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Synthesis of Neoglycolipid Analogues of the Oligosaccharide Portion of Ganglioside GM3[#]

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ABSTRACT

In order to elucidate the molecular specificity of the antimelanoma response induced by GM3 included in proteoliposome preparation, we designed syntheses of a series of neoglycolipids containing the trisaccharide portion of GM3 or its fragment. In the present paper, we synthesized two neoglycolipids containing as a lipid, a racemic glycerol unit substituted by two aliphatic octadecyl ether chains. The di- and trisaccharide derivatives were prepared as glycosides of the spacer by a sequence of isopropylidene-benzylation-hydrolysis followed by sialylation. The condensation between the oligosaccharides and the lipid was performed by an amidation reaction.

Key Words: Neoglycolipids; Proteoliposome; GM3 analogues.

[#]See Ref. ^[1]

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INTRODUCTION

Gangliosides are important targets for passive or active specific immunotherapy (ASI) of tumors.^[2,3] Gangliosides exhibit low immunogenicity, therefore several glycoprotein conjugates or proteoliposomal constructs are usually employed in order to promote an immune response useful for ASI.^[4,5] The molecular specificity of the response induced by this kind of conjugate and the role of the oligosaccharidic and lipidic parts in defining their cross recognition of the parent gangliosides in tumors are not fully understood.

Despite the limited knowledge currently available, the use of carbohydrate tumor-associated antigens as glycolipids^[6,7] for the specific activation of natural killer T cells seems more promising now. The conventional use of neoglycoprotein conjugates for the induction of a specific antibody response was very useful in vaccines against bacterial pathogens but did not give the result expected^[8] in the fight against tumor cells.

We selected GM3^[9] as the ganglioside with the shortest oligosaccharide chain in order to determine the individual contribution of each segment of the molecule to recognition. Additionally, GM3 included in Very Small Size Proteoliposome (VSSP) vesicles containing the *Neisseria meningitidis* outer membrane protein complex have been shown^[5] to induce a strong immune response against the parent ganglioside in animals and also in melanoma patients. These antibodies failed to recognize the trisaccharide Neu5Ac α -(2-3)-Gal β -(1-4)-Glc- β that represents the oligosaccharide portion of GM3 in neoglycoproteins.^[10]

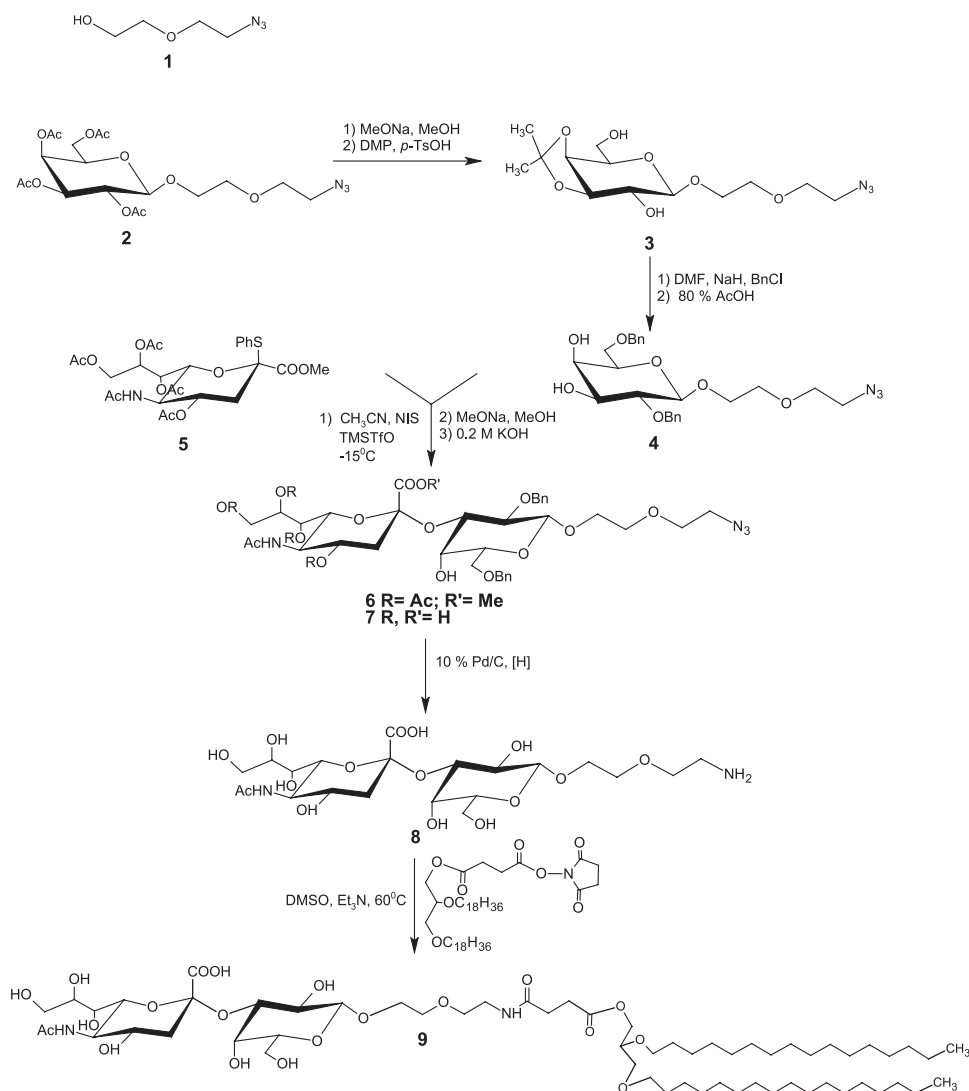
In the present paper, we describe the synthesis of neoglycolipids containing the trisaccharide or its fragments for further understanding of the specificity of anti-GM3 antibodies and also to elucidate the involvement of the CD1-receptor in the anti-melanoma response.

RESULTS AND DISCUSSION

The disaccharide NeuAc α -(2-3)-Gal- β was selected as the first candidate for the synthesis of neoglycolipids. The azido spacer **1** at the reducing end of the oligosaccharide has been shown to allow not only an efficient conjugation to a protein, but also to provide an easy route to the neoglycolipid having the intact oligosaccharide structure, as previously reported.^[11] The reaction of acetobromogalactose with 5-azido-3-oxapentanol (**1**) in the presence of mercuric cyanide afforded the corresponding glycoside **2** in 83% yield (Scheme 1).^[18] The presence of the spacer in glycoside **2** and also in the other derivatives was confirmed by the observation of a triplet at 3.4 ppm in the ¹H NMR spectrum. The ¹³C NMR spectrum also contained a characteristic signal for the CH₂N₃ residue at 50.0 ppm.

The glycoside **2** was subsequently deacetylated by the Zemplen procedure with sodium methoxide in methanol followed by isopropylideneation at the 3', 4'-O-positions by the method of Catelani et al.^[12] Pure compound **3** was isolated by column chromatography in 70% yield. The position of the isopropylidene group was ascertained by the absence of substitution at position C-6 as indicated by the observation of a signal at 61.9 ppm in the ¹³C NMR spectrum, typical of an unsubstituted primary carbon atom. The corresponding glycosyl acceptor **4** was obtained from **3** in 65% yield





Scheme 1. Synthesis of disaccharide NeuAc α (2-3)-Gal neoglycolipid analogue **9**.

after benzylation with benzyl chloride and sodium hydride in *N,N*-dimethylformamide followed by the hydrolysis of the isopropylidene group with 80% acetic acid.

The sialylation of acceptor **4** with the thioglycoside **5**^[13] was performed with *N*-iodosuccinimide and trimethylsilyl triflate^[14] in acetonitrile at -15°C to afford the α -D-linked disaccharide **6** in an acceptable 35% yield. The structure was assigned by observation of a signal in the ^1H NMR spectrum at 2.52 ppm for the equatorial H-3, characteristic^[13] of α -linked sialic acid.

The protecting groups of compound **6** were removed in two steps prior to the introduction of the lipid moiety. First, deacetylation with sodium methoxide in



methanol followed by saponification with aqueous 0.2 M KOH afforded disaccharide derivative **7** that was further hydrogenolyzed with Pd/C in methanol-acetic acid.

Disaccharide derivative **8** was condensed with 2,3-di-*O*-octadecyloxypropyl succinimidyl butanedioate^[11] in the presence of triethylamine in dimethyl sulfoxide at 60°C. The desired neoglycolipid **9** was obtained in 50% yield as a racemic mixture after purification by reverse phase column chromatography. The ¹H NMR spectrum contained a signal for the CH₂NHCO group at 3.30 ppm.

Once the strategy had been proven to be effective for the disaccharide Neu5Ac (2 → 3)-Gal, it was also applied to a lactose acceptor. The attempt to prepare the lactoside **11** using acetobromolactose in the presence of mercuric cyanide gave the orthoester as the main product. Therefore, the known trichloroacetimidate **10**^[15] was synthesized from peracetylated lactose by selective removal of the anomeric acetyl group with ethanolamine,^[16] followed by reaction with trichloroacetonitrile in the presence of potassium carbonate. The resulting product was an anomeric mixture that contained mainly the α-anomer.

Coupling of this imidate^[15] with spacer **1** proceeded smoothly under trimethylsilyl triflate catalysis (Scheme 2). The desired lactoside **11** was obtained in 60% yield.

The product was de-*O*-acetylated under Zemplén conditions and then reacted with 2,2-dimethoxypropane and *p*-toluenesulphonic acid. The initial product contained some mixed acetal attached to O-6. Hydrolysis afforded the 3',4'-*O*-isopropylidene derivative **12** in 70% yield. Observation of signals for C-6 and C-6' at chemical shifts characteristic of unsubstituted primary alcohols in the ¹³C NMR spectrum, together with the deshielding of the C-3' signal to 78.7 ppm indicated that the substitution took place as expected, on the 3',4'-diol.

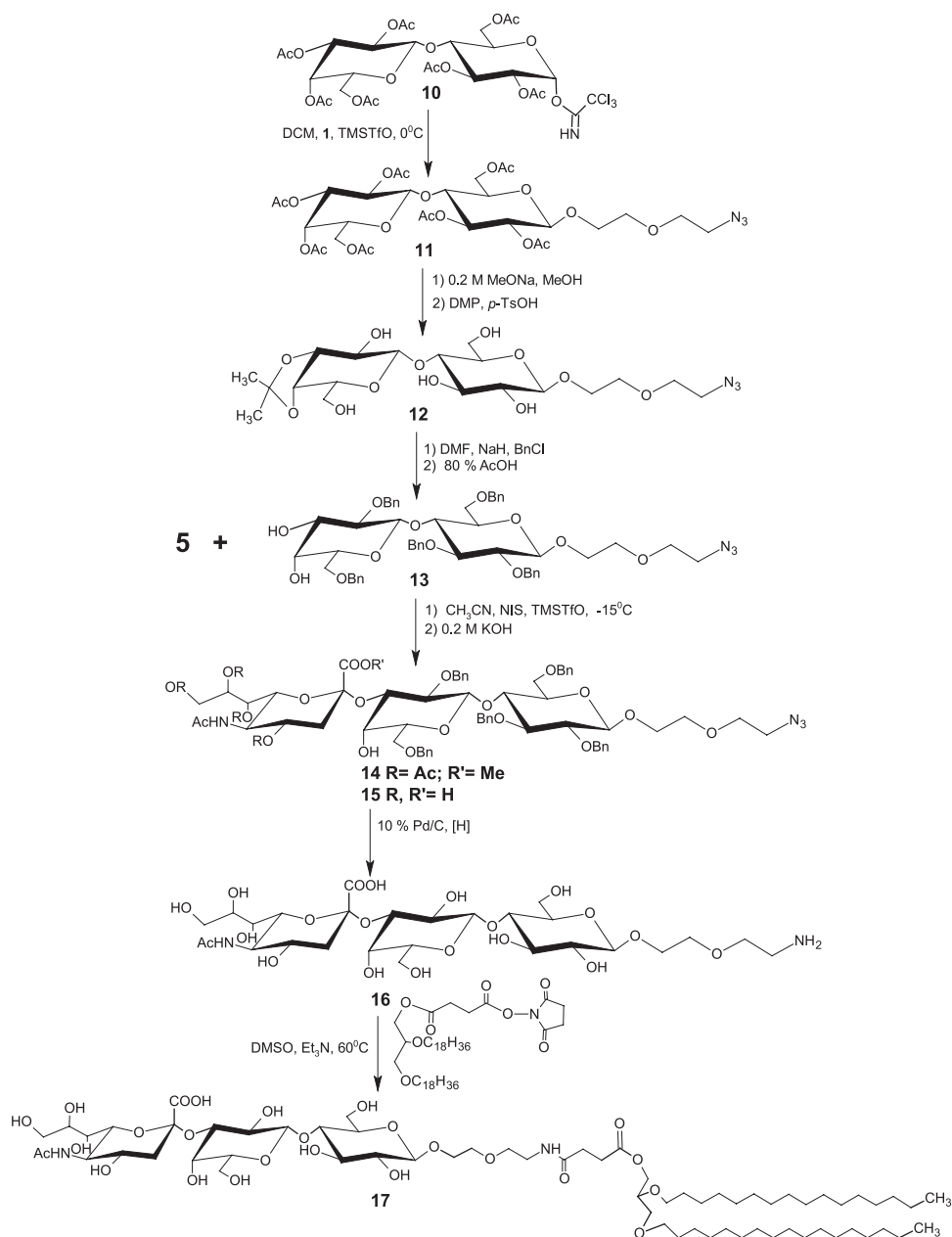
In a similar fashion as for the galactoside acceptor **4**, benzylation of **12** with benzyl chloride and sodium hydride in *N,N*-dimethylformamide followed by the hydrolysis of the isopropylidene group with 80% acetic acid gave acceptor **13** in 60% yield. Sialylation of acceptor **13** with thioglycoside **5** was performed with *N*-iodosuccinimide and trimethylsilyl triflate^[14] in acetonitrile at -15°C to afford the α-linked disaccharide **14** in 25% yield. The ¹H NMR spectrum contained the typical signal for sialic acid H-3eq^[17] at 2.55 ppm.

The protecting groups of compound **14** were removed in a similar fashion to those of **6**, by deacetylation with sodium methoxide in methanol followed by saponification with aqueous 0.2 M KOH to give the intermediate **15**, that was further hydrogenolyzed with Pd/C in methanol-acetic acid to give the spaced trisaccharide **16** in a combined 74% yield.

The trisaccharide derivative **16** was condensed with 2,3-di-*O*-octadecyloxypropyl succinimidyl butanedioate^[11] in the presence of triethylamine in dimethyl sulfoxide at 60°C. The desired racemic neoglycolipid **17** was obtained in 60% yield after purification by reverse phase column chromatography. The ¹H NMR spectrum contained signals corresponding to the oligosaccharide portion, two anomeric doublets at 4.52 and 4.38 ppm, and the signal for the equatorial H-3 of sialic acid at 2.61 ppm. The characteristic pattern of 2,3-di-*O*-octadecyloxypropyl butanedioate at 1.30 and 0.90 ppm was also present.

In a preliminary experiment compounds **9** and **17** bind very well to ELISA plates. The results of their recognition by anti-GM3 antibodies will be published in due course.





Scheme 2. Synthesis of trisaccharide NeuAc α (2-3)-Gal β (1-3) Glc neoglycolipid analogue **17**.



EXPERIMENTAL

General procedures. Optical rotations were measured at 25°C with a Polamat A automatic polarimeter, using a 5-cm 5-mL cell. NMR spectra were recorded at 25°C with a BRUKER AC-250F spectrometer. Chemical shifts (δ) are given in ppm relative to the signal for internal tetramethylsilane for ^1H NMR spectra and indirectly to CDCl_3 , δ 77.03 for ^{13}C NMR spectra. Assignments were performed on the basis of homo- and heteronuclear correlation experiments. The following notation was used to define the NMR signals, when more than one monosaccharide is present in the molecule: ' for galactose and '' for the sialyl moiety.

All compounds were purified by column chromatography on Kieselgel 60 or reverse phase C18 silica gel and fractions were monitored by TLC on Kieselgel 60 F₂₅₄ (Merck). Detection was effected by charring with a 5% solution of concentrated sulfuric acid in ethanol after examination under UV light. Evaporations were conducted under reduced pressure at 40°C (bath).

5-Azido-3-oxa-1-pentanol (1). Compound **1** was synthesized following a published procedure.^[18]

5-Azido-3-oxapentyl 2,3,4,6-tetra-O-acetyl- β -D-galactopyranoside (2). A solution of **1** (1.72 g, 13.1 mmol) and 2,3,4,6-tetra-O-acetyl α -D-galactopyranosyl bromide (5.85 g, 13.7 mmol) in anhydrous acetonitrile (10 mL) was stirred in the presence of molecular sieves (4Å, 5.85 g) at rt for 5 min. Then mercury(II) cyanide (3.56 g, 14.1 mmol) was added. After 12 h, the mixture was filtered and the filtrate concentrated under reduced pressure. The syrup was dissolved in dichloromethane (50 mL) and the solution was successively washed with 10% KI (15 mL \times 2), M NaHCO₃ (15 mL) and water (15 mL), dried (Na₂SO₄) and concentrated. The residue was purified by column chromatography (hexane/ethyl acetate 5:1 v/v) to give **2** (5.15 g, 83%); $[\alpha]_{\text{D}} + 33^\circ$ (*c* 1.0, chloroform); ^1H NMR (CDCl_3) δ 5.40 (m, 1H, H-4'), 5.21 (dd, 1H, $J_{2,3} = 9.6$ Hz, H-2'), 5.05 (dd, 1H, $J_{3,4'} = 3.3$ Hz, H-3'), 4.59 (d, 1H, $J_{1',2'} = 7.7$ Hz, H-1'), 4.19 (m, 2H, H-5', H-6'a), 3.89 (m, 1H, H-6'b), 3.8–3.6 (m, 6H, CH₂O), 3.37 (t, 2H, CH₂N₃), 2.18, 2.15, 2.05, 1.98 (4s, CH₃); ^{13}C NMR (CDCl_3) δ 169.8–168.9 (C = O), 100.7 (C-1), 70.6 (C-3), 70.0 (C-2), 69.9 (C-4), 69.0 (CH₂O), 68.9 (CH₂O), 68.7 (CH₂O), 66.6 (C-5), 61.2 (C-6), 50.7 (CH₂-N₃), 20.6–20.4 (CH₃).

Anal. Calcd for C₁₈H₂₇O₁₁N₃ (461.2): C, 46.85; H, 5.90; N, 9.11. Found: C, 46.99; H, 5.95; N 9.00.

5-Azido-3-oxapentyl 3,4-O-isopropylidene- β -D-galactopyranoside (3). To a solution of **2** (5.15 g, 11.2 mmol) in methanol (50 mL) was added sodium methoxide (200 mg) and the reaction was stirred for 1 h at rt. The reaction mixture was neutralized with Dowex-50 (H⁺) resin, filtered and the filtrate concentrated. The syrup was dissolved in 2,2-dimethoxypropane (184 mL) containing *p*-toluenesulfonic acid (430 mg). The mixture was stirred for 48 h. Triethylamine (0.5 mL) was then added and the reaction mixture was stirred for 15 min. The mixture was concentrated to dryness and coevaporated with toluene to remove traces of triethylamine. A solution of the crude product in 10:1 MeOH-H₂O (278 mL) was boiled under reflux for 3 h. The solvents were evaporated and the residue was purified by flash chromatography (ethyl



acetate/methanol 20:1 v/v) to give **3** (2.6 g, 70%); $[\alpha]_D + 23^\circ$ (*c* 1.0, chloroform); ^1H NMR (D_2O) δ 4.32 (d, 1H, $J_{1,2}$ 7.6 Hz, H-1), 4.15–3.80 (m, 6H), 3.80–3.52 (m, 6H, CH_2O), 3.40 (t, 2H, $\text{CH}_2\text{-N}_3$), 1.55, 1.30 (2s, 6H, CH_3); ^{13}C NMR (CDCl_3) δ 110.0 [$\text{C}(\text{CH}_3)_2$], 102.3 (C-1), 78.7 (C-3), 73.5 (C-4), 73.3 (C-5), 70.1, 69.6 (CH_2O), 68.3 (C-2), 61.9 (C-6), 50.3 ($\text{CH}_2\text{-N}_3$), 27.8, 26.1 (CH_3).

Anal. Calcd for $\text{C}_{13}\text{H}_{23}\text{O}_7\text{N}_3$ (333.3): C, 46.83; H, 6.96; N, 12.61. Found: C, 46.98; H, 7.01; N, 12.51.

5-Azido-3-oxapentyl 2,6-di-O-benzyl- β -D-galactopyranoside (4). Compound **3** (2.6 g, 7.8 mmol) was dissolved in *N,N*-dimethylformamide (17 mL) and sodium hydride (1.25 g, 31.2 mmol) was added at 0°C . After 30 min, benzyl chloride (3.6 mL, 31.2 mmol) was added at 0°C and the mixture was stirred for an additional 24 h at 25°C . Methanol was then added to destroy the excess sodium hydride and the mixture was concentrated. The resulting syrup was dissolved in dichloromethane (50 mL) and washed with water (15 mL), dried (Na_2SO_4) and the organic solvent evaporated. The residue was dissolved in 80% acetic acid solution (130 mL) and was boiled under reflux for 3 h. The mixture was concentrated to dryness and coevaporated with toluene. The residue was purified by flash chromatography (toluene/acetone 8:1 v/v) to give **4** (1.56 g, 65%); $[\alpha]_D + 13^\circ$ (*c* 1.0, chloroform); ^1H NMR (CDCl_3) δ 7.45–7.20 (m, 10H, Ph), 4.98 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 4.55 (AB, 2H, $J_{\text{AB}} = 12.1$ Hz, CH_2Ph), 4.52 (s, 2H, CH_2Ph), 4.05–3.75 (m, 6H), 3.70–3.51 (CH_2O), 3.35 (t, 2H, $\text{CH}_2\text{-N}_3$); ^{13}C NMR (CDCl_3) δ 103.8 (C-1), 78.9 (C-2), 75.0 (C-5), 74.8 (CH_2Ph), 74.1 (CH_2Ph), 71.2 (CH_2O), 70.9, 70.0 (C-3, C-4), 69.2, 69.0 (CH_2O), 68.5 (C-6), 50.1 ($\text{CH}_2\text{-N}_3$).

Anal. Calcd for $\text{C}_{24}\text{H}_{31}\text{O}_7\text{N}_3$ (473.5): C, 60.88; H, 6.60; N, 8.87. Found: C, 61.04; H, 6.66; N, 8.90.

Methyl (phenyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-D-glycero- α -D-galacto-2-nonulopyranosid) onate (5). Compound **5** was synthesized following a published procedure.^[13,19]

5-Azido-3-oxapentyl 3-O-[methyl (5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero- α -D-galacto-2-nonulopyranosyl) onate]-2,6-di-O-benzyl- β -D-galactopyranoside (6). A solution of compounds **4** (0.5 g, 1.06 mmol) and **5** (0.74 g, 1.27 mmol) and *N*-iodosuccinimide (0.43 g, 1.9 mmol) in dry acetonitrile (10 mL) was stirred in the presence of molecular sieves (3\AA , 0.75 g) for 15 min. The solution was cooled to -15°C under nitrogen. Trimethylsilyl triflate^[14] (0.1 mL, 0.57 mmol) was added and the mixture was stirred for 5 h. The mixture was diluted with dichloromethane (10 mL), filtered and the filtrate was successively washed with *M* NaHCO_3 (15 mL), aqueous sodium thiosulphate (15 mL) and water (15 mL), dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography (toluene/acetone 10:1 v/v) to give **6** (0.35 g, 35%); $[\alpha]_D + 12^\circ$ (*c* 1.0, chloroform); ^1H NMR (CDCl_3) δ 7.45–7.11 (m, 10H, Ph), 5.40 (m, 1H, H-8''), 5.32 (m, 2H, H-7'', NH), 4.88 (m, 1H, H-4'), 4.75 (AB, 2H, $J_{\text{AB}} = 12.6$ Hz, CH_2Ph), 4.61 (s, 2H, CH_2Ph), 4.51 (d, 1H, $J_{1,2'} = 7.5$ Hz, H-1'), 4.15 (m, 2H, H-3', H-5''), 3.85–3.52 (m, 10H, OCH_3 , H-2', CH_2O), 3.39 (t, 2H, $\text{CH}_2\text{-N}_3$), 2.52 (dd, 1H, H-3eq''), 2.18–1.82 (5s, 15H, CH_3).

Anal. Calcd for $\text{C}_{44}\text{H}_{58}\text{O}_{19}\text{N}_4$ (947.0): C, 55.79; H, 6.18. Found: C, 56.02; H, 6.22.



5-Azido-3-oxapentyl 3-O-(5-acetamido-3,5-dideoxy-D-glycero- α -D-galacto-2-nonulopyranosylonic acid)-2,6-di-O-benzyl- β -D-galactopyranoside (7). A solution of **6** (0.35 g, 0.46 mmol) in 0.74 M sodium methoxide (16.5 mL) was stirred at room temperature. After 24 h, a 0.2 M KOH solution (4.6 mL) was added. The reaction mixture was stirred for another 24 h, then neutralized with Dowex-50 (H⁺) resin, filtered and the filtrate was concentrated. The residue was purified by reverse phase column chromatography (H₂O/MeOH 5:1 v/v) to give **7** (0.27 g, 95%): $[\alpha]_D + 22^\circ$ (*c* 1.0, methanol); ¹H NMR (CD₃OD) δ 7.55–7.32 (m, 10H, Ph), 4.50 (d, 1H, $J_{1',2'} = 7.0$ Hz, H-1'), 3.75 (m, 2H, CH₂O), 3.65 (m, 1H, H-4''), 3.52–3.40 (m, 4H, CH₂O), 3.28 (CH₂-N₃), 2.65 (dd, 1H, $J_{3\text{eq}'',3\text{ax}''} = 12.9$ Hz, $J_{3\text{eq}'',4''} = 4.6$ Hz, H-3''eq), 1.98 (s, 3H, CH₃), 1.85 (dd, 1H, $J_{3\text{ax}'',4'} = 12.9$ Hz, H-3ax'').

Anal. Calcd for C₃₅H₄₈O₁₅N₄ (764.8): C, 54.97; H, 6.33; N, 7.33. Found: C, 54.78; H, 6.36; N, 7.37.

5-Amino-3-oxapentyl 3-O-(5-acetamido-3,5-dideoxy-D-glycero- α -D-galacto-2-nonulopyranosylonic acid)- β -D-galactopyranoside (8). A solution of compound **7** (0.25 g, 0.34 mmol) in methanol (10 mL) and acetic acid (0.1 mL) was hydrogenated in presence of 10% Pd-C (40 mg) for 12 h, then filtered and concentrated to give **8** (0.14g, 75% yield): $[\alpha]_D + 8^\circ$ (*c* 1.0, H₂O); ¹H NMR (D₂O) δ 4.49 (d, 1 H, H-1'), 3.15 (m, 2H, CH₂-NH₂), 2.61 (dd, 1H, $J_{3\text{eq}'',3\text{ax}''} = 12.9$ Hz, $J_{3\text{eq}'',4''} = 4.6$ Hz, H-3''eq), 1.98 (s, 3H, CH₃), 1.75 (dd, 1H, $J_{3\text{ax}'',4'} = 12.9$ Hz, H-3''ax).

5-(3-[(2,3-Dioctadecyloxypropyl)oxycarbonyl]propanoyl)-5-aza-3-oxapentyl 3-O-[(5-acetamido-3,5-dideoxy-D-glycero- α -D-galacto-2-nonulopyranosylonic acid)]- β -D-galactopyranoside (9). To a mixture of **8** (40 mg, 0.07 mmol) and 2,3-di-O-octadecyloxypropyl succinimidyl butanedioate (56.7 mg, 0.07 mmol) was added dimethyl sulfoxide (1 mL) and triethylamine (10 μ L, 0.07 mmol) and the solution was vigorously stirred at 60°C for 4 h. Then water (5 mL) was added and the resulting suspension was centrifuged. The supernatant liquid was discarded and the pellet was dissolved in chloroform/methanol 4:1 (v/v) and chromatographed using a small C 18 column eluting first with methanol-H₂O 1:1 (v/v), then with methanol and methanol-chloroform 3:1 (v/v). The fractions containing the desired compounds were concentrated: yield 50%; $[\alpha]_D + 4^\circ$ (*c* 1, MeOH); ¹H NMR (CD₃OD-CDCl₃) δ 4.45 (d, 1 H, $J_{1,2} = 7.0$ Hz, H-1') 3.30 (m, 2 H, CH₂NH₂), 2.78 (t, 2H, CH₂CO), 2.61 (m, 3 H, CH₂CO and H-3''eq), 1.89 (s, 3 H, Ac), 1.70 (m, 5 H, CH₂CH₂CO and H-3''ax), 1.30 (s, 60H, CH₂), 0.90 (t, 6H, CH₃).

Anal. Calcd for C₆₄H₁₁₇O₂₀N₂ (1234.6): C, 62.26; H, 9.55; N, 2.27. Found: C, 62.43; H, 9.60; N, 2.00.

4-O-(2,3,4,6-Tetra-O-acetyl- β -D-galactopyranosyl)-2,3,6-tri-O-acetyl- α -D-glucopyranosyl trichloroacetimidate (10). 2-Aminoethanol (8 mL) was added to a solution of lactose octaacetate (35.7 g, 52.6 mmol) in ethyl acetate (280 mL) and the solution was stirred for 24 h, then was washed with aqueous NaCl and water until neutral. The organic phase was dried (Na₂SO₄ anhydrous) and filtered, and the filtrate was concentrated and dried in vacuo. The resulting syrup was stirred in the presence of K₂CO₃ (17.6 g, 127.7 mmol) and trichloroacetonitrile (49.5 mL, 491.2 mmol) in dry dichloromethane (100 mL) for 24 h. Then the suspension was filtered through Celite and the filtrate concentrated in vacuo to give **10** (32.5 g, 85%): $[\alpha]_D + 7.0^\circ$ (*c* 1.0,



chloroform); ^1H NMR (CDCl_3) δ 8.65 (s, 1H, NH), 6.73 (d, 1H, $J_{1,2} = 2.2$ Hz, H-1), 6.13 (dd, 1H, $J_{2',3'} = 9.1$ Hz, $J_{3',4'} = 9.0$ Hz, H-3'), 5.78 (m, 2H, H-2', H-4'), 5.59 (dd, 1H, $J_{2,3} = 9.1$ Hz, H-2), 5.43 (dd, 1H, $J_{3,4} = 2.3$ Hz, H-3), 4.96 (d, 1H, $J_{1',2'} = 9.3$ Hz, H-1'), 4.62–3.71 (m, 6H, H-6a, H-6b, H-6'a, H-6'b, H-5, H-5'), 2.12–1.90 (7s, 21H, CH_3); ^{13}C NMR (CDCl_3) δ 170.2–168.8 (C = O), 160.5 (C-Cl₃), 100.8 (C-1'), 92.5 (C-1), 20.0–20.1 (CH_3).

5-Azido-3-oxapentyl 4-O-(2,3,4,6-tetra-O-acetyl- β -D-galactopyranosyl)-2,3,6-tri-O-acetyl- β -D-glucopyranoside (11). A solution of **10** (32.5 g, 41.7 mmol) and 5-azido-3-oxa-1-pentanol **1** (9.8 g, 75 mmol) in anhydrous dichloromethane (100 mL) was stirred in the presence of molecular sieves (4Å, 37 g). The solution was cooled to 0°C under nitrogen and trimethylsilyl triflate (7.5 mL, 41.7 mmol) was added. The mixture was stirred for 20 h, then diluted with dichloromethane (50 mL) and filtered. The filtrate was washed successively with 1 M NaHCO_3 (30 mL), and water (30 mL), dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography (toluene/acetone 10:1 v/v) to give **11** (18.6g, 60%): $[\alpha]_{\text{D}} + 26^\circ$ (c 1.0, chloroform); ^1H NMR (CDCl_3) δ 5.81 (dd, 1H, $J_{1',2'} = 9.3$ Hz, $J_{2',3'} = 9.1$ Hz, H-2'), 5.72 (m, 2H, H-2, H-4'), 5.48 (dd, 1H, $J_{3,4} = 9.2$ Hz, H-3), 5.37 (dd, 1H, $J_{2',3'} = 9.1$ Hz, $J_{3',4'} = 2.3$ Hz, H-3'), 4.88 (d, 1H, $J_{1,2} = 9.1$ Hz, H-1), 4.81 (d, 1H, H-1'), 4.52 (m, 2H, H-6a, H-6'a), 4.26 (dd, 1H, $J_{4,5} = 9.2$ Hz, H-4), 3.82–3.65 (m, 6H, CH_2O), 3.35 (m, 2H, CH_2N_3), 2.15–1.95 (7s, 21H, CH_3); ^{13}C NMR (CDCl_3) δ 170.2–168.8 (C = O), 100.8 (C-1'), 100.6 (C-1), 77.2 (C-4), 71.4 (C-3'), 70.4 (C-2, C-3), 70.0 (CH_2O), 69.9 (C-2', C-4'), 69.0 (C-5'), 68.9, 68.7 (CH_2O), 68.4 (C-5), 62.1 (C-6), 61.2 (C-6'), 20.6–20.4 (CH_3).

Anal. Calcd for $\text{C}_{30}\text{H}_{43}\text{O}_{19}\text{N}_3$ (749.7): C, 48.05; H, 5.78; N, 5.61. Found: C, 48.20; H, 5.82; N, 5.64.

5-Azido-3-oxapentyl 4-O-(3,4-O-isopropylidene- β -D-galactopyranosyl)- β -D-glucopyranoside (12). Compound **12** was synthesized following the procedure for compound **3** using **11** (7.29 g, 9.73 mmol), as starting material in methanol (150 mL) and 0.2 M sodium methoxide (80 mL). The syrup was dissolved in 2,2-dimethoxypropane (468 mL) containing *p*-toluenesulfonic acid (600 mg). The crude product was dissolved in 10:1 MeOH-H₂O (400 mL) and the residue was purified by flash chromatography (ethyl acetate/methanol 20:1 v/v) to give **12** (5.5 g, 70%): $[\alpha]_{\text{D}} + 21^\circ$ (c 1.0, chloroform); ^1H NMR (CD_3Cl) δ 4.35 (d, 1H, $J_{1',2'} = 7.6$ Hz, H-1'), 4.08 (d, 1H, $J_{1,2} = 7.6$ Hz, H-1), 4.05–3.65 (m, 10H), 3.65–3.50 (m, 6H, CH_2O), 3.41 (t, 2H, CH_2N_3), 1.55, 1.32 (2s, 6H, CH_3); ^{13}C NMR (CDCl_3) δ 110.2 [$\text{C}(\text{CH}_3)_2$], 102.3 (C-1'), 101.0 (C-1), 78.7 (C-3'), 74.8 (C-3), 74.5 (C-4), 73.5 (C-4'), 73.3 (C-5'), 72.3, 69.9 (CH_2O), 68.6, 68.3 (C-2', C-2), 62.0, 61.9 (C-6', C-6), 50.3 (CH_2N_3), 27.9–26.1 (CH_3).

Anal. Calcd for $\text{C}_{19}\text{H}_{33}\text{O}_{12}\text{N}_3$ (495.5): C, 46.04; H, 6.72; N, 8.48. Found: C, 46.15; H, 6.77; N, 8.51.

5-Azido-3-oxapentyl 4-O-(2,6-di-O-benzyl- β -D-galactopyranosyl)-2,3,6-tri-O-benzyl- β -D-glucopyranoside (13). Compound **13** was synthesized following the procedure for compound **4** using **12** (0.6 g, 1.21 mmol) in *N,N*-dimethylformamide (6 mL), sodium hydride (0.97 g, 24.2 mmol) and benzyl chloride (2.8 mL, 24.2 mmol). The resulting syrup was dissolved in dichloromethane (60 mL) and washed with water (20 mL), dried (Na_2SO_4) and evaporated. The residue was dissolved in 80% acetic acid aqueous solution (60 mL). The residue was purified by flash chromatography (toluene/



acetone 10:1 v/v) to give **13** (0.59 g, 60%): $[\alpha]_D + 11^\circ$ (c 1.0, chloroform); $^1\text{H NMR}$ (CDCl_3) δ 7.38–7.20 (m, 25H, Ph), 4.65 (d, 1H, $J_{1',2'} = 8.1$ Hz, H-1'), 4.60 (d, 1H, $J_{1,2} = 7.8$ Hz, H-1), 4.13 (dd, 1H, $J_{4',5'} = 1.4$ Hz, H-4'), 4.11 (dd, 1H, $J_{3',4'} = 7.7$ Hz, H-3'), 3.86 (dd, 1H, $J_{4,5} = 9.7$ Hz, H-4) 3.84 (m, 1H, $J_{5',6'a} = 7.1$ Hz, $J_{5',6'b} = 5$ Hz, H-5'), 3.76 (AB, 2H, 6a, 6b), 3.72–3.63 (m, 6H, CH_2O), 3.62 (dd, 1H, H-6'b), 3.60 (dd, 1H, $J_{3,4} = 9.3$ Hz, H-3), 3.59 (m, 1H, H-5), 3.47 (dd, 1H, $J_{6'a,6'b} = 10.3$ Hz, H-6'a), 3.40 (dd, 1H, $J_{2,3} = 9.1$ Hz, H-2), 3.32 (dd, 1H, $J_{2',3'} = 6.3$ Hz, H-2'), 3.25 (t, 2H, CH_2N_3); $^{13}\text{C NMR}$ (CDCl_3) δ 138.8–138.2 (Ph), 128.2–126.7 (Ph), 103.5 (C-1'), 102.4 (C-1), 82.4, 81.3, 79.8 (C-2, C-3, C-2'), 78.4 (C-4), 75.5, 74.2, 74.0, 73.9, 70.2 (CH_2Ph), 73.4, 72.4, 72.3, 71.6 (C-5, C-3', C-4', C-5'), 68.7 (C-6'), 67.9 (C-6), 50.4 (CH_2N_3).

Anal. Calcd for $\text{C}_{51}\text{H}_{57}\text{O}_{12}\text{N}_3$ (903.4): C, 67.74; H, 6.36; N, 4.65. Found: C, 67.86; H, 6.42; N, 4.50.

5-Azido-3-oxapentyl 4-O-[3-O-[methyl (5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero- α -D-galacto-2-nonulopyranosyl) onate]-(2,6-di-O-benzyl- β -D-galactopyranosyl)]-2,3,6-tri-O-benzyl- β -D-glucopyranoside (14**).** Compound **14** was synthesized following the procedure for compound **6** using **13** (0.06 g, 0.07 mmol), sialyl donor **5** (0.05 g, 0.08 mmol), *N*-iodosuccinimide (0.03 g, 0.12 mmol) in dry acetonitrile (1 mL), molecular sieves (3Å, 0.05 g) and trimethylsilyl triflate (2 μL , 0.012 mmol). The residue was purified by flash chromatography (toluene/acetone 10:1 v/v) to give **14** (0.04 g, 25%): $[\alpha]_D + 9^\circ$ (c 1.0, chloroform); $^1\text{H NMR}$ (CDCl_3) δ 7.40–7.15 (m, 25H, Ph), 5.75 (m, 1H, H-7''), 5.50 (m, 1H, H-8''), 5.32 (m, 1H, H-4''), 4.97 (dd, 2H, $J_{\text{AB}} = 12.5$ Hz, CH_2Ph), 4.87 (t, 2H, $J = 10.4$ Hz, CH_2Ph), 4.74 (dd, 2H, $J_{\text{AB}} = 10$ Hz, CH_2Ph), 4.69 (bb, 1H, NH), 4.62 (dd, 2H, $J_{\text{AB}} = 12.5$ Hz, CH_2Ph), 4.56 (d, 1H, $J_{1',2'} = 7.3$ Hz, H-1'), 4.42 (m, 3H, CH_2Ph , H-1), 4.25 (m, 1H, H-5''), 3.81 (s, 3H, OCH_3), 3.76–3.60 (m, 6H, CH_2O), 3.28 (t, 2H, $\text{CH}_2\text{-N}_3$), 2.55 (dd, 1H, $J_{3''\text{eq},3''\text{ax}} = 12.5$ Hz, $J_{3''\text{eq},4''} = 4.6$ Hz, H-3eq''), 2.20–1.88 (m, 13H, 4 CH_3 , H-3ax'').

Anal. Calcd for $\text{C}_{71}\text{H}_{86}\text{O}_{29}\text{N}_4$ (1459.5): C, 58.43; H, 5.94; N, 3.84. Found: C, 58.57; H, 6.00; N, 3.86.

5-Azido-3-oxapentyl 4-O-[3-O-(5-acetamido-3,5-dideoxy-D-glycero-D-galacto-2-nonulopyranosylonic acid)-2,6-di-O-benzyl- β -D-galactopyranosyl]-2,3,6-tri-O-benzyl- β -D-glucopyranoside (15**).** Compound **15** was synthesized following the procedure for compound **7** using **14** (0.04 g, 0.027 mmol) in 0.74 M sodium methoxide (2 mL) and 0.2 M KOH solution (0.5 mL). The residue was purified by column reverse phase chromatography ($\text{H}_2\text{O}/\text{MeOH}$ 5:1 v/v) to give **7** (0.03 g, 92%): $[\alpha]_D + 12^\circ$ (c 1.0, chloroform); $^1\text{H NMR}$ (CD_3OD) δ 7.45–7.11 (m, 25H, Ph), 4.51 (d, 1H, $J_{1',2'} = 7.8$ Hz, H-1'), 4.45 (m, 3H, CH_2Ph , H-1), 4.18 (d, 2H, $J_{\text{AB}} = 12.8$ Hz, CH_2Ph), 3.28 (t, 2H, $\text{CH}_2\text{-N}_3$), 2.65 (dd, 1H, $J_{3''\text{eq},3''\text{ax}} = 13.2$ Hz, $J_{3''\text{eq},4''} = 4.15$ Hz, H-3''eq), 1.95 (m, 4H, H-3''ax, CH_3).

Anal. Calcd for $\text{C}_{62}\text{H}_{76}\text{O}_{20}\text{N}_4$ (1197.3): C, 62.18; H, 6.40; N, 4.68. Found: C, 62.33; H, 6.36; N, 4.72.

5-Amino-3-oxapentyl 4-O-[3-O-(5-acetamido-3,5-dideoxy-D-glycero- α -D-galacto-2-nonulopyranosylonic acid)- β -D-galactopyranosyl]- β -D-glucopyranoside (16**).** A solution of compound **15** (30 mg, 0.025 mmol) in MeOH (2 mL) and CH_3COOH (0.01 mL) was hydrogenated in the presence of 10% Pd-C (25 mg) for 12 h, then filtered and

concentrated to give **16** in a 80% yield. $[\alpha]_{\text{D}}^{+4}$ (c 1.0, MeOH); $^1\text{H NMR}$ (D_2O) δ 4.49 (d, 1H, $J_{1,2} = 7.8$ Hz, H-1), 4.40 (d, 1H, $J_{1',2'} = 8.0$ Hz, H-1'), 3.18 (m, 2 H, CH_2NH_2), 2.61 (dd, 1 H, $J_{3''\text{eq},4''} = 3.2$, $J_{3''\text{eq},3''\text{ax}} = 13.0$ Hz, H-3''eq), 1.89 (s, 3 H, Ac), 1.75 (dd, 1 H, $J_{3''\text{ax},4''} = 13.0$ Hz, H-3''ax).

Anal. Calcd for $\text{C}_{27}\text{H}_{48}\text{O}_{20}\text{N}_2$ (720.7): C, 45.00; H, 6.71; N, 3.89. Found: C, 45.38; H, 6.75; N, 4.02.

5-(3-[(2,3-Dioctadecyloxypropyl)oxycarbonyl]propanoyl)-5-aza-3-oxapentyl 4-O-[3-O-(5-acetamido-3,5-dideoxy-D-glycero- α -D-galacto-2-nonulopyranosylonic acid)- β -D-galactopyranosyl]- β -D-glucopyranoside (17). To a mixture of **16** (35 mg, 0.025 mmol) and 2,3-di-*O*-octadecyloxypropyl succinimidyl butanedioate (19.8 mg, 0.025 mmol) was added dimethyl sulfoxide (1 mL) and triethylamine (3.6 μL , 0.025 mmol) and the solution was vigorously stirred at 60°C for 4 h. Then water (5 mL) was added and the resulting suspension was centrifuged. The supernatant liquid was discarded and the pellet was dissolved in chloroform/methanol 4:1 (v/v) and chromatographed in a small C18 column using methanol- H_2O , methanol and methanol-chloroform as eluent. The fractions containing the desired compounds were concentrated: yield 60%; $[\alpha]_{\text{D}}^{+4}$ (c 1.0, MeOH); $^1\text{H NMR}$ (CD_3OD) δ 4.52 (d, 1H, $J_{1,2} = 7.5$ Hz, H-1), 4.38 (d, 1H, $J_{1',2'} = 7.0$ Hz, H-1'), 3.30 (m, 2 H, CH_2NH_2), 2.78 (t, 2H, CH_2CO) 2.61 (m, 3 H, CH_2CO , H-3''eq), 1.89 (s, 3 H, Ac), 1.60 (m, 5 H, $\text{CH}_2\text{CH}_2\text{CO}$, H-3''ax), 1.30 (s, 60H, CH_2), 0.90 (t, 6H, CH_3).

Anal. Calcd for $\text{C}_{70}\text{H}_{127}\text{O}_{25}\text{N}_2$ (1396.8): C, 60.19; H, 9.16; N, 2.01. Found: C, 59.87; H, 9.29; N, 2.04.

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