18]: A solution of **19** (208 mg, 0.242 mmol), compound **5** (260 mg, 0.262 mmol), and EEDQ (67 mg, 0.271 mmol) in dry ethanol (4 mL) was stirred at 60–70 °C and then concentrated under reduced pressure. The residue was purified by chromatography on silica gel ($CHCl_3$/MeOH/water 70:30:3) to give (L)-**18** (250 mg, 55%) as a colorless lyophylisate (dioxane/water 4:1). $R_f = 0.41$ [$CHCl_3$/MeOH/$CaCl_2$ (2% in water) 55:45:10]; $[\alpha]_D = -11.2$ ($c = 1$, dioxane); the ^{1}H NMR specta showed two sets of signals (rotamers). Only significant signals are given: ^{1}H NMR (600 MHz, [D$_6$]DMSO/D$_2$O 95:5): $\delta = 0.77-0.80$ (m, 12H; $CH_2(CH_2)_9CH_3$, $CH_2(CH_2)_{16}CH_3$, $CH(CH_3)_2$), 1.07–2.29 (m, 76H; $CH_2(CH_2)_9CH_3$, $CH_2(CH_2)_{16}CH_3$, $CH_2CH(CH_3)_2$, $CH_2(H_2)_6CH_2CON$, 3′d-H, 2 $COCH_3$ [1.77, 1.85], 2.54 (m, 1H; 3d-H), 2.87–3.77 (m, 34H; 2a-H, 3a-H, 4a-H, 5a-H, 6a-H, 6′a-H, 2b-H, 3b-H, 4b-H, 5b-H, 6b-H, 6′b-H, 2c-H, 3c-H, 5c-H, 6c-H, 6′c-H, 4d-H, 5d-H, 6d-H, 7d-H, 8d-H, 9d-H, 9′d-H, 2e-H, 3e-H, 4e-H, 5e-H, 6e-H, 6′e-H, $CH_2(CH_2)_{16}CH_3$, $CH_2(CH_2)_6CH_2CON$), 3.88–3.91 (m, 2H; 4c-H, $CHCH_2$), 4.12 (d, $J(1,2) = 7.4$ Hz, 1H; 1b-H), 4.27 (d, $J(1,2) = 7.7$ Hz, 1H; 1c-H), 4.69 (d, $J(1,2) = 8.3$ Hz, 1H; 1e-H), 4.94–4.97 (m, 2H; $CH_2C_6H_5$), 5.03, 5.47 (2 d, $J(1,2) = 7.0$ Hz, 1H; 1a-H, rotamers), 7.27–7.30 (m, 5H; C_6H_5); MS (FAB, positive mode, matrix: DMSO/3-nitrobenzylalcohol): m/z: 1872 $[M+K+H]^+$ for $C_{90}H_{155}KN_6O_{32}$ (1872.3).

6-[9-*O*-[(2-Acetamido-2-deoxy-β-D-galactopyranosyl)-(1→4)-[5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-D-nonulopyranosylonat-(2→3)]-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranosyl]nonylamido-2-*N*-L-leucyl-2,6-dideoxy-β-D-glucopyranosyl]-*N*-octadecyldodecanamide triethylammonium salt (1): A mixture of (L)-**18** (20 mg, 0.011 mmol) in dioxane/water (4:1, 5 mL) and Pd/C (10%, 5 mg) was kept under hydrogen (1 atm) with vigorous stirring for 3 d. The catalyst was removed by filtration over Celite, and was washed with dioxane. The combined filtrates were concentrated in vacuo. The residue was purified by chromatography on silica gel ($CHCl_3$/MeOH/water/Et_3N 70:30:3:1) to give the final compound **1** (14 mg, 72%) as a colorless lyophylisate (dioxane). $R_f = 0.31$ [$CHCl_3$/MeOH/$CaCl_2$ (2% in H_2O) 55:45:10]; $[\alpha]_D = -11.9$ ($c = 1$, dioxane). The ^{1}H NMR spectra showed two sets of signals (rotamers). Only significant signals of the major rotamer are given: ^{1}H NMR (600 MHz, [D$_6$]DMSO/D$_2$O 90:10): $\delta = 0.79-0.82$ (m, 12H; $CH_2(CH_2)_9CH_3$, $CH_2(CH_2)_{16}CH_3$, $CH(CH_3)_2$), 1.11–2.31 (m, 82H; $CH_2(CH_2)_9CH_3$, $CH(CH_2)_{16}CH_3$, $CH_2CH(CH_3)_2$, $N(CH_2CH_3)_3$, $CH_2(CH_2)_6CH_2CON$) [1.75, 1.86 (2s, $COCH_3$)], 2.53 (m, 1H; 3′d-H), 2.89–3.75 (m, 42H; 2a-H, 3a-H, 4a-H, 5a-H, 6a-H, 6′a-H, 2b-H, 3b-H, 4b-H, 5b-H, 6b-H, 6′b-H, 2c-H, 3c-H, 5c-H, 6c-H, 6′c-H, 3d-H, 4d-H, 5d-H, 6d-H, 7d-H, 8d-H, 9d-H, 9′d-H, 2e-H, 3e-H, 4e-H, 5e-H, 6e-H, 6′e-H, $CHCH_2$, $N(CH_2CH_3)_3$, $(CH_2)_6CH_2CON$, $CH_2(CH_2)_9CH_3$), 3.90 (dd, $J(3,4) \approx J(4,5) < 2$ Hz, 1H; 4c-H), 4.12 (d, $J(1,2) = 7.8$ Hz, 1H; 1b-H), 4.24 (d, $J(1,2) = 8.6$ Hz, 1H; 1c-H), 4.78 (d, $J(1,2) = 8.7$ Hz, 1H; 1e-H), 5.13, 5.57 (2 d, $J(1,2) = 9.6$ Hz, 1H; 1a-H, rotamers); MS (MALDI, negative mode, matrix: ATT); m/z: 1696 $[M - Et_3N]^-$ for $C_{82}H_{149}N_6O_{30} \cdot C_6H_{15}N$ (1698.3 + 101.2).

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