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Glycosyltransferase catalyzed assemblage of sialyl-Lewis^a-saccharopeptides

Gabi Baisch, Reinhold Öhrlein*

Novartis Pharma AG, Postfach, CH-4002 Basle, Switzerland

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Abstract

A series of methyl hexopyranosiduronic acids are coupled to type I disaccharide amines to give 'trisaccharides' which have the natural *N*-acetyl group of the type I disaccharides replaced by uronic acids (→ saccharopeptides). These saccharopeptides are surprisingly good substrates for α-2,3-sialyltransferase and fucosyltransferase III. The enzymes transfer *N*-acetylneuraminic acid and fucose, respectively, onto these acceptor substrates, despite the far reaching alterations, regio- and stereospecifically in the expected manner to yield sialyl-Lewis^a-saccharopeptides.

Keywords: α-2,3-Sialyltransferase; Fucosyltransferase III; Enzymatic glycosylation; Sialyl-Lewis^a-saccharopeptide

1. Introduction

Oligosaccharides and peptides serve as receptor determinants in numerous biological reactions and infectious processes. Consequently, there have been a number of attempts recently to combine the features of both classes of compounds. Sugar amides (e.g. uronamides) have been designed [1,2] and integrated into synthetic concepts to serve as oligosaccharide [3–5] or peptide mimetics [6]. These 'peptidosaccharides' or 'saccharopeptides' have also been considered replacements of natural oligonucleotide backbones [7] in the anti-sense field.

Our interest in this quite unusual hybrid-class of compounds resulted from the search for analogues of the natural sialyl-Lewis^a (SLe^a)- and sialyl-Lewis^x (SLe^x)-tetrasaccharides (Scheme 1) with altered physiological properties [8,9]. Moreover we were interested if the glycosyltransferase methodology can be applied to assemble highly altered oligosaccharide structures predictably.

2. Results and discussion

Enhanced binding of SLe^a and SLe^x can e.g. be achieved by the introduction of hydrophobic groups [10] or replacements of the *N*-acetyl group of the glucosamine moiety with aromatic or heteroaromatic amides [9]. There has also been an intensive search for structurally simpler SLe^a and SLe^x mimetics where one or two of the monosaccharide units (Scheme 1) are replaced by various surrogates [11–13].

The replacement of the *N*-acetyl group of the *N*-acetylglucosamine unit by uronic acids, as described in this paper, represents a novel class of SLe^a

* Corresponding author;
e-mail: reinhold.oehrlein@pharma.novartis.com

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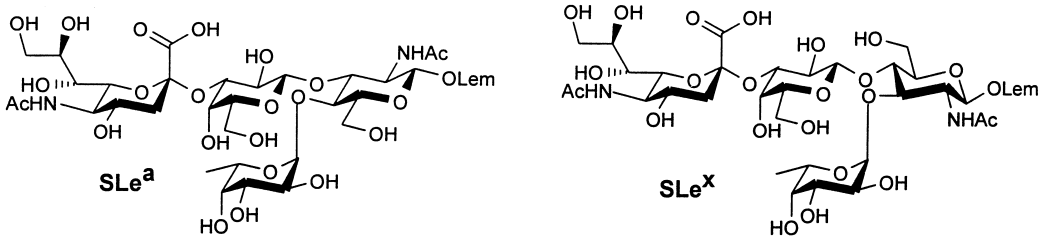


Scheme 1. Sialyl-Lewis^a- and sialyl-Lewis^x-tetrasaccharides (Lem = $(CH_2)_8COOMe$).

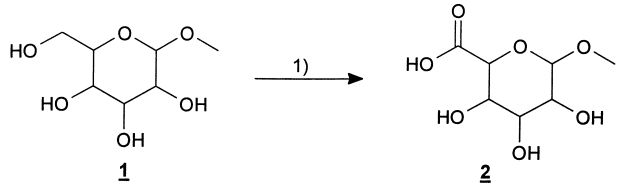
mimetics. The additional sugar moiety is thought to affect the selectin-ligand interaction [14].

The pathway for the preparation of the SLe^a-saccharopeptides is outlined in Scheme 2 and Scheme 3.

Non-commercially available methyl hexopyranosiduronic acids **2** are obtained from their corresponding α- or β-glycosides **1** via TEMPO-catalyzed oxidation of the CH_2OH group in water containing sodium hypochlorite (Scheme 2) [15,16]. The methyl α- or β-glycopyranosiduronic acids **2** are then coupled to the per-*O*-acetylated aminosugar **7** employing standard peptide coupling protocols [17,18] to give the desired saccharopeptides **8** after a final sodium methanolate treatment in order to remove the acetyl protection. We did not improve these couplings as sufficient material has been produced in all cases for the ensuing enzymatic glycosylations (Scheme 3).

The amine **7** is obtained from the known allyloxycarbonyl sugar **3** [19]. Compound **3** is 4,6-*O*-benzylidenated to give derivative **4** which is galactosylated with commercial galactosyl bromide **5**, promoted by mercury cyanide [20] to give disaccharide **6**. Subsequent protecting group manipulations according to the protocols of the parent *N*-acetyl congener followed by a Pd^o-catalyzed deallylation [21] give the free amine **7**.

The most convenient way to accomplish further glycosylations toward the desired SLe^a-structure without any tedious protecting group manipulations on the 'trisaccharide' **8** is to apply glycosyltransferases [22]. These enzymes transfer a monosaccharide unit from nucleotide-activated

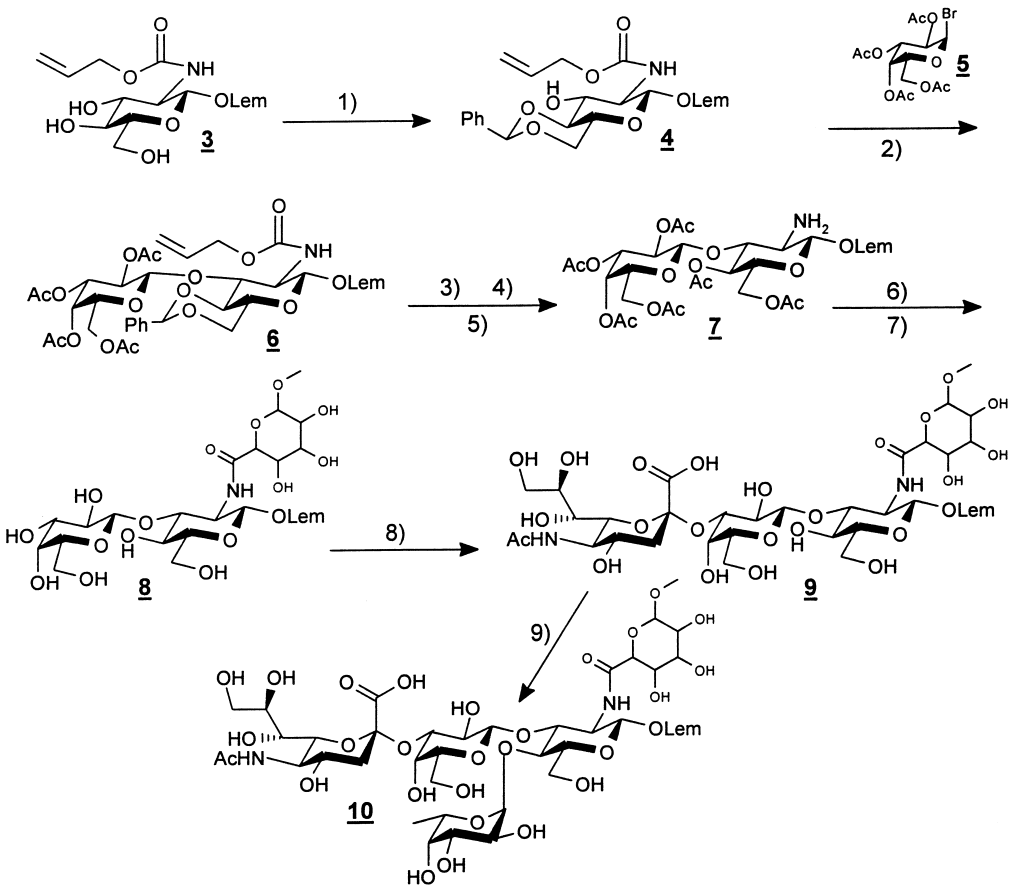


Scheme 2. (1) NaOCl, TEMPO, NaBr, H_2O, rt, 50–74%.

donor substrates regio- and stereospecifically onto a hydroxyl group of a growing oligosaccharide chain in vivo and in vitro. So, the various saccharopeptides **8** are incubated first with recombinant α-2,3-sialyltransferase (α-2,3-SiaT) [23] and CMP-Neu5Ac and then with fucosyltransferase III (FucT III) [24] and GDP-Fuc (Scheme 3 and Table 1).

Surprisingly, the incubations of all the described non-natural saccharopeptides **8** with SiaT give oligosaccharides whose ^1H and ^{13}C NMR spectra show the required signals for an additional *N*-acetylneuraminic acid, e.g, a singulet (3 H) at about 2.00 ppm for the additional *N*-acetyl group and a doublet of doublets (1 H) at about 2.75 ppm for the equatorial H-3, respectively. The attachment of Neu5Ac is further confirmed by signals in the ^{13}C NMR spectrum at about 41.0 ppm (C-3) and 102.0 ppm (C-2). Extensive NMR investigations (COSY, TOCSY, HMBC, and HSQC) of the starting compounds **8** and the sialylated saccharides **9** confirmed that the Neu5Ac residue is attached to the HO-3 group of the terminal Gal unit in an α-mode. A downfield shift of the C-3 signals of Gal in **8** from about 71 ppm to about 77 ppm in **9** is significant in all cases [25] (Table 2) and verifies the site of enzymatic sialylation. This is corroborated by a downfield shift of about 0.7 ppm (dd) of the corresponding Gal H-3 signal. No sialylation of the glycuronic acid part is observed, although compounds **8a** and **8b** bear D-GalA residues.

Finally, the sialylated sugars **9** are incubated with GDP-Fuc [26] and recombinant FucT III [24]. NMR data of the isolated materials indicate the presence of a Fuc moiety. All ^1H NMR spectra show additional doublets at about 1.2 ppm (3 H), a quartet at about 4.9 ppm, and a doublet at 5.1 ppm ($J\sim$ 4.5 Hz, 1 H). These signals are characteristic for H-6, H-5 and H-1, respectively, of an α-linked Fuc unit. This is corroborated by additional ^{13}C signals in the ^{13}C NMR spectra at about 16.6 ppm and 99.5 ppm, characteristic for C-6 and C-1 of an



Scheme 3. (1) THF, $PhCH(OMe)_2$, CSA, 95%; (2) toluene-nitromethane, 4 Å MS, $Hg(CN)_2$, 65%; (3) DCM, 90% -TFA, 100%; (4) Ac_2O, pyr, 76%; (5) THF, diethyl malonate, $Pd(PPh_3)_4$, 89%; (6) DMF, HBPyU, TEA, glycuronic acids **2**; (7) MeOH, MeONa; (8) 2,3-SiaT, CMP-Neu5Ac; (9) FucT III, GDP-Fuc; yields steps (6)–(9) see Table 1.

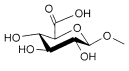
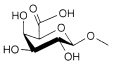
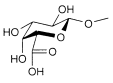
α-linked Fuc moiety. HMBC spectra and additional ROESY data of the saccharopeptides **10** confirm the attachment of Fuc to O-4 of the *N*-acylglucosamine unit. Also in this case no fucosylation of the glycuronic acid part is observed and none of the compounds seems to inhibit the transferase as even acceptors are glycosylated in high yield, which bear a L-GalA unit (**9d** and **9e**) closely resembling a fucose moiety.

3. Conclusion

In addition to former findings [19,23,27] these investigations underscore—the previously unexpected—high tolerance of recombinant SiaT and FucT III with respect to their acceptor substrates. Both enzymes accept a multitude of aliphatic and aromatic replacements of the *N*-acetyl group of their natural *N*-acetyl acceptor substrate [9,23] in vitro. As is shown here, even highly polar and bulky uronamides are tolerated at this position. *N*-acetylneuraminic acid is exclusively transferred onto O-3 of the terminal Gal residue in an α-mode like in the parent *N*-acetyl compound. Also fucosylations take place in the expected manner at O-4 of the GlcN moiety in an α-mode, although this sugar part carries the non-natural bulky uronamides close to the OH group which has to be approached closely by the enzyme in order to attach Fuc correctly.

These findings and previous ones show that the scope of the transferase methodology [27] has not been fully exploited yet. The pronounced ease of the synthetic use of these catalysts and their high regio- and stereochemical fidelity make the glycosyltransferases a valuable tool for the carbohydrate chemist. Thus naturally occurring oligosaccharides and a wealth of non-natural congeners can be prepared efficiently reducing the cumbersome manipulation of protecting groups, which is usually necessary in the classical chemical oligosaccharide synthesis, to a minimum. This rapid and unambiguous access to large numbers of carbohydrates makes available

Table 1
Yields of enzymatic glycosylations; *coupled to the fully de-*O*-acetylated amino sugar with HBTU (see Experimental)

Acid **2a–e**	Structure of coupled acid	Couple % **8a–e**	Neu5Ac % (mg) **9a–e**	Fuc % (mg) **10a–e**
a β-D-GlcA		16	70 (24.6)	82 (18.8)
b β-D-GalA		36	90 (35.0)	63 (18.8)
c α-D-GalA		36	93 (29.6)	79 (20.1)
d β-L-GalA		15	63 (24.8)	56 (12.2)
e α-L-GalA		4*	74 (10.4)	75 (8.6)

this class of compounds for high-through-put screens for various biological applications.

4. Experimental

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AC 250 and/or a Varian Unity 500 spectrometer with multi-probe heads. COSY, TOCSY, HMBC and HSQC experiments were performed using the manufacturers' softwares. ^{1}H NMR shifts were measured in CD_3OD and referenced to internal D_2O (4.80 ppm) and ^{13}C NMR shifts were referenced to CD_3OD (49.00 ppm) unless otherwise stated. 1H NMR and ^{13}C NMR shift assignments are tentative; shifts marked with an asterik (*) may be interchanged.

TLC was performed on silica gel $60F_{254}$ glass sheets (Merck), and sugars were stained with *p*-anisaldehyde/H_2SO_4 (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. Commercial L-Gal (Fluka) was used. 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, 7500 U/498 μL). Recombinant α-2,3-SiaT (~100 U/L) [23,28] (EMBL accession no. M97754) and recombinant FucT III (~11.8 U/L) [24,29] (EMBL accession no. X53578) were obtained from transfected insect cells. FABMS spectra were recorded on a FO25FAB instrument with Cs or Xe as bombarding gas, and with thioglycerol as matrix.

General procedure A: Preparation of methyl glycopyranosiduronic acids [16].—Methyl α- or β-glycoside **1** (2 g, 10.3 mmol), TEMPO (2,2,6,6-tetramethyl-1-piperidinyl oxy radical; 16 mg, 0.1 mmol) and NaBr (0.53 g, 5.1 mmol) were dissolved at room temperature in water (30 mL) and the solution cooled to 0 °C. To this mixture aq 5% NaOCl (15 mL) was added dropwise with vigorous stirring. During the addition the pH is kept at 10–11 with 0.5 M NaOH using a pH-dosimeter. When TLC (6:4:1 CH_2Cl_2–MeOH–H_2O) showed the complete consumption of the starting sugar (usually 2–7 h), MeOH (30 mL) was added. The resulting precipitates were filtered off and washed with MeOH. The combined filtrates were concentrated, and the residue chromatographed on silica gel (elute first with 3:1:0.2 CH_2Cl_2–MeOH–HOAc to remove side-products and then with 6:4:1 CH_2Cl_2–MeOH–H_2O) to give the methyl pyranosiduronic acids **2**.

General procedure B: Coupling of methyl glycopyranosiduronic acids (**2**) *to glucosamine residues* (**7**).—According to general peptide coupling protocols [18] amine **7** (0.33 mmol), methyl

Table 2
Selected ^{13}C NMR data of saccharopeptides **10**

entry	Fuc		Neu5Ac		Gal		GlcN		MeO	Uronic acid	
	C-1	C-6	C-2	C-3	C-1	C-3	C-1	C-2	OCH_3	C-1	C-6
10a	99.6	16.6	102.1	41.4	103.9	77.6	102.4	56.9	57.8	105.4	172.2
10b	99.6	16.6	101.8	41.7	104.2	77.7	102.2	57.7	58.1	105.8	171.6
10c	99.5	16.6	101.4	41.4	103.9	77.5	101.7	58.3	56.6	102.1	172.0
10d	99.5	16.6	102.6	40.6	103.2	77.2	102.6	56.9	57.8	106.0	171.7
10e	99.5	16.6	101.8	40.7	103.2	77.3	102.6	56.8	56.3	101.9	172.3

glycopyranosiduronic acid **2** (0.36 mmol) and HBPyU ([*O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-bis(tetramethylen)-uronium-hexafluourophosphate 0.36 mmol) were dissolved at room temperature in dry DMF (5 mL). The clear solution was treated with Et_3N (0.36 mmol) and stirred at room temperature for 2–4 days (even after this period the starting amine was not completely consumed). The mixture was then concentrated to dryness and the residue chromatographed over silica gel (10:0.5 CH_2Cl_2–MeOH) to give an acetylated saccharopeptide intermediate. This compound was dissolved in dry MeOH (4 mL), containing 0.1% NaOMe, and stirred at room temperature for 1–2 h until TLC (10:3 CH_2Cl_2–MeOH) showed complete consumption of the starting material. Evaporation of the solvent left a solid which was purified over silica gel (10:3 CH_2Cl_2–MeOH).

General procedure C: Enzymatic sialylation of type I saccharopeptides (**8**).—Following standard sialylation protocols [23,30] compound **8** (32.2 μmol) and CMP-Neu5Ac (44.6 μmol) [31] were dissolved in a mixture of 2 mL 0.05 M sodium cacodylate buffer (pH 6.5), 2 mL 0.06 M $MnCl_2$ and 1.3 mL deionized water containing 2.1 mg BSA. The mixture was diluted with Me_2SO (0.5 mL) and incubated at 37 °C with CIAP (3 μL) and recombinant SiaT (200 μL, 1.4 U) [28]. When TLC (10:4:0.8 CH_2Cl_2–MeOH–H_2O) showed the disappearance of the starting acceptor—usually overnight—the turbid solution was centrifuged and the supernatant passed over a C-18 reversed phase column, washed with water and eluted with MeOH. The MeOH was concentrated and the resulting residue was chromatographed over silica gel (10:4:0.8 CH_2Cl_2–MeOH–H_2O) to give the pure sialylated sugars **9** as white powders after lyophilization from water-dioxane.

General procedure D: Enzymatic fucosylation of saccharides (**9**).—Following general fucosylation protocols [24,30] compound **9** (22.2 μmol), GDP-Fuc (34.2 μmol) [26] and BSA (2 mg) were added to a mixture of 450 μL 0.25 M sodium cacodylate buffer (pH 6.5) and 150 μL 0.25 M $MnCl_2$. The mixture was diluted with deionized water (600 μL) and incubated at 37 °C with CIAP (3 μL) and recombinant FucT III (100 μL, 600 mU) [24,29] for 24–36 h until TLC (10:4:0.8 CH_2Cl_2–MeOH–H_2O) showed the consumption of the starting acceptor **9**. The mixture was then centrifuged and the supernatant passed over a C-18 reversed phase column, washed with water and eluted with MeOH. The MeOH was concentrated and the resulting residue was chromatographed on silica gel (10:4:0.8 CH_2Cl_2–MeOH–H_2O) to give the pure saccharides **10** as white powders after lyophilization from water.

Methyl β-D-*glucopyranosiduronic acid* (**2a**).—This was available from Sigma as the sodium salt.

Methyl β-D-*galactopyranosiduronic acid* (**2b**).—According to general procedure A, methyl *β*-D-galactopyranoside **1b** (2 g, 10 mmol) was oxidized to give **2b** (1.04 g, 50%). ^{1}H NMR (CD_3OD, 250 MHz): δ 3.40–3.59 (m, 5 H, H-2,3 and OCH_3), 3.89 (bs, 1 H, H-5), 4.06–4.18 (m, 2 H, H-1,4); ^{13}C NMR (CD_3OD, 62.9 MHz): δ 58.22 (OCH_3), 71.74 (C-4), 71.89 (C-2), 74.51 (C-3), 77.02 (C-5), 105.24 (C-1), 175.72 (C = O). MS Calcd for $C_7H_{12}O_7$: 208; found $[M-H]^-$ 207.

Methyl α-D-*galactopyranosiduronic acid* (**2c**).—According to general procedure A methyl *α*-D-galactopyranoside **1c** (2 g, 10 mmol) was oxidized to give **2c** (1.2 g, 57%). ^{1}H NMR (CD_3OD, 250 MHz): δ 3.38 (s, 3 H, OCH_3), 3.74–3.81 (m, 2 H, H-2,3), 4.11–4.20 (m, 2 H, H-4,5); ^{13}C NMR (CD_3OD, 62.9 MHz): δ 56.21 (OCH_3), 69.34 (C-2), 71.11 (C-3), 72.23 (C-4), 72.91 (C-5), 101.08 (C-1), 176.28 (C = O). MS Calcd for $C_7H_{12}O_7$: 208; found $[M-H]^-$ 207.

Methyl β-L-*galactopyranosiduronic acid* (**2d**).—According to general procedure A methyl *β*-L-galactopyranoside **1d** (0.54 g, 2.8 mmol) was oxidized to give **2d** (0.43 g, 74%) after a final passage over a weakly acid Amberlite resin (H^+-form). ^{1}H NMR (CD_3OD, 400 MHz): δ 3.52 (dd, 1 H, $J_{1,2}$ 7.2, $J_{2,3}$ 9.6 Hz, H-2), 3.60 (s, 3 H, OCH_3), 3.71 (dd, 1 H, $J_{3,4}$ 4.8, $J_{2,3}$ 9.6 Hz, H-3), 4.06 (d, 1 H, $J_{4,5}$ 1.2 Hz, H-5), 4.22 (dd, 1 H, $J_{4,5}$ 1.2, $J_{3,4}$ 4.8 Hz, H-4), 4.31 (d, 1 H, $J_{1,2}$ 7.2 Hz, H-1); ^{13}C NMR (CD_3OD, 100.6 MHz): δ 58.50 (OCH_3), 71.63 (C-4), 71.85 (C-2), 74.30 (C-3), 76.55 (C-5), 105.64 (C-1), 175.78 (C = O). MS calcd for $C_7H_{12}O_7$: 208; Found $[M-H]^-$ 207.

Methyl α-L-*galactopyranosiduronic acid* (**2e**).—According to general procedure A methyl *α*-L-galactopyranside **1e** (0.97 g, 5 mmol) was oxidized to give **2e** (0.65 g, 64%) after a final passage over a weakly acid Amberlite resin (H^+-form). ^{1}H NMR (CD_3OD, 400 MHz): δ 3.55 (s, 3 H, OCH_3), 3.95–4.04 (m, 2 H, H-2,3), 4.39–4.41 (m, 2 H, H-4,5), 5.06 (d, 1 H, $J_{1,2}$ 4.1 Hz, H-1); ^{13}C NMR (CD_3OD, 100.6 MHz): δ 56.21 (OCH_3), 69.44 (C-2), 71.21 (C-3), 72.31 (C-4), 72.99 (C-5), 101.21 (C-1), 176.22 (C = O). MS Calcd for $C_7H_{12}O_7$: 208; Found $[M-H]^-$ 207.

*8-Methoxycarbonyloctyl 2-*N*-allyloxycarbonyl-4,6-*O*-benzylidene-2-deoxy-*β*-*D*-glucopyranoside* (**4**).— To dry THF (100 mL) were added successively monosaccharide derivative **3** (9.7 g, 22.4 mmol) [19], benzaldehyde dimethylacetal (6 mL, 40 mmol; Fluka) and ± camphorsulfonic acid (0.25 g; Fluka). This mixture was heated to 50 °C and stirred overnight until complete consumption of the starting sugar. The mixture was then cooled to room temperature, treated with Et_3N (0.5 mL) and concentrated to dryness. The resulting residue was chromatographed on silica gel (20:1 CH_2Cl_2–MeOH) to give **4** (11 g, 95%). ^{1}H NMR ($CDCl_3$, 400.13 MHz): δ 1.23 and 1.51 (m, 8 H and 4 H, -$(CH_2)_6$-), 2.23 (t, 2 H, *J* 7.6 Hz, CH_2-C=O), 3.25–3.50 (m, 6 H, H-1,2,3,6a,6b and -CH*H*O-), 3.60 (s, 3 H, OCH_3), 3.70 (t, 1 H, $J_{3,4}$ 9.7 Hz, H-4), 3.78 (dt, 1 H, *J* 4.8, 9.7 Hz, -CH*H*O-), 4.25 (broad dd, 1 H, $J_{5,6a}$ 4.8, $J_{5,6b}$ 10.9 Hz, H-5), 4.50 (m, 2 H, allylic CH_2), 5.12 (m, 1 H, =CH_2), 5.23 (dq, *J* 1.2, 16.3 Hz, 1 H, =CH*H*), 5.45 (s, 1 H, -$CH(O)_2$-), 5.84 (m, 1 H, =CH-), 7.30 (m, 3 H, phenyl), 7.42 (m, 2 H, phenyl); ^{13}C NMR ($CDCl_3$, 100.61 MHz): δ 24.77, 25.63, 28.91, 29.00, 29.35, and 34.16 (all aglycon -CH_2-), 51.46 (OMe), 58.60 (C-2), 65.73, 66.04, 68.59, 70.21, 70.69, and 72.27 (C-3,4,5,6, -CH_2-, and allylic CH_2), 81.49 (-$CH(O)_2$-), 101.75 (C-1), 117.60 (=CH_2), 126.21 (2 C, phenyl), 128.23 (2 C, phenyl), 129.17 (phenyl), 132.46 (=CH_1-), 159.16 and 174.53 (each C=O).

*8-Methoxycarbonyloctyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→3)-2-*N*-allyloxycarbonyl-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside* (**6**).—To 1:1 dry toluene-nitromethane (260 mL), containing finely ground 4 Å molecular sieves (ca. 5 g), were added compound **4** (8.7 g, 17 mmol) and mercury cyanide (5.5 g, 22 mmol). The mixture was stirred for 30 min at room temperature. Then a solution of peracetylated galactose **5** (10.3 g, 25 mmol) in 1:1 dry toluene–nitromethane (35 mL) was dropped to this mixture with vigourous stirring, and the mixture was finally heated at 50 °C for 18 h. After complete consumption of compound **4** the mixture was carefully filtered over Celite and concentrated. The residue was chromatographed over silica gel (2:1 hexane–EtOAc) to yield **6** (9.1 g, 64%). ^{1}H NMR ($CDCl_3$, 400.13 MHz): δ 1.22 and 1.51 (m, 8 H and 4 H, $(CH_2)_6$-), 1.88, 1.89, 1.91, and 2.05 (4 s, each 3 H, 4 Ac), 2.23 (t, 2 H, *J* 7.6 Hz, CH_2-C=O), 3.06 (bs, 1 H, NH), 3.41, 3.59, and 3.71 (m, 2 H, 6 H, 2 H, H-1,2,3,4,6a,6b, OMe, and -CH*H*O-), 3.78 (dt, 1 H, *J* 6.1, 9.1 Hz, -CH*H*O-), 3.98 (dd, 1 H, $J_{5',6a'}$ 6.6, $J_{6a',6b'}$ 11.4 Hz, H-6a′), 4.25 (dd, 1 H, $J_{5',6b'}$ 6.1, $J_{6a',6b'}$ 11.4 Hz, H-6b′), 4.39 (m, 1 H, H-5), 4.50 (m, 2 H, allylic CH_2), 4.59 (d, 1 H, $J_{1,2}$ 7.3 Hz, H-1′), 4.89 (dd, 1 H, $J_{3',4'}$ 3.6, $J_{2',3'}$ 10.9 Hz, H-3′), 5.05 (m, 1 H, H-5′), 5.13 (dd, 1 H, $J_{1',2'}$ 7.3, $J_{2',3'}$ 10.9 Hz, H-2′), 5.19 (dq, 1 H, *J* 1.2, 11.5 Hz, =CH*H*), 5.22 (dd, 1 H, $J_{4',3'}$ 0.6, $J_{4',5'}$ 3.6 Hz, H-4′), 5.27 (m, 1 H, =CH*H*), 5.47 (s, 1 H, -$CH(O)_2$-), 5.86 (m, 1 H, =CH-), 7.30 (m, 3 H, phenyl), 7.40 (m, 2 H, phenyl); ^{13}C NMR ($CDCl_3$, 62.90 MHz): δ 20.52 (2 Ac), 20.62 (2 Ac), 24.80, 25.65, 28.93, 29.00 (2 C), 29.38, and 33.99 (all aglycon -CH_2-), 51.44 (OMe), 58.08 (C-2), 60.70, 65.60, 65.87, 66.73, 68.70, 69.06, 70.27, 70.40, 70.97, 76.49, and 78.63 (C-3,4,5,6,2′,3′,4′,5′,6′, allylic-C, and -CH_2O-), 80.18 (-$CH(O)_2$-), 101.01 (C-1′), 101.33 (C-1), 117.88 (=CH_2), 126.03 (2 C, phenyl), 128.15 (2 C, phenyl), 129.14 (phenyl), 137.04 (=CH_1-), 155.43, 169.40, 170.06, 170.11, 170.24, and 174.42 (each C=O).

*8-Methoxycarbonyloctyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→3)-4,6-di-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranoside* (**7**).—To a solution of disaccharide **6** (9.1 g, 10.7 mmol) in CH_2Cl_2 (100 mL) at room temperature were added aq 90% CF_3CO_2H (5 mL). After 6 h the mixture was neutralized with satured $NaHCO_3$, diluted with EtOAc and successively extracted with water and brine. The organic phase was dried (Na_2SO_4) and concentrated to give a syrup which is dissolved in pyridine (7 mL) and Ac_2O (3.5 mL). The mixture was stirred overnight at room temperature, then diluted with EtOAc and successively extracted with 4 M HCl, water, and satured $NaHCO_3$. Evaporation of the organic solvent left a yellow syrup which was chromatographed over silica gel (2:1 petrolether–EtOAc) to yield the *N*-allyloxycarbonyl intermediate (6.9 g, 76%). ^{1}H NMR ($CDCl_3$, 400.13 MHz): δ 1.22 and 1.51 (m, 8 H and 4 H, -$(CH_2)_6$-), 1.93, 1.98, 2.00, 2.01, 2.09, and 2.17 (6 s, each 3 H, 6 Ac), 2.24 (t, 2 H, *J* 7.6 Hz, -CH_2C=O); ^{13}C NMR ($CDCl_3$, 62.90 MHz): δ 20.50, 20.60 (3 C), 20.67, and 20.79 (each Ac), 24.79, 25.63, 28.91, 28.97, 29.01, 29.30, and 33.99 (aglycon -CH_2-), 51.43 (OMe), 58.02 (C-2), 60.98, 62.44, 65.59, 66.76 (2 C), 69.00, 69.15, 70.00, 70.42, 70.95, and 71.65 (C-3,4,5,6,2′,3′,6′,5′,6′ and allyl -CH_2-), 100.55 (C-1′), 101.02 (C-1), 117.91 (C=CH_2), 137.50 (C=CH_1-), 155.55, 169.15, 169.27, 170.11, 170.19, 170.32, 170.75 and 174.29 (each C=O).

To a solution of this intermediate (4 g, 4.7 mmol) in dry THF (60 mL) in an argon atmosphere at room temperature were added malonic acid diethyl ester (5.6 mL) and tetrakis-(triphenyl phosphino)-palladium (0.4 g, 0.3 mmol; Fluka). After 1 h the solvent was removed and the residue chromatographed on silica gel (2:1 petrolether–EtOAc) to give the amine **7** (3.1 g, 89%). ^{1}H NMR ($CDCl_3$, 250.13 MHz): δ 1.33 and 1.60 (m, 8 H and 4 H, aglycon -CH_2-), 1.99 (s, 3 H, Ac), 2.05 (m, 12 H, 4 Ac), 2.13 (s, 3 H, Ac), 2.29 (t, 2 H, J 7.6 Hz, -CH_2C=O aglycon), 2.92 (dd, 1 H, $J_{1,2}$ 7.6, $J_{2,3}$ 8.2 Hz, H-2), 3.46 (dt, 1 H, J 6.9, 10.3 Hz, -CH*H*O- aglycon), 3.58 (m, 1 H, H-5), 3.67 (s, 3 H, OMe), 3.89 (m, 2 H, H-3 and -CH*H*O- aglycon), 4.14 (m, 6 H, H-6a,6b,2′,5′,6a′,6b′), 4.73 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1), 4.99 (m, 2 H, H-1′,3′); 5.15 (dd, 1 H, $J_{3,4}$ 7.6, $J_{4,5}$ 11.7 Hz, H-4), 5.35 (m, 1 H, H-4′); ^{13}C NMR ($CDCl_3$, 62.90 MHz): δ 20.50 (Ac), 20.60 (3 Ac), 20.77 (Ac), 20.81 (Ac), 24.82, 25.83, 28.98, 29.09 (2 C), 29.42, and 33.99 (all aglycon-$(CH_2)_6$-), 51.40 (OMe), 57.05 (C-2), 60.91, 62.51, 66.74, 68.70, 69.52, 70.16, 70.58, 70.97, and 72.04 (C-3,4,5,6,2′,3′,5′,6′ and -CH_2O- aglycon), 83.53 (C-4′), 101.45 (C-1′), 103.12 (C-1), 169.03 (C=O), 169.30 (C=O), 170.13 (C=O), 170.29 (2 C=O), 170.75 (C=O), 174.44 (C=O). MS Calcd for $C_{34}H_{53}NO_{18}$: 763; Found $[M+H]^+$ 764.

Alternatively, the fully deacetylated amine **7a** [*8-methoxycarbonyloctyl ([2-amino-2-deoxy-β-*D*-galactopyranosyl)-(1→3)-β-*D*-glucopyranoside*] has been used in some cases. It was obtained by stirring the acetylated intermediate of the above reaction, after removal of the benzylidene group, at room temperature in dry MeOH, containing 0.1% MeONa, for 1 h. The mixture was neutralized with Dowex 50×8 (H^+-form), filtered, and concentrated. The resulting residue was chromatographed on silica gel (9:1 CH_2Cl_2–MeOH) to give the deacetylated *N*-allyloxy compound which was fully deprotected as follows: to the *N*-protected disaccharide (4.08 g, 6.8 mmol), dissolved in a mixture of dry THF (160 mL) and dry MeOH (60 mL) were added at room temperature 1,4-bis(diphenylphosphino)butane (0.50 g, 1.2 mmol), sodium thiophenolate (1.5 g, 11.3 mmol), and tris(dibenzylideneacetone)-dipalladium-0 adduct (0.3 g, 0.3 mmol; Aldrich). The mixture was stirred overnight in an argon atmosphere, then concentrated and chromatographed on silica gel (5:1 CH_2Cl_2–MeOH) to give the fully deacetylated amine **7a** (2.2 g, 64%). ^{1}H NMR (CD_3OD–$CDCl_3$, 400.13 Hz): δ 1.35 (m, 8 H, aglycon -CH_2-), 1.64 (m, 4 H, aglycon -CH_2-), 2.33 (t, 2 H, J 7.5 Hz, -CH_2C=O aglycon), 2.80 (bt, 1 H, $J_{1,2}$ 7.4 Hz, H-2), 3.35 (m, 1 H, H-5), 3.44–3.96 (m, 15 H, -CH_2O- aglycon, OMe, and H-3,4,6a,6b,2′,3′,4′,5′,6a′,6b′), 4.31 (d, 1 H, $J_{1',2'}$ 8.6 Hz, H-1′), 4.43 (d, 1 H, $J_{1,2}$ 8.6 Hz, H-1).

8-Methoxycarbonyloctyl β-D-*galactopyranosyl-(1→3)-2-deoxy-2-(methyl* β-D-*glucopyranosyluronamide)*-β-D-*glucopyranoside* (**8a**).—According to general procedure B, from the commercial sodium (methyl *β*-D-glucopyranosid)uronate **2a** (83 mg, 0.36 mmol) and compound **7** (250 mg, 0.33 mmol) was obtained the acetylated intermediate (96.9 mg, 31%) which was deacetylated to give the title compound (35 mg, 50%). ^{1}H NMR (Me_2SO-d_6, 500.13 MHz): GlcN (from H-1 to H-6a,6b): δ 4.12 (d, 1 H, $J_{1,2}$ ~ 7.6 Hz), 3.34, 3.60, 3.50, 3.67, 3.16, 3.33; Gal (from H-1 to H-6a,6b): δ 4.07 (d, 1 H, $J_{1,2}$ ≈ 7.4 Hz), 2.97, 3.27, 3.56, 3.15, 3.47, 3.68; GlcA (from H-1 to H-5, OCH_3): δ 4.45 (d, 1 H, $J_{1,2}$ ≈ 8.0 Hz), 3.23, 3.12, 3.30, 3.21, 3.39; aglycon: δ 2.25, 1.48, 1.20, 1.42, 3.44, 3.69, 3.60; ^{13}C NMR (Me_2SO-d_6): GlcN (from C-1 to C-6): δ 103.6, 55.9, 83.7, 75.0, 75.5, 60.7; Gal (from C-1 to C-6): δ 104.0, 72.9, 70.4, 68.1, 75.9, 60.4; GlcA (from C-1 to C-6, OCH_3): δ 100.3, 72.9, 76.4, 71.5, 73.0, 169.2, 56.0; aglycon: δ 173.4, 33.1, 24.2, 28.3, 25.0, 28.8, 68.3, 53.6. MS calcd for $C_{29}H_{51}NO_{18}$: 701; found $[M+Na]^+$ 724. Anal. Calcd for $C_{29}H_{51}NO_{18}$: C, 46.64; H, 7.33. Found: C, 46.53; H, 7.48.

8-Methoxycarbonyloctyl β-D-*galactopyranosyl-(1→3)-2-deoxy-2-(methyl* β-D-*galactopyranosyluronamide)*-β-D-*glucopyranoside* (**8b**).—According to general procedure B, from methyl *β*-D-galactopyranosiduronate **2b** (75 mg, 0.36 mmol) and compound **7** (250 mg, 0.33 mmol) was obtained the acetylated intermediate (230 mg, 74%) which was deacetylated to give the title compound (82.7 mg, 49%). ^{1}H NMR (Me_2SO-d_6, 500.13 MHz): GlcN (from H-1 to H-6a,6b): δ 4.77 (d, 1 H, $J_{1,2}$ ≈ 7.6 Hz), 3.41, 3.76, 3.37, 3.14, 3.67; Gal (from H-1 to H-6a,6b): δ 4.14 (d, 1 H, $J_{1,2}$ ≈ 7.4 Hz), 3.25, 3.14, 3.56, 3.24, 3.46; GalA (from H-1 to H-5, OCH_3): δ 4.05 (d, 1 H, $J_{1,2}$ ≈ 8.0 Hz), 3.30, 3.30, 3.91, 3.85, 3.45; aglycon: δ 2.26, 1.48, 1.21, 1.43, 3.37, 3.68, 3.55; ^{13}C NMR (Me_2SO-d_6): GlcN (from C-1 to C-6): δ 99.6, 54.8, 83.4, 75.4, 76.6, 60.7; Gal (from C-1 to C-6): δ 103.8, 73.0, 68.7, 68.1, 70.5, 60.5; GalA (from C-1 to C-6, OCH_3): δ 104.2, 69.9, 73.0, 68.9, 74.7, 168.5, 56.0; aglycon: δ 173.3, 33.1, 24.3, 28.4, 25.1, 28.9, 68.4, 51.0. MS calcd for $C_{29}H_{51}NO_{18}$: 701; found $[M+Na]^+$ 724.

Anal. Calcd for $C_{29}H_{51}NO_{18}$: C, 46.64; H, 7.33. Found: C, 46.71; H, 7.58.

8-Methoxycarbonyloctyl β-D-*galactopyranosyl-(1→3)-2-deoxy-2-(methyl* α-D-*galactopyranosyluronamide)*-β-D-*glucopyranoside* (**8c**).—According to general procedure B, from methyl α-D-galactopyranosiduronate **2c** (75 mg, 0.36 mmol) and compound **7** (250 mg, 0.33 mmol) was obtained the acetylated intermediate (131 mg, 42%) which was deacetylated to give the title compound (86 mg, 86%). ^{1}H NMR (Me_2SO-d_6, 500.13 MHz): GlcN (from H-1 to H-6a,6b): δ 4.76 (d, 1 H, $J_{1,2} \approx$ 7.6 Hz), 3.41, 3.69, 3.38, 3.42, 3.65; Gal (from H-1 to H-6a,6b): δ 4.11 (d, 1 H, $J_{1,2} \approx$ 7.4 Hz), 3.24, 3.24, 3.12, 3.11, 3.44, 3.48; GalA (from H-1 to H-5, OCH_3): 4.67 (d, 1 H, $J_{1,2} \approx$ 4.9 Hz), 3.55, 3.52, 3.99, 3.97, 3.28; aglycon: δ 2.25, 1.47, 1.20, 1.41, 3.35, 3.67, 3.55; ^{13}C NMR (Me_2SO-d_6): GlcN (from C-1 to C-6): δ 99.5, 54.7, 84.2, 75.5, 79.9, 60.7; Gal (from C-1 to C-6): 103.8, 72.8*, 70.7*, 68.7, 76.4, 60.4; GalA (from C-1 to C-6, OCH_3): δ 104.2, 69.9, 73.0, 68.9, 74.7, 168.5, 56.0; aglycon: δ 173.3, 33.1, 24.3, 28.4, 25.1, 28.9, 68.4, 51.0. MS calcd for $C_{29}H_{51}NO_{18}$: 701; found $[M-H]^-$ 700. Anal. Calcd for $C_{29}H_{51}NO_{18}$: C, 46.64; H, 7.33. Found: C, 46.67; H, 7.56.

8-Methoxycarbonyloctyl β-D-*galactopyranosyl-(1→3)-2-deoxy-2-(methyl* β-L-*galactopyranosyluronamide)*-β-D-*glucopyranoside* (**8d**).—According to general procedure B, from methyl β-L-galactopyranosiduronate **2d** (75 mg, 0.36 mmol) and compound **7** (250 mg, 0.33 mmol) was obtained the acetylated intermediate (104 mg, 33%) which was deacetylated to give the title compound (34.1 mg, 45%). ^{1}H NMR (Me_2SO-d_6, 500.13 MHz): GlcN (from H-1 to H-6a,6b): δ 4.53 (d, 1 H, $J_{1,2} \approx$ 7.6 Hz), 3.70, 3.70, 3.16, 3.10, 3.66; Gal (from H-1 to H-6a,6b): δ 4.16 (d, 1 H, $J_{1,2} \approx$ 7.4 Hz), 3.27, 3.27, 3.57, 3.39, 3.48; GalA (from H-1 to H-5, OCH_3): δ 4.04 (d, 1 H, $J_{1,2} \approx$ 7.4 Hz), 3.31, 3.34, 3.92, 3.81, 3.44; aglycon: δ 2.26, 1.45, 1.19, 1.40, 3.34, 3.65, 3.55; ^{13}C NMR (Me_2SO-d_6): GlcN (from C-1 to C-6): δ 100.3, 53.4, 83.8, 69.0, 76.2, 60.7; Gal (from C-1 to C-6): δ 103.5, 72.7*, 70.6*, 68.1, 75.5, 60.5; GalA (from C-1 to C-6, OCH_3): δ 104.2, 70.0, 72.6, 69.1, 75.3, C-6 not resolved, 56.1; aglycon: δ 173.3, 33.1, 24.2, 28.4, 25.0, 28.6, 68.5, 51.0. MS calcd for $C_{29}H_{51}NO_{18}$: 701; found $[M+Na]^+$ 724. Anal. Calcd for $C_{29}H_{51}NO_{18}$: C, 46.64; H, 7.33. Found: C, 46.31; H, 6.99.

8-Methoxycarbonyloctyl β-D-*galactopyranosyl-(1→3)-2-deoxy-2-(methyl* α-L-*galactopyranosyluronamide)*-β-D-*glucopyranoside* (**8e**).—To a solution of amine **7a** (0.2 g, 0.39 mmol) in DMF (5 mL) at room temperature was added sugar acid **2e** (0.1 g, 0.47 mmol), *O*-(1H-benzotriazole-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (0.18 g, 0.47 mmol; Fluka), and Et_3N (65 μL). This heterogenous mixture was stirred for 5 days then worked up according general procedure B to give compound **8e** (10.8 mg, 4%). ^{1}H NMR (Me_2SO-d_6, 400.13 MHz): GlcN (from H-1 to H-6a,6b): δ 4.52 (d, 1 H, $J_{1,2} \approx$ 7.6 Hz), 4.00, 3.80, 3.50, 3.40, 3.78, 3.91; Gal (from H-1 to H-6a,6b): δ 4.45 (d, 1 H, $J_{1,2} \approx$ 7.4 Hz), 3.52, 3.50, 3.82, 3.65, 3.71, 3.74; GalA (from H-1 to H-5, OCH_3): 4.93 (d, 1 H, $J_{1,2} \approx$ 4.0 Hz), 3.86, 3.83, 4.30, 4.22, 3.45; aglycon: δ 2.38, 1.60, 1.31, 1.53, 3.49, 3.89, 3.69; ^{13}C NMR (Me_2SO-d_6, 100.61 MHz): GlcN (from C-1 to C-6): δ 102.3, 55.4, 83.9, 73.8, 77.0, 62.3; Gal (from C-1 to C-6): δ 104.7, 72.5, 70.5, 70.1, 76.9, 62.5; GalA (from C-1 to C-6, OCH_3): δ 101.4, 69.4, 71.1, 71.3, 72.8, C-6 not resolved, 56.4; aglycon: δ C=O not resolved, 34.8, 25.8, 30.0, 26.8, 30.4, 70.0, 52.5. MS calcd for $C_{29}H_{51}NO_{18}$: 701; found $[M+Na]^+$ 724. Anal. Calcd for $C_{29}H_{51}NO_{18}$: C, 46.64; H, 7.33. Found: C, 46.73; H, 7.58.

8-Methoxycarbonyloctyl 5-N-*acetyl*-α-*neuraminyl-(2→3)*-β-D-*galactopyranosyl-(1→3)-2-deoxy-2-(methyl* β-D-*glucopyranosyluronamide)*-β-D-*glucopyranoside* (**9a**).—According to general procedure C, compound **8a** (25 mg, 35.6 μmol) was incubated with CMP-Neu5Ac (33.1 mg, 50.2 μmol) for 1 day to give the title compound (24.6 mg, 70%). ^{1}H NMR (CD_3OD, 500.13 MHz): GlcN (from H-1 to H-6a,6b): 4.54 (d, 1 H, $J_{1,2} \approx$ 7.6 Hz), 3.85, 3.81, 3.44, 3.32, 3.74, 3.89; Gal (from H-1 to H-6a,6b): δ 4.44 (d, 1 H, $J_{1,2} \approx$ 7.4 Hz), 3.58, 3.98, 3.93, 3.56, 3.64, 3.71; Neu5Ac (from H-3ax,3eq to H-9a,9b, $HNCOCH_3$): δ 1.83 (t, 1 H, $J_{3ax,4} \approx$ 12.1 Hz), 2.77 (dd, 1 H, $J_{3eq,4} \approx$ 4.2, $J_{3ax,3eq} \approx$ 12.1 Hz), 3.74, 3.71, 3.54, 3.51, 3.85, 3.63, 3.84, 2.02; GlcA (from H-1 to H-5, OCH_3): δ 4.28 (d, 1 H, $J_{1,2} \approx$ 8.0 Hz), 3.28, 3.46, 3.57, 3.73, 3.55; aglycon: δ 2.30, 1.58, 1.29, 1.31, 1.53, 3.47, 3.86, 3.64; ^{13}C NMR (CD_3OD, 100.61 MHz): GlcN (from C-1 to C-6): 102.2, 55.8, 83.9, 70.6, 77.5, 62.7; Gal (from C-1 to C-6): δ 104.6, 70.7, 77.1, 69.5, 76.8, 62.7; Neu5Ac (from C-1 to C-9, $HNCOCH_3$): δ C-1 not resolved, C-2 not resolved, 41.1, 69.4, 53.9, 74.8, 70.1, 72.8, 64.4, 175.5, 22.5; GlcA (from C-1 to C-6, OCH_3): δ 105.3, 74.3, 77.3, 73.2, 76.8, 172.2, 57.5; aglycon: δ C=O not resolved, 34.6, 25.9, 30.1, 26.8, 30.4, 70.6, 51.9. MS Calcd for $C_{40}H_{68}N_2O_{26}$: 992; found $[M-H]^-$ 991.

8-Methoxycarbonyloctyl-N-*acetyl*-α-*neuraminyl*-*(2→3)*-β-D-*galacto-pyranosyl*-*(1→3)*-*2-deoxy-2-(methyl* β-D-*galactopyranosyluronamide)*-β-D-*glucopyranoside* (**9b**).—According to general procedure C, compound **8b** (27.5 mg, 39.2 μmol) was incubated with CMP-Neu5Ac (34.8 mg, 52.8 μmol) for one day to give the title compound (35 mg, 90%). ^{1}H NMR (CD_3OD, 500.13 MHz): GlcN (from H-1 to H-6a,6b): δ 5.01 (d, 1 H, $J_{1,2} \approx$ 7.6 Hz), 3.42, 3.99, 3.38, 3.33, 3.67, 3.87; Gal (from H-1 to H-6a,6b): δ 4.47 (d, 1 H, $J_{1,2} \approx$ 7.4 Hz), 3.55, 3.95, 3.94, 3.59, 3.67, 3.74; Neu5Ac (from H-3ax,3eq to H-9a,9b, HNCOCH_3): δ 1.92 (t, 1 H, $J_{3ax,4} \approx$ 12.1 Hz), 2.75 (dd, 1 H, $J_{3eq,4} \approx$ 4.2, $J_{3ax,3eq} \approx$ 12.1 Hz), 3.79, 3.70, 3.48, 3.49, 3.79, 3.60, 3.82, 2.00; GalA (from H-1 to H-5, OCH_3): δ 4.24 (d, 1 H, $J_{1,2} \approx$ 8.0 Hz), 3.54, 3.56, 4.18, 4.08, 3.60; aglycon: δ 2.30, 1.58, 1.30, 1.55, 3.51, 3.85, 3.64; ^{13}C NMR (CD_3OD, 100.61 MHz): GlcN (from C-1 to C-6): δ 100.9, 57.9, 84.3, 70.7, 77.5, 62.7; Gal (from C-1 to C-6): δ 105.1, 71.7, 76.8, 70.4, 76.5, 62.6; Neu5Ac (from C-1 to C-9, HN*C*O*C*H$_3$): δ 175.3, 102.5, 40.5, 69.7, 54.0, 74.7, 70.1, 72.8, 64.4, 176.7, 22.5; GalA (from C-1 to C-6, OCH_3): δ 105.8, 71.0, 74.6, 71.1, 76.4, 171.2, 57.7; aglycon: δ 176.0, 34.6, 25.8, 30.2, 26.8, 30.4, 70.6, 51.9. MS Calcd for $C_{40}H_{68}N_2O_{26}$: 992; Found [M−H]$^-$ 991.

8-Methoxycarbonyloctyl 5-N-*acetyl*-α-*neuraminyl*-*(2→3)*-β-D-*galactopyranosyl*-*(1→3)*-*2-deoxy-2-(methyl* α-D-*galactopyranosyluronamide)*-β-D-*glucopyranoside* (**9c**).—According to general procedure C, compound **8c** (22.6 mg, 32.2 μmol) was incubated with CMP-Neu5Ac (29.4 mg, 44.6 μmol) for one day to give the title compound (29.6 mg, 93%). ^{1}H NMR (CD_3OD, 500.13 MHz): GlcN (from H-1 to H-6a,6b): δ 4.88 (d, 1 H, $J_{1,2} \approx$ 7.6 Hz), 3.34, 4.02, 3.36, 3.35, 3.68, 3.89*; Gal (from H-1 to H-6a,6b): δ 4.48 (d, 1 H, $J_{1,2} \approx$ 7.4 Hz), 3.57, 3.95, 3.95, 3.62, 3.75, 3.88*; Neu5Ac (from H-3ax,3eq to H-9a,9b, HNCOCH_3): δ 1.95 (t, 1 H, $J_{3ax,4} \approx$ 12.1 Hz), 2.72 (dd, 1 H, $J_{3eq,4} \approx$ 4.2, $J_{3ax,3eq} \approx$ 12.1 Hz), 3.81, 3.73, 3.51, 3.51, 3.78, 3.62, 3.81, 2.00; GalA (from H-1 to H-5, OCH_3): δ 5.11 (d, 1 H, $J_{1,2} \approx$ 4.9 Hz), 3.79, 3.78, 4.26, 4.23, 3.45; aglycon: δ 2.29, 1.58, 1.30, 1.32, 1.53, 3.50, 3.87, 3.64; ^{13}C NMR (CD_3OD, 100.61 MHz): GlcN (from C-1 to C-6): δ 101.7, 56.4, 84.3, 70.8, 77.5, 62.6; Gal (from C-1 to C-6): δ 105.1, 71.1, 76.6, 70.6, 76.4, 62.6; Neu5Ac (from C-1 to C-9, HN*C*O*C*H$_3$): δ C-1 not resolved, C-2 not resolved, 40.2, 69.4, 53.8, 74.8, 70.0, 72.7, 64.4, 175.5, 22.6; GalA (from C-1 to C-6, OCH_3): δ 100.6, 69.4, 71.0, 71.6, 72.3, 171.5, 56.4; aglycon: δ 176.3, 34.6, 25.9, 30.2, 26.9, 30.4, 70.5, 51.5. MS Calcd for $C_{40}H_{68}N_2O_{26}$: 992; Found [M−H]$^-$ 991.

8-Methoxycarbonyloctyl 5-N-*acetyl*-α-*neuraminyl*-*(2→3)*-β-D-*galactopyranosyl*-*(1→3)*-*2-deoxy-2-(methyl* β-L-*galactopyranosyluronamide)*-β-D-*glucopyranoside* (**9d**).—According to general procedure C, compound **8d** (28 mg, 39.9 μmol) was incubated with CMP-Neu5Ac (36.1 mg, 54.8 μmol) for 1 day to give the title compound (24.8 mg, 63%). ^{1}H NMR (CD_3OD, 500.13 MHz): GlcN (from H-1 to H-6a,6b): δ 4.51 (d, 1 H, $J_{1,2} \approx$ 7.6 Hz), 3.96, 3.86, 3.42, 3.34, 3.72, 3.90; Gal (from H-1 to H-6a,6b): 4.53 (d, 1 H, $J_{1,2} \approx$ 7.4 Hz), 3.57, 3.99, 3.94, 3.60, 3.65, 3.75; Neu5Ac (from H-3ax,3eq to H-9a,9b, HNCOCH_3): δ 1.88 (t, 1 H, $J_{3ax,4} \approx$ 12.1 Hz), 2.73 (dd, 1 H, $J_{3eq,4} \approx$ 4.2, $J_{3ax,3eq} \approx$ 12.1 Hz), 3.74, 3.72, 3.51, 3.50, 3.83, 3.63, 3.83, 2.00; GalA (from H-1 to H-5, OCH_3): δ 4.20 (d, 1 H, $J_{1,2} \approx$ 7.4 Hz), 3.59*, 3.56*, 4.21, 3.99, 3.59; aglycon: δ 2.30, 1.58, 1.29, 1.31, 1.52, 3.46, 3.85, 3.64; ^{13}C NMR (CD_3OD, 100.61 MHz): GlcN (from C-1 to C-6): δ 102.4, 55.7, 83.4, 70.5, 77.4, 62.7; Gal (from C-1 to C-6): δ 104.1, 70.8*, 76.7*, 69.9, 76.7, 62.8; Neu5Ac (from C-1 to C-9, HN*C*O*C*H$_3$): δ C-1 not resolved, C-2 not resolved, 40.8, 69.5, 53.9, 74.7, 70.0, 72.8, 64.4, 175.5, 22.4; GalA-unit (from C-1 to C-6, OCH_3): δ 105.8, 72.0*, 74.5, 70.8, 77.1*, 171.5, 57.1; aglycon: δ 175.9, 34.6, 25.4, 30.2, 26.9, 30.4, 70.7, 51.9. MS Calcd for $C_{40}H_{68}N_2O_{26}$: 992; Found [M−H]$^-$ 991.

8-Methoxycarbonyloctyl 5-N-*acetyl*-α-*neuraminyl*-*(2→3)*-β-D-*galacto-pyranosyl*-*(1→3)*-*2-deoxy-2-(methyl* α-L-*galactopyranosyluronamide)*-β-D-*glucopyranoside* (**9e**).—According to general procedure C, compound **8e** (10 mg, 14.3 μmol) was incubated with CMP-Neu5Ac (14.8 mg, 22.5 μmol) for 1 day to give the title compound (10.4 mg, 74%). ^{1}H NMR (CD_3OD, 400.13 MHz): GlcN (from H-1 to H-6a,6b): δ 4.49 (d, 1 H, $J_{1,2} \approx$ 7.6 Hz), 3.97, 3.85, 3.44, 3.34, 3.72, 3.91; Gal (from H-1 to H-6a,6b): δ 4.54 (d, 1 H, $J_{1,2} \approx$ 7.4 Hz), 3.56, 4.01, 3.94, 3.58, 3.65, 3.78; Neu5Ac (from H-3ax,3eq to H-9a,9b, HNCOCH_3): δ 1.88 (t, 1 H, $J_{3ax,4} \approx$ 12.1 Hz), 2.79 (dd, 1 H, $J_{3eq,4} \approx$ 4.2, $J_{3ax,3eq} \approx$ 12.1 Hz), 3.75, 3.72, 3.52, 3.50, 3.85, 3.64, 3.87, 2.02; GalA (from H-1 to H-5, OCH_3): δ 4.88 (d, 1 H, $J_{1,2} \approx$ 4.0 Hz), 3.90, 3.77, 4.29, 4.20, 3.43; aglycon: δ 2.30, 1.60, 1.31, 1.55, 3.47, 3.89, 3.66. ^{13}C NMR (CD_3OD, 100.61 MHz): GlcN (from C-1 to C-6): δ 101.3, 54.3, 82.2, 70.0, 76.5, 61.5*; Gal (from C-1 to C-6):

δ 103.8, 70.0, 75.5, 68.5, 75.5, 62.2*; Neu5Ac (from C-1 to C-9, HN*C*O*C*H$_3$): δ C-1 not resolved, C-2 not resolved, 40.4, 68.8, 52.9, 73.7, 69.4, 72.0, 63.7, C=O not resolved, 21.4; GalA (from C-1 to C-6, O*C*H$_3$): δ 100.9, 68.8, 70.3, 70.6, 72.3, C-6 not resolved, 55.2; aglycon: δ C=O not resolved, 34.1, 25.2, 29.1, 26.1, 29.6, 69.4, 51.2. MS Calcd for $C_{40}H_{68}N_2O_{26}$: 992; Found $[M-H]^-$ 991.

8-Methoxycarbonyloctyl 5-N-*acetyl*-α-*neuraminyl*-(2→3)-β-D-*galacto-pyranosyl*-(1→3)-[α-L-*fucopyranosyl*-(1→4)]-2-*deoxy*-2-(*methyl* β-D-*glucopyranosyluronamide*)-β-D-*glucopyranoside* (**10a**).—According to general procedure D, compound **9a** (20 mg, 20.1 μmol) was incubated with GDP-Fuc (18.4 mg, 29 μmol) for 1 day to give the title compound (18.8 mg, 82%). ^{1}H NMR (CD_3OD, 500.13 MHz): GlcN (from H-1 to H-6a,6b): δ 4.50 (d, 1 H, $J_{1,2} \approx 7.6$ Hz), 4.01, 4.09, 3.77, 3.42, 3.65*, 3.88; Gal (from H-1 to H-6a,6b): δ 4.59 (d, 1 H, $J_{1,2} \approx 7.4$ Hz), 3.52, 3.88, 3.91, 3.54, 3.64*, 3.78; Neu5Ac (from H-3ax,3eq to H-9a,9b HNCOC*H*$_3$): δ 1.91 (t, 1 H, $J_{3ax,4} \approx 12.1$ Hz), 2.80 (dd, 1 H, $J_{3eq,4} \approx 4.2$, $J_{3ax,3eq} \approx 12.1$ Hz), 3.72, 3.74, 3.64, 3.52, 3.83, 3.65, 3.88, 2.03; Fuc (from H-1 to H-6): δ 5.06 (d, 1 H, $J_{1,2} \approx 3.8$ Hz), 3.74, 3.86, 3.65, 4.85, 1.18 (d, 3 H, $J_{5,6} \approx 6.4$ Hz); GlcA (from H-1 to H-5, O*C*H$_3$): δ 4.36 (d, 1 H, $J_{1,2} \approx 8.0$ Hz), 3.28, 3.50, 3.58, 3.68, 3.59; aglycon: δ 2.35, 1.62, 1.32, 1.38, 1.58, 3.47, 3.85, 3.68; ^{13}C NMR (CD_3OD, 100.61 MHz): GlcN (from C-1 to C-6): δ 102.4, 56.9, 77.6, 77.3, 77.1, 63.3; Gal (from C-1 to C-6): δ 103.9, 70.9, 77.6, 69.4, 77.4, 61.3; Neu5Ac (from C-1 to C-9, HN*C*O*C*H$_3$): δ 175.2, 102.1, 41.4, 70.0, 54.0, 73.9, 70.9, 72.9, 64.6, 175.5, 22.7; Fuc (from C-1 to C-6): δ 99.6, 69.6, 71.1, 73.6, 67.6, 16.6; GlcA (from C-1 to C-6, O*C*H$_3$): δ 105.4, 74.6, 77.4, 73.5, 74.4, 172.2, 57.8; aglycon: δ 176.1, 34.8, 26.0, 30.2, 26.9, 30.6, 70.7, 52.0. MS Calcd for $C_{46}H_{78}N_2O_{30}$: 1138; Found $[M-H]^-$ 1137. Anal. Calcd for $C_{46}H_{78}N_2O_{30}$: C, 48.50; H, 6.90. Found: C, 48.15; H, 7.21.

8-Methoxycarbonyloctyl 5-N-*acetyl*-α-*neuraminyl*-(2→3)-β-D-*galacto-pyranosyl*-(1→3)-[α-L-*fucopyranosyl*-(1→4)]-2-*deoxy*-2-(*methyl* β-D-*galactopyranosyluronamide*)-β-D-*glucopyranoside* (**10b**).—According to general procedure D, compound **9b** (26 mg, 26.2 μmol) was incubated with GDP-Fuc (22.6 mg, 35.6 μmol) for one day to give the title compound (18.8 mg, 63%). A TLC of the crude mixture still showed some starting sugar. ^{1}H NMR (CD_3OD, 400.13 MHz): GlcN (from H-1 to H-6a,6b): δ 4.70 (d, 1 H, $J_{1,2} \approx 7.6$ Hz), 3.85, 3.89, 3.74, 3.42, 3.91; Gal (from H-1 to H-6a,6b): δ 4.61 (d, 1 H, $J_{1,2} \approx 7.4$ Hz), 3.52, 3.89, 3.90, 3.41, 3.63, 3.76; Neu5Ac (from H-3ax,3eq to H-9a,9b, HNCOC*H*$_3$): δ 1.87 (t, 1 H, $J_{3ax,4} \approx 12.1$ Hz), 2.72 (dd, 1 H, $J_{3eq,4} \approx$ Hz, $J_{3ax,3eq} \approx 12.1$ Hz), 3.72*, 3.74, 3.65, 3.56, 3.74, 3.65, 3.87, 2.02; Fuc (from H-1 to H-6): δ 5.05 (d, 1 H, $J_{1,2} \approx 3.8$ Hz), 3.73*, 3.88, 3.66, 4.88, 1.17 (d, 3 H, $J_{5,6} \approx 6.4$ Hz); GalA (from H-1 to H-5, O*C*H$_3$): δ 4.35 (d, 1 H, $J_{1,2} \approx 8.0$ Hz), 3.59, 3.65, 4.18, 4.16, 3.57; aglycon: δ 2.31, 1.62, 1.31, 1.58, 3.51, 3.81, 3.59; ^{13}C NMR (CD_3OD, 100.61 MHz): GlcN (from C-1 to C-6): δ 102.2, 57.7, 78.0, 77.4, 77.4, 61.4; Gal (from C-1 to C-6): δ 104.2, 71.0, 77.7, 69.3, 76.8, 63.2; Neu5Ac (from C-1 to C-9, HN*C*O*C*H$_3$): δ 175.0, 101.8, 41.7, 69.6*, 53.9, 74.9, 70.0, 73.0, 64.6, 176.0, 22.6; Fuc (from C-1 to C-6): 99.6, 70.0*, 71.1, 73.7, 67.6, 16.6; GlcA (from C-1 to C-6, O*C*H$_3$): δ 105.8, 72.0, 74.5, 71.1, 76.4, 171.6, 58.1; aglycon: δ 175.4, 34.8, 26.0, 30.3, 26.9, 30.6, 70.2, 52.0. MS calcd for $C_{46}H_{78}N_2O_{30}$: 1138; found $[M-H]^-$ 1137. Anal. Calcd for $C_{46}H_{78}N_2O_{30}$: C, 48.50; H, 6.90. Found: C, 48.31; H, 6.68.

8-Methoxycarbonyloctyl 5-N-*acetyl*-α-*neuraminyl*-(2→3)-β-D-*galacto-pyranosyl*-(1→3)-[α-L-*fucopyranosyl*-(1→4)]-2-*deoxy*-2-(*methyl* α-D-*galactopyranosyluronamide*)-β-D-*glucopyranoside* (**10c**).—According to general procedure D, compound **9c** (22.0 mg, 22.2 μmol) was incubated with GDP-Fuc (21.7 mg, 34.2 μmol) for 1 day to give the title compound (20.1 mg, 79%). ^{1}H NMR (CD_3OD, 400.13 MHz): GlcN (from H-1 to H-6a,6b): δ 4.84 (d, 1 H, $J_{1,2} \approx 7.6$ Hz), 3.71, 3.89, 3.75, 3.42, 3.91; Gal (from H-1 to H-6a,6b): δ 4.68 (d, 1 H, $J_{1,2} \approx 7.4$ Hz), 3.52, 3.91*, 3.92*, 3.50, 3.64, 3.75; Neu5Ac (from H-3ax,3eq to H-9a,9b, HNCOC*H*$_3$): δ 1.88 (t, 1 H, $J_{3ax,4} \approx 12.1$ Hz), 2.79 (dd, 1 H, $J_{3eq,4} \approx 4.2$, $J_{3ax,3eq} \approx 12.1$ Hz), 3.74, 3.76, 3.57, 3.50, 3.83, 3.65, 3.88, 2.01; Fuc (from H-1 to H-6): δ 5.06 (d, 1 H, $J_{1,2} \approx 3.8$ Hz), 3.73, 3.86, 3.65, 4.86, 1.17 (d, 3 H, $J_{5,6} \approx 6.4$ Hz); GalA (from H-1 to H-5, O*C*H$_3$): δ 4.91 (d, 1 H, $J_{1,2} \approx 4.9$ Hz), 3.92, 3.83, 4.29, 4.25, 3.50; aglycon: δ 2.34, 1.60, 1.32, 1.57, 3.50, 3.83, 3.69; ^{13}C NMR (CD_3OD, 100.61 MHz): GlcN (from C-1 to C-6): δ 101.7, 58.3, 77.4, 77.2, 76.1, 61.4; Gal (from C-1 to C-6): δ 103.9, 71.0, 77.5, 69.9, 77.2, 63.1; Neu5Ac (from C-1 to C-9, HN*C*O*C*H$_3$): δ 175.2, 101.4, 41.4, 69.4*, 53.8, 74.7, 69.9, 72.8, 64.4, 175.6, 22.7; Fuc (from C-1 to C-6): δ 99.5, 69.5*, 70.9, 73.6, 67.7, 16.6; GalA (from C-1 to C-6, O*C*H$_3$): 102.1, 69.2, 72.8, 71.2, 72.6, 172.0, 56.6; aglycon: δ 176.4, 34.8, 26.0, 30.1, 30.2, 26.9,

30.6, 70.9, 52.2. MS Calcd for $C_{46}H_{78}N_2O_{30}$: 1138; Found $[M-H]^-$ 1137. Anal. Calcd for $C_{46}H_{78}N_2O_{30}$: C, 48.50; H, 6.90. Found: C, 48.78; H, 6.69.

8-Methoxycarbonyloctyl 5-N-*acetyl*-α-*neuraminyl*-(2→3)-β-D-*galacto-pyranosyl*-(1→3)-[α-L-*fucopyranosyl*-(1→4)]-2-*deoxy*-2-(*methyl* β-L-*galactopyranosyluronamide*)-β-D-*glucopyranoside* (**10d**).—According to general procedure D, compound **9d** (19 mg, 19.1 μmol) was incubated with GDP-Fuc (16.8 mg, 26.4 μmol) for 4 days to give the title compound (12.2 mg, 56%). The crude mixture still showed some starting sugar. ^{1}H NMR (CD_3OD, 400.13 MHz): GlcN (from H-1 to H-6a,6b): δ 4.46 (d, 1 H, $J_{1,2} \approx 7.6$ Hz), 4.11, 4.12, 3.78, 3.40, 3.92; Gal (from H-1 to H-6a,6b): δ 4.74 (d, 1 H, $J_{1,2} \approx 7.4$ Hz), 3.52, 3.93, 3.91, 3.54, 3.64, 3.77; Neu5Ac (from H-3ax,3eq to H-9a,9b, HNCOCH_3): δ 1.85 (t, 1 H, $J_{3ax,4} \approx 12.1$ Hz), 2.72 (dd, 1 H, $J_{3eq,4} \approx 4.2$, $J_{3ax,3eq} \approx 12.1$ Hz), 3.72, 3.74, 3.61, 3.47, 3.83, 3.62, 3.85, 2.02; Fuc (from H-1 to H-6): δ 5.06 (d, 1 H, $J_{1,2} \approx 3.8$ Hz), 3.73, 3.88, 3.63, 4.89, 1.19 (d, 3 H, $J_{5,6} \approx 6.4$ Hz); GalA (from H-1 to H-5, OCH_3): δ 4.20 (d, 1 H, $J_{1,2} \approx 7.4$ Hz), 3.61, 3.55, 4.29, 3.98, 3.62; aglycon: δ 2.33, 1.60, 1.31, 1.55, 3.47, 3.85, 3.68. ^{13}C NMR (CD_3OD, 100.61 MHz): GlcN (from C-1 to C-6): δ 102.6, 56.9, 77.4*, 74.6, 77.4, 61.4; Gal (from C-1 to C-6): δ 103.2, 71.0, 77.2, 70.0, 76.1, 63.3; Neu5Ac (from C-1 to C-9, HNCOC$_H$3): δ 173.7, 102.6, 40.6, 70.2, 54.0, 74.7, 70.9, 73.7, 64.7, 175.6, 22.6; Fuc (from C-1 to C-6): δ 99.5, 69.8, 71.9, 73.7, 67.7, 16.6; GalA (from C-1 to C-6, OCH_3): δ 106.0, 71.9, 74.7, 70.9, 77.3*, 171.7, 57.8; aglycon: δ 176.0, 34.8, 26.0, 30.2, 30.4, 27.0, 30.6, 70.9, 52.0. MS calcd for $C_{46}H_{78}N_2O_{30}$: 1138; found $[M-H]^-$ 1137. Anal. Calcd for $C_{46}H_{78}N_2O_{30}$: C, 48.50; H, 6.90. Found: C, 48.24; H, 6.61.

8-Methoxycarbonyloctyl 5-N-*acetyl*-(-*neuraminyl*-(2→3)-β-D-*galacto-pyranosyl*-(1→3)-[α-L-*fucopyranosyl*-(1→4)]-2-*deoxy*-2-(*methyl* α-L-*galactopyranosyluronamide*)-β-D-*glucopyranoside* (**10e**).—According to general procedure D, compound **9e** (10 mg, 10.0 μmol) is incubated with GDP-Fuc (14.5 mg, 22.8 μmol) for 5 days to give the title compound (8.6 mg, 75%). The crude mixture still showed some starting sugar. ^{1}H NMR (CD_3OD, 400.13 MHz): GlcN (from H-1 to H-6a,6b): δ 4.45 (d, 1 H, $J_{1,2} \approx 7.6$ Hz), 4.11, 4.11, 3.75, 3.40, 3.93; Gal (from H-1 to H-6a,6b): δ 4.73 (d, 1 H, $J_{1,2} \approx 7.4$ Hz), 3.52, 3.92, 3.89, 3.52, 3.68, 3.79; Neu5Ac (from H-3ax,3eq to H-9a,9b, HNCOCH_3): δ 2.04 (t, 1 H, $J_{3ax,4} \approx 12.1$ Hz), 2.71 (dd, 1 H, $J_{3eq,4} \approx 4.2$, $J_{3ax,3eq} \approx 12.1$ Hz), 3.71, 3.72, 3.48, 3.44, 3.83, 3.63, 3.87, 2.01; Fuc (from H-1 to H-6): 5.04 (d, 1 H, $J_{1,2} \approx 3.8$ Hz), 3.74, 3.88, 3.72, 4.86, 1.19 (d, 3 H, $J_{5,6} \approx 6.4$ Hz); GalA (from H-1 to H-5, OCH_3): δ 4.91 (d, 1 H, $J \approx 4.0$ Hz), 3.94, 3.76, 4.36, 4.17, 3.52; aglycon: δ 2.32, 1.59, 1.29, 1.59, 3.47, 3.87, 3.68. ^{13}C NMR (CD_3OD, 100.61 MHz): GlcN (from C-1 to C-6): δ 102.6, 56.5, 76.9, 73.4, 76.0, 61.1; Gal (from C-1 to C-6): 103.8, 70.6, 76.6, 70.6, 76.1, 63.0; Neu5Ac (from C-1 to C-9, HNCOCH_3): δ C-1 not resolved, C-2 not resolved, 40.2, 69.5, 52.7, 75.7, 70.6, 72.8, 64.4, C=O not resolved, 22.7; Fuc (from C-1 to C-6): 99.5, 69.5, 70.6, 74.4, 67.6, 16.6; GalA (from C-1 to C-6, OCH_3): 101.8, 69.6, 71.1, 71.1, 72.8, 172.0, 55.8; aglycon: δ 176.5, 34.8, 25.9, 30.0, 30.2, 27.0, 30.6, 70.7, 52.1. MS Calcd for $C_{46}H_{78}N_2O_{30}$: 1138; found $[M-H]^-$ 1137. Anal. Calcd for $C_{46}H_{78}N_2O_{30}$: C, 48.50; H, 6.90. Found: C, 48.87; H, 7.19.

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