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Systematic Synthesis of *N*-Methyl-1-Deoxynojirimycin-Containing, Le^x, Le^a, Sialyl-Le^x And Sialyl-Le^a Epitopes Recognized by Selectins†

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Abstract—A systematic synthesis of the *N*-methyl-1-deoxynojirimycin-containing oligosaccharides related to the Lewis x, Lewis a, sialyl-Lewis x and sialyl-Lewis a antigens has been achieved. The couplings of the suitably protected 1-deoxynojirimycin derivative **10** with methyl-1-thioglycosides (glycosyl donors) of L-fucose (**11**), D-galactose (**15**) and α-sialyl-(2→3)-D-galactose (**27**) were carried out by using dimethyl(methylthio)sulfonium triflate (DMTST) or *N*-iodosuccinimide/trifluoromethanesulfonic acid (NIS/TfOH) as the glycosyl promoter. The resulting di- and tri-saccharides were each converted, by further cross glycosylations with **11**, **15** or **27**, to the desired tri- and tetra-saccharides **3**-**6** that inhibit the recognition between sialyl-Lewis x and selectins, a family of leukocyte cell adhesion molecules.

Introduction

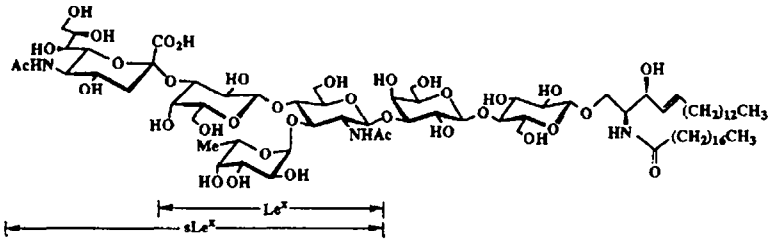
The Lewis x (Le^x) carbohydrate determinant identified as the stage-specific embryonic antigen-1 (SSEA-1) is one of the prominent tumor-associated antigens,[2] and its 2,3-sialylation leads to another major tumor-associated antigen sialyl-Lewis X (sLe^x; sialyl SSEA-1). Since 1990, the sLe^x antigen, found on neutrophils, monocytes, and cancer cells, has been identified[3,4] as a minimal carbohydrate ligand recognized not only by endothelial-leukocyte adhesion molecule 1 (ELAM-1, E-selectin) but also by other LEC-CAMs, such as platelet activation-dependent granule external membrane protein (PADGEM, P-selectin) and LECAM-1 (L-selectin). The Lewis a (Le^a) determinant is a blood group antigen,[5] and the sialyl-Lewis a (sLe^a) determinant has often been detected on cancer cells of digestive organs.[6] It has also been demonstrated that the sLe^a antigen is one of the possible ligands recognized by selectins.[3d-f,7] These findings suggest that both sLe^x and sLe^a antigens may be involved in the processes not only of leukocyte trafficking and recruitment to the site of inflammation but also of hematogeneous metastasis of cancer cells.[8]

In the course of synthetic studies on sialoglycoconjugates,[9] we have succeeded in the first total syntheses of sLe^x and sLe^a gangliosides (**1** and **2** in Scheme I).[10] The cell adhesion studies of selectins have been accomplished by employing those chemically synthesized gangliosides and analogues.[11] On the other hand, 1-deoxynojirimycin (DNJ) and related compounds have been shown not only to be potent inhibitors of glycosidases and glycoprotein-processing enzymes, but also to be of potential clinical value as antidiabetic, antineoplastic, and anti-HIV agents.[12] Focusing on the biological significance of both sialic acid and DNJ, we have synthesized a variety of DNJ-containing sialo-oligosaccharides designed as novel biofunctional carbohydrates.[13] In this course, it has been found[14] that the *N*-benzyloxycarbonyl derivatives of DNJ can be readily hydrogenolyzed in MeOH to yield the corresponding *N*-methyl-DNJ derivatives accompanied by the dramatic conformational change. This paper describes the systematic synthesis of *N*-methyl-1-deoxynojirimycin-containing Le^x (**3**), Le^a (**4**), sialyl-Le^x (**5**), and sialyl-Le^a (**6**) carbohydrate antigens (Scheme II) recognized by selectins (LEC-CAMs).

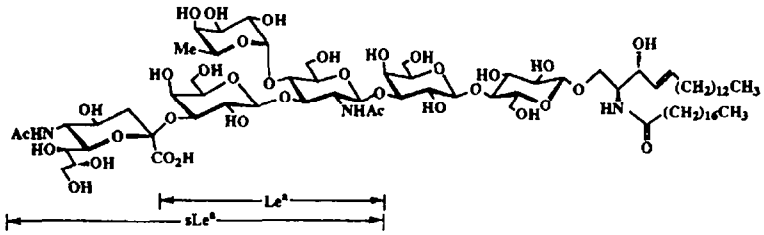
Results and Discussion

4,6-*O*-Benzylidene-*N*-benzyloxycarbonyl-1,5-dideoxy-1,5-imino-D-glucitol (**7**)[14a] was treated with chloroacetyl chloride (1.2 mol equiv.), to give a 70 % yield of the 3-*O*-chloroacetyl derivative **8**, regioselectively. Following 2-*O*-acetylation of **8**, dechloroacetylation with wet pyridine gave the first glycosyl acceptor **10** (Scheme III). The $^4C_1$ conformation of **10** was determined by ^1H NMR data ($J_{1ax,2}$ = 8, $J_{2,3} = J_{3,4} = J_{4,5}$ = 9–10 Hz). Glycosylation of **10** with methyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-fucopyranoside (**11**)[10a] in the presence of dimethyl(methylthio)sulfonium triflate (DMTST)[15] and molecular sieves 4 Å in benzene for 2.5 h, with warming from 7 °C to room temperature, gave the desired disaccharide **12** in 92 % yield (Scheme IV). The conformation of the DNJ moiety in **12** seems to be half-chair ($J_{1ax,2}$ = 5.5 Hz) rather than typical $^4C_1$ to avoid the *gauche* interaction between C-2 and C-3 substituents, but after deacetylation (**13**), the $^4C_1$ conformation was again favored. Reductive ring-

†Synthetic studies on sialoglycoconjugates, Part 62. For Part 61, see Ref. 1.

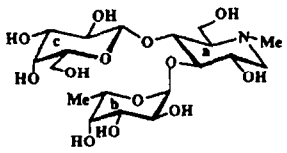


1 Sialyl-Le^x(sLe^x) ganglioside

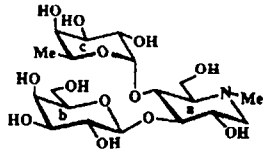


2 Sialyl-Le^a(sLe^a) ganglioside

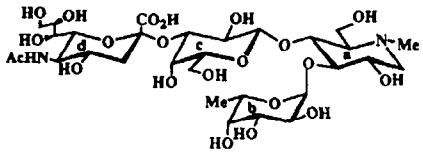
Scheme I.



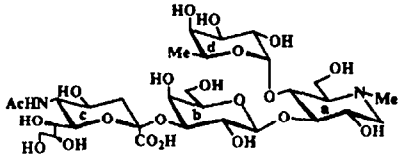
3 Le^x type



4 Le^a type

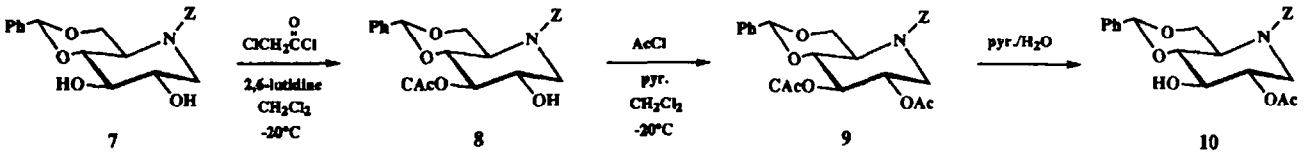


5 sLe^x type



6 sLe^a type

Scheme II.



7 8 9 10

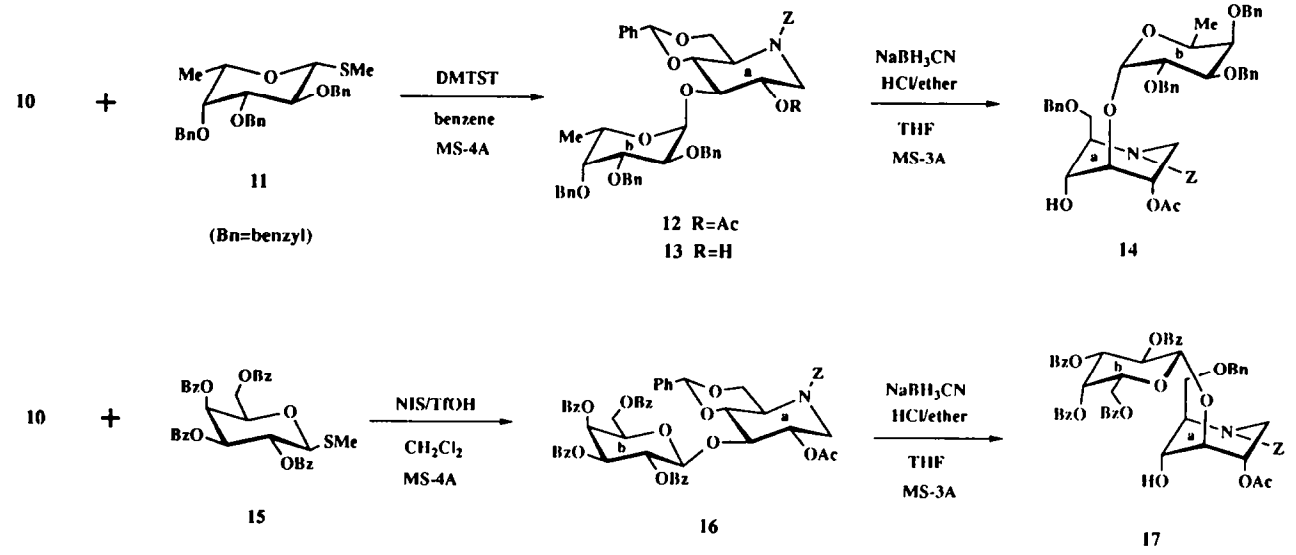
Scheme III.

opening[16] of the benzylidene group of **12** with $NaBH_3CN$ and HCl/ether afforded the next disaccharide glycosyl acceptor **14**, in which the conformation of the DNJ moiety changed dramatically from $^4C_1$ to $^1C_4$ ($J_{1ax,2} = 3$, $J_{1eq,2} < 2$, $J_{2,3} = J_{3,4} = 2$–3 Hz) as reported in our previous papers.[14]

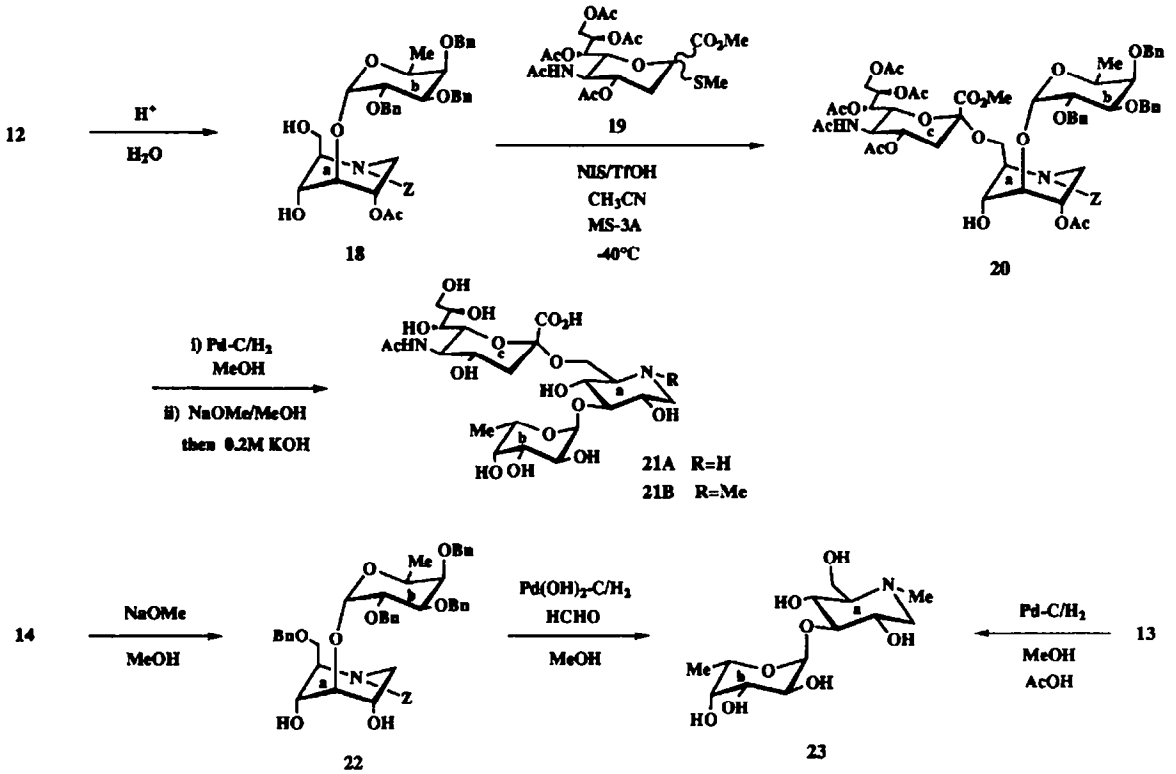
The glycosylation of **10** by methyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-galactopyranose (**15**) was performed in the presence of *N*-iodosuccinimide (NIS) and trifluoromethanesulfonic acid (TfOH)[17] in CH_2Cl_2 to give **16** (quant.), which was then treated with $NaBH_3CN$ and HCl/ether as just described for **14** to yield another disaccharide glycosyl acceptor **17** in 90 % yield. The dramatic change in conformation of the DNJ moiety ($^4C_1 \rightarrow {}^1C_4$) was again caused by the reductive ring opening of the benzylidene group (Scheme IV).

Debenzylidenation of **12** with 80 % AcOH gave **18** that was successively glycosylated[18] by **19**[19] using NIS/TfOH in CH_3CN at –40 °C to afford **20** as the major product (Scheme V). This trisaccharide **20** was then hydrogenolyzed over Pd/C for 3–7 days in MeOH/AcOH, the reaction being monitored by TLC. The palladium-catalyzed *N*-methylation had already started in the initial stage and then gradually proceeded. The resulting product was successively deprotected by treatment with NaOMe/MeOH and then 0.2 M KOH to yield **21B** as the major product, which, in the ion-spray MS and MS/MS spectra, gave the molecular ion peak at m/z 615.5 $(M + H)^+$ and a significant daughter ion peak at m/z 177.7 (protonated *N*-methyl-DNJ fragment) providing the unambiguous evidence for the *N*-methylation.[14c] The minor product **21A** gave the molecular ion peak at m/z 601.1 that corresponds to the *N*-H free derivative. The disaccharide **22**, prepared by deacetylation of **14**, was effectively converted to **23** by hydrogenation under the coexistence of formalin in MeOH.

The further glycosylations of **14** with **15** and of **17** with **11** were each performed by using NIS/TfOH or DMTST



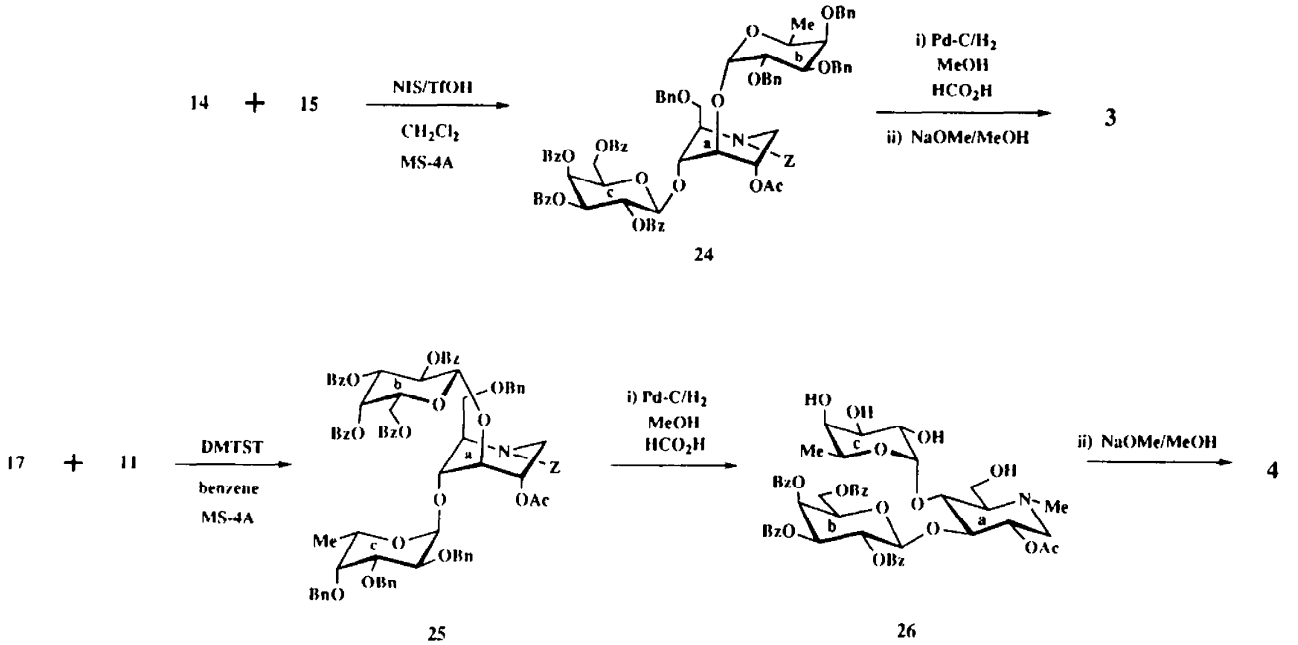
Scheme IV.



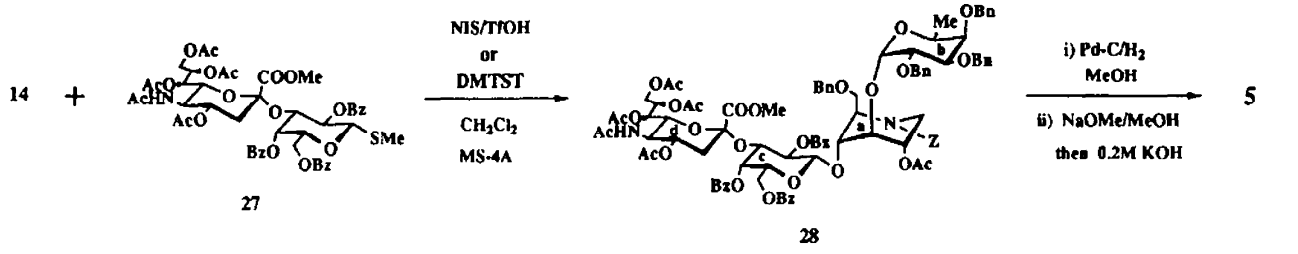
Scheme V.

as the glycosyl promoter to give **24** (70 %) and **25** (quant.), respectively (Scheme IV). Hydrogenolysis of **24** over Pd/C in MeOH/HCO_2H for 10 days and following deacylation with NaOMe in MeOH afforded the Le^x type antigen **3** in a quantitative yield. Similarly, **25** was hydrogenolyzed to give **26** (quant.) which was then converted by treatment with methanolic NaOMe to the desired Le^a type antigen **4**. The ion-spray MS and MS/MS spectra of **3** and **4** are shown in Figure 1 and Figure 2, respectively. In both spectra, a significant molecular ion $(M + H)^+$ was clearly detected as the base peak at m/z 486.5 for **3** and 486.6 for **4**, respectively, showing the average molecular weight of $C_{19}H_{35}NO_{13}$ (485.49) [Fig. 1 (A) and Fig. 2 (A)]. In the MS/MS spectra, four significant daughter ions were detected at m/z 340 $(M - Fuc + H)^+$, 324 $(M - Gal + H)^+$, 178 (protonated *N*-methyl-DNJ fragment) and 160 (178 – H_2O), providing the unambiguous evidence for the *N*-methylated structure assigned. [Fig. 1 (B) and Fig. 2 (B)]. Although an enzymatic approach for synthesizing the Le^x epitope analogue containing DNJ has also been tried,[20] GDP-fucose could not be transfered to the Galβ(1→4)-DNJ disaccharide showing the inhibitory activity against both α1,3- and α1,3/4-fucosyl transferases. This finding suggests that the oligosaccharides containing aza-sugars such as DNJ may affect recognitions not only by glycosyltransferases but also by glycosidases.

For the synthesis of sialyl-Le^x type antigen **5**, the disaccharide acceptor **14** was coupled with methyl *O*-(methyl-5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4,6-tri-*O*-benzoyl-1-thio-β-D-galactopyranoside (**27**)[21] (Scheme VII). DMTST is usually favored as the promoter for **27**, but in this coupling NIS/TfOH was also effective to give **28** (61 %), which was then hydrogenolyzed over Pd/C in MeOH/AcOH and successively treated with methanolic NaOMe and 0.2 M KOH as described for **21**, to give **5** in a quantitative yield. On the other hand, coupling of **10** with **27** under the similar reaction condition employed for **28** provided the desired trisaccharide **29** in 90 % yield (Scheme VIII). Reductive ring opening of the benzylidene group in **29** gave **30**, in which the DNJ moiety had the $^1C_4$ conformation similar to those of **14** and **17**. This trisaccharide glycosyl acceptor **30** was then coupled with **11** as described for **25** to give **31** in 89 % yield. Hydrogenolysis of **31** over Pd/C in MeOH/HCO_2H, and following deacylation with methanolic NaOMe and saponification of the methyl ester with 0.2 M KOH as just described for **5** yielded the sialyl-Le^a type antigen **6**, quantitatively.



Scheme VI.



Scheme VII.

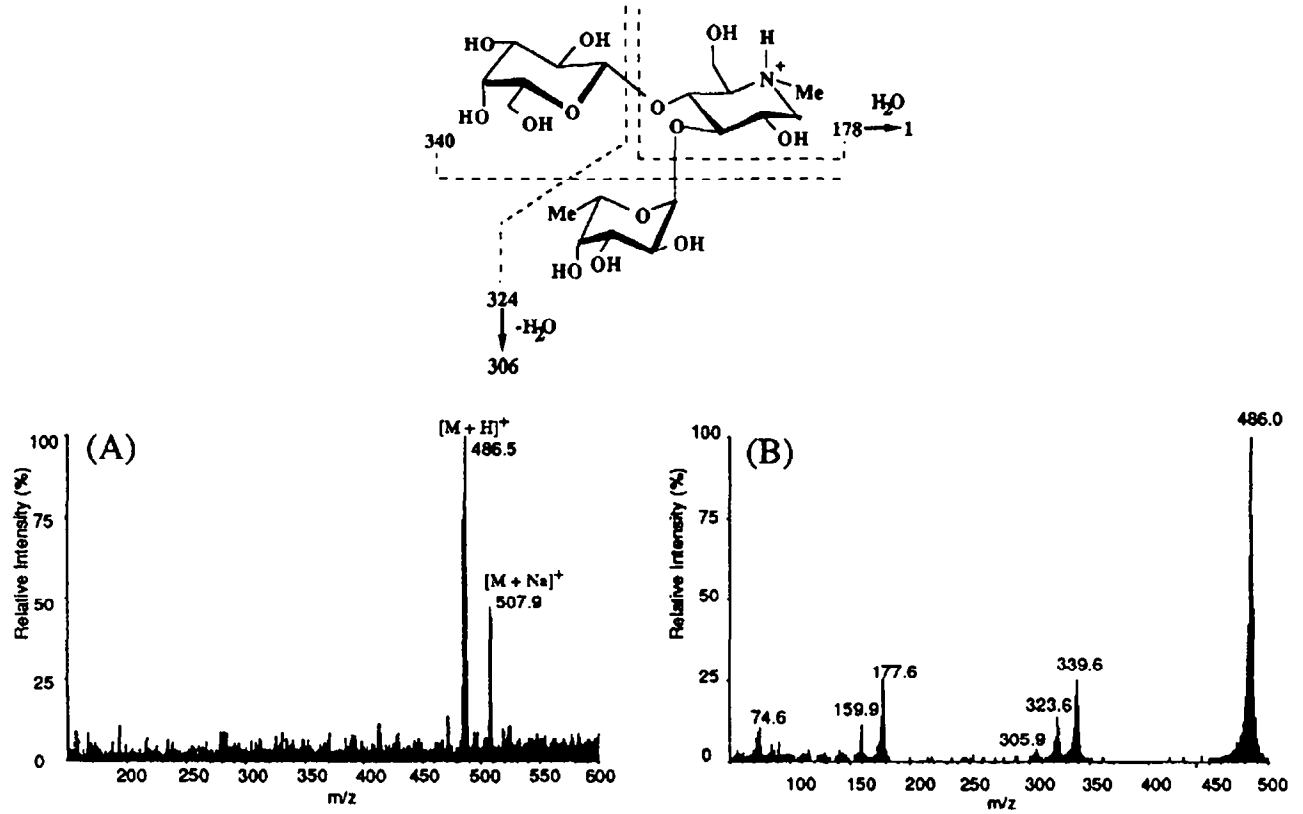


Figure 1. Ion-spray MS and MS/MS spectra for ca. 10 pmol of compound 3, infusion rate 3 mL/min in 1:1 CH_3CN-H_2O with 0.05 % TFA. (A) Positive-ion spectrum; sum of 10 scans (m/z 150–600, step 0.05, 9.9 s/scan), orifice voltage = 70 V. (B) MS/MS spectrum from CAD of the precursor-ion (m/z 486.5); sum of 20 scans (m/z 50–500, step 0.05, 9.65 s/scan), orifice volatage = 120 V.

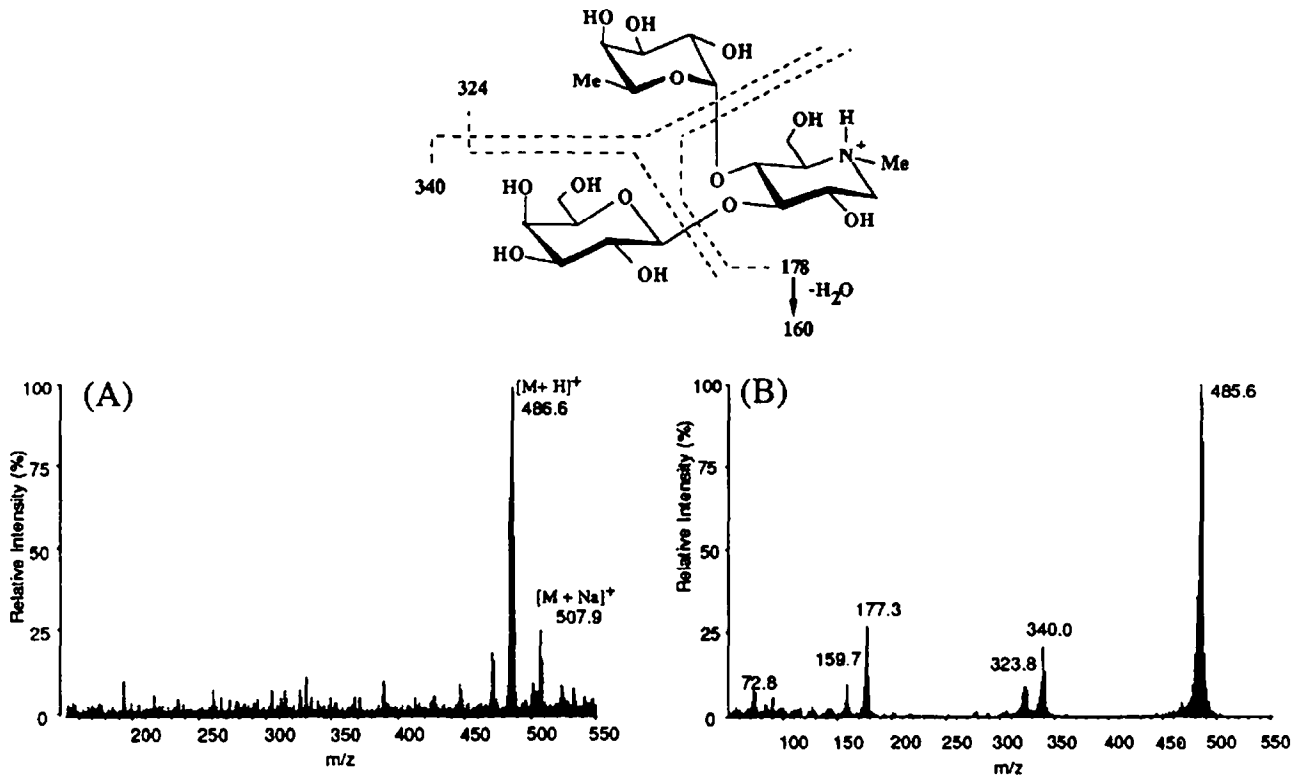
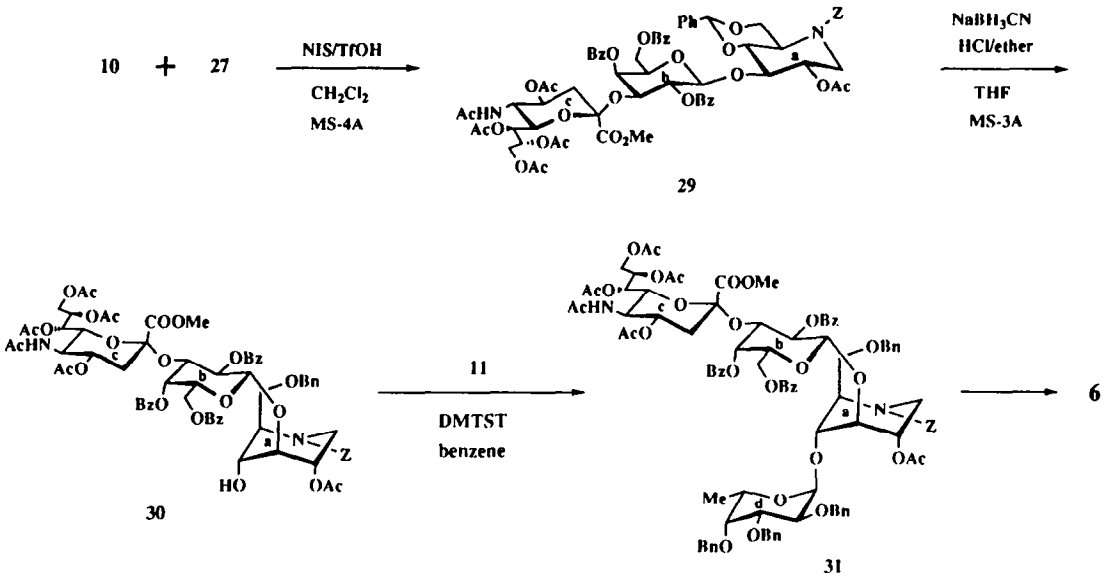
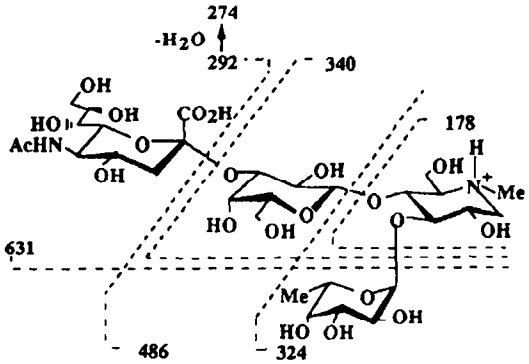


Figure 2. Ion-spray MS and MS/MS spectra for ca. 1 nmol of compound 4, infusion rate 3 mL/min in 1:1 CH_3CN-H_2O with 0.05 % TFA. (A) Positive-ion spectrum; sum of 10 scans (m/z 150–550, step 0.05, 8.8 s/scan), orifice voltage = 70 V. (B) MS/MS spectrum from CAD of the precursor-ion (m/z 486.5); sum of 20 scans (m/z 50–550, step 0.1, 4.95 s/scan), orifice volatage = 100 V.



Scheme VIII.

The structures of **5** and **6** thus obtained were analyzed in detail by ion-spray MS, MS/MS, ^1H and ^{13}C NMR spectrometry. As shown in Figure 3 and Figure 4, the clear molecular ion peak $(M + H)^+$ was detected at m/z 777.2, which gave the seven significant daughter ions at m/z 631 $(M - Fuc + H)^+$, 486 $(M - Neu5Ac + H)^+$, 340 $(M - Fuc - Neu5Ac + H)^+$, 324 $(M - Neu5Ac - Gal + H)^+$, 292 (Neu5Ac fragment), 274 (292 – H_2O), and 178 (protonated *N*-methyl-DNJ fragment), respectively [Fig.3 (B) and Fig. 4 (B)]. Thus, the ion-spray MS[22] and tandem MS (MS/MS), which permit the formation of gas phase ions directly from solution at atmospheric pressure, were extremely useful for analyzing the structures of DNJ-containing sialo-oligosaccharides. In



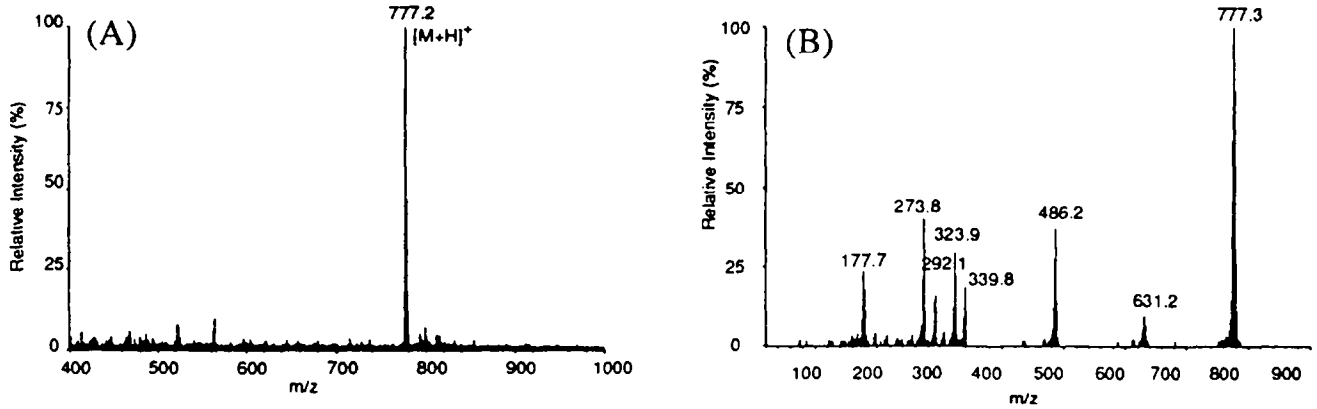


Figure 3. Ion-spray MS and MS/MS spectra for ca. 100 pmol of compound 5, infusion rate 5 mL/min in 1:1 CH_3CN-H_2O with 0.05 % TFA. (A) Positive-ion spectrum; sum of 10 scans (m/z 200–1000, step 0.1, 8.7 s/scan), orifice voltage = 90 V. (B) MS/MS spectrum from CAD of the precursor-ion (m/z 777.2); sum of 10 scans (m/z 20–900, step 0.1, 9.6 s/scan), orifice volatage = 130 V.

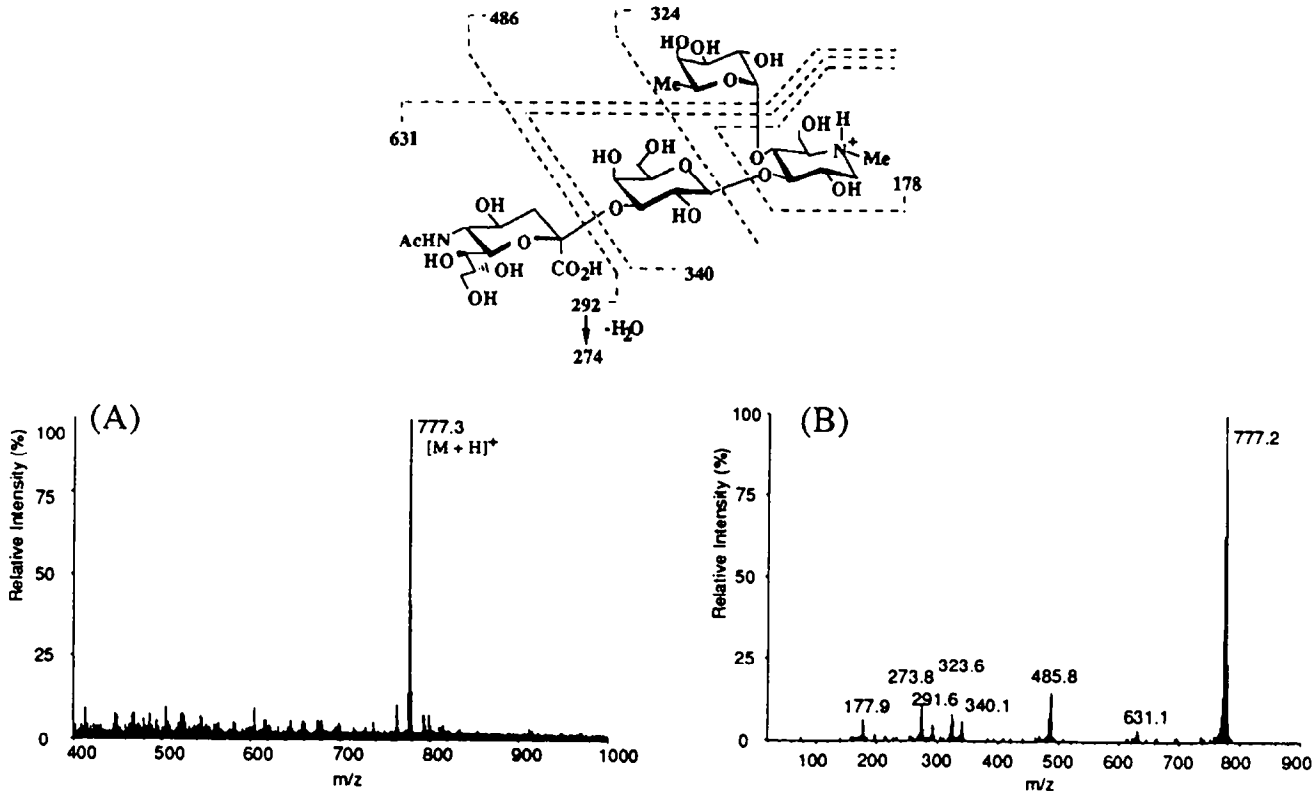


Figure 4. Ion-spray MS and MS/MS spectra for ca. 100 pmol of compound 6, infusion rate 5 mL/min in 1:1 CH_3CN-H_2O with 0.05 % TFA. (A) Positive-ion spectrum; sum of 10 scans (m/z 200–1000, step 0.1, 9.0 s/scan), orifice voltage = 100 V. (B) MS/MS spectrum from CAD of the precursor-ion (m/z 777.2); sum of 10 scans (m/z 20–900, step 0.1, 9.9 s/scan), orifice volatage = 100 V.

the ^{1}H NMR spectra, the two anomeric protons of Gal and Fuc residues bound to the DNJ moiety appeared at δ 4.61 ($J_{1,2}$ = 8.8 Hz, H-1 of Gal) and 5.38 (near s, H-1 of Fuc) for **5**, and δ 4.80 ($J_{1,2}$ = 8 Hz, H-1 of Gal) and 5.10 ($J_{1,2}$ = 3 Hz, H-1 of Fuc) for **6**, respectively, showing the desired β- and α-glycosidic linkages. The *N*-C*H*$_3$ signals of the DNJ moiety were each observed[23] at δ 3.00 for **5** and 2.96 for **6** as relatively broad singlets. The ^{13}C NMR spectra were extremely similar to each other and clearly provided three anomeric carbons at around δ 100, 101, and 103 or 104, and two carbonyl carbons (CO of AcN and CO_2H) at around δ 175 and 176 ppm, respectively.

The mechanism of the palladium-catalyzed *N*-methylation of DNJ in MeOH has been investigated,[14c] and it is believed[24] to involve the dehydrogenation of methanol by palladium to form formaldehyde, which then reacts with the free amine derived from the *N*-benzyloxycarbonyl derivative of DNJ. The resulting Schiff base is finally reduced to yield the corresponding *N*-methyl-DNJ derivative.

A number of studies on selectin binding have been achieved[11,25] by using synthetic oligosaccharide derivatives related to the sLe^x and sLe^a determinants. Both sLe^x (**5**) and sLe^a (**6**) epitope analogues described here exhibited potential inhibitory activity against selectin binding to the immobilized sialyl-Le^x (hexasaccharide) ganglioside (**1**),[26] and they may become useful in biomedical applications.

Experimental

General methods

Optical rotations were determined using a Union PM-201 polarimeter at 25 °C, and IR spectra were recorded on a JASCO IRA-100 spectrophotometer. ^{1}H NMR spectra were recorded on JEOL JNM-GX 270 (270 MHz) or JNM-GX 400 (400 MHz) spectrometers using deuterated solvents ($CDCl_3$, CD_3OD or D_2O) with TMS (δ = 0.00 ppm) or acetone (δ = 2.225 ppm) as the internal standards. ^{13}C NMR spectra were recorded on a JEOL JNM-GX 400 (100 MHz) spectrometer. All reactions were monitored by TLC (Merck silica gel aluminum plates 60 F-254) and preparative column chromatography was performed on silica gel (Wako Chemical Co., 200 mesh) with the solvent systems specified. Concentrations were conducted *in vacuo*.

Mass spectrometry

Electroscopy mass spectra were recorded on an API–III triplequadrupole mass spectrometer (Perkin–Elmer Sciex Instruments, Thornhill, Canada) fitted with an atmospheric pressure ionization source. The mass spectrometer was operated in the positive mode; the ion-spray voltage was set to 4500 V and the interface plate voltage was 650 V. The orifice voltage was 70–100 V. The pressure of the nebulizing gas was 30 psi and the flow rate was 0.8 L/min. The collisionally activated dissociated (CAD) spectrum was measured

with argon as the collision gas, and the collision gas pressure was set at 300×10^{12} atoms/cm^2. The collision energy was 120 eV.

4,6-O-Benzylidene-N-benzyloxycarbonyl-3-O-chloroacetyl-1,5-dideoxy-1,5-imino-D-glucitol (**8**)

A solution of chloroacetyl chloride (0.25 mL) in CH_2Cl_2 (20 mL) was added at –20 °C to a stirred solotion of 4,6-*O*-benzylidene-*N*-benzyloxycarbonyl-1,5-dideoxy-1,5-*imino*-D-glucitol (**7**, 1 g) in CH_2Cl_2 (80 mL) and 2,6-lutidine (0.6 mL). The mixture was stirred for 1.5 h at –20 °C and then washed with ice-cold 2 M HCl and water, dried (Na_2SO_4), and concentrated. The residue was chromatographed on a column of silica gel (CH_2Cl_2:MeOH 500:1) to give **8** (70 %) as an amorphous mass; $[\alpha]_D$ –5.2° (*c* 0.6, CH_2Cl_2); IR 3500, 1770, 1730, 770, 720 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.99 (dd, 1H, J_{gem} = 13.6, $J_{1ax,2}$ = 9.5 Hz, H-1*ax*), 3.40 (m, 1H, $J_{4,5} = J_{5,6ax}$ = 10, $J_{5,6eq}$ 4.4 Hz, H-5), 4.12 (s, 2H, COCH_2Cl), 4.17 (dd, 1H, $J_{1eq,2}$ = 4.4 Hz, H-1*eq*), 4.31 (t, 1 H, $J_{gem} = J_{5,6ax}$ = 10–11 Hz, H-6*ax*), 4.80 (dd, 1H, H-6*eq*), 5.04 (t, 1H, $J_{2,3} = J_{3,4}$ = 8.4 Hz, H-3), 5.1 (s, 2H, OCH_2Ph), 5.51 (s, 1 H, C*H*Ph of benzylidene), 7.3–7.4 (m, 10H, Ph-*H*). Found: C, 60.03; H, 4.98; N, 3.29; calcd for $C_{23}H_{24}NO_7Cl$: C, 59.81; H, 5.24; N, 3.03 %.

2-O-Acetyl-4,6-O-benzylidene-N-benzyloxycarbonyl-3-O-chloroacetyl-1,5-dideoxy-1,5-imino-D-glucitol (**9**)

To a stirred solution of **8** (0.41 g) in CH_2Cl_2 (4 mL) and pyridine (2 mL) was added acetyl chloride (0.1 mL) at –20 °C, and the mixture was stirred for 4.5 h at –20 °C – 0 °C. Dichloromethane (50 mL) was added and the mixture was successively washed with ice-cold 2 M HCl and water, dried (Na_2SO_4), and concentrated to yield an almost pure **9** (0.45 g); $[\alpha]_D$ –19.5° (*c* 1, CH_2Cl_2); IR 1750, 1720, 750, 700 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.00 (s, 3H, AcO), 3.23 (dd, 1H, J_{gem} = 14, $J_{1ax,2}$ = 9 Hz, H-1*ax*), 3.45 (m, 1H, H-5), 3.92 (t, 1H, *J* = 10 Hz, H-4), 4.05 (s, 2H, COCH_2Cl), 4.18 (dd, 1H, $J_{1eq,2}$ = 4.4 Hz, H-1*eq*), 4.30 (t, 1H, $J_{gem} = J_{5,6ax}$ = 10–11 Hz, H-6*ax*), 4.83 (dd, 1H, J_{gem} = 11, $J_{5,6eq}$ = 4.4 Hz, H-6*eq*), 4.97 (m, 1H, H-2), 5.11, 5.17 (2 d, 2H, OCH_2Ph), 5.24 (dd, 1H, $J_{2,3}$ = 7, $J_{3,4}$ = 9 Hz, H-3), 5.53 (s, 1H, C*H*Ph of benzylidene), 7.3–7.5 (m, 10H, Ph-*H*). Found: C, 59.69; H, 5.14; N, 3.05; calcd for $C_{25}H_{26}NO_8Cl$: C, 59.59; H, 5.20; N, 2.78 %.

2-O-Acetyl-4,6-O-benzylidene-N-benzyloxycarbonyl-1,5-dideoxy-1,5-imino-D-glucitol (**10**)

To a solution of **9** (0.25 g) in pyridine (10 mL) was added water (2 mL), and the mixture was stirred overnight at room temperature. Dichloromethane was added and the mixture was washed with ice-cold 2 M HCl and water, dried (Na_2SO_4) and concentrated. The syrupy residue was chromatographed on a column of silica gel (CH_2Cl_2:MeOH, 500:1) to afford **10** as an amorphous mass; $[\alpha]_D$ –12.5° (*c* 1, CH_2Cl_2); IR 3450, 1740, 1710, 750, 700 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.04 (s, 3H, AcO), 3.17 (dd, 1H, J_{gem} = 14, $J_{1ax,2}$ = 8 Hz, H-1*ax*), 3.37 (m, 1H, H-5), 3.70 (t, 1H, *J* = 9 Hz, H-4), 3.79 (near t, 1H, $J_{3,4}$ = 9, $J_{2,3}$ = 6.6 Hz, H-3), 4.11 (dd, 1H, $J_{1eq,2}$ = 4.4 Hz, H-1*eq*), 4.21 (t, 1H, *J* = 10–11 Hz, H-6*ax*), 4.79–4.86 (m, 2H, H-2 and H-6), 5.09, 5.16 (2 d, 2H, J_{gem} = 12 Hz, OCH_2Ph), 5.55 (s, 1H, C*H*Ph of benzylidene), 7.28–7.4 (m, 10H, Ph-*H*). Found: C, 64.72; H, 5.70; N, 3.38; calcd for $C_{23}H_{25}NO_7$: C, 64.63; H, 5.90; N, 3.28 %.

O-(2,3,4-Tri-O-benzyl-α-L-fucopyranosyl)-(1→3)-2-O-acetyl-4,6-O-benzylidene-N-benzyloxycarbonyl-1,5-dideoxy-1,5-imino-D-glucitol (12)

A mixture of **10** (30 mg), methyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-fucopyranoside (**11**, 39 mg, 1.2 equiv.), and powdered molecular sieves 4 Å (100 mg) in benzene (6 mL) was stirred overnight at room temperature then cooled to 7 °C. Dimethyl(methylthio)sulfonium triflate (DMTST, 97 mg, 4 equiv.) was added and the reaction mixture was stirred for 2.5 h at 7 °C – room temperature. After dilution with CH_2Cl_2 (50 mL), MeOH (2 mL) and Et_3N (40 μL) were added and the solids were removed by filtration. The filtrate was concentrated and the residual syrup was taken-up in CH_2Cl_2. The CH_2Cl_2 solution was successively washed with sat. $NaHCO_3$ and water, dried (Na_2SO_4), and concentrated. The residue was chromatographed on a column of silica gel (AcOEt:hexane, 1:4) to give **12** (55 mg, 92 %) as a syrup; $[\alpha]_D$ –94° (*c* 1, CH_2Cl_2); IR 1750, 1720, 740, 700 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.83 (d, 3H, $J_{5,6}$ = 6.6 Hz, H-6b), 1.87 (s, 3H, AcO), 3.57 (dd, 1H, J_{gem} = 13, $J_{1ax,2}$ = 5.5 Hz, H-1a(*ax*)), 4.57, 4.70, 4.71, 4.76, 4.83, 4.93, 5.13, 5.17 (8 d, 8H, OCH_2Ph), 4.85 (dd, 1H, J_{gem} = 11, $J_{5,6eq}$ = 4.4 Hz, H-6a(*eq*)), 4.98 (m, 1H, H-2a), 5.21 (d, 1H, $J_{1,2}$ = 4 Hz, H-1b), 5.52 (s, 1H, C*H*Ph of benzylidene), 7.2–7.45 (m, 25 H, Ph-*H*). Found: C, 71.34; H, 6.54; N, 1.69; calcd for $C_{50}H_{53}NO_{11}$: C, 71.16; H, 6.33; N, 1.66 %.

O-(2,3,4-Tri-O-benzyl-α-L-fucopyranosyl)-(1→3)-4,6-O-benzylidene-N-benzyloxycarbonyl-1,5-dideoxy-1,5-imino-D-glucitol (13)

Compound **12** (2.1 g) was treated with a catalytic amount of NaOMe in dry MeOH (100 mL) for 2 h at 0 °C. The solution was neutralized with Amberlite IR-120 (H^+) ion exchange resin and then filtered. The filtrate was concentrated to give **13** almost quantitatively; $[\alpha]_D$ –60° (*c* 0.5, CH_2Cl_2); IR 3500, 1700, 750, 700 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.81 (d, 3H, *J* = 6.6 Hz, H-6b), 2.72 (dd, 1H, J_{gem} = 13, $J_{1ax,2}$ = 11 Hz, H-1a(*ax*)), 3.24 (dt, 1H, $J_{4,5} = J_{5,6ax}$ = 10–11, $J_{5,6eq}$ = 4.8 Hz, H-5a), 3.48 (t, 1H, $J_{2,3} = J_{3,4}$ = 8.6 Hz, H-3a), 4.00, 4.10 (2 dd, 2H, $J_{2,3}$ = 10, $J_{3,4}$ = 3 Hz, H-3b and H-4b), 4.36 (dd, 1H, J_{gem} = 13, $J_{1eq,2}$ = 4.9 Hz, H-1a(*eq*)), 4.50 (near t, 1H, $J_{gem} = J_{5,6ax}$ = 10–11 Hz, H-6a(*ax*)), 4.81 (dd, 1H, H-6a(*eq*)), 4.99 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1b).

O-(2,3,4-Tri-O-benzyl-α-L-fucopyranosyl)-(1→3)-2-O-acetyl-6-O-benzyl-N-benzyloxycarbonyl-1,5-dideoxy-1,5-imino-D-glucitol (14)

To a solution of **12** (1 g) in dry THF (30 mL) were added molecular sieves 3 Å (2 g), the mixture was stirred for 2 h at room temperature, and $NaBH_3CN$ (1.2 g) was gradually added. After the reagent had dissolved, saturated HCl in ether was added dropwise at room temperature until the evolution of gas ceased. The reaction mixture was stirred for 30 min at room temperature and neutralized with Et_3N. The solids were removed by filtration, and washed with MeOH. The combined filtrate and washings were concentrated then extracted with CH_2Cl_2. The extract was washed with water, dried (Na_2SO_4), and concentrated. Column chromatography (AcOEt:hexane 1:2) of the residue on silica gel afforded **13** (0.81 g, 81 %) as an amorphous mass; $[\alpha]_D$ −51° (*c* 0.8, CH_2Cl_2); IR 3500, 1760, 1700, 750, 710 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.05 (d, 3H, J = 6.6 Hz, H-6b), 1.85 (s, 3H, AcO), 3.08 (broad d, 1H, J = 7 Hz, 4a-O*H*), 3.38 (dd, 1H, J_{gem} = 15, $J_{1ax,2}$ = 3 Hz, H-1a(*ax*)), 4.13 (near d, 1H, J_{gem} = 15 Hz, H-1a(*eq*)), 4.91 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1b), 4.88–4.94 (narrow m, 1H, H-2a), 4.37, 4.44, 4.62, 4.64, 4.68, 4.77, 4.78, 4.95, 5.08, 5.18 (10 d, 10H, OC*H*$_2$Ph), 7.2–7.4 (m, 25 H, Ph-*H*). Found: C, 71.06; H, 6.81; N, 1.70; calcd for $C_{50}H_{55}NO_{11}$: C, 70.99; H, 6.55; N, 1.66 %.

O-(2,3,4,6-Tetra-O-benzoyl-β-D-galactopyranosyl)-(1→3)-2-O-acetyl-4,6-O-benzylidene-N-benzyloxycarbonyl-1,5-dideoxy-1,5-imino-D-glucitol (16)

To a solution of **10** (1 g) and methyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-galactopyranoside (**15**, 2.9 g) in dry CH_2Cl_2 (30 mL) was added molecular sieves 4 Å (4 g), and the mixture was stirred for 6 h at room temperature, then cooled to −20 °C. To the cooled mixture were added, with stirring, *N*-iodosuccinimide (NIS, 2.11 g) and trifluoromethanesulfonic acid (TfOH, 83 μL), and the stirring was continued overnight at −20 °C – room temperature. The solids were filtered off and washed with CH_2Cl_2. The combined filtrate and washings were successively washed with M Na_2CO_3, M $Na_2S_2O_3$ and water, dried (Na_2SO_4), and concentrated. Column chromatography (AcOEt:hexane 1:4) of the residue on silica gel gave **16** (2.35 g, quant.); $[\alpha]_D$ +20° (*c* 1, CH_2Cl_2); IR 1750, 1700, 740, 710 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.70 (s, 3H, AcO), 3.34 (dd, 1H, J_{gem} = 14, $J_{1ax,2}$ = 7 Hz, H-1a(*ax*)), 3.45 (dt, 1H, $J_{4,5}$ = $J_{5,6ax}$ = 10, $J_{5,6eq}$ = 4.6 Hz, H-5a), 3.70 (dd, 1H, J_{gem} = 14, $J_{1eq,2}$ = 3.3 Hz, H-1a(*eq*)), 4.36, 4.52 (2 dd, 2H, J_{gem} = 11, $J_{5,6}$ = 7.6, $J_{5,6'}$ = 6 Hz, H-6b), 4.81 (m, 1H, H-2a), 4.86 (dd, 1H, J_{gem} = 11, $J_{5,6eq}$ = 4.6 Hz, H-6a(*eq*)), 5.08, 5.13 (2 d, 2H, OC*H*$_2$Ph), 5.16 (d, 1H, $J_{1,2}$ = 8 Hz, H-1b), 5.57, 5.76 (2 dd, 2H, $J_{2,3}$ = 10.3, $J_{3,4}$ = 3.4 Hz, H-3b and H-2b), 5.62 (s, 1H, C*H*Ph of benzylidene), 5.95 (near d, 1H, J = 3–4 Hz, H-4b), 7.2–8.1 (m, 30 H, Ph-*H*). Found: C, 67.96; H, 5.21; N, 1.31; calcd for $C_{57}H_{51}NO_{16}$: C, 68.05; H, 5.11; N, 1.39 %.

O-(2,3,4,6-Tetra-O-benzoyl-β-D-galactopyranosyl)-(1→3)-2-O-acetyl-6-O-benzyl-N-benzyloxycarbonyl-1,5-dideoxy-1,5-imino-D-glucitol (17)

Reductive ring opening of the benzylidene group of **16** (2.35 g) was carried out in THF (50 mL) using $NaBH_3CN$ (2.5 g) and molecular sieves 3 Å (4 g) as just described for **14**. The product was purified by column chromatography (AcOEt:hexane, 1:2) on silica gel to give **17** (2.11 g, 90 %) as an amorphous mass; $[\alpha]_D$ +62° (*c* 1, CH_2Cl_2); IR 3500, 1740, 1700, 740, 720, 700 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.59 (s, 3H, AcO), 3.27 (dd, 1H, J_{gem} = 15.6, $J_{1ax,2}$ = 2.7 Hz, H-1a(*ax*)), 3.75–3.80 (narrow m, 2H, H-3a and H-4a), 3.99 (near d, 1H, J_{gem} = 15.6 Hz, H-1a(*eq*)), 4.47, 4.53 (2 d, 2H, OC*H*$_2$Ph of Bn), 4.70 (narrow m, 1H, H-2a), 4.94 (d, 1H, $J_{1,2}$ = 8 Hz, H-1b), 5.01, 5.14 (2 d, 2H, OC*H*$_2$Ph of Z), 5.63, 5.79 (2 dd, 2H, $J_{2,3}$ = 10, $J_{3,4}$ = 3.5 Hz, H-3b and H-2b), 5.98 (near d, 1H, J = 3–4 Hz, H-4b), 7.2–8.1 (m, 30 H, Ph-*H*). Found: C, 68.02; H, 5.57; N, 1.41; calcd for $C_{57}H_{53}NO_{16}$: C, 67.92; H, 5.30; N, 1.39 %.

O-(2,3,4-Tri-O-benzyl-α-L-fucopyranosyl)-(1→3)-2-O-acetyl-N-benzyloxycarbonyl-1,5-dideoxy-1,5-imino-D-glucitol (18)

To a solution of **12** (0.45 g) in AcOH (8 mL) was added water (2 mL). The mixture was stirred overnight at 45 °C and then concentrated. Column chromatography (AcOEt:hexane, 2:1) of the residue on silica gel gave **18** (96 %) as an amorphous mass; $[\alpha]_D$ −53° (*c* 1, CH_2Cl_2); IR 3450, 1750, 1690, 750, 700 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.12 (d, 3H, H-6b), 1.83 (s, 3 H, AcO), 4.64, 4.65, 4.73, 4.80, 4.81, 4.97, 5.09, 5.20 (8 d, 8H, OC*H*$_2$Ph), 4.89 (m, 1H, H-2a), 4.92 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1b), 7.2–7.4 (m, 20H, Ph-*H*). Found: C, 68.36; H, 6.76; N, 1.96; calcd for $C_{43}H_{49}NO_{11}$: C, 68.33; H, 6.53; N, 1.85 %.

O-(Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→6)-O-[(2,3,6-tri-O-benzyl-α-L-fucopyranosyl)-(2→3)]-2-O-acetyl-N-benzyloxycarbonyl-1,5-dideoxy-1,5-imino-D-glucitol (20)

A mixture of **18** (0.3 g), methyl(methyl-5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-D-*galacto*-2-thio-2-nonulopyranosid)onate (**19**, 0.35 g), and molecular sieves 3 Å (0.7 g) in dry CH_3CN (20 mL) was stirred for 6 h at room temperature, then cooled to −40 °C. To the cooled mixture were added, with stirring, NIS (0.304 g) and TfOH (12 μL), and the stirring was continued overnight at −40 °C. The solids were filtered off and washed with CH_2Cl_2. The combined filtrate and washings were washed with sat. $NaHCO_3$, M $Na_2S_2O_3$ and water, dried (Na_2SO_4), and concentrated. Column chromatography (AcOEt:hexane 2:1) of the residue on silica gel afforded the α-glycoside **20** (56 %) as an amorphous mass; $[\alpha]_D$ −38° (*c* 1, CH_2Cl_2); IR 3400, 1750, 1700, 1650, 1550, 740, 700 cm^{-1}; ^{1}H NMR

($CDCl_3$): δ 1.14 (d, 3H, J = 6.6 Hz, H-6b), 1.87 (s, 3H, AcN), 1.97, 2.02, 2.05, 2.09, 2.12 (5 s, 15H, AcO), 2.55 (dd, 1H, J_{gem} = 13, $J_{3eq,4}$ = 4.6 Hz, H-3c(*eq*)), 3.40 (dd, 1H, J_{gem} = 15, $J_{1ax,2}$ = 3.7 Hz, H-1a(*ax*)), 3.68 (s, 3H, CO_2CH_3), 7.2–7.4 (m, 20 H, OCH_2Ph). Found: C, 61.43; H, 6.36; N, 2.05; calcd for $C_{63}H_{76}N_2O_{23}$: C, 61.56; H, 6.23; N, 2.28 %. The minor product (10–20 %, β-glycoside) had ^1H NMR ($CDCl_3$): δ 1.13 (d, 3H, J = 6.4 Hz, H-6b), 2.37 (dd, 1H, J_{gem} = 13, $J_{3eq,4}$ = 4-5 Hz, H-3c(*eq*)), 3.43 (dd, 1H, J_{gem} = 15, $J_{1ax,2}$ = 2-3 Hz, H-1a(*ax*)).

Deprotection of compound ***20***

A solution of **20** (0.1 g) in MeOH (10 mL) and AcOH (10 mL) was hydrogenolyzed in the presence of palladium-black catalyst for 3–7 days at room temperature, then filtered and concentrated to dryness. The residue was dissolved in MeOH and treated with a catalytic amount of NaOMe, then 0.2 M KOH (2 mL) for 2 days with stirring at room temperature. The solution was neutralized with Amberlite IR-120 (H^+) ion-exchange resin, filtered and concentrated. The product was purified by chromatography (EtOH:H_2O 3:2) on a column of Sephadex LH-20 to give *O*-(5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-*O*-[(α-L-fucopyranosyl)-(1→3)]-1,5-dideoxy-1,5-imino-*N*-methyl-D-glucitol (**21B**) as the major product; $[\alpha]_D$ −13° (*c* 0.5, EtOH:H_2O 1:3); ^1H NMR (D_2O/CD_3OD): δ 1.21 (d, 3H, J = 6.6 Hz, H-6b), 1.77 (t, 1H, J_{gem} = $J_{3ax,4}$ = 12 Hz, H-3c(*ax*)), 2.05 (s, 3H, AcN), 2.74 (dd, 1H, J_{gem} = 12, $J_{3eq,4}$ = 4 Hz, H-3c(*eq*)), 3.03 (broad t, 1H, J_{gem} = $J_{1ax,2}$ = 12 Hz, H-1a(*ax*)), 3.45 (broad dd, 1H, J_{gem} 12, $J_{1eq,2}$ = 2–3 Hz, H-1a(*eq*)), 5.33 (d, 1H, $J_{1,2}$ = 3.9 Hz, H-1b); Ion-spray MS m/z 615.5 $(M + H)^+$; MS/MS m/z 469 $(M - Fuc + H)^+$, 324 $(M - Neu5Ac + H)^+$, 291.7 (Neu5Ac fragment), 273.9 (Neu5Ac fragment − H_2O), 177.7 (protonated *N*-methyl-DNJ fragment), and for further details, see Ref. 14c, average molecular weight 614.59 ($C_{24}H_{42}N_2O_{16}$). The minor product **21A** gave the molecular ion peak at m/z 601.1 $(M + H)^+$, corresponding to $C_{23}H_{40}N_2O_{16}$.

*O-(2,3,4-Tri-O-benzyl-α-L-fucopyranosyl)-(1→3)-6-O-benzyl-N-benzyloxycarbonyl-1,5-dideoxy-1,5-imino-D-glucitol (***22***)*

Compound **14** (65 mg) was treated with a catalytic amount of NaOMe in dry MeOH for 2.5 h. The solution was neutralized with Amberlite IR-120 (H^+), then filtered and concentrated. The product was purified by column chromatography (CH_2Cl_2:MeOH, 180:1) on silica gel to afford **22** quantitatively; $[\alpha]_D$ −76° (*c* 1, CH_2Cl_2); IR 3500–3400, 1700, 750, 700 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.02 (d, 3H, J = 6.6 Hz, H-6b), 3.43 (near d, 1H, J_{gem} = 14 Hz, H-1a(*ax*)), 3.50 (d, 1H, $J_{3,4}$ = 1.8 Hz, H-4b), 4.02 (dd, 1H, $J_{2,3}$ = 9.9 Hz, H-2b), 4.85 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1b), 4.27–4.95 (8 d, 8H, OCH_2Ph of Bn), 5.13 (s, 2H, OCH_2Ph of Z), 5.13 (s, 2H, OCH_2Ph of Z), 7.15–7.40 (m, 25H, Ph-*H*). Found: C, 71.49; H, 6.50; N, 1.69; calcd for $C_{48}H_{53}NO_{10}$: C, 71.71; H, 6.65; N, 1.74 %.

*O-(α-L-Fucopyranosyl)-(1→3)-1,5-dideoxy-1,5-imino-N-methyl-D-glucitol (***23***)*

A solution of **22** (48 mg) in MeOH (10 mL) was hydrogenolyzed in the presence of formalin (89 μL) and palladium hydroxide-on-carbon (50 mg) for 3 days at room temperature. The catalyst was filtered off and washed with MeOH. The combined filtrate and washings were concentrated. The product was purified by chromatography (MeOH) on a column of Sephadex LH-20 to give **23** (84 %) as an amorphous mass; $[\alpha]_D$ −84° (*c* 0.32, MeOH:H_2O, 1:1); ^1H NMR (D_2O): δ 1.19 (d, 3H, J = 6.6 Hz, H-6b), 2.41 (t, 1H, J_{gem} = $J_{1ax,2}$ = 11–12 Hz, H-1a(*ax*)), 2.47 (s, 3H, *N*-CH_3), 3.05 (dd, 1H, J_{gem} = 12, $J_{1eq,2}$ = 5 Hz, H-1a(*eq*)), 3.44, 3.54 (2 t, 2H, $J_{2,3}$ = $J_{3,4}$ = $J_{4,5}$ = 9 Hz, H-3a and H-4a), 4.37 (near q, 1H, J = 6–7 Hz, H-5b), 5.30 (d, 1H, $J_{1,2}$ = 4 Hz, H-1b). Found: C, 48.23; H, 7.59; N, 4.08; calcd for $C_{13}H_{25}NO_8$: C, 48.29; H, 7.79; N, 4.43 %.

A solution of **13** (130 mg) in MeOH (6 mL) and AcOH (6 mL) was also hydrogenolyzed over palladium-black catalyst for 10 days at room temperature. Work-up and purification on Sephadex LH-20 as just described afforded **23** in an 82 % yield.

*O-(2,3,4,6-Tetra-O-benzoyl-β-D-galactopyranosyl)-(1→4)-O-[(2,3,6-tri-O-benzyl-α-L-fucopyranosyl)-(1→3)]-2-O-acetyl-6-O-benzyl-N-benzyloxycarbonyl-1,5-dideoxy-1,5-imino-D-glucitol (***24***)*

To a solution of **14** (0.13 g) and **15** (0.222 g, 2 equiv.) in dry CH_2Cl_2 (12 mL) was added molecular sieves 4 Å (0.4 g), and the mixture was stirred overnight at room temperature, then cooled to 0 °C. To the cooled mixture were added NIS (0.16 g) and TfOH (6 μL), and the stirring was continued overnight at 0 °C – room temperature. Work-up as described for **16** and column chromatography (AcOEt:hexane, 1:2) gave **24** (70 %) as an amorphous mass; $[\alpha]_D$ −10° (*c* 1.2, CH_2Cl_2); IR 1750, 1700, 740, 710, 700 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.04 (d, 3H, J = 6.6 Hz, H-6b), 3.27 (near d, 1H, J_{gem} = 13 Hz, H-1a(*ax*)), 3.66, 3.98 (2 dd, 2H, $J_{2,3}$ = 10, $J_{3,4}$ = 2.2 Hz, H-3b and H-2b), 4.98 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1b), 5.53 (dd, 1H, $J_{2,3}$ = 10, $J_{3,4}$ = 3 Hz, H-3c), 5.94 (near d, 1H, J = 3 Hz, H-4c), 7.2–8.1 (m, 45H, Ph-*H*). Found: C, 70.68; H, 5.90; N, 0.80; calcd for $C_{84}H_{81}NO_{20}$: C, 70.82; H, 5.73; N, 0.98 %.

*O-(β-D-Galactopyranosyl)-(1→4)-O-[(α-L-fucopyranosyl)-(1→3)]-1,5-dideoxy-1,5-imino-N-methyl-D-glucitol (***3***)*

A solution of **24** (30 mg) in MeOH (3 mL) and formic acid (3 mL) was hydrogenolyzed in the presence of palladium-black catalyst (30 mg) for 10 days at room temperature. The catalyst was filtered off and washed

with MeOH. The combined filtrate and washings were concentrated to dryness. The residue was dissolved in dry MeOH (10 mL) and treated with a catalytic amount of NaOMe overnight at room temperature. The solution was neutralized with Amberlite IR-120 (H^+) ion-exchange resin. The resin was removed by filtration and washed with MeOH. The combined filtrate and washings were concentrated, and the product was purified by chromatography (EtOH:H_2O, 3:1) on a column of Sephadex LH-20 to give **3** in a quantitative yield; $[\alpha]_D$ −24° (*c* 0.5, H_2O:EtOH, 2:1); ^1H NMR (D_2O/CD_3OD): δ 1.14 (d, 3H, J = 6.8 Hz, H-6b), 2.27 (near t, 1H, $J_{gem} = J_{1ax,2}$ = 11–12 Hz, H-1a(*ax*)), 2.38 (s, 3H, *N*-CH_3), 2.97 (dd, 1H, J_{gem} = 12, $J_{1eq,2}$ = 4–5 Hz, H-1a(*eq*)), 4.51 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1c), 5.37 (d, 1H, $J_{1,2}$ = 3.9 Hz, H-1b); Ion-spray MS m/z 507.9 $(M + Na)^+$, 486.5 $(M + H)^+$ (base peak); MS/MS (P = 486.5) m/z 339.6 $(M - Fuc + H)^+$, 323.6 $(M - Gal + H)^+$, 305.9 $(M - Gal - H_2O + H)^+$, 177.6 (protonated *N*-methyl-DNJ fragment), 159.9 (protonated *N*-methyl-DNJ fragment − H_2O), for further details, see Figure 1, average. molecular weight 485.49. Found: C, 46.98; H, 6.97; N, 2.81; calcd for $C_{19}H_{35}NO_{13}$: C, 47.01; H, 7.27; N, 2.89 %.

O-(2,3,4,6-Tetra-O-benzoyl-β-D-galactopyranosyl)-(1→3)-O-[(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1→4)]-2-O-acetyl-6-O-benzyl-N-benzyloxycarbonyl-1,5-dideoxy-1,5-imino-D-glucitol (25)

A mixture of **17** (0.5 g), **11** (0.346 g, 1.5 equiv.) and molecular sieves 4 Å (1 g) in benzene (10 mL) was stirred overnight at room temperature, then cooled to 7 °C. DMTST (0.682 g, 4 equiv.) was added and the mixture was stirred for 3.5 h at 7 °C – room temperature. Work-up and chromatographic purification (AcOEt: hexane, 1:3) on silica gel as described for **12** gave **25** in a quantitative yield; $[\alpha]_D$ −25° (*c* 1, CH_2Cl_2); IR 1740, 1710, 740, 720, 700 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.13 (d, 3H, J = 6.4 Hz, H-6c), 1.83 (s, 3H, AcO), 2.99 (broad d, H-1a(*ax*)), 5.65, 5.77 (2 dd, 2H, $J_{1,2}$ = 8, $J_{2,3}$ = 10.8, $J_{3,4}$ = 2.9 Hz, H-3b and H-2b), 5.99 (d, 1H, $J_{3,4}$ = 2.9 Hz, H-4b), 7.1–8.1 (m, 45H, Ph-*H*). Found: C, 70.83; H, 5.74; N, 1.12; calcd for $C_{84}H_{81}NO_{20}$: C, 70.82; H, 5.73; N, 0.98 %.

O-(2,3,4,6-Tetra-O-benzoyl-β-D-galactopyranosyl)-(1→3)-O-[(α-L-fucopyranosyl)-(1→4)]-2-O-acetyl-1,5-dideoxy-1,5-imino-N-methyl-D-glucitol (26)

A solution of **25** (0.1 g) in MeOH (13 mL) and formic acid (3 mL) was hydrogenolyzed in the presence of palladium-black catalyst (0.1 g) for 10 days at room temperature. Work-up and the resulting crude product was purified by chromatography (CH_2Cl_2:MeOH, 25:1–20:1) on silica gel to give **26** (quant.) as an amorphous mass; $[\alpha]_D$ −13° (*c* 1.6, MeOH); ^1H NMR (CD_3OD): δ 1.41 (d, 3H, J = 6.6 Hz, H-6c), 2.14 (s, 3H, AcO), 2.31 (s, 3H, *N*-CH_3), 2.94 (dd, 1H, J_{gem} = 12, $J_{1ax,2}$ = 4.6 Hz, H-1a(*ax*)), 4.41 (dd, 1H, J_{gem} = 11, $J_{5,6}$ = 6.8 Hz, H-6b), 4.49 (t, 1H, $J_{5,6} = J_{5,6'}$ = 6.3 Hz, H-5b), 4.75 (m, 1H, H-2a), 4.80 (dd, J_{gem} = 11, $J_{5,6'}$ = 6 Hz, H-6'b), 5.17 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1c), 5.30 (d, 1H, $J_{1,2}$ = 7.5 Hz, H-1b), 5.69, 5.75 (2 dd, 2H, $J_{2,3}$ = 10, $J_{3,4}$ = 3 Hz, H-3b and H-2b), 6.01 (d, 1H, J = 3, H-4b), 7.2–8.1 (m, 20H, Ph-*H*). Found: C, 62.43; H, 5.42; N, 1.46; calcd for $C_{49}H_{53}NO_{18}$: C, 62.35; H, 5.66; N, 1.48 %.

O-(β-D-Galactopyranosyl)-(1→3)-O-[(α-L-fucopyranosyl)-(1→4)]-1,5-dideoxy-1,5-imino-N-methyl-D-glucitol (4)

Treatment of **26** (47 mg) with a catalytic amount of NaOMe in dry MeOH as described for **3**, and purification of the product by chromatography (EtOH:H_2O 2:1) on Sephadex LH-20 afforded **4** (quant.) as an amorphous mass; $[\alpha]_D$ +1° (*c* 1, H_2O:EtOH, 2:1); ^1H NMR (D_2O/CD_3OD): δ 1.21 (d, 3H, J = 6.4 Hz, H-6a), 2.58 (s, 3H, *N*-CH_3 overlapped with H-1a(*ax*)), 3.20 (broad dd, 1H, J_{gem} = 12, $J_{1eq,2}$ = 4 Hz, H-1a(*eq*)), 4.79 (d, 1H, $J_{1,2}$ = 7.2 Hz, H-1b), 5.15 (d, 1H, $J_{1,2}$ = 3 Hz, H-1c); Ion-spray MS m/z 507.9 $(M + Na)^+$, 486.6 $(M + H)^+$ (base peak); MS/MS (P = 486.6) m/z 340.0 $(M - Fuc + H)^+$, 323.8 $(M - Gal + H)^+$, 177.3 (protonated *N*-methyl-DNJ fragment), 159.7 (protonated *N*-methyl-DNJ fragment − H_2O), for further details, see Figure 2. Average molecular weight 485.49. Found: C, 46.98; H, 6.97; N, 2.81; calcd for $C_{19}H_{35}NO_{13}$: C, 47.01; H, 7.27; N, 2.89 %.

O-(Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-O-(2,4,6-tri-O-benzoyl-β-D-galactopyranosyl)-(1→4)-O-[(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1→3)]-2-O-acetyl-6-O-benzyl-N-benzyloxycarbonyl-1,5-dideoxy-1,5-imino-D-glucitol (28)

To a solution of **14** (70 mg) and **27** (0.124 g, 1.5 equiv.) in dry CH_2Cl_2 (10 mL) was added molecular sieves 4 Å (0.25 g), and the mixture was treated with NIS (56 mg) and TfOH (2.2 μL) at 0 °C – room temperature as described for **24**. Work-up and column chromatography (AcOEt:hexane, 3:2) on silica gel gave **28** (61 %) as a syrup; $[\alpha]_D$ −11° (*c* 0.7, CH_2Cl_2); IR 3400, 1750, 1700, 1660, 1540, 740, 710, 700 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.95 (d, 3H, J = 6 Hz, H-6b), 2.45 (dd, 1H, J_{gem} = 13, $J_{3eq,4}$ = 4.6 Hz, H-3d(*eq*)), 3.81 (s, 3H, CO_2CH_3), 5.23 (dd, 1H, H-7d), 5.41 (d, 1H, J = 3 Hz, H-4c), 5.61 (m, 1H, H-8d), 7.1–7.6, 7.95–8.2 (m, 40H, Ph-*H*). Found: C, 64.75; H, 5.56; N, 1.58; calcd for $C_{97}H_{104}N_2O_{31}$: C, 64.95; H, 5.84; N, 1.56 %. When this glycosylation was performed by using DMTST as the glycosyl promoter, the title compound **28** was obtained in 59 % yield.

O-(5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→3)-O-(β-D-galactopyranosyl)-(1→4)-O-[(α-L-fucopyranosyl)-(1→3)]-1,5-dideoxy-1,5-imino-N-methyl-D-glucitol (5)

A solution of **28** (70 mg) in MeOH (6 mL) and AcOH (6 mL) was hydrogenolyzed in the presence of

palladium-black catalyst (70 mg) for 9 days at room temperature, and then worked up as described for **3**. The product was treated with a catalytic amount of NaOMe in dry MeOH (10 mL), then with 0.2 M KOH (2 mL) for 2 days, and the solution was neutralized with Amberlite IR-120 (H^+) ion-exchange resin. The resin was filtered off and washed with MeOH/H_2O. The combined filtrate and washings were concentrated to a residue which was chromatographed (EtOH:H_2O, 1:1) on a column of Sephadex LH-20 to give **5** (quant.) as an amorphous mass; $[\alpha]_D$ −14° (*c* 0.83, H_2O:EtOH, 3:1); ^1H NMR (D_2O, at 27 °C): δ 1.19 (d, 3H, $J_{5,6}$ = 6.6 Hz, H-6b), 1.79 (t, 1H, $J_{gem} = J_{3ax,4}$ = 12 Hz, H-3d(*ax*)), 2.03 (s, 3H, AcN), 2.77 (dd, 1H, J_{gem} = 12, $J_{3eq,4}$ = 4.4 Hz, H-3d(*eq*)), 3.00 (s, 3H, *N*-CH_3), 3.18 (t, 1H, $J_{gem} = J_{1ax,2}$ = 11 Hz, H-1a(*ax*)), 4.61 (d, 1H, $J_{1,2}$ = 8.8 Hz, H-1c), 5.38 (near s, 1H, H-1b); ^{13}C NMR (D_2O, at 27 °C): δ 16.59 (C-6b), 23.32 (Me of AcN), 31.53, 41.15, 53.00, 62.59, 63.96, 68.06, 68.53, 69.23, 69.42, 69.52, 70.47, 70.57, 73.18, 74.23, 76.46, 76.93, 99.96, 100.97, 103.42, 175.04 (CO of AcN), 176.37 (C-1d); Ion-spray MS m/z 777.2 $(M + H)^+$; MS/MS (P = 777.2) m/z 631.2 $(M - Fuc + H)^+$, 486.2 $(M - Neu5Ac + H)^+$, 339.8 $(M - Fuc - Neu5Ac + H)^+$, 323.9 $(M - Neu5Ac - Gal + H)^+$, 292.1 (Neu5Ac fragment), 273.8 (Neu5Ac fragment − H_2O), 177.7 (protonated *N*-methyl-DNJ fragment), for further details, see Figure 3. Average. molecular weight 776.73. Found: C, 46.19; H, 6.47; N, 3.63; calcd for $C_{30}H_{52}N_2O_{21}$: C, 46.39; H, 6.75; N, 3.61 %.

O-(Methyl-5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-O-(2,4,6-tri-O-benzoyl-β-D-galactopyranosyl)-(1→3)-2-O-acetyl-N-benzyloxycarbonyl-1,5-dideoxy-1,5-imino-D-glucitol (29)

To a solution of **10** (0.2 g) and **27** (0.7 g, 1.2 equiv.) in dry CH_2Cl_2 (15 mL) was added molecular sieves 4 Å (1 g), and the mixture was stirred overnight at room temperature, then cooled to −20 °C. NIS (0.316 g) and TfOH (13 μL) were added and stirring continued overnight at −20 °C – room temperature. Work-up and column chromatography (CH_2Cl_2:MeOH, 250:4) on silica gel as described for **16** afforded **29** (90 %) as an amorphous mass; $[\alpha]_D$ +9° (*c* 1, CH_2Cl_2); IR 3400, 1750, 1700, 1660, 1550, 750, 710, 700 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.65, 1.66, 1.77, 1.92, 1.99, 2.18 (6 s, 18H, AcN and AcO), 2.47 (dd, 1H, J_{gem} = 12, $J_{3eq,4}$ = 4 Hz, H-3c(*eq*)), 3.26 (dd, 1H, J_{gem} = 13, $J_{1ax,2}$ = 6 Hz, H-1a(*ax*)), 3.44 (dt, 1H, $J_{4,5} = J_{5,6ax}$ = 10–11, $J_{5,6eq}$ = 4.6 Hz, H-6a(*ax*)), 3.83 (s, 3H, CO_2CH_3), 4.84 (d, 1H, *J* = 8 Hz, H-1b), 4.75–4.90 (2 m, 2H, H-2a and H-4c), 4.88 (dd, 1H, $J_{5,6}$ = 10, $J_{6,7}$ = 3 Hz, H-6c), 4.95 (d, 1H, *J* = 9.7 Hz, N*H*), 5.06, 5.11 (2 d, 2H, OCH_2Ph), 5.19 (dd, 1H, $J_{6,7}$ = 3, $J_{7,8}$ = 10 Hz, H-7c), 5.36 (d, 1H, $J_{3,4}$ = 3 Hz, H-4b), 5.41 (dd, 1H, $J_{1,2}$ = 8, $J_{2,3}$ = 10 Hz, H-2b), 5.58 (s, 1H, C*H*Ph of benzylidene), 5.62 (m, 1H, H-8c), 7.2–7.6, 7.9–8.2 (m, 25 H, Ph-*H*). Found: C, 60.87; H, 5.23; N, 1.89; calcd for $C_{70}H_{74}N_2O_{27}$: C, 61.13; H, 5.42; N, 2.04 %.

O-(Methyl-5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-O-(2,4,6-tri-O-benzoyl-β-D-galactopyranosyl)-(1→3)-2-O-acetyl-6-O-benzyl-N-benzyloxycarbonyl-1,5-dideoxy-1,5-imino-D-glucitol (30)

To a solution of **29** (0.2 g) in THF (30 mL) were added molecular sieves 3 Å (0.4 g), the mixture was stirred for 4 h at room temperature, and then $NaBH_3CN$ (0.15 g) was added. After the reagent had dissolved, the mixture was cooled to 0 °C. Saturated HCl in ether was added dropwise at 0 °C and the mixture was stirred for 1.5 h. Work-up as described for **14** and column chromatography (AcOEt:hexane, 4:1) of the product on silica gel gave **30** (quant.) as an amorphous mass; $[\alpha]_D$ +26° (*c* 0.92, CH_2Cl_2); IR 3500, 3300, 1750, 1700, 1660, 1550, 740, 710, 700 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.66, 1.78, 1.91, 2.07, 2.18, 2.35 (6 s, 18H, AcN and AcO), 2.45 (dd, 1H, J_{gem} = 13, $J_{3eq,4}$ = 4.4 Hz, H-3c(*eq*)), 3.17 (near d, 1H, J_{gem} = 15.6, $J_{1ax,2}$ = 2 Hz, H-1a(*ax*)), 3.63 (dd, 1H, $J_{2,3}$ = 10, $J_{3,4}$ = 3 Hz, H-3b), 3.85 (s, 3H, CO_2CH_3), 4.70 (narrow m, 1H, H-2a), 4.81 (m, 1H, H-4c), 4.91 (dd, 1H, $J_{5,6}$ = 10, $J_{6,7}$ = 3 Hz, H-6c), 5.00 (d, 1H, $J_{1,2}$ = 8 Hz, H-1b), 5.01, 5.13 (2 d, 2H, OCH_2Ph), 5.23 (dd, 1H, $J_{6,7}$ = 3, $J_{7,8}$ = 10 Hz, H-7c), 5.35 (d, 1H, $J_{3,4}$ = 3 Hz, H-4b), 5.46 (dd, 1H, $J_{1,2}$ = 8, $J_{2,3}$ = 10 Hz, H-2b), 5.62 (m, 1H, H-8c), 7.15–7.6, 8.0–8.2 (m, 25H, Ph-*H*). Found: C, 61.24; H, 5.42; N, 1.91; calcd for $C_{70}H_{76}N_2O_{27}$: C, 61.04; H, 5.56; N, 2.03 %.

O-(Methyl-5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-O-(2,4,6-tri-O-benzoyl-β-D-galactopyrano-syl)-(1→3)-O-[(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1→4)]-2-O-acetyl-6-O-benzyl-N-benzyloxycarbonyl-1,5-dideoxy-1,5-imino-D-glucitol (31)

To a solution of **30** (0.2 g) and **11** (0.101 g, 1.5 equiv.) was added molecular sieves 4 Å (0.5 g), and the mixture was stirred overnight at room temperature, then cooled to 7 °C. To the cooled mixture was added DMTST (0.2 g, 4 equiv.) and the mixture was stirred for 4 h at 7 °C – room temperature. MeOH (10 mL) was added at 0 °C and the mixture was neutralized with Et_3N. The solids were filtered off and the filtrate was concentrated. The residual syrup was taken up in CH_2Cl_2 and washed with water, dried (Na_2SO_4), and concentrated. Column chromatography (AcOEt:hexane 4:1) of the residue on silica gel afforded **31** (89 %) as an amorphous mass; $[\alpha]_D$ −16.5° (*c* 1, CH_2Cl_2); IR 3350, 1750, 1700, 1650, 1550, 740, 710, 700 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.06 (d, 3H, *J* = 6.2 Hz, H-6d), 1.64, 1.78, 1.91, 2.04, 2.07, 2.17 (6 s, 18H, AcN and AcO), 2.46 (dd, 1H, J_{gem} = 13, $J_{3eq,4}$ = 4.8 Hz, H-3c(*eq*)), 2.92 (near d, 1H, H-1a(*ax*)), 3.83 (s, 3H, CO_2CH_3), 5.20 (dd, 1H, $J_{6,7}$ = 2.2, $J_{7,8}$ = 9.5 Hz, H-7c), 5.37 (near d, 1H, $J_{3,4}$ = 3.5 Hz, H-4b), 5.43 (dd, 1H, $J_{1,2}$ = 8, $J_{2,3}$ = 10 Hz, H-2b), 5.64 (m, 1H, H-8c), 7.1–7.6, 8.0–8.24 (m, 40H, Ph-*H*). Found: C, 64.70; H, 5.86; N, 1.36; calcd for $C_{97}H_{104}N_2O_{31}$: C, 64.95; H, 5.84; N, 1.56 %.

O-(5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→3)-O-(β-D-galactopyranosyl)-(1→3)-O-[(α-L-fucopyranosyl)-(1→4)]-1,5-di-deoxy-1,5-imino-N-methyl-D-glucitol (6)

A solution of **31** (60 mg) in MeOH (10 mL) and formic acid (10 mL) was hydrogenolyzed over palladium-black catalyst (60 mg) for 10 days at room temperature as described for **3**. The product (40 mg) was treated with NaOMe in dry MeOH (10 mL) overnight at room temperature, and then with 0.2 M KOH (10 mL). The solution was neutralized with Amberlite IR-120 (H^+) and worked-up. Column chromatography (EtOH:H_2O, 1:2) on Sephadex LH-20 gave **6** (quant.) as an amorphous mass; $[\alpha]_D$ −4° (*c* 0.74, H_2O:EtOH, 3:1); ^1H NMR (D_2O, at 27 °C): δ 1.20 (d, 3H, J = 6.6 Hz, H-6d), 1.80 (t, 1H, $J_{gem} = J_{3ax,4}$ = 12 Hz, H-3c(*ax*)), 2.03 (s, 3H, AcN), 2.76 (dd, 1H, J_{gem} = 12, $J_{3eq,4}$ = 4.4 Hz, H-3c(*eq*)), 2.96 (s, 3H, *N*-CH_3), 3.13 (broad t, 1H, $J_{gem} = J_{1ax,2}$ = 11 Hz, H-1a(*ax*)), 5.10 (near s, 1H, H-1d); ^1H NMR (D_2O, at 45 °C): δ 4.80 (d, 1H, $J_{1,2}$ = 8 Hz, H-1b), 5.10 (d, 1H, $J_{1,2}$ = 3 Hz, H-1d); ^{13}C NMR (D_2O, at 27 °C): δ 16.71 (C-6d), 23.32 (Me of AcN), 31.54, 40.99, 52.99, 62.70, 63.91, 68.61, 68.74, 69.09, 69.40, 69.59, 70.40, 70.53, 73.14, 74.15, 76.08, 77.04, 100.12, 101.10, 103.91, 175.12 (CO of AcN), 176.35 (C-1c); Ion-spray MS m/z 777.2 $(M + H)^+$; MS/MS (P = 777.2) m/z 631.1 $(M - Fuc + H)^+$, 485.8 $(M - Neu5Ac + H)^+$, 340.1 $(M - Fuc - Neu5Ac + H)^+$, 323.6 $(M - Neu5Ac - Gal + H)^+$, 291.6 (Neu5Ac fragment), 273.8 (Neu5Ac fragment $- H_2O$), 177.9 (protonated *N*-methyl-DNJ fragment), for further details, see Figure 4. Average molecular weight 776.73. Found: C, 46.29; H, 6.91; N, 3.62; calcd for $C_{30}H_{52}N_2O_{21}$: C, 46.39; H, 6.75; N, 3.61 %.

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