Synthesis of 3-aminopropyl β-glycoside of sialyl-3´-lactose and derived neoglycoconjugates as a tumor vaccine prototype and artificial antigens for the control of immune response

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Starting from the biotechnologically available trisaccharide sialyl-3´-lactose, representing the carbohydrate portion of the tumor-associated ganglioside GM3, the corresponding 3-aminopropyl β-glycoside (**1**) and 3-(4-maleimidobutanoylamino)propyl glycoside were synthesized. The reaction of the latter with a thiolated derivative of the *Megathura crenulata* hemocyanine (KLH) afforded a carbohydrate—protein conjugate, a tumor vaccine prototype containing about 330 trisaccharide ligands attached to KLH. N-Stearoylation of ligand **1** gave the model neoglycolipid for comparative study of the activity of mono- and polyvalent immunogens and the natural ganglioside GM3. A monovalent conjugate, in which ligand **1** is linked to biotin through an oligo(ethylene glycol) spacer and a polyvalent conjugate with a polyacrylamide carrier were also prepared. These conjugates are meant as covering antigens to assess the specificity and efficiency of the immune response in the ELISA assay.

Key words: sialyl-3´-lactose, KLH, polyacrylamide, biotin, neoglycoconjugate, tumor vaccine.

On the surface of tumor cells, oligosaccharides with unique structures, the so-called tumor-associated carbohydrate antigens, which are absent on the surface of normal cells, are expressed.[1] This feature of cancer cells can be successfully used for typing or determination of the stage of the disease and mortality; it can also serve as a fundamental for the development of cancer vaccines (tumor vaccines). It is assumed that immunization of patients with the oligosaccharide antigen typical of a particular kind of cancer cells may induce the formation of cytotoxic antibodies able to recognize and destroy cancer cells. However, the development of such vaccine is held up by low immunogenicity of oligosaccharide antigens.[2] On the one hand, this is caused by the tolerance of the immune system to autoantigens, and on the other hand, by inability of oligosaccharide antigens to activate one of the components of the immune system, *viz.*, T-lymphocytes, which is necessary for antibody production. Therefore, in the development of synthetic tumor vaccines, oligosaccharide fragments are conjugated with natural highly immunogenic proteins capable of initiating T-cell immune response,[3] such as hemocyanin from *Megathura crenulata* (keyhole limpet hemocyanin, KLH) or tetanus toxin. Previously, KLH conjugates with tumor-associated antigens such as the tetrasaccharide Le^Y (see Ref. 4) and the ganglioside GD3 tetrasaccharide (see Ref. 5) have been prepared.

The ganglioside GM3, whose carbohydrate chain is represented by the β-anomer of the N-acetylated trisaccharide sialyl-3´-lactose, is a frequently encountered tumor-associated carbohydrate antigen, expressed in a large number of tumor types, such as malignant melanoma or neuroectodermal tumors.[6] However, the ganglioside GM3 is also present on the surface of normal cells; therefore, the use of this carbohydrate chain as a ligand for the design of specific conjugated tumor vaccine seems problematic. Meanwhile, the *N*-glycolyl analog of the ganglioside GM3 (*N*-glycolyl-GM3)[7] expressed specifically on tumor cells (melanoma, breast cancer, *etc.*)[8] has not been detected in normal tissues[9] and, hence, conjugates of trisaccharide *N*-glycolylneuraminyl-3´-lactose with

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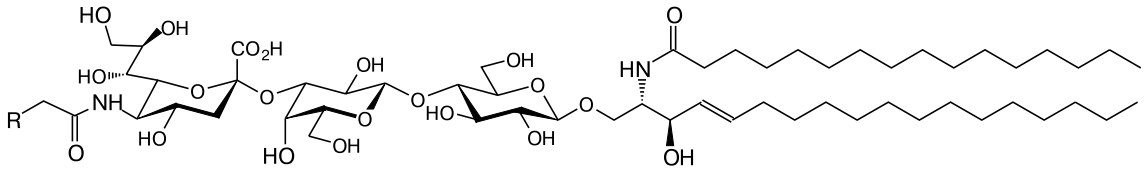
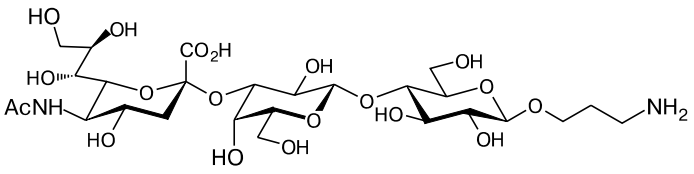


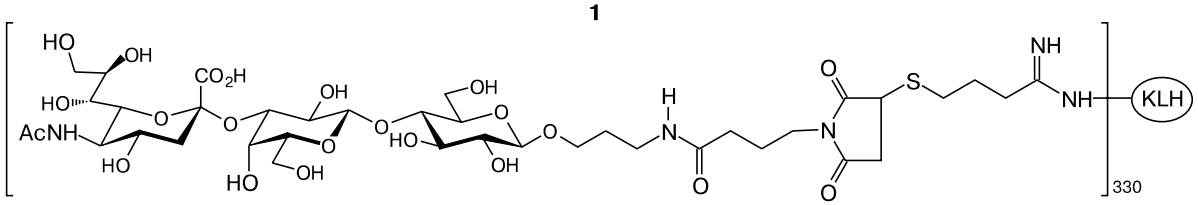
GM3 (R = H), *N*-glycolyl-GM3 (R = OH)

proteins appear promising candidates for the development of cancer vaccines.

This study is devoted to the synthesis of the β-(3-aminopropyl) glycoside of the trisaccharide sialyl-3´-lactose (**1**), which represents the carbohydrate part of the ganglioside GM3, and its use for the preparation of a tumor-vaccine prototype, a conjugate with a thiolated derivative of KLH **2**. The goal of this study is to optimize the conjugation conditions for their subsequent use in the synthesis of analogous derivatives based on the carbohydrate chain of the *N*-glycolyl-GM3 ganglioside. In this study, we prepared the *N*-stearoylated neoglycolipide **3**, a model compound for a comparative investigation of the activities of mono- and polyvalent immunogens and the natural ganglioside GM3. In addition, we synthesized a monovalent conjugate **4** in which ligand **1** is linked to



1



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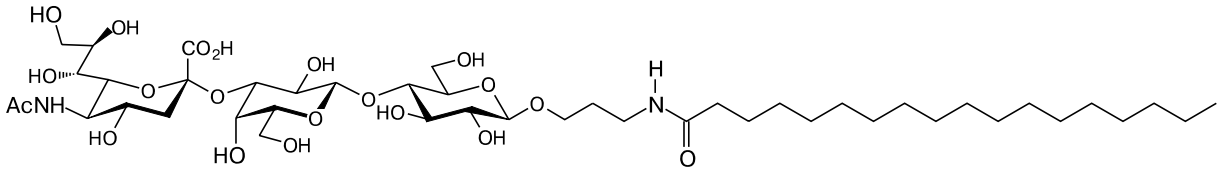

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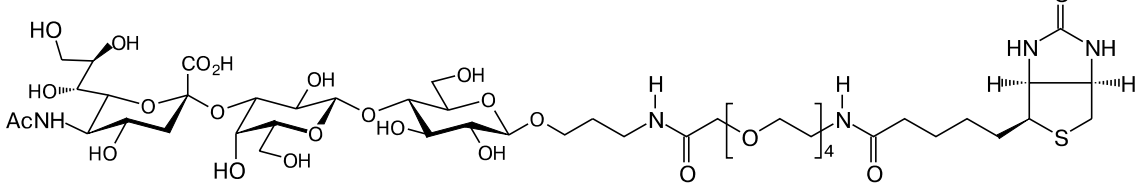

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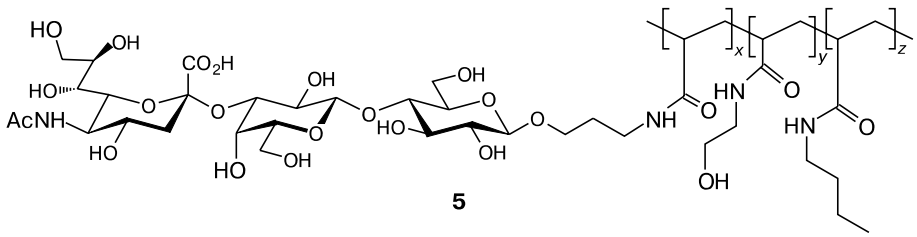

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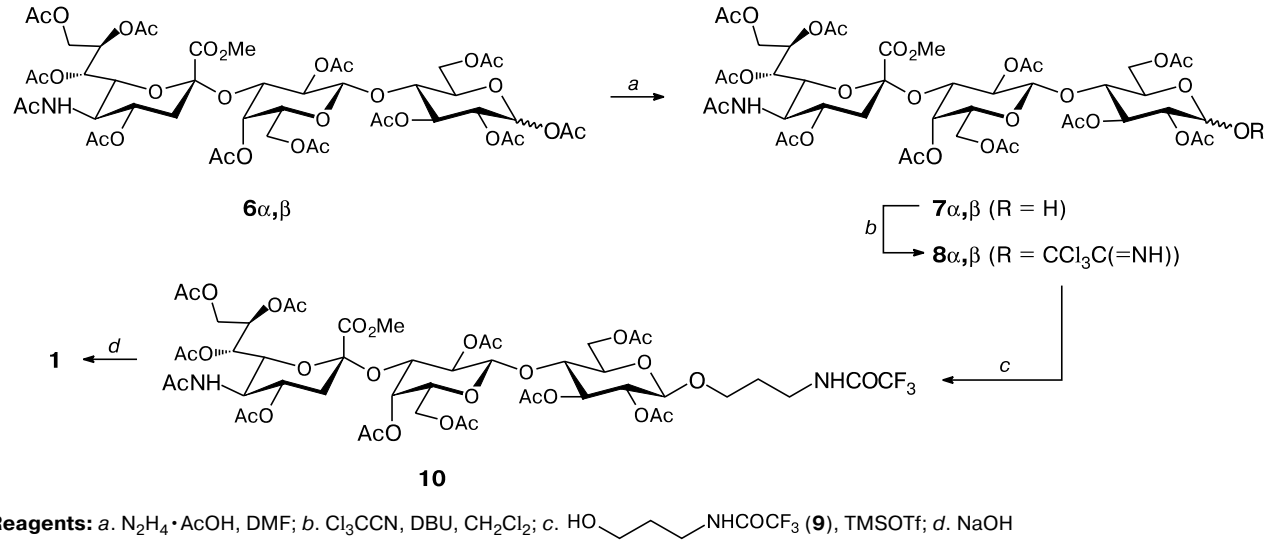
$x = 0.2$, $y = 0.4$, $z = 0.4$

Scheme 1



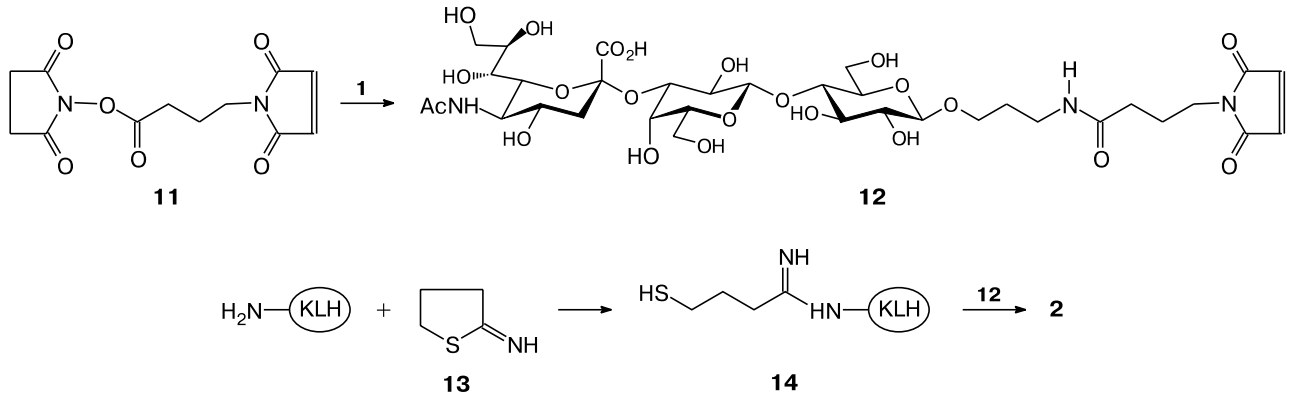
Reagents: *a*. $N_2H_4 \cdot AcOH$, DMF; *b*. Cl_3CCN, DBU, CH_2Cl_2; *c*. HO–(CH2)3–NHCOCF3 (**9**), TMSOTf; *d*. NaOH

biotin through an oligo(ethylene glycol) spacer and a polyvalent conjugate with polyacrylamide carrier **5** as covering antigens to control the specificity and efficiency of the immune response in the enzyme-linked immunosorbent assay (ELISA).

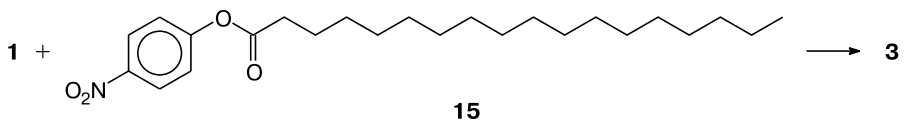
An anomeric mixture of sialyl-3′-lactose peracetates **6α,β** described in our previous study[10] served as the starting compound in the preparation of aminopropyl glycoside **1**. This mixture was synthesized from the biotechnologically available trisaccharide sialyl-3′-lactose.[11] Removal of the anomeric acetyl group from peracetate **6α,β** by treatment with hydrazine acetate (Scheme 1) afforded a mixture of hemiacetals **7α,β**, which was treated with trichloroacetonitrile in the presence of DBU to give an anomeric mixture of trichloroacetimidates **8α,β** (overall yield 69%). Glycosylation of 3-trifluoroacetamidopropan-1-ol (**9**) with the anomeric mixture of trichloroacetimidates **8α,β** in the presence of TMSOTf proceeded stereoselectively resulting in β-glycoside **10** in 52% yield. The β-configuration of the glucosidic bond in compound **10** was unambiguously confirmed by the corresponding spin coupling constant ($J_{1,2}$ = 8.0 Hz) in the ^{1}H NMR spectrum. Complete deprotection of compound **10** by alkaline hydrolysis resulted in target aminopropyl glycoside **1** in 88% yield.

The method used for conjugation of trisaccharide **1** with KLH was based on the addition of the thiol group to the maleimide double bond. For the introduction of the maleimide fragment, the amino group of trisaccharide **1** was acylated by active ester of 4-maleimidobutyric acid **11** to give maleimide derivative **12** in 85% yield (Scheme 2). To increase the number of thiol groups in the protein molecule able to react with maleimide, free amino groups of the lysine residues in native KLH were modified by 2-iminothiolane (**13**) to give thiolated KLH **14** (see Ref. 4b). The conjugation of maleimide derivative **12** with

Scheme 2



Scheme 3



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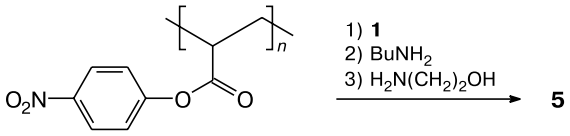
Scheme 4



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thiolated protein **14** for 24 h yields conjugate **2**, which was separated from monomeric components by dialysis. Quantitative determination of neuraminic acid in conjugate **2** was carried out by the Svennerholm method[12] and of the protein content, using the bicinchoninic acid reagent. According to the results of analysis, conjugate **2** contained ~330 trisaccharide residues per protein molecule.

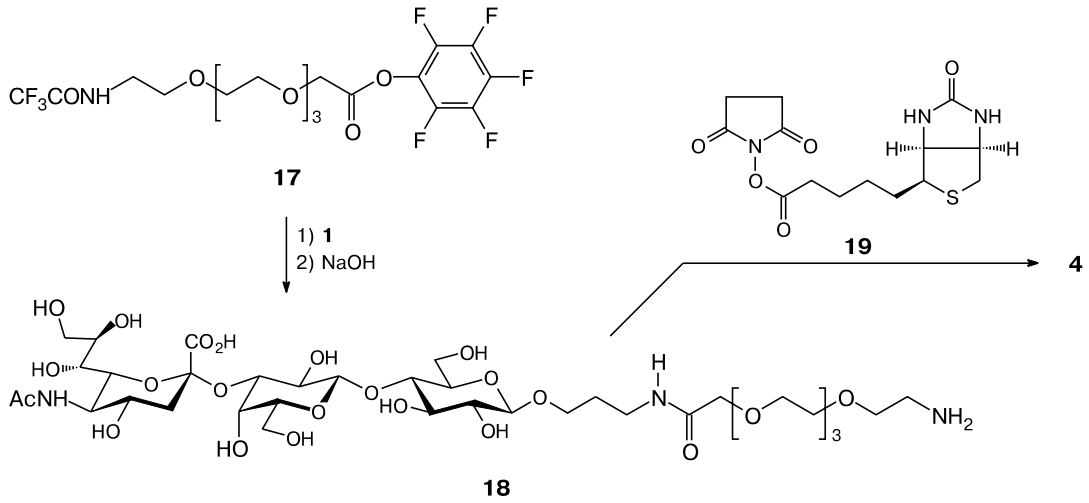
Acylation of the amino group in trisaccharide **1** with *p*-nitrophenyl stearate (**15**) (see Ref. 13) gave neoglycolipid **3** (Scheme 3) (yield 80%), which mimics the natural ganglioside GM3 and was necessary for comparative analysis of the immunogenicities of mono- and polyvalent antigens.

To study the antibody specificity against conjugate **2** by ELISA, we synthesized two covering antigens, namely, a polyvalent conjugate with polyacrylamide and a monovalent biotinylated derivative starting from trisaccharide **1**. Poly(*p*-nitrophenyl acrylate) (**16**) (see Ref. 14) was treated with 20 mol.% of aminopropyl glycoside **1** and then with 40 mol.% of *n*-butylamine to impart hydrophobic properties to the conjugate in order to enhance its adsorption on the surface of polystyrene plates for the ELISA assay (Scheme 4). The nonconsumed *p*-nitrophenyl ester groups were subsequently converted into *N*-(2-hydroxyethyl)amide groups by treatment with excess ethanolamine. This gave polyacrylamide conjugate **5**.

Biotin is known to form an exceptionally strong complex with streptavidin; therefore, biotinylation of oligosaccharide ligands is used to bind them to streptavidin-coated ELISA plates. Preliminarity, the spacer group of trisaccharide **1** was elongated by a hydrophilic tetraethylene glycol fragment in order to ensure the optimum distance between the oligosaccharide ligand and the plate surface, which is essential for recognition of the carbohydrate ligand by antibodies. For this purpose, the amino group in compound **1** was acylated by active ester **17** prepared from tetraethylene glycol (the synthesis of compound **17** will be published elsewhere). The removal of the *N*-trifluoroacetyl group by alkaline hydrolysis gave amino derivative **18**. Its acylation with active biotin ester **19** afforded the target biotinylated trisaccharide **4**.

Immunological assays have shown that immunization of mice by conjugate **2** induced a humoral immune response; the titers of IgG antibodies against the trisaccharide sialyl-3′-lactose were essentially higher than upon immunization with the natural ganglioside GM3.[15] (Detailed results of the immunization by conjugate **2** and

Scheme 5



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19

18

neoglycolipid **3** and study of the specificity of the immue response using biotinylated derivative **4** and polyacrylamide conjugate **5** will be published elsewhere.)

Experimental

Triethylamine, *N*-hydroxysuccinimide ester of 4-maleimidobutyric acid (Fluka), trichloroacetonitrile, TMSOTf, hydrazine acetate (Acros), DBU (Merck), KLH, 2-iminothiolane hydrochloride, and *N*-hydroxysuccinimide ester of biotin (Sigma) were used as received. Dichloromethane was distilled twice from P_2O_5 and then from CaH_2 under argon. Dimethylformamide was distilled *in vacuo* (oil pump) from phthalic anhydride and then from CaH_2. Molecular sieves (MS 4 Å) were activated by calcination at 180 °C *in vacuo* (oil pump) for 2 h. The ^1H and ^{13}C NMR spectra were recorded on Bruker DRX-500 and Bruker AM-300 instruments at 25 °C. The signals were assigned by homo- and heteronuclear 2D COSY, TOCSY, and HSQC spectra. Optical rotation was measured on a PU-07 digital polarimeter (State Research and Engineering Center of Scientific Instrument Making) at 18—25 °C. Thin layer chromatography was carried out on silica gel Kieselgel-60 plates (Merck), and compounds were visualized by treatment with a 10% (v/v) solution of orthophosphoric acid in ethanol or (for amines) with a ninhydrin solution (3 g L^{-1} in a butanol—acetic acid mixture, 30 : 1) followed by heating at ~150 °C. Column chromatography was carried out on Silica gel 60 (Merck), 0.040—0.063 mm. A Knauer 98.00 refractometer was used as the detector for gel chromatography.

[Methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-α,β-D-glycopyranose (7α,β). A solution of acetate mixture **6α,β** (0.876 g, 0.79 mmol) and hydrazine acetate (0.109 g, 1.18 mmol) in anhydrous DMF (10 mL) was stirred for 3 h at ~20 °C, diluted with chloroform (50 mL), washed with water (50 mL) and brine (20 mL), and concentrated; then DMF was co-evaporated with toluene. Column chromatography of the residue (toluene—methanol, 95 : 5 → 9 : 1) gave 0.786 g (89%) of anomeric mixture **7α,β** in 2 : 1 ratio as a white foam, R_f 0.18 (acetone—toluene, 2 : 3), $[\alpha]_D$ +18 (*c* 1, $CHCl_3$). ^1H NMR ($CDCl_3$), δ: 5.55—5.47 (m, H(8)-Neu, **7α,β**, H(3)-Glc, **7α**); 5.41 (dd, H(7)-Neu, **7α,β**, $J_{7,6}$ = 2.7 Hz, $J_{7,8}$ = 9.4 Hz); 5.37 (d, H(1)-Glc, **7α**, $J_{1,2}$ = 3.5 Hz); 5.22 (dd, H(3)-Glc, **7β**, $J_{3,2}$ = $J_{3,4}$ = 9.5 Hz); 5.12 (d, NH-Neu, **7α,β**, $J_{5,NH}$ = 10.0 Hz); 4.94 (dd, H(2)-Gal, **7α,β**, $J_{2,1}$ = 8.0 Hz, $J_{2,3}$ = 10.0 Hz); 4.92—4.87 (m, H(4)-Gal, **7α,β**, H(4)-Neu, **7α,β**); 4.86 (dd, H(2)-Glc, **7α**, $J_{2,1}$ = 3.5 Hz, $J_{2,3}$ = 10.5 Hz); 4.80 (dd, H(2)-Glc, **7β**, $J_{2,1}$ = $J_{2,3}$ = 9.5 Hz); 4.73 (d, H(1)-Glc, **7β**, $J_{1,2}$ = 9.5 Hz); 4.67 (d, H(1)-Gal, **7α,β**, $J_{1,2}$ = 8.0 Hz); 4.52 (m, H(3)-Gal, **7α,β**); 4.47 (d, H(6a)-Glc, **7β**); 4.43 (m, H(6a)-Glc, **7α**, H(9a)-Neu, **7α,β**); 4.25—4.14 (m, H(5)-Glc, **7α**, H(6b)-Glc, **7α,β**); 4.08—3.97 (m, H(5)-Neu, **7α,β**, H(6a)-Gal, H(6b)-Gal, **7α,β**, H(9b)-Neu, **7α,β**); 3.88—3.82 (m, H(4)-Glc, **7α,β**, H(5)-Gal, **7α,β**, OCH_3 **7α,β**); 3.66 (m, H(5)-Glc, **7β**); 3.64 (m, H(6)-Neu, **7α,β**); 2.58 (dd, H_{eq}(3)-Neu, **7α,β**, $J_{3eq,4}$ = 4.4 Hz, $J_{3eq,3ax}$ = 12.7 Hz); 1.82—2.30 (m, CH_3CO, **7α,β**); 1.78 (dd, H_{ax}(3)-Neu, **7α,β**, $J_{3ax,3eq}$ = $J_{3ax,4}$ = 12.5 Hz). ^{13}C NMR ($CDCl_3$), δ: 170.0—170.8 (CH_3CO, CH_3CON); 168.0 (C(1)-Neu, **7α,β**); 101.0 (C(1)-Gal, **7α,β**); 96.8 (C(2)-Neu, **7α,β**); 95.2 (C(1)-Glc, **7β**); 90.2 (C(1)-Glc, **7α**); 76.3 (C(4)-Glc, **7α**); 76.2 (C(4)-Glc, **7β**); 73.6 (C(2)-Glc, **7β**); 73.0 (C(5)-Glc, **7β**); 72.5 (C(3)-Glc, **7β**); 72.0 (C(6)-Neu, **7α,β**); 71.4 (C(2)-Glc, **7α**, C(3)-Gal, **7α,β**); 70.4 (C(5)-Gal, **7α,β**); 70.1 (C(3)-Glc, **7α**); 69.9 (C(2)-Gal, **7α,β**); 69.3 (C(4)-Neu, **7α,β**); 68.2 (C(5)-Glc, **7α**); 67.8 (C(7)-Neu, **7α,β**); 67.3 (C(4)-Gal, **7α,β**); 66.8 (C(8)-Neu, **7α,β**); 62.2 (C(6)-Gal, **7α,β**); 62.1 (C(6)-Glc, **7α,β**); 61.5 (C(9)-Neu, **7α,β**); 53.1 ($COOCH_3$, **7α,β**); 49.2 (C(5)-Neu, **7α,β**); 37.4 (C(3)-Neu, **7α,β**); 23.1—20.5 (CH_3CO). Found (%): C, 49.25; H, 5.79; N, 1.27. $C_{44}H_{61}NO_{29}$. Calculated (%): C, 49.49; H, 5.79; N, 1.31.

[Methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-α,β-D-glucopyranosyl trichloroacetimidate (8α,β). Trichloroacetonitrile (0.53 mL, 5.3 mmol) was added to a solution of hemiacetal **7α,β** (0.565 g, 0.529 mmol) in anhydrous CH_2Cl_2 (10 mL), the mixture was cooled to −30 °C, DBU (0.08 mL) was added, and the mixture was stirred for 1 h at −30 °C. The mixture was applied onto a column with silica gel (50 g) prewashed with toluene containing 0.1% of triethylamine. Elution with a toluene—acetone mixture (7 : 3, +0.1% of triethylamine) gave 0.492 g (77%) of anomeric mixture **8α,β** in a 2 : 1 ratio as a white foam, R_f 0.35 (acetone—toluene, 2 : 3). ^1H NMR ($CDCl_3$), δ: 6.50 (d, H(1)-Glc, **8α**, $J_{1,2}$ = 3.3 Hz); 5.95 (d, H(1)-Glc, **8β**, $J_{1,2}$ = 8.2 Hz); 5.55 (dd, H(3)-Glc, **8α**, $J_{3,2}$ = $J_{3,4}$ = 9.6 Hz); 5.50 (m, H(8)-Neu, **8α,β**); 5.40 (dd, H(7)-Neu, **8α,β**, $J_{7,6}$ = 2.7 Hz, $J_{7,8}$ = 9.4 Hz); 5.30 (dd, H(3)-Glc, **8β**, $J_{3,2}$ = $J_{3,4}$ = 8.9 Hz); 5.16 (d, NH-Neu, **8β**, $J_{5,NH}$ = 10.0 Hz); 5.12 (d, NH-Neu, **8α**, $J_{5,NH}$ = 10.0 Hz); 5.08 (dd, H(2)-Glc, **8α**, $J_{2,1}$ = 3.5 Hz, $J_{2,3}$ = 10.0 Hz); 4.96 (dd, H(2)-Gal, **8α,β**, $J_{2,1}$ = $J_{2,3}$ = 8.5 Hz); 4.90 (m, H(4)-Gal, **8α,β**, H(4)-Neu, **8α,β**, H(2)-Glc, **8β**, H(6a)-Glc, **8β**); 4.68 (d, H(1)-Gal, **8α,β**, $J_{1,2}$ = 8.0 Hz); 4.52 (dd, H(3)-Gal, **8α,β**, $J_{2,3}$ = 8.5 Hz, $J_{3,4}$ = 2.7 Hz); 4.46—4.39 (m, H(6a)-Glc, **8α**, H(9a)-Neu, **8α,β**); 4.23 (dd, H(6b)-Glc, **8α**, $J_{6b,6a}$ = 12.0 Hz, $J_{6b,5}$ = 4.0 Hz); 4.23 (m, H(6b)-Glc, **8β**); 4.17—3.91 (m, H(4)-Glc, **8α,β**, H(5)-Glc, **8α**, H(6a)-Gal, H(6b)-Gal, **8α,β**, H(5)-Neu, **8α,β**, H(9b)-Neu, **8α,β**); 3.88—3.82 (m, H(5)-Gal, **8α,β**, OCH_3, **8α,β**); 3.68 (m, H(5)-Glc, **8β**); 3.64 (H(6)-Neu, **8α,β**); 2.58 (dd, H_{eq}(3)-Neu, **8α,β**, $J_{3eq,4}$ = 4.4 Hz, $J_{3eq,3ax}$ = 12.7 Hz); 1.82—2.30 (m, CH_3CO, **8α,β**); 1.78 (dd, H_{ax}(3)-Neu, **8α,β**, $J_{3ax,3eq}$ = $J_{3ax,4}$ = 12.5 Hz). ^{13}C NMR ($CDCl_3$), δ: 170.0—170.8 (CH_3CO, CH_3CON); 155.9 ($OC(CCl_3)N$); 168.0 (C(1)-Neu, **8α,β**); 101.2 (C(1)-Gal, **8α,β**); 96.8 (C(2)-Neu, **8α,β**); 96.3 (C(1)-Glc, **8β**); 93.2 (C(1)-Glc, **8α**); 75.9 (C(4)-Glc, **8α,β**); 72.0 (C(5)-Glc, **8β**, C(6)-Neu, **8α,β**); 71.6 (C(3)-Gal, **8α,β**); 71.0 (C(2)-Glc, **8β**, C(5)-Glc, **8α**); 70.6 (C(5)-Gal, **8α,β**); 70.1 (C(2)-Glc, **8α**); 70.0 (C(3)-Glc, **8α**, C(2)-Gal, **8α,β**); 69.4 (C(4)-Neu, **8α,β**); 68.5 (C(3)-Glc, **8β**); 67.8 (C(7)-Neu, **8α,β**); 67.3 (C(4)-Gal, **8α,β**); 66.8 (C(8)-Neu, **8α,β**); 62.2 (C(6)-Gal, **8α,β**); 62.0 (C(9)-Neu, **8α,β**); 61.7 (C(6)-Glc, **8α,β**); 53.1 ($COOCH_3$, **8α,β**); 49.2 (C(5)-Neu, **8α,β**); 37.4 (C(3)-Neu, **8α,β**); 23.1—20.5 (CH_3CO). Found (%): C, 44.69; H, 5.07; N, 2.37. $C_{46}H_{61}Cl_3N_2O_{29}$. Calculated (%): C, 45.57; H, 5.07; N, 2.31.

3-Trifluoroacetamidopropyl [methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (10). A solution of imidate **8α,β** (0.482 g, 0.398 mmol) and 3-trifluoroacetamidopropan-1-ol **9** (0.136 g, 0.795 mmol) in anhydrous

CH_2Cl_2 (3 mL) with activated molecular sieves MS-4A (100 mg) was stirred for 2 h under argon and cooled to −20 °C. Then TMSOTf (0.1 mL, 0.54 mmol) was added and the mixture was stirred for 2 h at this temperature. After completion of the reaction, Et_3N (0.05 mL) was added, the temperature was brought to ~20 °C, and the mixture was filtered through Celite and concentrated. The residue was chromatographed on silica gel (toluene→toluene—acetone, 1 : 1) to give 250 mg (52%) of compound **10** as a white foam, $[\alpha]_D$ −7.8 (*c* 1, $CHCl_3$), R_f 0.13 (acetone—toluene, 2 : 3). ^{1}H NMR ($CDCl_3$), δ: 5.52 (m, 1 H, H(8)-Neu); 5.33 (dd, 1 H, H(7)-Neu, $J_{7,6} < 1$, $J_{7,8} = 9.1$ Hz); 5.18 (d, 1 H, NH-Neu, $J_{5,NH} = 8.0$ Hz); 5.15 (dd, 1 H, H(3)-Glc, $J_{3,2} = J_{3,4} = 9.3$ Hz); 4.90 (dd, 1 H, H(2)-Gal, $J_{2,1} = 8.0$ Hz, $J_{2,3} = 9.8$ Hz); 4.84 (d, 1 H, H(4)-Gal, $J_{3,4} = 2.7$ Hz, $J_{4,5} < 1$ Hz); 4.82 (m, 1 H, H(4)-Neu); 4.80 (dd, 1 H, H(2)-Glc, $J_{2,3} = 9.3$ Hz); 4.66 (d, 1 H, H(1)-Gal, $J_{1,2} = 8.0$ Hz); 4.46—4.51 (m, 2 H, H(3)-Gal, H(6a)-Glc); 4.43 (d, 1 H, H(1)-Glc, $J_{1,2} = 8.0$ Hz); 4.38 (dd, 1 H, H(9a)-Neu, $J_{9a,8} = 2$ Hz, $J_{9a,9b} = 12.5$ Hz); 4.13 (dd, 1 H, H(6b)-Glc, $J_{6a,6b} = 12.0$ Hz, $J_{6b,5} = 4.9$ Hz); 3.89—4.40 (m, 4 H, H(5)-Neu, H(6a)-Gal, H(6b)-Gal, H(9b)-Neu); 3.80—3.87 (m, 3 H, H(4)-Glc, H_a-OCH$_2$CH$_2$CH$_2$N, H(5)-Gal); 3.79 (s, 3 H, COOCH$_3$); 3.67 (m, 1 H, H_b-OCH$_2$CH$_2$CH$_2$N); 3.61 (d, 1 H, H(6)-Neu, $J_{6,7} < 1$ Hz, $J_{6,5} = 10.8$ Hz); 3.57 (m, 1 H, H(5)-Glc); 3.45 (m, 1 H, H_a-OCH$_2$CH$_2$CH$_2$N); 3.35 (m, 1 H, H_b-OCH$_2$CH$_2$CH$_2$N); 2.54 (dd, 1 H, H_{eq}(3)-Neu, $J_{3eq,4} = 4.4$ Hz, $J_{3eq,3ax} = 12.7$ Hz); 1.70—2.20 (m, 33 H, CH$_3$CO); 1.82 (m, 2 H, OCH$_2$CH$_2$CH$_2$N); 1.62 (dd, 1 H, H_{ax}(3)-Neu, $J_{3ax,3eq} = J_{3ax,4} = 12.5$ Hz). ^{13}C NMR ($CDCl_3$), δ: 169.6—170.5 (CH_3CO, CH_3CON); 167.9 (C(1)-Neu); 100.9 (C(1)-Gal); 100.6 (C(1)-Glc); 96.8 (C(2)-Neu); 75.9 (C(4)-Glc); 73.2 (C(3)-Glc); 73.0 (C(5)-Glc); 71.9 (C(2)-Glc); 71.7 (C(6)-Neu); 71.3 (C(3)-Gal); 70.5 (C(5)-Gal); 69.9 (C(2)-Gal); 69.3 (C(4)-Neu); 68.1 (OCH$_2$CH$_2$CH$_2$N); 67.7 (C(8)-Neu); 67.2 (C(4)-Gal); 67.0 (C(7)-Neu); 62.3 (C(9)-Neu); 61.9 (C(6)-Glc); 61.4 (C(6)-Gal); 53.1 (COOCH$_3$); 49.0 (C(5)-Neu); 37.8 (OCH$_2$CH$_2$CH$_2$N); 37.4 (C(3)-Neu); 28.3 (OCH$_2$CH$_2$CH$_2$N); 23.1—20.5 (CH_3CO). Found (%): C, 47.64; H, 5.53; N 2.32. $C_{49}H_{67}F_3N_2O_{30}$. Calculated (%): C, 48.20; H, 5.53; N, 2.29.

3-Aminopropyl [(5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl)onic acid]-(2→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (1). A solution of protected derivative **10** (240 mg, 0.196 mmol) in a mixture of methanol (8.3 mL) and 3 *M* aqueous NaOH (1.7 mL) was kept at room temperature for 6 h, AcOH was added to pH 7, and the mixture was concentrated. The residue was subjected to gel chromatography on a column with TSK HW-40(S) gel (1.5×90 cm) in 0.05 *M* NH_4HCO_3, the carbohydrate-containing fractions were concentrated and freeze-dried from water to give 120 mg (88%) of compound **1**, $[\alpha]_D$ +2.0 (*c* 1, H_2O), R_f 0.43 (butanol—propanol—hydrochloric acid—acetonitrile—methanol—water, 2 : 2 : 2 : 3 : 3 : 3). ^{1}H NMR (D_2O), δ: 4.43 (d, 1 H, H(1)-Gal, $J_{2,1} = 10.0$ Hz); 4.41 (d, 1 H, H(1)-Glc, $J_{2,1} = 10.0$ Hz); 4.01 (dd, 1 H, H(3)-Gal, $J_{3,2} = 9.9$ Hz, $J_{3,4} = 2.5$ Hz); 3.95 (m, 1 H, H_a-OCH$_2$CH$_2$CH$_2$NH$_2$); 3.90 (dd, 1 H, H(6a)-Glc, $J_{6a,6b} = 12.0$ Hz, $J_{6a,5} < 1$); 3.87 (d, 1 H, H(4)-Gal, $J_{3,4} = 2.5$ Hz, $J_{4,5} < 1$ Hz); 3.82—3.68 (m, 5 H, H(5)-Neu, H(6)-Neu, H(9a)-Neu, H_b-OCH$_2$CH$_2$CH$_2$NH$_2$, H(6a)-Glc, H(6b)-Glc); 3.68—3.58 (m, 5 H, H(4)-Neu, H(2)-Gal, H(5)-Gal, H(6a)-Gal, H(6b)-Gal); 3.58—3.44 (m, 6 H, H(7)-Neu, H(8)-Neu, H(9b)-Neu, H(3)-Glc, H(4)-Glc, H(5)-Glc); 3.24 (dd, 1 H, H(2)-Glc, $J_{2,3} = 10.0$ Hz); 3.07 (t, 2 H, OCH$_2$CH$_2$CH$_2$NH$_2$, $J = 7.0$ Hz); 2.66 (dd, 1 H, H_{eq}(3)-Neu, $J_{3e,3a} = 4.5$ Hz, $J_{3e,4} = 12.5$ Hz); 1.94 (s, 3 H, CH$_3$CO); 1.92 (m, 2 H, OCH$_2$CH$_2$CH$_2$NH$_2$); 1.70 (t, 1 H, H_{ax}(3)-Neu, $J_{3a,3e} = J_{3a,4} = 12.5$ Hz). ^{13}C NMR (D_2O), δ: 176.2 (CH_3CON); 175.0 (C(1)-Neu); 103.8 (C(1)-Gal); 103.3 (C(1)-Glc); 101.0 (C(2)-Neu); 79.4 (C(4)-Glc); 76.6 (C(3)-Gal); 76.3 (C(5)-Gal); 75.9 (C(8)-Neu); 75.4 (C(5)-Glc); 74.0 (C(3)-Glc); 73.9 (C(2)-Glc); 72.9 (C(6)-Neu); 70.5 (C(2)-Gal); 69.4 (C(4)-Neu); 69.3 (C(7)-Neu); 69.0 (OCH$_2$CH$_2$CH$_2$NH$_2$); 68.7 (C(4)-Gal); 63.8 (C(9)-Neu); 62.1 (C(6)-Gal); 61.2 (C(6)-Glc); 52.9 (C(5)-Neu); 40.8 (C(3)-Neu); 38.7 (OCH$_2$CH$_2$CH$_2$NH$_2$); 27.8 (OCH$_2$CH$_2$CH$_2$NH$_2$); 23.2 (CH_3CON).

3-(4-Maleimidobutanoylamino)propyl [(5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl)onic acid]-(2→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (12). Active ester **11** (44 mg, 0.158 mmol, 2.1 equiv.) was added to a suspension of aminopropyl glycoside **1** (51 mg, 0.074 mmol) in anhydrous DMF (2 mL), the mixture was stirred for 10 min, and a 2% solution of Et_3N in anhydrous DMF (0.8 mL) was added in portions. The reaction mixture was concentrated *in vacuo* (oil pump) at ~20 °C, and the residue was subjected to gel chromatography on a column with Sephadex G-15 (45×2.5 cm) in water. Freeze-drying of carbohydrate-containing fractions from water gave 54 mg (85%) of compound **12**. ^{1}H NMR (D_2O), δ: 6.83 (s, 2 H, CH=CH); 4.51 (d, 1 H, H(1)-Gal, $J_{2,1} = 7.8$ Hz); 4.45 (d, 1 H, H(1)-Glc, $J_{2,1} = 7.9$ Hz); 4.09 (dd, 1 H, H(3)-Gal, $J_{3,2} = 9.9$ Hz, $J_{3,4} = 2.6$ Hz); 3.97 (dd, 1 H, H(6a)-Glc, $J_{6a,6b} = 12.0$ Hz, $J_{6a,5} < 1$ Hz); 3.94 (d, 1 H, H(4)-Gal, $J_{3,4} = 3.1$ Hz, $J_{4,5} < 1$ Hz); 3.92 (m, 1 H, H_a-OCH$_2$CH$_2$CH$_2$NH); 3.89—3.76 (m, 5 H, H(5)-Neu, H(9a)-Neu, H_b-OCH$_2$CH$_2$CH$_2$NH, H(5)-Gal, H(6b)-Glc); 3.76—3.60 (m, 9 H, H(4)-Neu, H(6)-Neu, H(8)-Neu, H(9b)-Neu, H(3)-Glc, H(4)-Glc, H(5)-Glc, H(6a)-Gal, H(6b)-Gal); 3.60—3.54 (m, 2 H, H(7)-Neu, H(2)-Gal); 3.51 (t, 2 H, NHCOCH$_2$CH$_2$CH$_2$N, $J = 6.8$ Hz); 3.29 (dd, 1 H, H(2)-Glc, $J_{2,3} = J_{2,1} = 7.9$ Hz); 3.22 (m, 2 H, OCH$_2$CH$_2$CH$_2$NH); 2.74 (dd, 1 H, H_{eq}(3)-Neu, $J_{3e,3a} = 12.4$ Hz, $J_{3e,4} = 4.5$ Hz); 2.23 (t, 2 H, NHCOCH$_2$CH$_2$CH$_2$N, $J = 7.0$ Hz); 2.02 (s, 3 H, CH$_3$CO); 1.88 (m, 2 H, NHCOCH$_2$CH$_2$CH$_2$N); 1.79 (m, 2 H, OCH$_2$CH$_2$CH$_2$NH); 1.78 (dd, 1 H, H_{ax}(3)-Neu, $J_{3a,3e} = J_{3a,4} = 12.5$ Hz). ^{13}C NMR (D_2O), δ: 176—172 (CH_3CON, C(1)-Neu, COCH=CHCO, NHCOCH$_2$CH$_2$CH$_2$N); 135.6 (CH=CH); 103.9 (C(1)-Gal); 103.3 (C(1)-Glc); 101.0 (C(2)-Neu); 79.6 (C(4)-Glc); 76.7 (C(3)-Gal); 76.4 (C(5)-Gal); 76.0 (C(8)-Neu); 75.6 (C(5)-Glc); 74.1 (C(3)-Glc); 74.1 (C(2)-Glc); 73.0 (C(6)-Neu); 70.6 (C(2)-Gal); 69.5 (C(4)-Neu); 69.4 (C(7)-Neu); 69.1 (OCH$_2$CH$_2$CH$_2$NH); 68.7 (C(4)-Gal); 63.9 (C(9)-Neu); 62.2 (C(6)-Gal); 61.4 (C(6)-Glc); 52.9 (C(5)-Neu); 40.9 (C(3)-Neu); 38.2 (OCH$_2$CH$_2$CH$_2$NH); 34.4 (NHCOCH$_2$CH$_2$CH$_2$N); 37.6 (NHCOCH$_2$CH$_2$CH$_2$N); 29.6 (OCH$_2$CH$_2$CH$_2$NH); 24.9 (NHCOCH$_2$CH$_2$CH$_2$N); 23.3 (CH$_3$CO).

Thiolation of KLH. 2-Iminothiolane hydrochloride **13** (27 mg) was added under argon to a mixture of a solution of KLH (7 mL, ~37.1 mg of protein) in a PBS buffer (5 mL) (containing 1 *mM* $MgCl_2$ and 15 *mM* NaN_3, pH 7.2) and a buffer (7 mL) containing 50 *mM* triethanolamine, 0.15 *mM* NaCl, and 5 *mM* EDTA (pH 8.0). The mixture was kept for 2 h at ~20 °C and dialyzed under Ar against a buffer solution

(250 mL) containing 0.1 *M* sodium phosphate, Na_2H_2edta (0.1 mol), NaCl (0.15 mol), and 0.01% NaN_3 (buffer solution A, pH 7.2). The dialysis was repeated three times to give a solution of thiolated KLH **14**, which was used directly for conjugation with maleimide derivative **12**.

Conjugation of maleimide 12 with thiolated KLH 14. A solution of maleimide **12** (14.5 mg, 0.017 mmol) in buffer solution A (1 mL) was added with gentle shaking under Ar to the obtained solution of thiolated protein **14**. The mixture was kept for 24 h at ~20 °C and dialyzed under Ar against a PBS buffer (250 mL) containing 0.01% NaN_3. Dialysis was repeated three times to give a solution of conjugate **2**. The content of neuraminic acid in conjugate **2** was determined by the Svennerholm method and the protein content was determined by spectrophotometry using the bicinchoninic acid reagent (BCA Protein assay kit, Pierce) according to the manufacturer´s protocol.

3-(Octadecanoylamino)propyl [(5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl)onic acid]-(2→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (3). *p*-Nitrophenyl stearate **15** (3.2 mg, 0.008 mmol) and Et_3N (0.01 mL) were added to a solution of trisaccharide **1** (5.9 mg, 0.0078 mmol) in anhydrous DMF (0.4 mL), and the resulting mixture was stirred for 16 h at ~20 °C. The solvent was evaporated *in vacuo* (oil pump), and the residue was chromatographed on a column with Sephadex LH-20 (2×45 cm) in methanol. Carbohydrate-containing fractions were concentrated, and the residue was dissolved in water (1.5 mL) and applied onto the cartridge Sep-Pak C-18. The cartridge was washed with water (10 mL) and methanol (10 mL). The methanolic eluate was concentrated, and the residue was freeze-dried from water to give 6 mg (80%) of amide **3**, R_f 0.68 ($CHCl_3$—MeOH—water, 5 : 5 : 1). ^{1}H NMR (D_2O), δ: 2.15 (t, 2 H, NHCOC$\underline{H}_2$, J = 7.4 Hz); 1.57 (m, 2 H, NHCOCH$_2$C$\underline{H}_2$); 1.26 (m, 30 H, 15 CH_2); 0.87 (t, 3 H, CH_3, J = 7.1 Hz); the carbohydrate part of the spectrum did not virtually differ from the spectrum of starting **1**.

Conjugate of trisaccharide 1 with polyacrylamide (5). A solution of poly(*p*-nitrophenyl acrylate) **16** (10 mg, 0.052 mmol of nitrophenyl residues), aminopropyl glycoside **1** (7 mg, 0.01 mmol), and Et_3N (0.015 mL) in dry DMF (0.3 mL) was stirred for 24 h at 40 °C, then $BuNH_2$ (1.5 mg, 0.02 mmol) was added, the mixture was stirred for 48 h at ~20 °C, ethanolamine (0.02 mL) was added, and the mixture was stirred for an additional 48 h at 40 °C. The reaction mixture was concentrated at 40 °C *in vacuo* (oil pump). The product was isolated by chromatography on a column with Sephadex LH-20 in aqueous MeCN (1 : 1). Freeze-drying gave 9.5 mg of functionalized polyacrylamide **5**.

3-(14-Amino-3,6,9,12-tetraoxatetradecanoylamino)propyl [(5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl)onic acid]-(2→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (18). A solution of pentafluorophenyl 14-trifluoroacetamido-3,6,9,12-tetraoxatetradecanoate (**17**) (28 mg, 0.054 mmol) in anhydrous DMF (0.2 mL) and Et_3N (87 mg, 0.86 mmol) in anhydrous DMF (0.1 mL) were added with stirring to a suspension of aminopropyl glycoside **1** (15 mg, 0.022 mmol) in anhydrous DMF (0.2 mL). After complete dissolution of the starting **1**, water (0.2 mL) was added, and the solution was concentrated *in vacuo* (oil pump) at ~20 °C. The residue was dissolved in 0.5 *M* NaOH (1.2 mL) and kept for 10 min, then AcOH was added to pH 6.5—7, and the solution was concentrated and chromatographed on a column with TSK HW-40(S) in 0.05 *M* NH_4HCO_3. Freeze-drying of carbohydrate-containing fractions gave 17 mg (85%) of amino derivative **17**, R_f 0.38 (butanol—propanol—hydrochloric acid—acetonitrile—methanol—water, 2 : 2 : 2 : 3 : 3 : 3). ^{1}H NMR (D_2O), δ: 4.50 (d, 1 H, H(1)-Gal, $J_{2,1}$ = 7.5 Hz); 4.45 (d, 1 H, H(1)-Glc, $J_{2,1}$ = 8.0 Hz); 4.08 (dd, 1 H, H(3)-Gal, $J_{3,2}$ = 9.9 Hz, $J_{3,4}$ = 2.9 Hz); 4.05 (s, 2 H, NHCOC$\underline{H}_2$O); 3.95 (dd, 1 H, H(6a)-Glc, $J_{6a,6b}$ = 12.7 Hz, $J_{6a,5}$ = 1.1 Hz); 3.94 (m, 1 H, OC$\underline{H}_2$CH$_2$CH$_2$NH); 3.93 (d, 1 H, H(4)-Gal, $J_{3,4}$ = 3.2 Hz, $J_{4,5}$ < 1 Hz); 3.88—3.79 (m, 3 H, H(5)-Neu, H(9a)-Neu, H(5)-Gal); 3.79 (dd, 1 H, H(6b)-Glc, $J_{6b,6a}$ = 12.7 Hz, $J_{6b,5}$ = 4.8); 3.75—3.65 (m, 20 H, OC$\underline{H}_2$C$\underline{H}_2$O, OC$\underline{H}_2$CH$_2$NH$_2$, OC$\underline{H}_2$CH$_2$CH$_2$NH, H(6a)-Gal, H(6b)-Gal, H(4)-Neu, H(6)-Neu); 3.65—3.58 (m, 6 H, H(3)-Glc, H(4)-Glc, H(8)-Neu, H(9b)-Neu); 3.52—3.58 (m, 4 H, H(2)-Gal, H(5)-Gl), H(7)-Neu); 3.34 (t, 2 H, OCH$_2$CH$_2$C$\underline{H}_2$NH); 3.28 (dd, 1 H, H(2)-Glc, $J_{2,3}$ = $J_{2,1}$ = 8.5 Hz); 3.12 (t, 2 H, OCH$_2$C$\underline{H}_2$NH$_2$); 2.73 (dd, 1 H, H_{eq}(3)-Neu, $J_{3e,3a}$ = 12.5 Hz, $J_{3e,4}$ = 4.6 Hz); 2.00 (s, 3 H, C$\underline{H}_3$CON); 1.84 (m, 2 H, OCH$_2$C$\underline{H}_2$CH$_2$NH); 1.78 (dd, 1 H, H_{ax}(3)-Neu, $J_{3a,3e}$ = $J_{3a,4}$ = 12.5 Hz). ^{13}C NMR (D_2O), δ: 176—172 ($CH_3\underline{C}$ON, $CH_2\underline{C}$ON, C(1)-Neu); 103.9 (C(1)-Gal); 103.3 (C(1)-Glc); 101.0 (C(2)-Neu); 79.8 (C(4)-Glc); 76.7 (C(3)-Gal); 76.4 (C(5)-Gal); 76.0 (C(8)-Neu); 75.6 (C(5)-Glc); 74.1 (C(3)-Glc); 74.1 (C(2)-Glc); 73.0 (C(6)-Neu); 73.0—68.5 (C(8), O$\underline{C}H_2\underline{C}H_2O, O\underline{C}H_2CH_2NH_2$, O$\underline{C}H_2CH_2CH_2$NH, C(4)-Neu); 71.3 (NHCO$\underline{C}H_2$O); 70.6 (C(2)-Gal); 69.4 (C(7)-Neu); 68.5 (C(4)-Gal); 63.9 (C(9)-Neu); 62.2 (C(6)-Gal); 61.4 (C(6)-Glc); 52.9 (C(5)-Neu); 40.9 (C(3)-Neu); 40.3 (OCH$_2\underline{C}$H$_2$NH$_2$); 38.2 (OCH$_2$CH$_2\underline{C}$H$_2$NH); 30.4 (OCH$_2\underline{C}$H$_2$CH$_2$NH); 23.3 ($\underline{C}$H$_3$CO).

3-(14-Biotinylamino-3,6,9,12-tetraoxatetradecanoylamino)propyl [(5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl)onic acid]-(2→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (4). Triethylamine (0.03 mL) was added to a solution containing compound **18** (9.4 mg, 0.010 mmol) and active ester **19** (5 mg, 0.014 mmol) in anhydrous DMF (0.3 mL), the mixture was stirred for 10 min and concentrated *in vacuo* (oil pump) at ~20 °C. The residue was chromatographed on a column with TSK HW-40(S) in 0.05 *M* NH_4HCO_3. Freeze-drying of carbohydrate-containing fractions gave 9.4 mg (82%) of biotinylated product **5**. R_f 0.58 (butanol—propanol—hydrochloric acid—acetonitrile—methanol—water, 2 : 2 : 2 : 3 : 3 : 3). ^{1}H NMR (D_2O), δ: 4.58 (dd, 1 H, H(2)-biotin, $J_{2,3}$ = 7.8 Hz, $J_{2,1cis}$ < 1, $J_{2,1trans}$ = 4.2 Hz); 4.50 (d, 1 H, H(1)-Gal, $J_{2,1}$ = 7.5 Hz); 4.44 (d, 1 H, H(1)-Glc, $J_{2,1}$ = 7.8 Hz); 4.40 (dd, 1 H, H(3)-biotin, $J_{3,2}$ = 7.8 Hz, $J_{3,4}$ = 4.5 Hz); 4.08 (dd, 1 H, H(3)-Gal, $J_{3,2}$ = 9.9 Hz, $J_{3,4}$ = 2.7 Hz); 4.04 (s, 2 H, NHCOC$\underline{H}_2$O); 3.99—3.89 (m, 3 H, H(4)-Gal, H(6a)-Glc, OC$\underline{H}_2$CH$_2$CH$_2$NH); 3.89—3.76 (m, 6 H, H(5)-Gal, H(5)-Neu, H(6)-Neu, H(9a)-Neu, H(6b)-Glc); 3.51—3.76 (m, 25 H, OC$\underline{H}_2$C$\underline{H}_2$O, OC$\underline{H}_2$CH$_2$NH, OCH$_2$CH$_2$C$\underline{H}_2$NH, H(2)-Gal, H(6a)-Gal, H(6b)-Gal, H(4)-Neu, H(7)-Neu, H(8)-Neu, H(9b)-Neu, H(3)-Glc, H(4)-Glc, H(5)-Glc); 3.39—3.26 (6 H, OCH$_2$C$\underline{H}_2$NH, H(2)-Glc, H(4)-biotin); 2.96 (dd, 1 H, H(1)-*trans*-biotin, $J_{1cis,1trans}$ = 13.0 Hz, $J_{1trans,2}$ = 4.2 Hz); 2.75 (dd, 1 H, H(1)-*cis*-biotin, $J_{1cis,1trans}$ = 13.0 Hz, $J_{1cis,2}$ < 1 Hz); 2.73 (dd, 1 H, H_{eq}(3)-Neu, $J_{3e,3a}$ = 12.5 Hz, $J_{3e,4}$ = 4.3 Hz); 2.25 (dd, 2 H, H(8)-biotin, $J_{7,8}$ = $J_{7,6}$ = 7.1 Hz, $J_{2,1cis}$ < 1 Hz); 2.00 (s, 3 H, C$\underline{H}_3$CON); 1.84 (m, 2 H, NHCOCH$_2$C$\underline{H}_2$CH$_2$N); 1.78 (dd, 1 H, H_{ax}(3)-Neu, $J_{3a,3e}$ = $J_{3a,4}$ = 12.5 Hz); 1.73—1.50

(m, 2 H, H(5)-biotin, H(3)-biotin); 1.38 (m, 1 H, H(6)-biotin). ^{13}C NMR (D_2O), δ: 178.0 ($OCH_2CH_2N\underline{C}O$); 176.2 ($CH_3\underline{C}ON$); 174.8 (C(1)-Neu); 173.5 ($N\underline{C}OCH_2O$); 151.2 (HNC(O)NH); 103.9 (C(1)-Gal); 103.3 (C(1)-Glc); 101.0 (C(2)-Neu); 79.7 (C(4)-Glc); 76.8 (C(3)-Gal); 76.3 (C(6)-Neu); 76.0 (C(5)-Glc); 75.6 (C(8)-Neu); 74.2 (C(2)-Gal, C(3)-Gal); 73.0 (C(5)-Gal); 71.5—70.1 (C(10), $O\underline{C}H_2\underline{C}H_2O$, $O\underline{C}H_2CH_2NH$, C(2)-Gal, $NHCO\underline{C}H_2O$); 69.5 (C(4)-Neu); 69.4 (C(7)-Gal); 69.0 ($O\underline{C}H_2CH_2CH_2NH$); 68.7 (C(4)-Glc); 63.9 (C(9)-Neu); 63.3 (C(3)-biotin); 62.2 (C(6)-Gal); 61.4 (C(6)-Glc, C(2)-biotin); 56.5 (C(4)-biotin); 52.9 (C(5)-Neu); 40.9 (C(3)-Neu, C(1)-biotin); 40.1 ($OCH_2\underline{C}H_2NH$); 37.2 ($OCH_2CH_2\underline{C}H_2NH$); 36.7 (C(8)-biotin); 29.7 ($OCH_2\underline{C}H_2CH_2NH$); 29.1 (C(6)-biotin); 28.9 (C(5)-biotin); 26.3 (C(7)-biotin); 23.3 ($\underline{C}H_3CO$).

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