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Glycosyl-transferase Catalyzed Assemblage of Sialyl-Lewis^x-saccharopeptides

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Abstract—A series of glycohexopyranuronic acids are coupled to glucosamines to give 'disaccharides' which have the natural *N*-acetyl group of the glcNAc-moiety replaced by various sugar acids (→saccharopeptides). These saccharopeptides are surprisingly good substrates for β(1-4)galactosyl-transferase, α(2-3)sialyl-transferase, and fucosyl-transferase VI. The enzymes transfer successively galactose, sialic acid, and fucose from the corresponding donors onto these acceptor substrates—despite the far reaching alterations—regio- and stereospecifically in the expected manner to yield a new class of compounds, the sialyl-Lewis^x-saccharopeptides.

Introduction

Besides peptides, carbohydrates have been identified as major determinants in a variety of physiologically important adhesion phenomena.[1,2] These findings have spawned an intensive search for carbohydrates as potential anti-adhesion therapeutics.[3,4] Only recently has a hybrid-class of compounds (→ the peptidosaccharides or saccharopeptides) been designed[5,6] which is composed of hexosamine moieties and glycuronic acids. These compounds combine features of oligosaccharides (e.g. the glycosidic linkage) and peptides (e.g. the conformationally restricted amide-bond).

Our interest in this quite unusual hybrid-class of compounds stems from the search for analogues of the natural sialyl-Lewis^a (SLe^a)- and sialyl-Lewis^x (SLe^x)-tetrasaccharides. These sugars are involved in the early phases of pathological inflammatory responses.[7] The replacement of some of the monosaccharides of the natural tetrasaccharide or the introduction of additional lipophilic[8] or heteroaromatic moieties[9] has lead to SLe^x-mimetics with improved and/or altered physiological properties.[10] An efficient and versatile approach to assemble such complex structures would be the use of various glycosyl-transferases.

Results and Discussion

The pathway for the preparation of the novel SLe^x-saccharopeptides, which have the natural *N*-acetyl group of the glcNAc-subunit replaced by a number of glycuronamides, is outlined in Scheme 1 below.

The common precursor **1** is obtained from per-*O*-acetylated, *N*-allyloxycarbonyl-protected glucosamine[11] and 9-hydroxynonanoic acid methyl ester as a hydrophobic aglycon[12] according standard glycosylation protocols.[11] The allyloxycarbonyl protection is subsequently removed with a Pd^o-catalyst and diethyl malonate as an allylscavenger[13] to give amine **2**.

A series of α- and β-methylglycohexopyranuronic acids **3** are obtained from a TEMPO-oxidation of the corresponding glycosides.[14] These sugar acids **3** are then coupled to amine **2** employing standard peptide coupling protocols[15] to yield the saccharopeptides **4** after a final deacetylation (see Table 1). This amide-coupling can also be accomplished with the fully deacetylated amine **2a**, but has not been optimized as sufficient material of all the saccharopeptides **4a**–**e** has been easily produced for the ensuing enzymatic glycosylations.

Despite numerous known glycosylation procedures, the chemical synthesis of a complex oligosaccharide is still not a routine undertaking[16] and is often plagued by inefficient protecting group manipulations and

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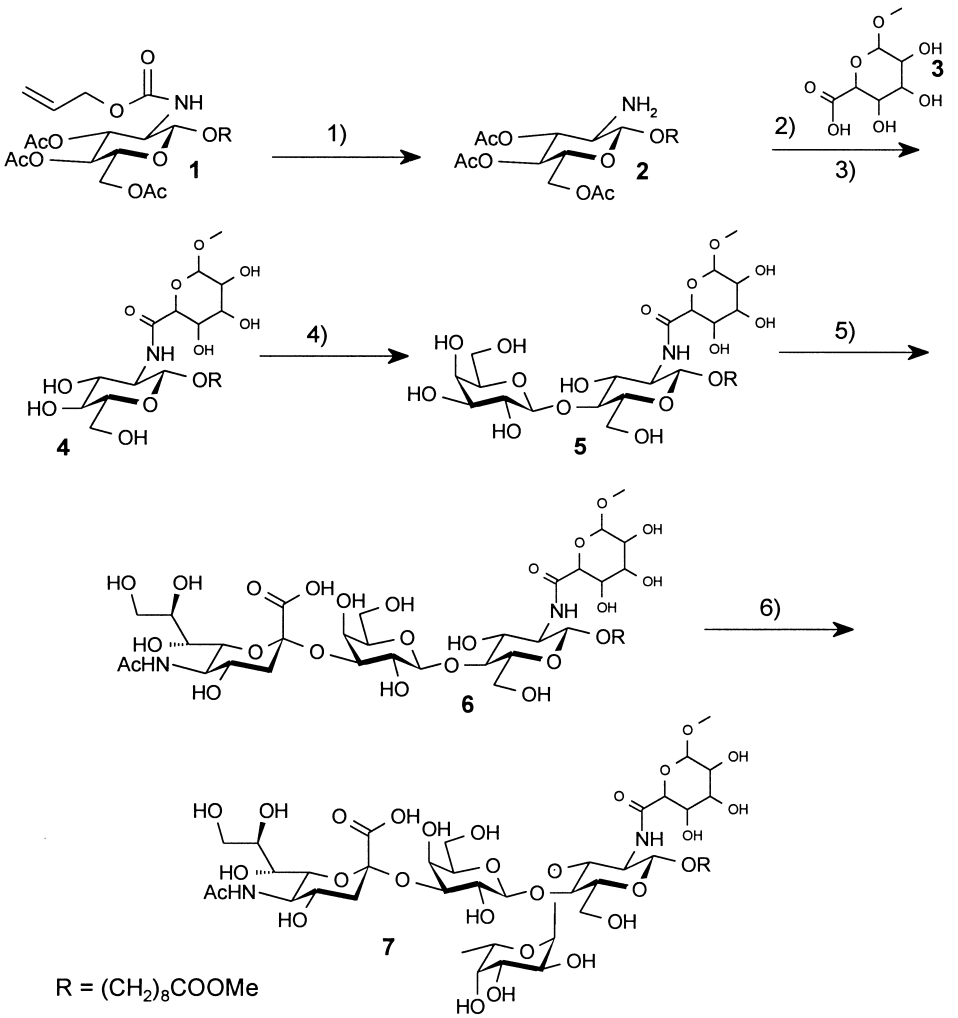
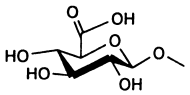
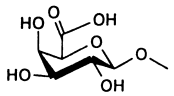
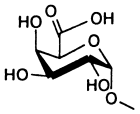
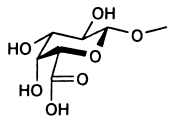
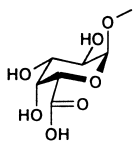


Scheme 1. (1) THF, diethyl malonate, dppb, $Pd_2(dba)_3$, 82%; (2) DMF, HBPyU, TEA, glycuronic acids **3**; (3) MeOH, MeONa; (4) β(1-4)gal-t, UDP-gal; (5) α(2-3)sia-t, CMP-sia; (6) fuc-t VI, GDP-fuc; yields steps 2–6 see Table 1.

cumbersome separations of the desired oligosaccharide from the glycosylation mixtures. A modern alternative to this approach is offered by the use of recombinant glycosyl-transferases.[17] These enzymes transfer a monosaccharide unit from a nucleotide-activated donor substrate regio- and stereospecifically onto an OH-group of a growing oligosaccharide chain in vivo and in vitro.

We could recently show that a number of recombinant glycosyl-transferases can be used to assemble non-natural SLe^x-analogues.[9,18] Consequently, we incubated (see Table 1) the non-natural saccharopeptides **4** first with commercial β(1-4)galactosyl-transferase and the donor UDP-galactose. Comparative NMR-investigations (COSY, TOCSY, HSQC and HMBC) of the starting sugar **4a–e** and the isolated materials **5a–e** show the attachment of an additional galactose-unit in all cases. Indicative for a β-linked galactose in **5** (see Table 2) are signals at about 105 ppm in the ^{13}C NMR-spectra for the galactose C-1 atoms corresponding to additional doublets in the ^1H NMR-spectra at about 4.5 ppm (J~6.8 Hz) for galactose H-1 atoms. The large down-field shift of the C-4 signal of the glucosamide moiety from about 72 ppm in **4** to about 80–81 ppm in **5** shows that galactose is linked to the 4-OH group.[19] The saccharopeptides **5** are then incubated with CMP-sialic acid[20] and recombinant α(2-3)sialyl-transferase.[21] This enzyme transfers a sialic acid unit from CMP-sia onto the 3-OH group of a terminal galactose in an α-mode in vivo. This same mode of action is observed with the non-natural substrates **5** to give the saccharopeptides **6** selectively. The presence of a sia-unit is again extracted from signals in the ^{13}C NMR-spectra of the compounds **6**. Additional signals at approximately 101 ppm and

Table 1. Yields of enzymatic glycosylations

Acid **3a–e**	Structure of coupled acid	Couple[a]% **4a–e**	Galactose%(mg) **5a–e**	Sialic%(mg) **6a–e**	Fucose%(mg) **7a–e**
a β-D-glc		31	53 (10.7)	95 (16.6)	60 (11.0)
b β-D-gal		49	61 (16.2)	100 (17.1)	32 (5.5)
c α-D-gal		38	58 (16.5)	87 (19.7)	25 (4.8)
d β-L-gal		26	96 (36.5)	35 (17.1)	35 (4.8)
e α-L-gal		12[b]	40 (12.1)	62 (10.5)	73 (8.3)

[a]Combined yield of coupling step and deacetylation.
[b]Coupled to the fully deacetylated amino-sugar **2a** with HBTU (see Experimental).

Table 2. Selected NMR-data of saccharopeptides

Entry	Fucose		Sialic acid		Galactose			Glucosamide		Sugar acid	
	C-1	C-6	C-2	C-3	H-3	C-1	C-3	C-1	C-4	OCH_3	C-1
5a					3.69	104.0	73.5	101.7	79.7	58.3	104.4
5b					3.50	104.8	74.6	102.0	80.8	58.0	105.8
5c					3.52	105.1	74.9	102.2	81.2	56.3	101.8
5d					3.52	104.8	74.8	102.3	80.4	57.8	105.8
5e					3.52	104.5	73.7	101.7	79.5	55.1	101.1
6a			101.2	41.9	4.04	104.8	77.5	102.2	81.2	57.4	105.2
6b			100.9	41.9	4.04	105.0	77.5	102.2	81.3	57.6	105.9
6c			101.2	41.9	4.04	105.0	77.6	102.2	81.4	56.1	101.8
6d			100.9	42.1	4.04	104.9	77.6	102.3	81.1	57.8	106.0
6e			101.1	42.1	4.07	104.9	77.7	102.5	81.1	56.3	101.9
7a	100.1	16.4	100.7	42.0	4.03	103.7	77.7	102.1	76.3	57.7	105.4
7b	100.3	16.5	n.r[a]	42.2	4.02	103.8	77.9	101.7	75.5	57.8	105.8
7c	100.2	16.6	100.7	42.2	4.03	103.7	77.7	101.6	76.5	56.1	102.1
7d	99.5	16.4	n.r[a]	42.2	3.95	103.9	77.7	102.2	76.0	57.7	106.0
7e	99.8	16.6	n.r[a]	42.2	4.03	103.8	77.9	102.4	76.3	56.4	101.9

[a]n.r. = not registered.

42 ppm are characteristic for the C-2 and C-3 atoms of an α-linked sialic acid.[22] Simultaneous down-field shifts (~3 ppm) of the galactose C-3 atom in the ^{13}C NMR-spectra and about 0.5 ppm of the galactose H-3 in the ^{1}H NMR-spectra confirm that sialic acid has been transferred onto the 3-OH group of the galactose moiety.[23] In a final step the saccharopeptides **6** are incubated with GDP-fucose[24] and recombinant fucosyltransferase VI.[25] This enzyme selectively transfers a fuc-unit from GDP-fuc onto the 3-OH group of a glcNAc

moiety in a sialylated or non-sialylated type II sugar chain in an α-mode.[26]

NMR-investigations of the isolated oligosaccharides show the successful attachment of a fucose to saccharopeptides **6**. Diagnostic for an α-linked fucose-moiety are a doublet at about 5.1 ppm (J∼4.5 Hz) for the fucose H-1 and a broad quartet at about 4.9 ppm (J∼6.7 Hz) for the fucose H-5 in all the ^{1}H NMR-spectra of compounds **7**. This is corroborated by additional signals in the ^{13}C NMR-spectra at about 100 ppm and 16 ppm which must be assigned to C-1, respectively, C-6 of the fuc-unit. Slight down-field shifts of the glucosamide C-3 signals, accompanied by slight up-field shifts of the glucosamide C-4 signals[22] prove that fucose has been transferred onto its 3-OH group.

The findings discussed above are quite surprising because it could not have been claimed in advance that all three glycosyl-transferases accept all the non-natural saccharopeptides **4** to **6** as substrates at all. The changes which have been introduced by the replacement of the natural *N*-acetyl group with a glycuronamide are significantly, albeit enzymatic glycosylations take place as expected for the parent acceptors. Furthermore, none of the investigated transferases seems to be inhibited by these non-natural substrates. Both sia-t and fuc-t VI catalyze the exclusive sugar-transfer from their respective donors onto the expected OH-groups of the acceptor substrates. So neither of the D- or L-galacturonamide residues is attacked by sia-t despite the close stereochemical resemblance with the terminal gal-unit in **5**. Even more surprisingly, fuc-t VI catalyzes the exclusive transfer of fucose onto the deeply buried 3-OH group of the glucosamide-residues neighboring the bulky, highly polar glycuronamide appendices. The modest, nonetheless preparative useful fucosylation-yields in some examples reflect those steric impediments.

Conclusion

These studies show in addition to previous reports[9,19,23] an unexpected high substrate promiscuity of β(1-4)gal-t, recombinant α(2-3)sia-t and fuc-t VI. All three enzymes tolerate the replacement of the stereochemically little conspicuous *N*-acetyl group of the acceptor substrates by bulky, highly polar D- and L-glycuronamides (→saccharopeptides) and give unambiguously SLe^x-saccharopeptides after three sequential incubations with their respective donor-substrates. Thus the preparative scope of the investigated transferases has been substantially extended. They proved to be versatile tools to get an easy and unexpected access to a novel class of compounds with a high and predictable regio- and stereochemical fidelity. This renders glycosyl-transferases an indispensable tool for the glycochemist, especially if future genetic engineering of the transferases is taken into account. This may further widen the synthetic potential of glycosyl-transferases.

The simultaneous, enzymatic conversion of non-natural donors with non-natural acceptors is currently investigated and will be reported in due course.

Experimental

^{1}H NMR and ^{13}C NMR spectra were recorded on a BRUKER AC 400 and/or a VARIAN Unity 500 spectrometer with multi-probe heads. COSY, TOCSY, HMBC and HSQC experiments were performed using the manufactures' software. Proton- and carbon-signals were assigned by the combined use of these spectra.[27–29] ^{1}H NMR-shifts are measured in CD_3OD and referenced to internal D_2O (4.80 ppm) and ^{13}C NMR-shifts are referenced to CD_3OD (49.00 ppm) unless otherwise stated. ^{1}H NMR and ^{13}C NMR-shift assignments are tentative; shifts marked with an asterisk (*) may be interchanged.

TLC was performed on silica gel $60F_{254}$ glass sheets (Merck), and sugars were stained with *p*-anisaldehyde sulfuric acid (Pernod-mixture). Flash chromatography was carried out with silica gel 60, 0.040–0.063 mm (Merck).

Solvents and chemicals used were of commercial quality unless otherwise stated. Bovine serum albumine (BSA) was obtained from Boehringer (no. 28031). Calf intestine alkaline phosphatase (E.C.3.1.3.1) (CIAP) was purchased from Boehringer (no 108146, 7500U/498 μL). Recombinant α(2,3)sialyl-transferase (∼100 U/l, from transfected insect cells)[21,23] (EMBL accession no. M97754) and recombinant fucosyl-transferase VI (∼16 U/l, from transfected CHO-cells)[9,25] (EMBL accession no. LO1698) were used. FABMS spectra were recorded on FO25FAB instrument with Cs or Xe as bombarding gas, and with thioglycerol as matrix.

General Procedures A–D

Procedure A

Coupling of glycopyranuronic acids 3 to glucosamine 2. According general peptide coupling protocols[30] 0.32 mmol of the amine **2**, 0.33 mmol of the glycopyranuronic acid **3** and 0.33 mmol HBPyU (*O*-(benzotriazole-1-yl)-*N*,*N*,*N*′,*N*′-bis(tetramethylene)uronium hexafluorophosphate) are dissolved at room temperature in 5 mL dry DMF. The clear solution is treated with

0.33 mmol triethyl amine and stirred at room temperature over night. The mixture is then evaporated to dryness and the residue chromatographed over silica gel (eluent: CH_2Cl_2/MeOH, 10/1) to give an acetylated saccharopeptide intermediate. This compound is dissolved in 4 mL dry methanol, containing 0.1% sodium methanolate, and stirred at room temperature for 1–8 h until TLC (CH_2Cl_2/MeOH, 10/3) shows complete consumption of the starting material. Evaporation leaves a solid that is purified over silica gel (eluent: CH_2Cl_2/MeOH, 10/3).

Procedure B

Enzymatic galactosylation of saccharopeptides 4. Following standard galactosylation protocols[19,31] 38.2 μmol of compound **4** and 47.5 μmol UDP-gal (FLUKA) are dissolved in a mixture of 1.4 mL sodium cacodylate-buffer (0.1 M, pH 7.4) containing 1.7 mg BSA, 5.7 mg (28.8 μmol) $MnCl_2 \times 4H_2O$ and 0.2 mL DMSO. This mixture is incubated at 37 °C with 2 μL CIAP and 600 μL β(1-4)gal-t (1.5 U). When TLC (CH_2Cl_2/MeOH/H_2O, 10/4/0.8) shows the disappearance of the starting acceptor, usually over night, the turbid solution is centrifuged and the supernatant passed over a C-18 reversed-phase column, washed with water and eluted with methanol. The methanol is evaporated and the resulting residue chromatographed over silica gel (eluent: CH_2Cl_2/MeOH/H_2O, 10/2/0.2→6/4/1) to give the pure galactosylated sugars **5** as white powders after lyophilization from water-dioxanes.

Procedure C

Enzymatic sialylation of saccharopeptides 5. Following standard sialylation protocols[23,31] 17.1 μmol of compound **5** and 26.7 μmol CMP-sialic acid[20] are dissolved in a mixture of 2 mL sodium cacodylate-buffer (0.05 M, pH 6.5), 2 mL of a $MnCl_2$-solution (0.06 M) and 1.3 mL deionized water containing 1.2 mg BSA. The mixture is incubated at 37 °C with 2 μL CIAP and 75 μL (525 mU) of recombinant sialyl-transferase.[21] When TLC (CH_2Cl_2/MeOH/H_2O, 10/2/0.2) shows the disappearance of the starting acceptor, usually over night, the turbid solution is centrifuged and the supernatant passed over a C-18 reversed-phase column, washed with water and eluted with methanol. The methanol is evaporated and the resulting residue chromatographed over silica gel (eluent: CH_2Cl_2/MeOH/H_2O, 10/2/0.2) to give the pure sialylated sugars **6** as white powders after lyophilization from water-dioxanes.

General procedure D

Enzymatic fucosylation of saccharides 6. Following general fucosylation protocols[26,31] 16.1 μmol of sugar **6**, 22.4 μmol of GDP-fucose[24] and 2.1 mg BSA are added to a mixture of 450 μL sodium cacodylate-buffer (250 mM, pH 6.5) and 150 μL of a $MnCl_2$-solution (250 mM). The mixture is diluted with 600 μL deionized water and incubated at 37 °C with 2 μL CIAP and 400 μL (800 mU) of recombinant fucosyl-transferase VI[25] for 24–72 h until TLC (CH_2Cl_2/MeOH/H_2O, 10/4/0.8) shows the consumption of the starting acceptor **6**. The mixture is then centrifuged and the supernatant passed over a C-18 reversed-phase column, washed with water and eluted with methanol. The methanol is evaporated and the resulting residue chromatographed on silica gel (eluent: CH_2Cl_2/MeOH/H_2O, 10/4/0.8) to give the pure saccharides **7** as white powders after lyophilization from water.

Individual protocols and data

8-Methoxycarbonyloctyl 3,4,6-tri-*O*-acetyl-2-*N*-allyloxycarbonyl-2-deoxy-β-D-glucopyranoside 1. An anomeric mixture of 30.9 g (71.6 mmol) per-*O*-acetylated-*N*-allyloxycarbonylated glucosamine[11] and 20.0 g (107.4 mmol) of 9-hydroxy-nonanoic acid methylester[12] as a hydrophobic aglycon are dissolved in 500 mL dry DCM containing 10 g powdered 4 Å molecular sieves. The mixture is cooled to −35 °C and treated with 36.4 mL (200.6 mmol) TMS-triflate (FLUKA) over night. The reaction mixture is then warmed to −20 °C and stirred for an additional 5 h at this temperature. When TLC (hexanes/ethyl acetate, 2/1) shows the complete consumption of the starting sugar, the mixture is neutralized with 50 mL triethylamine (pH 10), warmed to room temperature and filtered over a Celite-pad. The filtrate is then successively washed with satd sodium hydrogen carbonate solution, 1 N hydrogen chloride solution and water. Evaporation of the solvent leaves a residue which is chromatographed over silica gel (eluent: hexanes/ethyl acetate, 2/1) to give 30.8 g (77%) of the title compound. ^{1}H NMR ($CDCl_3$, 400.13 MHz) δ 1.23 (m, 8H), 1.51 (m, 4H), 2.01 (s, 3H), 2.03 (s, 3H), 2.10 (s, 3H), 2.23 (t, J = 7.6 Hz, 2H), 3.45 (dt, J = 11.0, 8.8 Hz, 1H), 3.55 (br, 5H), 3.66 (s, 3H), 3.69 (m, 1H), 3.86 (dt, J = 4.8 Hz, 9.7 Hz, 1H), 4.11 (dd, J = 13.4 Hz, 2.2 Hz, 1H), 4.26 (dd, J = 13.4 Hz, 5.5 Hz, 1H), 4.55 (m, 3H); 4.93 (br, 1H), 5.04 (t, J = 11.0 Hz, 1H), 5.19 (dq, J = 1.2 Hz, 16.3 Hz, 1H), 5.19 (br d, J = 16.5 Hz, 1H), 5.87 (m, 1H); ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 20.56; 20.62; 20.69; 24.75; 25.58; 28.88; 28.95; 29.00; 29.27; 33.94; 51.40; 56.09; 62.09; 65.61; 68.74; 70.09; 71.56; 72.09; 100.75; 117.45; 132.54; 155.55; 169.44; 170.57; 170.67; 174.28.

8-Methoxycarbonyloctyl 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-β-D-gluco-pyranoside 2. Under an argon atmosphere in 100 mL dry THF are dissolved 6.0 g (10.7 mmol) of sugar **1**, 12.9 mL (84.7 mmol) malonic

acid diethylester and 0.6 g (0.5 mmol) tetrakis-(triphenylphosphino)-palladium (Aldrich). The resulting yellow solution is stirred at room temperature until TLC (ethyl acetate/hexanes, 3/1) shows the complete consumption of the starting material, usually over night. The mixture is then evaporated to dryness and the residue passed over a silica gel column (eluent: ethyl acetate/hexanes, 3/1). The product-containing fractions are collected and rechromatographed over silica gel (eluent: DCM/methanol, 29/1) to give 3.9 g (76%) of the title amine as an amorphous solid sufficiently pure for the ensuing amidations. ^{1}H NMR ($CDCl_3$, 250.13 MHz) δ 1.34 (m, 8H), 1.64 (m, 4H), 2.06 (s, 3H), 2.11 (s, 6H), 2.32 (t, J = 7.6 Hz, 2 H), 2.96 (dd, J = 7.5 Hz, 8.2 Hz, 1H), 3.53 (dt, J = 6.9 Hz, 10.3 Hz, 1H), 3.71 (m, 4H); 3.83 (dt, J = 7.5 Hz, 8.3 Hz, 1 H), 4.14 (dd, J = 3.4 Hz, 13.8 Hz, 1H), 4.29 (d, J = 7.6 Hz, 1H), 4.34 (dd, J = 5.5 Hz, 13.8 Hz, 1H), 5.03 (m, 2H); ^{13}C NMR ($CDCl_3$, 62.90 MHz) δ 20.17; 20.25; 20.31; 24.37; 25.37; 28.51; 29.63 (2×C); 28.99; 33.48; 50.90; 55.48; 61.79; 68.47; 69.74; 71.26; 74.90; 103.57; 169.22; 170.06 (2×C); 173.59.

Alternatively, the fully deacetylated amine **2a** (8-methoxycarbonyloctyl-2-amino-2-deoxy-β-D-glucopyranoside) has been used in some cases. It is obtained by stirring 22.0 g (39.3 mmol) of the acetylated compound **1** at rt in dry methanol, containing 0.1% of sodium methanolate, for 1.5 h. The mixture is neutralized with DOWEX 50×8 (H^+-form), filtered and evaporated to give 16.9 g (99%) of the deacetylated allyloxy compound, which is fully deprotected as follows: to 4.5 g (10.3 mmol) of the deacetylated sugar, dissolved in a mixture of 25 mL dry THF, 10 mL dry dioxane and 50 mL of dry methanol are added at rt 0.20 g (0.5 mmol) 1,4-bis(diphenylphosphino)butane (FLUKA), 2.4 g (18.2 mmol) sodium thiophenolate (FLUKA), and 0.2 g (0.2 mmol) of tris(dibenzylideneacetone)-dipalladium-0 adduct (Aldrich). The mixture is stirred over night in an argon atmosphere. It is then evaporated and chromatographed on silica gel (eluent: methylenechloride:methanol, 7:1) to give 3.2 g (88%) of the fully deprotected amine **2a** as a syrup. ^{1}H NMR (CD_3OD, 250.13 MHz) δ 1.29 (m, 8H), 1.53 (m, 4H), 2.25 (t, J = 7.5 Hz, 2H), 2.52 (broad t, J = 7.4 Hz, 1H), 3.20 (m, 3H), 3.42 (dt, J = 10.3 Hz, 6.9 Hz, 1H), 3.59 (m, 4H), 3.72 (m, 2H), 4.15 (d, J = 8.6 Hz, 1H).

8-Methoxycarbonyloctyl 2-deoxy-2-(methyl-β-D-gluco-hexopyranosyluronamide)-β-D-glucopyranoside 4a. According general procedure A from 76.0 mg (0.33 mmol) of the commercial sodium salt of methyl-β-D-gluco-hexopyranosiduronic acid **3a** and 150.0 mg (0.32 mmol) of compound **2** are obtained 92.0 mg (44%) acetylated intermediate which is deacetylated to give 51.7 mg (71%) of the title sugar. ^{1}H NMR ($CDCl_3$:CD_3OD:D_2O, 400.13 MHz) glcN-unit (H-1 to H-6,6′) δ 4.51, 3.67, 3.52, 3.37, 3.37, 3.68 and 3.83; gluA-unit (H-1 to H-5, OCH_3) δ 4.33, 3.24, 3.46, 3.50, 3.79, 3.51; aglycon δ 2.32, 1.55, 1.26, 1.51, 3.51, 3.82, 3.64; ^{13}C NMR ($CDCl_3$:CD_3OD:D_2O) glcN-unit (C-1 to C-6) δ 101.8, 56.9, 74.8*, 70.9, 77.1, 62.0; gluA-unit (C-1 to C-6, O*C*H_3) δ 104.8, 73.9, 76.6, 72.7, 75.7*, 171.7, 57.9; aglycon δ 176.7, 34.8, 25.6, 29.8, 29.9, 26.4, 30.1, 71.1, 52.4; MS calcd for $C_{23}H_{41}NO_{13}$ 539: found MS + H 540.

8-Methoxycarbonyloctyl 2-deoxy-2-(methyl-β-D-galacto-hexopyranosyluronamide)-β-D-glucopyranoside 4b. According general procedure A from 68.2 mg (0.33 mmol) of methyl-β-D-galacto-hexopyranosiduronic acid **3b** and 150.0 mg (0.32 mmol) of compound **2** are obtained 131.2 mg (63%) acetylated intermediate which is deacetylated to give 81.9 mg (78%) of the title sugar. ^{1}H NMR (CD_3OD, 400.13 MHz) glcN-unit (H-1 to H-6,6′) δ 4.69, 3.57, 3.70, 3.34, 3.31, 3.71, 3.90; galA-unit (H-1 to H-5, OCH_3) δ 4.22, 3.55, 3.56, 4.18, 4.03, 3.62; aglycon δ 2.31, 1.61, 1.31, 1.57, 3.52, 3.83, 3.69; ^{13}C NMR (CD_3OD) glcN-unit (C-1 to C-6) δ 101.8, 58.2, 75.3, 72.1, 77.8, 62.8; galA-unit (C-1 to C-6, O*C*H_3) δ 106.0, 71.9*, 74.5*, 70.8, 76.6, 171.4, 57.8; aglycon δ 176.0, 34.8, 26.0, 30.1, 30.3, 26.9, 30.6; 70.8, 52.0; MS calcd for $C_{23}H_{41}NO_{13}$ 539: found MS + H 540.

8-Methoxycarbonyloctyl 2-deoxy-2-(methyl-α-D-galacto-hexopyranosyluronamide)-β-D-glucopyranoside 4c. According general procedure A from 109.0 mg (0.53 mmol) of methyl-α-D-galacto-hexopyranosiduronic acid **3c** and 240.0 mg (0.50 mmol) of compound **2** are obtained 171.0 mg (51%) acetylated intermediate, which is deacetylated to give 102.7 mg (74%) of the title sugar. ^{1}H NMR (CD_3OD, 400.13 MHz) glcN-unit (H-1 to H-6,6′) δ 4.62, 3.46, 3.64, 3.25, 3.24, 3.63, 3.82; galA-unit (H-1 to H-5, OCH_3) δ 4.81, 3.77, 3.73, 4.15, 4.15, 3.40; aglycon δ 2.26, 1.56, 1.31, 1.42, 3.44, 3.78, 3.59; ^{13}C NMR (CD_3OD) glcN-unit (C-1 to C-6) δ 101.8, 58.3, 75.3, 72.2, 77.9, 62.8; galA-unit (C-1 to C-6, O*C*H_3) δ 101.8, 69.7, 71.1, 71.4, 72.7, 171.9, 56.3; aglycon δ 176.0, 34.8, 26.0, 30.1, 30.3, 30.4, 27.0, 30.6; 70.7, 52.0; MS calcd for $C_{23}H_{41}NO_{13}$ 539: found MS + H 540.

8-Methoxycarbonyloctyl 2-deoxy-2-(methyl-β-L-galacto-hexopyranosyluronamide)-β-D-glucopyranoside 4d. According general procedure A from 50.0 mg (0.24 mmol) of methyl-α-D-galacto-hexopyranosiduronic acid **3d** and 100.0 mg (0.21 mmol) of compound **2** are obtained 58.0 mg (41%) acetylated intermediate, which is deacetylated to give 30.0 mg (64%) of the title sugar. ^{1}H NMR (CD_3OD, 400.13 MHz) glcN-unit (H-1 to H-6,6′) δ 4.41, 3.40, 3.52, 3.22, 3.20, 3.70, 3.79; galA-unit (H-1 to H-5, OCH_3) δ 4.15, 3.49, 3.51, 4.10, 3.96, 3.52; aglycon δ 2.31, 1.50, 1.24, 1.45, 3.49, 3.78, 3.58; ^{13}C NMR

(CD_3OD) glcN-unit (C-1 to C-6) δ 102.4, 57.4, 75.7, 71.7, 77.8, 62.7; galA-unit (C-1 to C-6, OCH_3) δ 106.0, 71.9, 74.3, 70.6, 76.7, 171.4, 57.8; aglycon δ 176.8, 34.7, 26.0, 30.3, 30.4, 30.5, 27.0, 30.1; 70.9, 52.5; MS calcd for $C_{23}H_{41}NO_{13}$ 539: found MS + H 540.

8-Methoxycarbonyloctyl 2-deoxy-2-(methyl-α-L-galactohexopyranosyluronamide)-β-D-glucopyranoside 4e. To 0.13 g (0.37 mmol) of amine **2a**, dissolved in 5 mL DMF at rt, are added 0.09 g (0.45 mmol) sugar acid **3e**, 0.17 g (0.45 mmol) *O*-(1*H*-benzotriazole-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexa-fluorophosphate (FLUKA) and 65 μL triethyl amine. This heterogenous mixture is stirred for 5 days and then worked up according general procedure A to give 23.4 mg (12%) of compound **4e**. ^1H NMR (CD_3OD, 400.13 MHz) glcN-unit (H-1 to H-6,6′) δ 4.46, 3.81, 3.60, 3.39, 3.35, 3.72 and 3.91; galA-unit (H-1 to H-5, OCH_3) δ 4.91, 3.84, 3.83, 4.29, 4.25, 3.43; aglycon δ 2.32, 1.62, 1.30, 1.57, 3.47, 3.85, 3.67; ^{13}C NMR (100.61 MHz) glcN-unit (C-1 to C-6) δ 101.8, 56.3, 74.4, 70.9, 77.1, 61.8; galA-unit (C-1 to C-6, OCH_3) δ 100.9, 68.1, 69.8, 70.5, 71.8, not resolved, 55.3; aglycon δ not resolved, 34.0, 25.3, 29.2, 26.1, 29.6, 70.1, 50.9; MS calcd for $C_{23}H_{41}NO_{13}$ 539: found MS + H 540.

8-Methoxycarbonyloctyl β-D-galactopyranosyl-(1→4)-2-deoxy-2-(methyl-β-D-glucohexopyranosyluronamide)-β-D-glucopyranoside 5a. According general procedure B 15.5 mg (28.7 μmol) of sugar **4a** are incubated with 19.6 mg (32.1 μmol) UDP-gal for 3 days to give 10.7 mg (53%) of the title compound. ^1H NMR (D_2O, 500.13 MHz) glcN-unit (H-1 to H-6,6′) δ 4.65, 3.83, 3.82, 3.75, 3.63, 3.86 and 4.02; gal-unit (H-1 to H-6,6′) δ 4.51, 3.58, 3.69, 3.95, 3.76, 3.78 and 3.78; glcA-unit (H-1 to H-5, OCH_3) δ 4.46, 3.35, 3.57, 3.62, 3.92, 3.60; aglycon δ 2.41, 1.62, 1.31, 1.32, 1.58, 3.64, 3.91, 3.71; ^{13}C NMR glcN-unit (C-1 to C-6) δ 101.7, 56.1, 73.3, 79.7, 75.8, 61.1; gal-unit (C-1 to C-6) δ 104.0, 72.2, 73.5, 69.5, 76.4, 62.0; glcA-unit (C-1 to C-6, OCH_3) δ 104.4, 73.6, 76.3, 72.3, 76.1, not resolved, 58.3; aglycon δ not resolved, 34.6, 25.2, 29.1, 25.8, 29.4, 71.5, 53.0; MS calcd for $C_{29}H_{51}NO_{18}$ 701: found MS + H 702 and MS + Na 724.

8-Methoxycarbonyloctyl β-D-galactopyranosyl-(1→4)-2-deoxy-2-(methyl-β-D-galactohexopyranosyluronamide)-β-D-glucopyranoside 5b. According to general procedure B 20.6 mg (38.2 μmol) of sugar **4b** are incubated with 29.0 mg (47.5 μmol) UDP-gal for 1 day to give 16.2 mg (61%) of the title compound. ^1H NMR (CD_3OD, 500.13 MHz) glcN-unit (H-1 to H-6,6′) δ 4.63, 3.72, 3.82, 3.62, 3.44, 3.86 and 3.92; gal-unit (H-1 to H-6,6′) δ 4.39, 3.51, 3.50, 3.82, 3.59, 3.70 and 3.77; galA-unit (H-1 to H-5, OCH_3) δ 4.22, 3.55, 3.55, 4.16, 4.03, 3.59; aglycon δ 2.31, 1.59, 1.30, 1.57, 3.52, 3.80, 3.65; ^{13}C NMR glcN-unit (C-1 to C-6) δ 102.0, 57.0, 73.8, 81.0, 76.3, 61.9; gal-unit (C-1 to C-6) δ 105.1, 72.5, 74.7, 70.2, 77.0, 62.3; galA-unit (C-1 to C-6, OCH_3) δ 105.9, 71.9, 74.5, 70.7, 76.7, 171.4, 57.6; aglycon δ 176.3, 34.5, 25.7, 29.9, 26.7, 30.4, 70.8, 51.8; MS calcd for $C_{29}H_{51}NO_{18}$ 701: found MS + H 702 and MS + Na 724.

8-Methoxycarbonyloctyl β-D-galactopyranosyl-(1→4)-2-deoxy-2-(methyl-α-D-galactohexopyranosyluronamide)-β-D-glucopyranoside 5c. According to general procedure B 22.0 mg (40.8 μmol) of sugar **4c** are incubated with 32.1 mg (52.6 μmol) UDP-gal for 1 day to give 16.5 mg (58%) of the title compound. ^1H NMR (CD_3OD, 500.13 MHz) glcN-unit (H-1 to H-6,6′) δ 4.62, 3.71, 3.80, 3.61, 3.44, 3.87 and 3.92; gal-unit (H-1 to H-6,6′) δ 4.39, 3.55, 3.52, 3.82, 3.59, 3.70 and 3.75; galA-unit (H-1 to H-5, OCH_3) δ 4.86, 3.83, 3.78, 4.23, 4.23, 3.45; aglycon δ 2.32, 1.60, 1.31, 1.58, 3.52, 3.78, 3.65; ^{13}C NMR glcN-unit (C-1 to C-6) δ 102.0, 56.9, 73.8, 81.1, 76.4, 61.9; gal-unit (C-1 to C-6) δ 105.2, 72.5, 74.8, 70.2, 77.0, 62.4; galA-unit (C-1 to C-6, OCH_3) δ 101.8, 69.7, 71.0, 71.2, 72.1, 171.8, 56.1; aglycon δ 176.2, 34.5, 25.7, 29.9, 26.7, 30.3, 71.0, 51.8; MS calcd for $C_{29}H_{51}NO_{18}$ 701: found MS + H 702 and MS + Na 724.

8-Methoxycarbonyloctyl β-D-galactopyranosyl-(1→4)-2-deoxy-2-(methyl-β-L-galactohexopyranosyluronamide)-β-D-glucopyranoside 5d. According to general procedure B 29.0 mg (53.7 μmol) of sugar **4d** are incubated with 43.1 mg (70.6 μmol) UDP-gal for 3 days to give 36.5 mg (96%) of the title compound. ^1H NMR (CD_3OD:D_2O, 500.13 MHz) glcN-unit (H-1 to H-6,6′) δ 4.52, 3.84, 3.78, 3.65, 3.45, 3.85 and 3.92; gal-unit (H-1 to H-6,6′) δ 4.40, 3.55, 3.52, 3.82, 3.60, 3.68 and 3.76; galA-unit (H-1 to H-5, OCH_3) δ 4.22, 3.55, 3.57, 4.16, 4.02, 3.59; aglycon δ 2.32, 1.58, 1.29, 1.52, 3.50, 3.78, 3.65; ^{13}C NMR glcN-unit (C-1 to C-6) δ 102.3, 56.6, 73.8, 80.4, 76.3, 61.7; gal-unit (C-1 to C-6) δ = 104.8, 72.8, 74.8, 70.1, 76.9, 62.3; galA-unit (C-1 to C-6, OCH_3) δ 105.8, 71.8, 74.2, 70.7, 76.9, 171.5, 57.8; aglycon δ 176.5, 34.7, 25.8, 30.0, 26.8, 30.3, 70.9, 52.2; MS calcd for $C_{29}H_{51}NO_{18}$ 701: found MS + H 702 and MS + Na 724.

8-Methoxycarbonyloctyl β-D-galactopyranosyl-(1→4)-2-deoxy-2-(methyl-α-L-galactohexopyranosyluronamide)-β-D-glucopyranoside 5e. According to general procedure B 23.0 mg (42.6 μmol) of sugar **4e** are incubated with 36.0 mg (59.0 μmol) UDP-gal for 3 days to give 12.1 mg (40%) of the title compound. ^1H NMR (CD_3OD, 400.13 MHz) glcN-unit (H-1 to H-6,6′) δ 4.53, 3.88, 3.79, 3.66, 3.47, 3.90 and 3.93; gal-unit (H-1 to H-6,6′) δ 4.45, 3.55, 3.52, 3.84, 3.64, 3.72 and 3.78; galA-unit (H-1 to H-5, OCH_3) δ 4.92, 3.86, 3.81, 4.29, 4.25, 3.45; aglycon δ 2.34, 1.61, 1.31, 1.55, 3.47, 3.85, 3.68; ^{13}C NMR glcN-unit (C-1 to C-6) δ 101.7, 55.5, 73.0, 79.5, 75.5, 60.9; gal-unit (C-1 to C-6) δ 104.5, 71.6, 73.7, 69.2, 76.2,

61.4; galA-unit (C-1 to C-6, O*C*H$_3$) δ 101.1, 68.8, 70.0, 70.4, 71.9, not resolved, 55.1; aglycon δ not resolved, 34.1, 25.3, 29.4, 26.3, 29.5, 70.0, 51.4; MS calcd for $C_{29}H_{51}NO_{18}$ 701: found MS + H 702 and MS + Na 724.

8-Methoxycarbonyloctyl 5-*N*-acetyl-α-neuraminyl-(2→3)-β-D-galactopyranosyl-(1→4)-2-deoxy-2-(methyl-β-D-glucohexopyranosyluronamide)-β-D-glucopyranoside 6a. According to general procedure C 12.0 mg (17.1 μmol) of sugar **5a** are incubated with 17.6 mg (26.7 μmol) CMP-sialic acid for 2 days to give 16.0 mg (95%) of the title compound. ^{1}H NMR (CD_3OD, 500.13 MHz) glcN-unit (H-1 to H-6,6′) δ 4.50, 3.83, 3.70, 3.62, 3.42, 3.89 and 3.91; gal-unit (H-1 to H-6,6′) δ 4.45, 3.56, 4.04, 3.91, 3.57, 3.64 and 3.73; sia-unit (H-3 to H-9, HNCOC*H*$_3$) δ 1.72, 2.84, 3.71, 3.71, 3.60, 3.48, 3.85, 3.61 and 3.83, 2.00; glcA-unit (H-1 to H-5, OC*H*$_3$) δ 4.25, 3.22, 3.42, 4.48, 3.73, 3.58; aglycon δ 2.31, 1.59, 1.31, 1.55, 3.48, 3.84, 3.64; ^{13}C NMR glcN-unit (C-1 to C-6) δ 102.2, 56.1, 74.0, 81.2, 76.3, 61.9; gal-unit (C-1 to C-6) δ 104.8, 70.2, 77.5, 68.8, 76.9, 62.5; sia-unit (C-1 to C-9, HN*C*O*C*H$_3$) δ 175.0, 101.2, 41.9, 69.2, 53.8, 74.9, 70.0, 72.8, 64.4, 22.4; glcA-unit (C-1 to C-6, O*C*H$_3$) δ 105.2, 74.4, 77.5, 73.6, 76.8, 172.4, 57.4; aglycon δ 176.0, 34.6, 25.6, 30.1, 26.6, 30.4, 70.5, 51.8; MS calcd for $C_{40}H_{68}N_2O_{26}$ 992: found MS + H 993.

8-Methoxycarbonyloctyl 5-*N*-acetyl-α-neuraminyl-(2→3)-β-D-galactopyranosyl-(1→4)-2-deoxy-2-(methyl-β-D-galactohexopyranosyluronamide)-β-D-glucopyranoside 6b. According to general procedure C 12.2 mg (17.4 μmol) of sugar **5b** are incubated with 17.4 mg (26.4 μmol) CMP-sialic acid for 1 day to give 17.1 mg (100%) of the title compound. ^{1}H NMR (CD_3OD, 500.13 MHz) glcN-unit (H-1 to H-6,6′) δ 4.59, 3.75, 3.78, 3.61, 3.42, 3.89 and 3.92; gal-unit (H-1 to H-6,6′) δ 4.45, 3.56, 4.04, 3.91, 3.56, 3.63 and 3.73; sia-unit (H-3 to H-9, HNCOC*H*$_3$) δ 1.72, 2.84, 3.71, 3.70, 3.60, 3.48, 3.85, 3.60 and 3.83, 2.00; galA-unit (H-1 to H-5, OC*H*$_3$) δ 4.20, 3.55, 3.52, 4.15, 4.02, 3.58; aglycon δ 2.30, 1.58, 1.29, 1.56, 3.50, 3.79, 3.64; ^{13}C NMR glcN-unit (C-1 to C-6) δ 102.2, 56.8, 73.9, 81.3, 76.3, 62.0; gal-unit (C-1 to C-6) δ 105.0, 70.8, 77.5, 68.9, 77.0, 62.6; sia-unit (C-1 to C-9, HN*C*O*C*H$_3$) δ 174.9, 100.9, 41.9, 69.2, 53.8, 74.9, 70.0, 72.8, 64.4, 175.4, 22.5; galA-unit (C-1 to C-6, O*C*H$_3$) δ 105.9, 71.9, 74.5, 70.7, 76.6, 171.3, 57.6; aglycon δ 176.0, 34.7, 25.8, 30.1, 26.8, 30.4, 70.9, 51.8; MS calcd for $C_{40}H_{68}N_2O_{26}$ 992: found MS − H 991 and MS + Na 1015.

8-Methoxycarbonyloctyl 5-*N*-acetyl-α-neuraminyl-(2→3)-β-D-galactopyranosyl-(1→4)-2-deoxy-2-(methyl-α-D-galactohexopyranosyluronamide)-β-D-glucopyranoside 6c. According general procedure C 16.0 mg (22.8 μmol) of sugar **5c** are incubated with 21.8 mg (33.1 μmol) CMP-sialic acid for 1 day to give 19.7 mg (87%) of the title compound. ^{1}H NMR (CD_3OD, 500.13 MHz) glcN-unit (H-1 to H-6,6′) δ 4.57, 3.74, 3.78, 3.61, 3.42, 3.89 and 3.92; gal-unit (H-1 to H-6,6′) δ 4.45, 3.55, 4.04, 3.91, 3.56, 3.63 and 3.72; sia-unit (H-3 to H-9, HNCOC*H*$_3$) δ 1.73, 2.84, 3.71, 3.67, 3.61, 3.48, 3.85, 3.61 and 3.83, 2.00; galA-unit (H-1 to H-5, OC*H*$_3$) δ 4.84, 3.82, 3.76, 4.21, 4.21, 3.43; aglycon δ 2.30, 1.58, 1.29, 1.55, 3.50, 3.78, 3.64; ^{13}C NMR glcN-unit (C-1 to C-6) δ 102.2, 56.8, 73.9, 81.4, 76.4, 62.0; gal-unit (C-1 to C-6) δ 105.0, 70.8, 77.6, 69.0, 77.0, 62.6; sia-unit (C-1 to C-9, HN*C*O*C*H$_3$) δ 174.9, 101.2, 41.9, 69.2, 53.8, 74.9, 70.0, 72.8, 64.4, 175.4, 22.5; galA-unit (C-1 to C-6, O*C*H$_3$) δ 101.8, 69.7, 71.1, 71.3, 72.7, 171.8, 56.1; aglycon δ 176.0, 34.7, 25.8, 30.1, 26.8, 30.5, 70.8, 51.9; MS calcd for $C_{40}H_{68}N_2O_{26}$ 992: found MS − H 991 and MS Ê 2H + Na 1013.

8-Methoxycarbonyloctyl 5-*N*-acetyl-α-neuraminyl-(2→3)-β-D-galactopyranosyl-(1→4)-2-deoxy-2-(methyl-β-L-galactohexopyranosyluronamide)-β-D-glucopyranoside 6d. According to general procedure C 35.0 mg (49.9 μmol) of sugar **5d** are incubated with 42.8 mg (65.0 μmol) CMP-sialic acid for 3 days to give 17.1 mg (35%) of the title compound. ^{1}H NMR (CD_3OD, 500.13 MHz) glcN-unit (H-1 to H-6,6′) δ 4.51, 3.84, 3.75, 3.63, 3.43, 3.91 and 3.96; gal-unit (H-1 to H-6,6′) δ 4.45, 3.56, 4.04, 3.89, 3.56, 3.65 and 3.74; sia-unit (H-3 to H-9, HNCOC*H*$_3$) δ 1.70, 2.84, 3.70, 3.70, 3.60, 3.47, 3.86, 3.60 and 3.82, 2.00; galA-unit (H-1 to H-5, OC*H*$_3$) δ 4.20, 3.54, 3.54, 4.14, 3.99, 3.58; aglycon δ 2.29, 1.57, 1.29, 1.52, 3.46, 3.84, 3.64; ^{13}C NMR glcN-unit (C-1 to C-6) δ 102.3, 56.5, 73.9, 81.1, 76.4, 62.0; gal-unit (C-1 to C-6) δ 104.9, 70.7, 77.6, 68.9, 77.1, 62.7; sia-unit (C-1 to C-9, HN*C*O*C*H$_3$) δ 175.0, 100.9, 42.1, 69.3, 53.9, 74.8, 70.1, 72.9, 64.5, 176.7, 22.5; galA-unit (C-1 to C-6, O*C*H$_3$) δ 106.0, 71.9*, 74.4*, 70.9, 77.0, 171.6, 57.8; aglycon δ 176.0, 34.7, 25.9, 30.1, 26.8, 30.4, 70.7, 51.9; MS calcd for $C_{40}H_{68}N_2O_{26}$ 992: found MS − H 991 and MS - 2H + Na 1013.

8-Methoxycarbonyloctyl 5-*N*-acetyl-α-neuraminyl-(2→3)-β-D-galactopyranosyl-(1→4)-2-deoxy-2-(methyl-α-L-galactohexopyranosyluronamide)-β-D-glucopyranoside 6e. According to general procedure C 12.0 mg (17.1 μmol) of sugar **5e** are incubated with 17.4 mg (26.4 μmol) CMP-sialic acid for 3 days to give 10.5 mg (62%) of the title compound. ^{1}H NMR (CD_3OD, 400.13 MHz) glcN-unit (H-1 to H-6,6′) δ 4.48, 3.87, 3.76, 3.65, 3.46, 3.79 and 3.80; gal-unit (H-1 to H-6,6′) δ 4.46, 3.58, 4.07, 3.92, 3.62, 3.67 and 3.76; sia-unit (H-3 to H-9, HNCOC*H*$_3$) δ 1.74, 2.88, 3.72, 3.71, 3.61, 3.50, 3.87, 3.64 and 3.86, 2.03; galA-unit (H-1 to H-5, OC*H*$_3$) δ 4.85, 3.85, 3.79, 4.24, 4.20, 3.45; aglycon δ 2.32, 1.62, 1.32, 1.55, 3.44, 3.87, 3.68; ^{13}C NMR glcN-unit (C-1 to C-6) δ 102.5, 56.4, 73.9, 81.1, 76.5, 62.1; gal-unit (C-1 to

C-6) δ 104.9, 70.8, 77.7, 69.0, 77.1, 62.7; sia-unit (C-1 to C-9, HN*C*O*C*H$_3$) δ 174.3, 101.1, 42.1, 69.4, 53.9, 74.9, 70.1, 72.9, 64.6, 175.5, 22.6; galA-unit (C-1 to C-6, O*C*H$_3$) δ 101.9, 69.7, 71.1, 71.4, 73.1, 172.0, 56.3; aglycon δ 176.0, 34.8, 26.0, 30.1, 27.1, 30.7, 70.8, 51.9; MS calcd for $C_{40}H_{68}N_2O_{26}$ 992: found MS + H 993 and MS + Na 1015.

8-Methoxycarbonyloctyl 5-*N*-acetyl-α-neuraminyl-(2→3)-β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl-(1→3)]-2-deoxy-2-(methyl-β-D-glucohexopyranosyluronamide)-β-D-glucopyranoside 7a. According to general procedure D 16.0 mg (16.1 μmol) of sugar **6a** are incubated with 14.2 mg (22.4 μmol) GDP-fucose for 4 days to give 11.0 mg (60%) of the title compound. ^{1}H NMR (CD$_3$OD-D$_2$O, 500.13 MHz) glcN-unit (H-1 to H-6,6′) δ 4.49, 3.99, 3.93, 3.88, 3.45, 3.92 and 3.95; gal-unit (H-1 to H-6,6′) δ 4.50, 3.53, 4.03, 3.88, 3.46, 3.65 and 3.72; sia-unit (H-3 to H-9, HNCOC*H*$_3$) δ 1.71, 2.84 3.70, 3.71, 3.63, 3.47, 3.88, 3.60 and 3.84, 2.01; fuc-unit (H-1 to H-6) δ 5.09, 3.61, 3.84, 3.71, 4.85, 1.14; glcA-unit (H-1 to H-5, OC*H*$_3$) δ 4.25, 3.23, 3.43, 3.54, 3.72, 3.52; aglycon δ 2.31, 1.58, 1.28, 1.54, 3.47, 3.82, 3.64; ^{13}C NMR glcN-unit (C-1 to C-6) δ 102.1, 56.8, 75.1, 76.3, 77.1, 61.13; gal-unit (C-1 to C-6) δ 103.7, 70.6, 77.7, 68.6, 76.4, 62.8; sia-unit (C-1 to C-9, HN*C*O*C*H$_3$) δ 176.7, 100.7, 42.0, 69.1, 53.7, 74.7, 69.9, 73.0, 64.4, 175.6, 22.5; fuc-unit (C-1 to C-6) δ 100.1, 69.7, 70.7, 73.5, 67.6, 16.4; glcA-unit (C-1 to C-6, O*C*H$_3$) δ 105.4, 74.4, 77.1, 73.1, 76.6, 172.2, 57.7; aglycon δ 175.1, 34.7, 25.8, 30.0, 26.7, 30.4, 70.8, 52.1; MS calcd for $C_{46}H_{78}N_2O_{30}$ 1138: found MS − H 1137.

8-Methoxycarbonyloctyl 5-*N*-acetyl-α-neuraminyl-(2→3)-β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl-(1→3)]-2-deoxy-2-(methyl-β-D-galactohexopyranosyluronamide)-β-D-glucopyranoside 7b. According to general procedure D 15.0 mg (15.1 μmol) of sugar **6b** are incubated with 14.3 mg (22.5 μmol) GDP-fucose for 3 days to give 5.5 mg (32%) of the title compound. A TLC of the crude reaction mixture still showed some starting sugar. ^{1}H NMR (CD$_3$OD, 500.13 MHz) glcN-unit (H-1 to H-6,6′) δ 4.64, 3.82, 3.88, 3.85, 3.43, 3.93 and 3.98; gal-unit (H-1 to H-6,6′) δ 4.50, 3.54, 4.02, 3.87, 3.44, 3.62 and 3.74; sia-unit (H-3 to H-9, HNCOC*H*$_3$) δ 1.70, 2.85, 3.69, 3.69, 3.62, 3.46, 3.88, 3.58 and 3.83, 1.99; fuc-unit (H-1 to H-6) δ 5.11, 3.60, 3.85, 3.70, 4.84, 1.13; galA-unit (H-1 to H-5, OC*H*$_3$) δ 4.18, 3.51, 3.52, 4.12, 4.02, 3.56; aglycon δ 2.30, 1.58, 1.29, 1.56, 3.49, 3.81, 3.63; ^{13}C NMR glcN-unit (C-1 to C-6) δ 101.7, 57.7, 75.4*, 75.5*, 76.6, 61.3; gal-unit (C-1 to C-6) δ 103.8, 70.8, 77.9, 68.8, 77.3, 62.9; sia-unit (C-1 to C-9, HN*C*O*C*H$_3$) δ 176.0, not resolved, 42.2, 69.3, 53.8, 74.9, 70.1, 72.9, 64.6, 175.4, 22.6; fuc-unit (C-1 to C-6) δ 100.3, 70.0, 70.9, 73.6, 67.5, 16.5; glcA-unit (C-1 to C-6, O*C*H$_3$) δ 106.0, 72.0, 74.6, 70.7, 76.9, 171.6, 57.8; aglycon δ 176.0, 34.7, 25.9, 30.2, 26.8, 30.6, 70.9, 51.9;MS calcd for $C_{46}H_{78}N_2O_{30}$ 1138: found MS − H 1137.

8-Methoxycarbonyloctyl 5-*N*-acetyl-α-neuraminyl-(2→3)-β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl-(1→3)]-2-deoxy-2-(methyl-α-D-galactohexopyranosyluronamide)-β-D-glucopyranoside 7c. According to general procedure D 17.0 mg (17.1 μmol) of sugar **6c** are incubated with 15.6 mg (24.6 μmol) GDP-fucose for 4 days to give 4.8 mg (25%) of the title compound. A TLC of the crude reaction mixture still showed some starting sugar. ^{1}H NMR (CD$_3$OD, 500.13 MHz) glcN-unit (H-1 to H-6,6′) δ 4.60, 3.91, 3.87, 3.96, 3.42, 3.92 and 3.98; gal-unit (H-1 to H-6,6′) δ 4.51, 3.55, 4.03, 3.88, 3.44, 3.62 and 3.72; sia-unit (H-3 to H-9, HNCOC*H*$_3$) δ 1.70, 2.86 3.69, 3.70, 3.63, 3.45, 3.87, 3.59 and 3.84, 1.99; fuc-unit (H-1 to H-6) δ 5.09, 3.59, 3.84, 3.69, 4.87, 1.14; galA-unit (H-1 to H-5, OC*H*$_3$) δ 4.88, 3.81, 3.76, 4.20, 4.24, 3.41; aglycon δ 2.30, 1.58, 1.30, 1.54, 3.54, 3.76, 3.63; ^{13}C NMR glcN-unit (C-1 to C-6) δ 101.6, 57.3, 75.3, 76.5, 76.6, 61.2; gal-unit (C-1 to C-6) δ 103.7, 70.8, 77.7, 68.9, 77.3, 62.8; sia-unit (C-1 to C-9, HN*C*O*C*H$_3$) δ 174.6, 100.7, 42.2, 69.2, 53.9, 74.9, 70.2, 72.9, 64.6, 175.7, 22.6; fuc-unit (C-1 to C-6) δ 100.2, 69.6, 71.0, 73.6, 67.4, 16.4; galA-unit (C-1 to C-6, O*C*H$_3$) δ 101.5, 69.8, 70.9, 71.0, 72.8, not resolved, 56.1; aglycon δ 176.1, 34.7, 25.6, 30.1, 26.8, 30.4, 70.8, 51.8; MS calcd for $C_{46}H_{78}N_2O_{30}$ 1138: found MS + Na 1161 and MS − H + 2Na 1183.

8-Methoxycarbonyloctyl 5-*N*-acetyl-α-neuraminyl-(2→3)-β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl-(1→3)]-2-deoxy-2-(methyl-β-L-galactohexopyranosyluronamide)-β-D-glucopyranoside 7d. According to general procedure D 12.0 mg (12.1 μmol) of sugar **6d** are incubated with 32.4 mg (51.0 μmol) GDP-fucose for 3 days to give 4.8 mg (35%) of the title compound. The crude reaction mixture still showed some starting sugar. ^{1}H NMR (CD$_3$OD, 400.13 MHz) glcN-unit (H-1 to H-6,6′) δ 4.38, 4.05, 3.87, 3.81, 3.35, 3.86 and 3.94; gal-unit (H-1 to H-6,6′) δ 4.44, 3.46, 3.95, 3.80, 3.38, 3.57 and 3.68; sia-unit (H-3 to H-9, HNCOC*H*$_3$) δ 1.66, 2.78 3.65, 3.65, 3.64, 3.47, 3.84, 3.53 and 3.81, 1.96; fuc-unit (H-1 to H-6) δ 5.12, 3.56, 3.77, 3.65, 4.71, 1.06; galA-unit (H-1 to H-5, OC*H*$_3$) δ 4.13, 3.49, 3.49, 4.17, 3.89, 3.56; aglycon δ 2.38, 1.54, 1.29, 1.48, 3.40, 3.83, 3.68; ^{13}C NMR glcN-unit (C-1 to C-6) δ 102.2, 56.7, 75.5, 76.0, 76.9, 61.2; gal-unit (C-1 to C-6) δ 103.9, 70.8, 77.7, 68.6, 76.9, 62.9; sia-unit (C-1 to C-9, HN*C*O*C*H$_3$) δ not resolved, not resolved, 42.2, 69.3, 53.7, 75.1, 70.3, 72.9, 64.2, not resolved, 22.4; fuc-unit (C-1 to C-6) δ 99.5, 69.9, 70.7, 73.4, 67.3, 16.2; galA-unit (C-1 to C-6, O*C*H$_3$) δ 105.9, 71.6*, 74.1*, 71.2, 76.9, not resolved, 57.7; aglycon δ not resolved, 34.7, 26.0, 30.1, 26.9, 30.8, 70.4, 52.0; MS calcd for $C_{46}H_{78}N_2O_{30}$ 1138: found MS − H 1137 and MS + Na 1161.

8-Methoxycarbonyloctyl 5-*N*-acetyl-α-neuraminyl-(2→3)-β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl-(1→3)]-2-deoxy-2-(methyl-α-L-galactohexopyranosyluronamide)-β-D-glucopyranoside 7e. According to general procedure D 10.0 mg (10.0 μmol) of sugar **6e** are incubated with 12.1 mg (19.0 μmol) GDP-fucose for 2 days to give 8.3 mg (73%) of the title compound. The crude reaction mixture still showed some starting sugar. ^{1}H NMR (CD_3OD, 400.13 MHz) glcN-unit (H-1 to H-6,6′) δ 4.47, 4.11, 3.89*, 3.91*, 3.45, 3.95 and 3.98; gal-unit (H-1 to H-6,6′) δ 4.52, 3.55, 4.03, 3.87, 3.44, 3.65 and 3.75; sia-unit (H-3 to H-9, HNCOCH_3) δ 1.72, 2.89 3.71, 3.72, 3.63, 3.48, 3.86, 3.63 and 3.87, 2.02; fuc-unit (H-1 to H-6) δ 5.19, 3.62, 3.88, 3.72, 4.89, 1.18; galA-unit (H-1 to H-5, OCH_3) δ 4.89, 3.85, 3.79, 4.32, 4.18, 3.43; aglycon δ 2.32, 1.60, 1.31, 1.52, 3.45, 3.88, 3.68; ^{13}C NMR glcN-unit (C-1 to C-6) δ 102.4, 56.8, 75.2, 76.3, 76.7, 61.2; gal-unit (C-1 to C-6) δ 103.8, 70.8, 77.9, 68.8, 77.1, 63.0; sia-unit (C-1 to C-9, HN*C*OCH_3) δ not resolved, not resolved, 42.2, 69.2, 53.9, 74.9, 70.1, 73.0, 64.6, not resolved, 22.6; fuc-unit (C-1 to C-6) δ 99.8, 69.7, 70.8, 73.5, 67.5, 16.6; galA-unit (C-1 to C-6, OCH_3) δ 101.7, 69.5, 70.7, 71.5, 72.8, 172.1, 55.4; aglycon δ 175.7, 34.8, 25.9, 30.2, 27.0, 30.6, 70.9, 52.2; MS calcd for $C_{46}H_{78}N_2O_{30}$ 1138: found MS − H 1137.

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