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Synthesis and NMR assignment of two repeating units (decasaccharide) of the type III group B *Streptococcus* capsular polysaccharide and its ^{13}C-labeled and *N*-propionyl substituted sialic acid analogues [1,2]

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Abstract

For the purpose of carrying out a comprehensive investigation into the nature of the conformational epitope of the type III group B *Streptococcus* polysaccharide, combined chemical and enzymatic methods were applied to the synthesis of three decasaccharide probes, namely β-D-Glc-(1 → 6)[α-NeuR-(2 → 3)-β-D-Gal-(1 → 4)]-β-D-GlcNAc-(1 → 3)-β-D-Gal-(1 → 4)-β-D-Glc-(1 → 6)[α-NeuR-(2 → 3)-β-D-Gal-(1 → 4)]-β-D-GlcNAc-(1 → 3)-β-D-Gal-OMe (**22** NeuR = NeuAc; **23** NeuR = NeuAc with 8% ^{13}C-labeling; **24** NeuR = NeuPr). The precursor core octasaccharide **21** was chemically synthesized from trisaccharide donor **11** and pentasaccharide acceptor **19** by block condensation. Sialylation of **21** with α-(2 → 3)-sialyltransferase and CMP-NeuAc afforded **22**. In the presence of CMP-sialic acid synthetase and α-(2 → 3)-sialyltransferase, **21** was sialylated with sialic acid derivatives (8% ^{13}C-labeled, or *N*-propionyl substituted) to give **23** and **24**, respectively. Complete assignments of the ^{1}H and ^{13}C NMR spectra of compounds **21**, **22** (**23**), and **24** are also presented. © 1996 Elsevier Science Ltd.

Keywords: Synthesis; Chemoenzymatic synthesis; Oligosaccharide; Type III group B *Streptococcus*; NMR spectroscopy

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[1] This is NRCC publication No. 39525.

[2] Dedicated to Professor Dr. Hans Paulsen on the occasion of his 75th birthday.

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1. Introduction

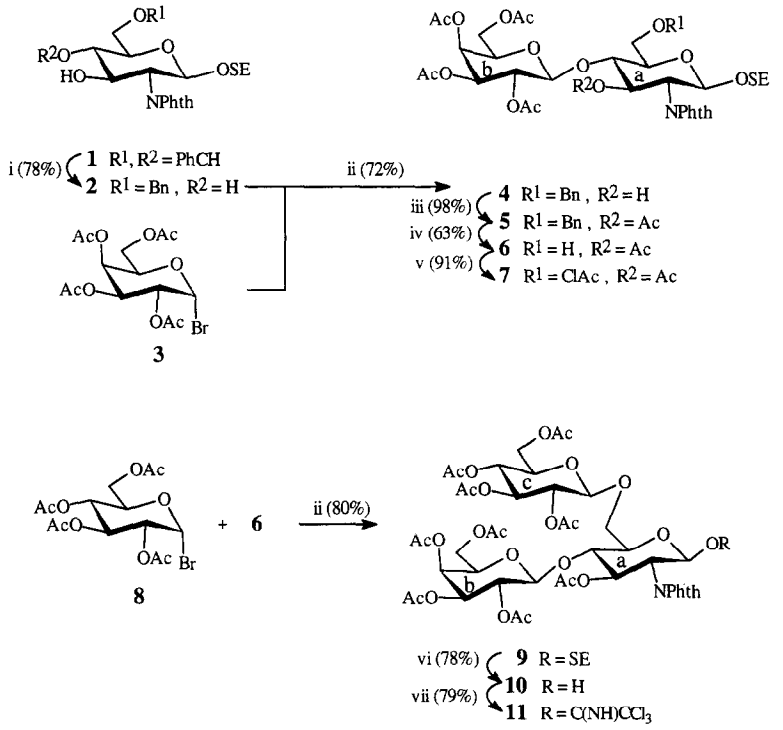
Antibodies to the specific capsular polysaccharide [1] of type III group B *Streptococcus* (GBS III), which causes a substantial amount of group B streptococcal bacterial meningitis and sepsis in newborn infants [2], have been demonstrated to be protective [3]. The role of this polysaccharide as a virulence factor in bacterial infections has also been established [4]. As part of our program aimed at developing diagnostics [5] for, and vaccines [3] against GBS infections in humans, we have been investigating the structural factors governing the binding of the native capsular polysaccharide to its homologous antibodies. This binding was not exhibited by either of two overlapping branched pentasaccharide single repeating units of the GBS III polysaccharide (GBSP III) prepared by enzymatic degradation [1] or by synthesis [6], and binding was not even optimal for a two repeating unit decasaccharide [1,7]. Thus only oligosaccharide fragments of two repeating units or more can assume a particular conformational feature inherent in the native polysaccharide [8]. In addition, the fact that neither the carboxyl-reduced nor desialylated GBSP III were able to bind to homologous antibody revealed that this conformational epitope is dependent for its existence on the carboxylate groups of the sialic acid residues [8]. In order to further define this epitope, this laboratory has undertaken the synthesis of a comprehensive range of oligosaccharides [6,7,9] and oligosaccharide mimics [10,11] related to the GBSP III. These will provide further molecular probes for mapping the antibody binding site and for estimating the minimum structural requirement for binding.

Attempts to prepare two repeating units (decasaccharide) of GBSP III by enzymatic degradation have proven to be difficult because of the extremely low yields obtained. Enzymatic digestion using *endo*-β-D-glactosidase of GBSP III gave predominantly one repeating unit. Therefore, we decided to use a combined chemical–enzymatic approach to this synthesis which has been widely used for sialo-oligosaccharides [12,13]. An added advantage of the enzymatic method is that it might also be possible to link modified sialic acids to the basal oligosaccharides by exploiting the low substrate specificity of α-(2 → 3)-sialyltransferase [14]. Obviously, two repeating units containing ^{13}C-labeled sialic acid and *N*-propionyl sialic acid would be useful probes to reveal whether, and if so, how the sialic acid is involved in the binding site.

Here, we describe a combined chemical–enzymatic synthesis of three decasaccharides that involves the chemical synthesis of core octasaccharide methyl glycoside **21**, with subsequent enzymatic transformation of **21** using sialic acid-CMP derivative into decasaccharide **22**, using ^{13}C-labeled sialic acid into **23**, and using *N*-propionyl sialic acid into **24**. Other related syntheses of various oligosaccharide fragments with part of the above structural elements have been reported [6,7,9–11,15].

2. Results and discussion

Chemical sialylation gives only modest yields, and stereoisomers and elimination products are always encountered [16]. In contrast, enzymatic coupling of NeuAc to oligosaccharides has proved to be highly successful using various bacterial or mam-



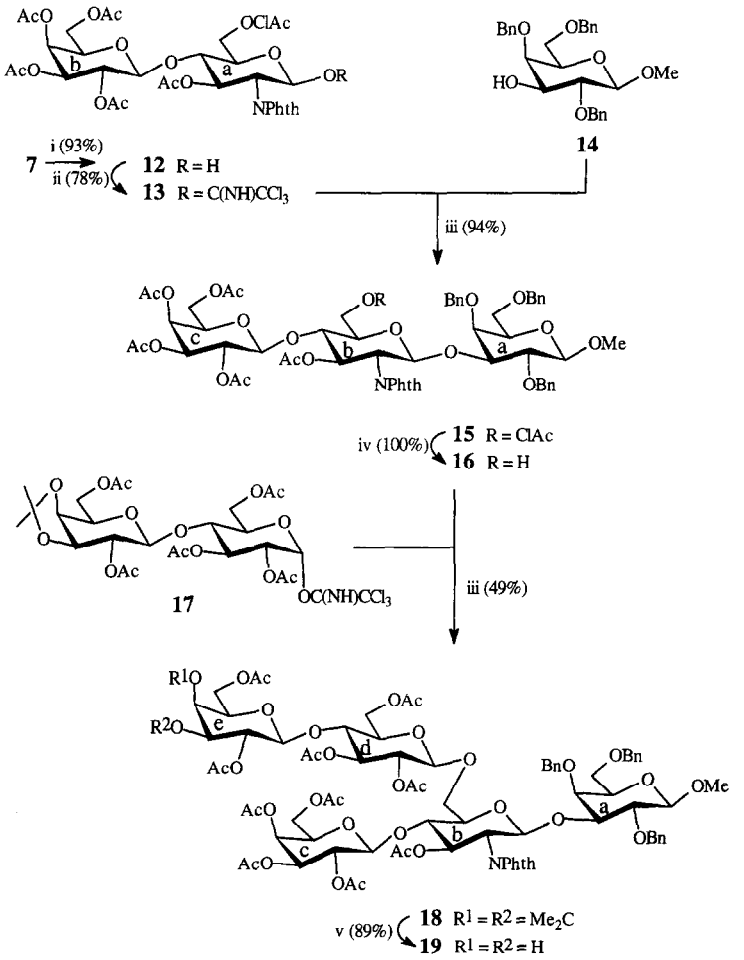
Scheme 1. Reagents and conditions: (i) $NaCNBH_3$ in THF, HCl, 4 h; (ii) $Hg(CN)_2$ in $C_6H_5CH_3$–CH_3NO_2, 16 h; (iii) pyridine–Ac_2O; (iv) H_2–Pd/C in MeOH, overnight; (v) $(ClCH_2CO)_2O$–2,6-lutidine in CH_2Cl_2, overnight; (vi) CF_3CO_2H in CH_2Cl_2; (vii) Cl_3CCN–DBU in CH_2Cl_2, 0 °C, 2 h.

malian sialytransferases [6,7,12,13]. Therefore, the overall strategy for the preparation of decasaccharides **22**, **23**, and **24** is based on the chemical synthesis of core octasaccharide methyl glycoside **21**, followed by enzymatic sialylation at both 3-OH groups of its terminal galactose residues.

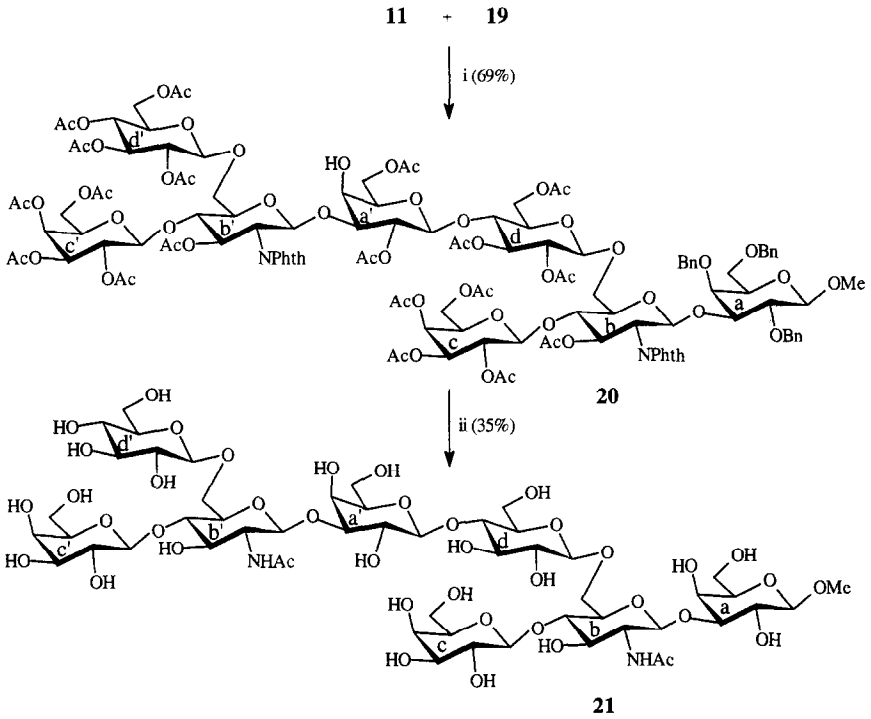
Our approach to the synthesis of **21** was guided by the methods previously developed for the synthesis of two related oligosaccharides [10,11]. A block procedure [3 + 2 + 3] was applied. Trisaccharide acceptor **16** was coupled with disaccharide donor **17** to afford pentasaccharide **18**. This was subsequently converted to pentasaccharide glycosyl acceptor **19**, which, when condensed with trisaccharide glycosyl donor **11**, afforded **21**.

Synthesis of trisaccharide glycosyl donor **11** is shown in Scheme 1. Reductive ring-opening of 4,6-di-*O*-benzylidene by treatment of compound **1** [17] with $NaBH_3CN$ and HCl [18] gave 2-(trimethylsilyl)ethyl 6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**2**) in 78% yield. Selective glycosylation at the 4-*O*-position of **2** with tetra-*O*-acetyl-D-galactopyranosyl bromide (**3**) could be achieved giving **4** by using either silver triflate (63%) or $HgCN_2$ (72%) as promoter. Compound **4** was converted to **5** by acetylation (Ac_2O–Py) in 98% yield, and to compound **6** from **5** by catalytic hydrogenation (10% Pd/C) in 63% yield.

Condensation of **6** with tetra-*O*-acetyl-D-glucopyranosyl bromide (**8**) was performed at 60 °C in 1:1 toluene–CH_3NO_2 using $HgCN_2$ as promoter, giving **9** in 80% yield. Attempts to use silver triflate–2,4,6-collidine as promoters led to the formation of a significant amount of orthoester product. In the ^1H NMR spectrum of **9**, three anomeric proton resonances appeared at δ_H 4.516 (H-1^c, $J_{1,2}$ 7.5 Hz); 4.695 (H-1^b, $J_{1,2}$ 7.4 Hz); 5.294 (H-1^a, $J_{1,2}$ 7.9 Hz). The 2-(trimethylsilyl)ethyl group of **9** was removed by its treatment with boron trifluoride etherate [17,19] in dichloromethane to furnish compound **10** (δ_H 5.491 ppm, H-1^a, $J_{1,2}$ 7.8 Hz) and its α anomer in 75% yield in a ratio of about 10:1 according to ^1H NMR integration. Trichloroacetimidate **11** was then obtained in 79% yield by the reaction of **10** with trichloroacetonitrile in the presence of DBU [20].



Scheme 2. Reagents and conditions: (i) CF_3CO_2H in CH_2Cl_2, 3 h; (ii) Cl_3CCN–K_2CO_3 in CH_2Cl_2, 4 h; (iii) $CF_3SO_3SiMe_3$ in CH_2Cl_2, −45 °C, 2 h; (iv) thiourea–2,6-lutidine in CH_2Cl_2–MeOH; (v) 1:10 90%CF_3CO_2H–CH_2Cl_2, 0 °C, 2 h.



Scheme 3. Reagents and conditions: (i) $CF_3SO_3SiMe_3$ in CH_2Cl_2, −45 °C, 2 h; (ii) $NH_2NH_2 \cdot H_2O$ in 95% EtOH, reflux, 16 h; then Ac_2O in MeOH–H_2O, overnight.

Synthesis of two glycosyl acceptors **16** and **19** is illustrated in Scheme 2. Chloroacetylation of compound **6** at its 6^b-*O*-position with chloroacetic anhydride–2,6-lutidine in dichloromethane gave compound **7**, which in turn was treated with trifluoroacetic acid [17] to remove the 2-(trimethylsilyl)ethyl group and thus to give the reducing disaccharide **12** in 93% yield. Reaction of **12** with trichloroacetonitrile in dichloromethane using anhydrous K_2CO_3 as base, gave trichloroacetimidate **13** in 78% yield. Coupling of **13** and **14** [21] in the presence of $CF_3SO_3SiMe_3$ [22] furnished trisaccharide derivative **15** in 94% yield. The 6^b-*O*-ClAc group of **15** was quantitatively removed using thiourea–2,6-lutidine [23] to yield **16** as glycosyl acceptor. Coupling of **16** and **17** [11] was performed under the same conditions as above to give pentasaccharide **18** in 49% yield. The NMR and MS data were in accordance with the assigned structure of **18**. The 3^e,4^e-di-*O*-isopropylidene group of **18** was then removed with 1:10 90% CF_3CO_2H–CH_2Cl_2 to give diol **19** in 89% yield.

Condensation of trisaccharide donor **11** and pentasaccharide acceptor **19** (Scheme 3) afforded protected octasaccharide **20** in 69% yield. $CF_3SO_3SiMe_3$ was used again as promoter. The ^1H NMR spectrum of this compound was complicated; however, its ^{13}C NMR spectrum showed eight anomeric carbon resonances at δ_C 98.11, 98.33, 100.51, 100.63 (2), 101.12, 101.48, 104.96 ppm, and FABMS also showed its molecular-ion peak at *m/z* 2662.25 [M + Li] as expected. As previously reported [11], we observed

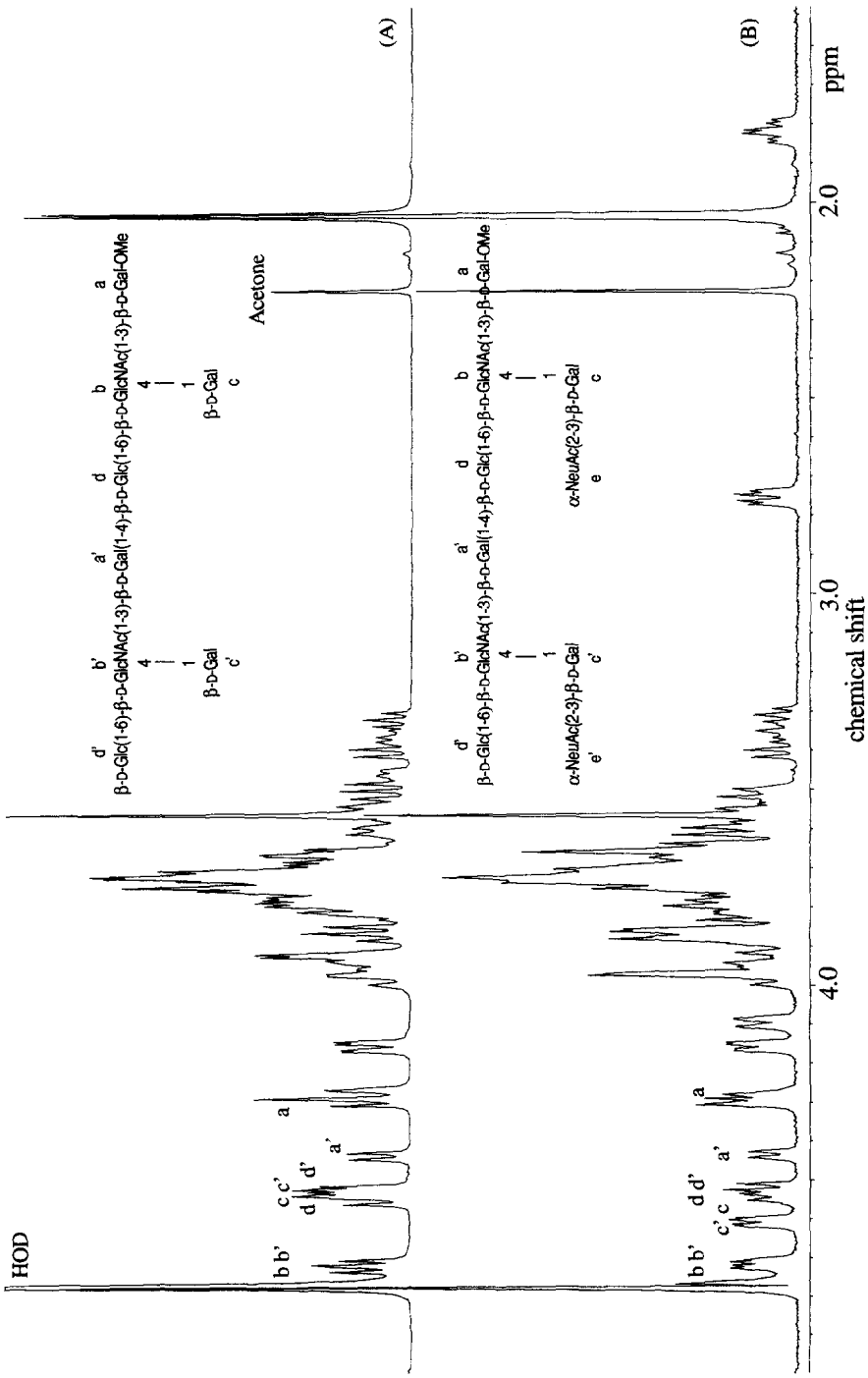


Fig. 1. 500 MHz ^{1}H NMR spectra of compounds **21** (A) and **22** (B) recorded in D_2O at 298 K.

Table 1
^{1}H NMR data for compounds **21**, **22** (**23**), and **24** [a]

H-atom	J (Hz)	chemical shifts [b]		
		21	**22** (**23**)	**24**
Gal-a(Gal-a′)				
1		4.297(4.437)	4.297(4.434)	4.297(4.434)
	$J_{1,2}$	7.8(7.8)	8.4(7.8)	8.4(7.8)
2		3.55(3.59)	3.55(3.60)	3.55(3.60)
33		3.71(3.73)	3.72(3.73)	3.72(3.73)
4		4.14(4.16)	4.14(4.16)	4.15(4.16)
5		3.68(3.71)	3.68(3.71)	3.68(3.71)
6		3.74(3.75)	3.70–3.80	3.70–3.80
6′		3.79(3.80)	3.70–3.80	3.70–3.80
GlcNAc-b(GlcNAc-b′)				
1		4.729(4.715)	4.719(4.713)	4.720(4.712)
	$J_{1,2}$	7.8(7.8)	7.8(7.8)	7.8(7.8)
2		3.80(3.81)	3.79(3.82)	3.79(3.82)
3		3.72(3.72)	3.72(3.74)	3.72(3.74)
4		3.86(3.87)	3.87(3.88)	3.87(3.88)
5		3.72(3.73)	3.73(3.73)	3.73(3.73)
6		4.28(4.28)	4.29(4.29)	4.29(4.29)
6′		3.96(3.96)	3.96(3.96)	3.96(3.96)
Gal-c(Gal-c′)				
1		4.530(4.530)	4.603(4.610)	4.603(4.611)
	$J_{1,2}$	8.4(8.4)	7.8(7.8)	7.8(7.8)
2		3.54(3.54)	3.57(3.57)	3.57(3.57)
3		3.67(3.67)	4.09(4.09)	4.09(4.09)
4		3.92(3.92)	3.97(3.97)	3.97(3.97)
5		3.70(3.70)	3.70(3.70)	3.70(3.70)
6		3.75(3.75)	3.70–3.80	3.70–3.80
6′		3.76(3.76)	3.70–3.80	3.70–3.80
Glc-d(Glc-d′)				
1		4.552(4.522)	4.542(4.517)	4.542(4.516)
	$J_{1,2}$	7.8(7.8)	7.8(7.8)	7.8(7.8)
2		3.36(3.32)	3.35(3.31)	3.35(3.31)
3		3.66(3.50)	3.65(3.52)	3.66(3.52)
4		3.66(3.40)	3.66(3.40)	3.65(3.40)
5		3.61(3.46)	3.60(3.51)	3.60(3.50)
6		3.80(3.72)	3.81(3.74)	3.81(3.74)
6′		3.98(3.92)	3.99(3.93)	3.99(3.92)
Neu-e(Neu-e′)				
3a			1.81(1.82)	1.81(1.82)
3e			2.76(2.76)	2.76(2.76)
4			3.70(3.70)	3.70(3.70)
5			3.85(3.85)	3.85(3.85)
6			3.65(3.65)	3.66(3.66)
7			3.61(3.61)	3.58(3.58)
8			3.88(3.88)	3.88(3.88)
9			3.87(3.87)	3.87(3.87)
9′			3.65(3.65)	3.65(3.65)

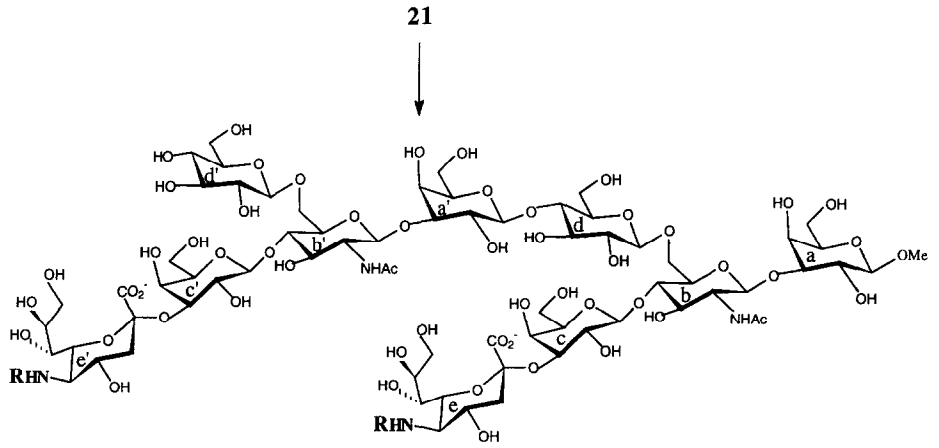
Table 1 (continued)

H-atom	J (Hz)	chemical shifts [b]		
		21	**22 (23)**	**24**
OMe		3.57	3.55	3.56
CH_3CON		2.03, 2.04	2.026, 2.033(3)	2.03, 2.04
CH_3CH_2CON				1.114(2), 2.298(2)

[a] At 600 MHz, in D_2O (pH 7.00) at 298 K.
[b] First-order data.

again the upfield shift of one *O*-Ac resonance at δ_H 1.389 ppm. The transformation of **20** into **21** was achieved by a three-step approach: (1) removal of *O*-acetyl and phthalimido groups by refluxing with hydrazine hydrate in 95% ethanol; (2) *N*-acetylation of obtained amino groups with acetic anhydride in 10:1 methanol–water; (3) removal of *O*-benzyl groups by catalytic hydrogenation over Pd/C in 20% aq acetic acid solution. The overall yield for the three steps was 35%. Compound **21** was purified by a Sephadex G-10 column and characterized by means of ^1H NMR (see Fig. 1 and Table 1), ^{13}C NMR (see Table 2) and FABMS analysis.

Enzymatic sialylation of **21** at 3^e-*O*-positions of both terminal galactose residues was achieved (Scheme 4) using recombinant α-(2 → 3)-sialyltransferase and CMP-NeuAc [13,24], to afford decasaccharide methyl glycoside **22** in 80% yield. Since neither ^{13}C-labeled nor *N*-propionyl substituted sialic acid-CMP derivative are commercially



22 **R** = CH_3CO
23 **R** = CH_3CO sialic acid with 8% ^{13}C-labeling
24 **R** = CH_3CH_2CO

Scheme 4. Reagents and conditions: for **22**, CMP-NeuAc, α-(2 → 3)-sialyltransferase, 80%; for **23**, [^{13}C]-NeuAc, CTP, sialic acid-CMP synthetase, α-(2 → 3)-sialyltransferase, 71%; for **24**, NeuPr, CTP, sialic acid-CMP synthetase, α-(2 → 3)-sialyltransferase, 75%.

Table 2
^{13}C NMR chemical shifts for compounds **21**, **22**, and **24** [a]

C-atom	**21**	**22**	**24**
Gal-a(Gal-a′)			
1	104.74(103.80)	104.81(103.89)	104.81(103.90)
2	70.60(70.83)	70.67(70.95)	70.50(70.68)
3	82.95(83.17)	82.97(83.24)	82.96(83.24)
4	69.15(69.09)	69.26(69.21)	69.21(69.15)
5	75.56(75.77)	75.64(75.84)	75.65(75.85)
6	61.79(61.84)	61.87(61.92)	61.88(61.94)
GlcNAc-b(GlcNAc-b′)			
1	103.66(103.60)	103.80(103.73)	103.80(103.73)
2	56.09(56.02)	56.13(56.06)	56.14(56.07)
3	72.94(72.94)	72.98(72.98)	72.98(72.98)
4	78.58(78.58)	78.24(78.24)	78.24(78.24)
5	74.20(74.14)	74.33(74.25)	74.34(74.26)
6	68.41(68.41)	68.57(68.48)	68.57(68.48)
Gal-c(Gal-c′)			
1	103.60(103.60)	103.12(103.12)	103.13(103.13)
2	71.77(71.77)	70.30(70.30)	70.31(70.31)
3	73.34(73.34)	76.60(76.60)	76.61(76.61)
4	69.43(69.43)	68.57(68.57)	68.57(68.57)
5	76.11(76.11)	75.94(75.94)	75.95(75.95)
6	61.90(61.90)	61.99(61.99)	61.99(61.99)
Glc-d(Glc-d′)			
1	103.42(103.55)	103.56(103.68)	103.55(103.68)
2	73.50(73.84)	73.60(73.94)	73.61(73.95)
3	75.11(76.51)	75.21(76.60)	75.21(76.61)
4	79.30(70.53)	79.23(70.58)	79.24(70.59)
5	75.56(76.72)	75.59(76.74)	75.60(76.75)
6	60.94(61.60)	60.98(61.67)	60.99(61.67)
Neu-e(Neu-e′)			
1		174.75(174.75)	174.77(174.77)
2		100.95(101.05)	100.96(101.06)
3		40.50(40.56)	40.58(40.64)
4		69.27(69.27)	69.21(69.21)
5		52.61(52.61)	52.48(52.48)
6		73.88(73.88)	73.91(73.91)
7		68.95(68.98)	68.96(68.99)
8		72.70(72.70)	72.71(72.71)
9		63.53(63.56)	63.52(63.52)
OMe	58.03	58.11	58.11
CH_3CON	23.02(2)	22.96(2), 23.10(2)	23.10(2)
CH_3CON	175.78, 175.74	175.81, 175.85, 175.93(2)	175.86(2)
CH_3CH_2CON			10.41, 30.15
CH_3CH_2CON			179.94(2)

[a] At 125 MHz, in D_2O (pH 7.00) at 298 K.

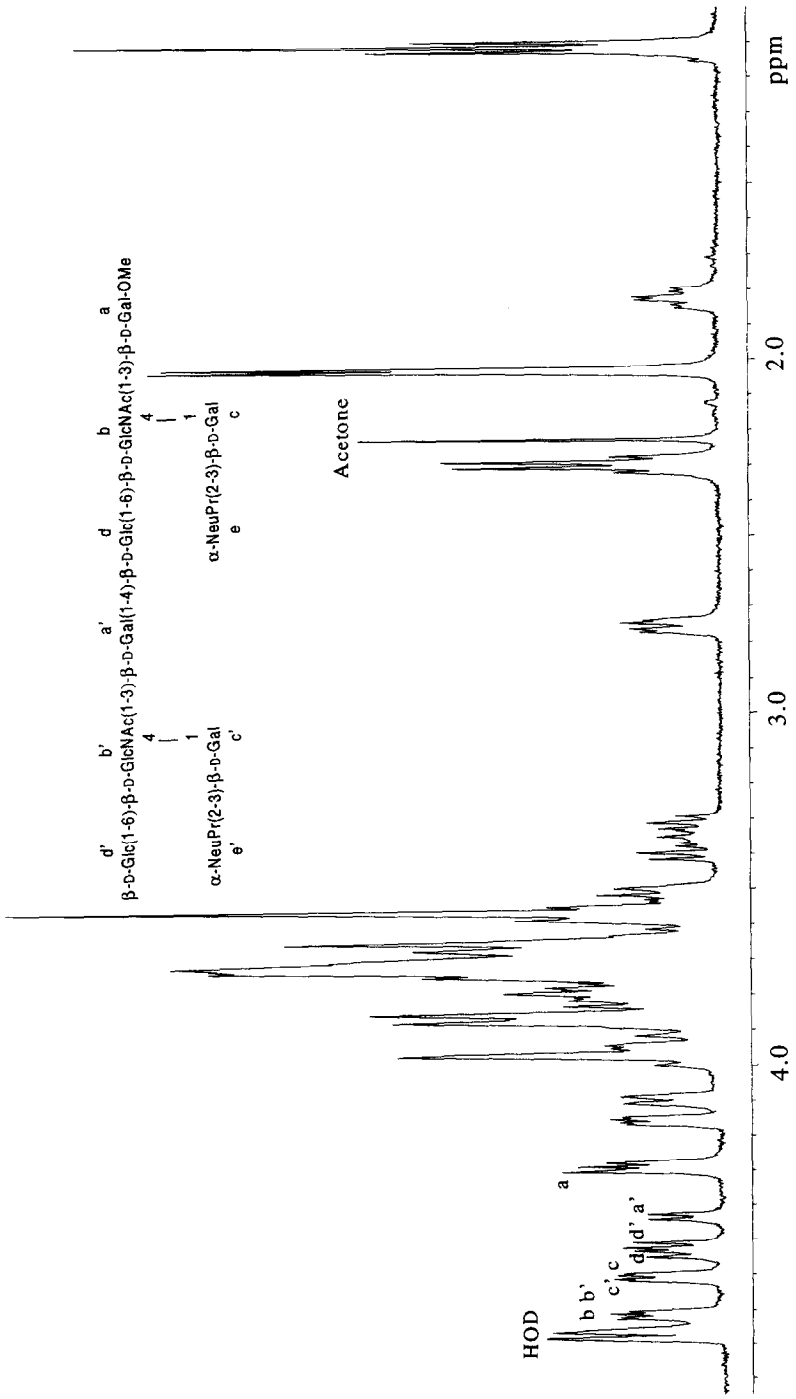


Fig. 2. 500 MHz ^{1}H NMR spectrum of compound **24** recorded in D_2O at 298 K.

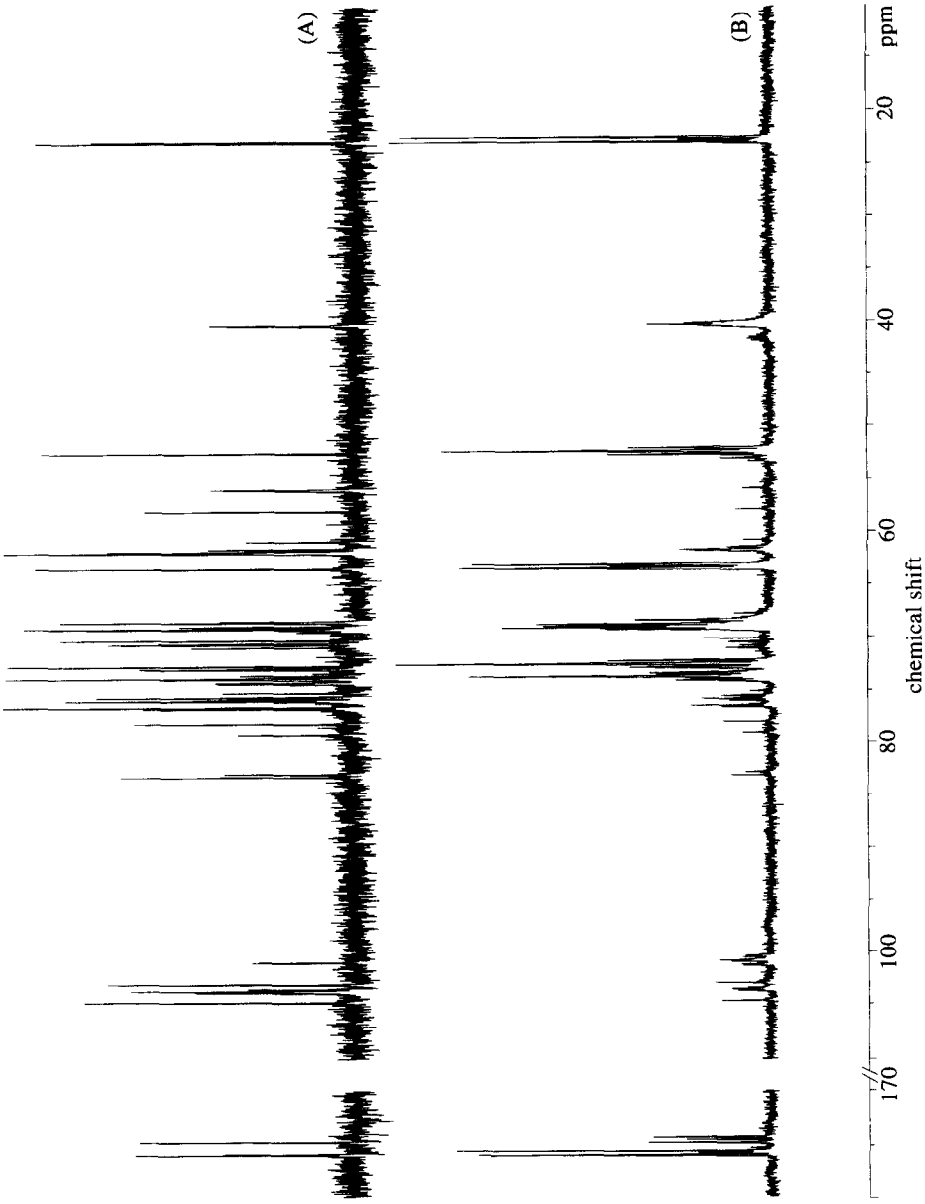


Fig. 3. 125 MHz ^{13}C NMR spectra of compounds **22** (A) and **23** (B) recorded in D_2O at 298 K.

available, a recombinant sialic acid-CMP synthetase was used in combination with α-(2 → 3)-sialyltransferase according to Paulson and Wong's procedures [24] for the preparation of decasaccharides **23** (71%) and **24** (75%). The 8% ^{13}C-labeled sialic acid was prepared by hydrolysis of *E. coli* K1 polysialic acid capsular polysaccharide which was previously labeled by adding ^{13}C-labeled glucose to the growth media of the bacteria [25]. The ^{13}C content of sialic acid was determined by measuring the relative intensity of the isotopic side bands by ^{1}H NMR spectroscopy. The *N*-propionylated sialic acid was obtained by hydrolysis of the *N*-propionylated group B meningococcal polysaccharide [26].

^{1}H NMR spectra of **21**, **22**, and **24** are shown in Figs. 1 and 2, respectively, and the assignment of these spectra was achieved by a combination of ^{1}H–^{1}H COSY, RELAY–COSY, TOCSY, ^{13}C–^{1}H heteronuclear correlation, and two-dimensional NOESY and ROESY spectroscopy. Their chemical shift data is summarized in Table 1. A difference in chemical shift caused by sialylation was observed on H-1 of galacto-pyranosyl residues attached by sialic acid. Both H-1^{c} and H-1$^{c'}$ resonances (δ_H 4.603 and 4.610 ppm) were shifted downfield by about 0.07–0.08 ppm. The ^{13}C NMR data of **21**, **22**, and **24** are presented in Table 2, and the ^{13}C NMR spectra of **22** and ^{13}C labeled **23** are shown in Fig. 3 for comparison. The multiplet in the spectrum of **23** was attributed to the carbon–carbon coupling.

3. Experimental

General methods.—Optical rotations were measured at room temperature with a Perkin–Elmer 243 polarimeter, using a 10 cm, 1 mL cell. ^{1}H and ^{13}C NMR spectra were recorded at 500 and 125 MHz, respectively, with a Bruker AMX 500 instrument at 300 K unless otherwise noted. Chemical shifts (δ) are given relative to the signal for internal Me_4Si or indirectly to solvent signal 7.25 ($CDCl_3$), 2.225 (acetone in D_2O) for ^{1}H NMR spectra, and to the solvent signals 76.9 ($CDCl_3$), 31.07 (internal acetone) for ^{13}C NMR spectra. The ^{1}H NMR resonances of oligosaccharides were assigned on the basis of 2D ^{1}H-homonuclear chemical-shift correlated (^{1}H–COSY) experiments. FAB mass spectroscopic analyses were performed with a JEOL JMS-AX505H mass spectrometer.

Column chromatography was performed on Silica Gel 60 (E. Merck, 230–400 mesh) and fractions were monitored by TLC on Silica Gel 60 F_{254} (E. Merck) unless otherwise noted. Detection was effected by examination under UV light and by charring with 5% sulfuric acid solution in ethanol. Solutions were concentrated at or below 40 °C and dried with anhydrous Na_2SO_4.

*2-(Trimethylsilyl)ethyl 6-O-benzyl-2-deoxy-2-phthalimido-β-*D*-glucopyranoside* (**2**). —To a suspension of compound **1** (5.0 g, 10.0 mmol), sodium cyanoborohydride (5.0 g, 79.6 mmol), and 3 Å molecular sieves (3.0 g) in dry THF at 0 °C, a saturated solution of HCl in ethyl ether was added dropwise until the evolution of gas ceased and the pH of the solution was kept at pH 3–4 for 2 h. Ethyl acetate (200 mL) was added, and the mixture was filtered through Celite. The filtrate was washed with aq $NaHCO_3$ and water, dried and concentrated. Purification by column chromatography (2:1 EtOAc–hexane) gave amorphous **2** (3.9 g, 78%): $[\alpha]_D$ − 14.1° (*c* 0.39, MeOH); ^{1}H NMR

($CDCl_3$) δ − 0.164 (s, 9 H, $SiMe_3$), 0.740 (m, 2 H, CH_2Si), 2.385 (bs, 1 H, 4-OH), 3.084 (bs, 1 H, 3-OH), 3.463 (m, 1 H, one of CH_2CH_2Si), 3.621 (m, 2 H, H-4, H-5), 3.776 (m, 1 H, H-6), 3.821 (m, 1 H, H-6′), 3.893 (m, 1 H, one of CH_2CH_2Si), 4.117 (dd, 1 H, H-2, $J_{2,3}$ 10.7 Hz), 4.296 (m, 1 H, H-3), 4.574 and 4.630 (2d, 1 H each, CH_2Ph, J 11.9 Hz), 5.217 (d, 1 H, H-1, $J_{1,2}$ 8.4 Hz), 7.289–7.345 (m, 5 H, Ph), 7.688–7.830 (m, 4 H, phth).

*2-(Trimethylsilyl)ethyl 2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl-(1 → 4)-6-*O*-benzyl-2-deoxy-2-phthalimido-β-*D*-glucopyranoside* (**4**).—A mixture of **2** (2.6 g, 5.2 mmol), galactosyl bromide **3** (3.0 g, 7.3 mmol), and $Hg(CN)_2$ (4.0 g, 15.8 mmol) in 1:1 toluene–CH_3NO_2 (40 mL) was stirred at room temperature for 12 h. The mixture was diluted with CH_2Cl_2 (100 mL), and was washed subsequently with 10% aq KI, water, aq $NaHCO_3$, and water, and the organic layer was dried and concentrated. Fractionation by column chromatography (3:2 EtOAc–hexane) gave starting material **2** (0.4 g, 15%) and amorphous **4** (3.1 g, 72%): $[\alpha]_D$ + 8.6° (*c* 1.6, CH_2Cl_2); ^1H NMR ($CDCl_3$) δ − 0.156 (s, 9 H, $SiMe_3$), 0.764 (m, 2 H, CH_2Si), 1.883, 1.944, 1.978, 2.087 (4s, 3 H each, 4 × OAc), 4.134 (dd, 1 H, H-2^a, $J_{2,3}$ 10.5 Hz), 4.359 (dd, 1 H, H-3^a, $J_{3,4}$ 9.7 Hz), 4.470 (d, 1 H, H-1^b, $J_{1,2}$ 8.0 Hz), 4.517 and 4.721 (2d, 1 H each, CH_2Ph, J 12.0 Hz), 4.910 (dd, 1 H, H-3^b, $J_{2,3}$ 10.4 Hz, $J_{3,4}$ 3.3 Hz), 5.161 (dd, 1 H, H-2^b), 5.191 (d, 1 H, H-1^a, $J_{1,2}$ 8.6 Hz), 5.298 (d, 1 H, H-4^b), 7.290–7.355 (m, 5 H, Ph), 7.672–7.813 (m, 4 H, phth); ^{13}C NMR ($CDCl_3$) δ − 1.48 ($SiMe_3$), 17.77 (CH_2Si), 20.32, 20.50, 20.60, 20.70 (4 × CH_3C=O), 56.09 (C-2^a), 97.78 (C-1^a), 101.54 (C-1^b), 169.16, 169.84, 170.15, 170.46 (4 × CH_3C=O).

*2-(Trimethylsilyl)ethyl 2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl-(1 → 4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-*D*-glucopyranoside* (**5**).—A solution of **4** (3.0 g, 3.6 mmol) in 2:1 pyridine–Ac_2O (45 mL) was stirred overnight. The solution was poured into cold water with stirring. After 10 min the precipitates were collected by filtration, thoroughly washed with water, and dried to give **5** as a solid (3.1 g, 98%): $[\alpha]_D$ + 18.8° (*c* 0.81, MeOH); ^1H NMR ($CDCl_3$) δ − 0.166 (s, 9 H, $SiMe_3$), 0.753 (m, 2 H, CH_2Si), 1.840, 1.923, 1.939, 2.026, 2.074 (5s, 3 H each, 5 × OAc), 4.630 (d, 1 H, H-1^b, $J_{1,2}$ 8.0 Hz), 4.958 (dd, 1 H, H-3^b, $J_{2,3}$ 9.8 Hz), 4.988 (dd, 1 H, H-2^b), 5.235 (bs, 1 H, H-4^b), 5.302 (d, 1 H, H-1^a, $J_{1,2}$ 8.5 Hz), 5.648 (t, 1 H, H-3^a, $J_{2,3}$ 9.5 Hz, $J_{3,4}$ 9.5 Hz), 7.131–7.360 (m, 5 H, Ph), 7.684–7.804 (m, 4 H, phth).

*2-(Trimethylsilyl)ethyl 2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl-(1 → 4)-3-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranoside* (**6**).—A mixture of **5** (3.0 g, 3.4 mmol) and 10% Pd/C (50% wet, 0.5 g) in 19:1 MeOH–AcOH (20 mL) was subjected to hydrogen pressure (30 psi) for 16 h. The filtrate was concentrated and the residue was purified by column chromatography (1:1 EtOAc–hexane) to give amorphous **6** (1.7 g, 63%): $[\alpha]_D$ + 12.6° (*c* 0.39, MeOH); ^1H NMR ($CDCl_3$) δ − 0.190 (s, 9 H, $SiMe_3$), 0.720 (m, 2 H, CH_2Si), 1.851, 1.918, 1.985, 2.036, 2.084 (5s, 3 H each, 5 × OAc), 4.110 (dd, 1 H, H-2^a, $J_{2,3}$ 9.0 Hz), 4.630 (d, 1 H, H-1^b, $J_{1,2}$ 7.5 Hz), 4.958 (dd, 1 H, H-3^b, $J_{2,3}$ 9.8 Hz), 5.081 (dd, 1 H, H-2^b), 5.289 (bs, 1 H, H-4^b), 5.361 (d, 1 H, H-1^a, $J_{1,2}$ 8.5 Hz), 5.688 (dd, 1 H, H-3^a, $J_{3,4}$ 9.5 Hz), 7.684–7.798 (m, 4 H, phth); HRFABMS: Calcd for $C_{35}H_{47}LiNO_{17}Si$ (M + Li): 788.2773. Found: 788.2759.

*2-(Trimethylsilyl)ethyl 2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl-(1 → 4)-3-*O*-acetyl-6-*O*-chloroacetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranoside* (**7**).—To a solu-

tion of **6** (1.1 g, 1.3 mmol) and 2,6-lutidine (3 mL) in CH_2Cl_2 (20 mL) chloroacetic anhydride (1.0 g, 5.8 mmol) was added. The mixture was stirred overnight and diluted with EtOAc (100 mL). The organic solution was subsequently washed with water, N HCl, and water, dried and concentrated. Purification by column chromatography (1:1 EtOAc–hexane) gave **7** as needles (1.1 g, 91%); mp 130–131 °C (EtOAc–hexane); $[\alpha]_D$ +5.3° (*c* 0.38, MeOH); ^{1}H NMR ($CDCl_3$) δ − 0.153 (s, 9 H, $SiMe_3$), 0.755 (m, 2 H, CH_2Si), 1.886, 1.948, 2.030, 2.054, 2.117 (5s, 3 H each, 5 × OAc), 4.572 (d, 1 H, H-1^b, $J_{1,2}$ 7.8 Hz), 4.953 (dd, 1 H, H-3^b, $J_{2,3}$ 9.8 Hz), 5.095 (dd, 1 H, H-2^b), 5.316 (d, 1 H, H-4^b, $J_{3,4}$ 3.4 Hz), 5.301 (d, 1 H, H-1^a, $J_{1,2}$ 8.5 Hz), 5.724 (dd, 1 H, H-3^a, $J_{3,4}$ 9.5 Hz), 7.711–7.828 (m, 4 H, phth); ^{13}C NMR ($CDCl_3$) d-1.54 ($SiMe_3$), 17.77 (CH_2Si), 20.62 (5 × CH_3C=O), 40.66 ($ClCH_2C$=O), 55.05 (C-2^a), 97.53 (C-1^a), 100.86 (C-1^b), 166.75 ($ClCH_2C$=O), 169.08, 169.79, 170.03, 170.13, 170.32 (5 × CH_3C=O); Anal. Calcd for $C_{37}H_{48}ClNO_{18}Si$: C 54.0; H 5.9; N 1.7. Found: C 53.7; H 5.8; N 1.8.

*2-(Trimethylsilyl)ethyl 2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl-(1 → 4)-[2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl-(1 → 6)]-3-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranoside* (**9**).—A mixture of **6** (1.5 g, 1.9 mmol), glucosyl bromide **8** (0.8 g, 2.0 mmol), and $Hg(CN)_2$ in 1:1 toluene–CH_3NO_2 (30 mL) was stirred at 60 °C overnight. The mixture was diluted with CH_2Cl_2 (100 mL), and the organic solution was subsequently washed with 10% aq KI, water, aq $NaHCO_3$, and water, dried and concentrated. Purification by column chromatography (1:1 EtOAc–hexane) gave amorphous **9** (1.7 g, 80%): $[\alpha]_D$ +10.4° (*c* 1.3, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ − 0.175 (s, 9 H, $SiMe_3$), 0.734 (m, 2 H, CH_2Si), 1.851, 1.935, 1.982, 2.001, 2.021, 2.033, 2.047, 2.088, 2.105 (9s, 3 H each, 9 × OAc), 4.516 (d, 1 H, H-1^c, $J_{1,2}$ 7.5 Hz), 4.695 (d, 1 H, H-1^b, $J_{1,2}$ 7.4 Hz), 5.294 (d, 1 H, H-1^a, $J_{1,2}$ 7.9 Hz), 5.310 (bs, 1 H, H-4^b), 5.673 (t, 1 H, H-3^a, $J_{2,3}$ 9.4 Hz, $J_{3,4}$ 9.4 Hz), 7.696–7.807 (m, 4 H, phth); ^{13}C NMR ($CDCl_3$) δ − 1.50 ($SiMe_3$), 17.65 (CH_2Si), 20.51–20.74 (9 × CH_3C=O), 54.97 (C-2^a), 97.40, 100.85, 101.10 (3 × C-1), 169.10, 169.17, 169.40, 169.81, 169.87, 170.16 (2), 170.32, 170.59 (9 × CH_3C=O); HRFABMS: Calcd for $C_{49}H_{65}LiNO_{26}Si$ (M + Li): 1118.3724. Found: 1118.3729.

*2,3,4,6-Tetra-*O*-acetyl-β-*D*-galactopyranosyl-(1 → 4)-[2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl-(1 → 6)]-3-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranose* (**10**).—To a solution of **9** (1.4 g, 1.3 mmol) in CH_2Cl_2 (14 mL) was added trifluoroacetic acid (4.0 mL). The mixture was stirred at room temperature for 3 h and concentrated. Purification by column chromatography (2:1 EtOAc–hexane) gave amorphous **10** (1.0 g, 78%): $[\alpha]_D$ +23.9° (*c* 0.79, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.868, 1.937, 2.017, 2.023, 2.027, 2.036, 2.102, 2.113, 2.178 (9s, 3 H each, 9 × OAc), 4.628 (d, 1 H, H-1^c, $J_{1,2}$ 7.0 Hz), 4.648 (d, 1 H, H-1^b, $J_{1,2}$ 7.4 Hz), 5.328 (d, 1 H, H-4^b, $J_{3,4}$ 3.2 Hz), 5.491 (d, 1 H, H-1^a, $J_{1,2}$ 7.8 Hz), 5.727 (t, 1 H, H-3^a, $J_{2,3}$ 9.8 Hz, $J_{3,4}$ 9.8 Hz), 7.686–7.863 (m, 4 H, phth); ^{13}C NMR ($CDCl_3$) δ 20.56–20.92 (9 × CH_3C=O), 56.92 (C-2^a), 92.59 (C-1^a), 100.85, 101.10 (C-1^b and C-1^c), 167.85, 168.89, 169.55, 169.84, 170.07, 170.22, 170.29, 170.37, 171.36 (9 × CH_3C=O).

*2,3,4,6-Tetra-*O*-acetyl-β-*D*-galactopyranosyl-(1 → 4)-[2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl-(1 → 6)]-3-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl trichloroacetimidate* (**11**).—To a solution of **10** (0.89 g, 0.88 mmol) and Cl_3CCN (3 mL) in CH_2Cl_2 (20 mL) was added DBU (0.14 mL) at 0 °C. The mixture was stirred at

0 °C for 2 h and concentrated. Purification by column chromatography (2:1 EtOAc–hexane) gave amorphous **11** (0.80 g, 79%): $[\alpha]_D$ +27.0° (*c* 0.79, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.879, 1.927, 1.977 (2), 2.013, 2.030, 2.057, 2.078, 2.101 (8s, 9 × OAc), 4.447 (dd, 1 H, H-2^a, $J_{2,3}$ 9.5 Hz), 4.578 (d, 1 H, H-1^c, $J_{1,2}$ 7.6 Hz), 4.695 (d, 1 H, H-1^b, $J_{1,2}$ 7.6 Hz), 5.320 (bs, 1 H, H-4^b), 5.804 (t, 1 H, H-3^a, $J_{3,4}$ 9.5 Hz), 6.568 (d, 1 H, H-1^a, $J_{1,2}$ 8.9 Hz), 7.681–7.790 (m, 4 H, phth), 8.664 (s, 1 H, C=NH); ^{13}C NMR ($CDCl_3$) δ 20.54–20.77 (9 × CH_3C=O), 53.81 (C-2^a), 93.32 (C-1^a), 100.81, 101.01 (C-1^b and C-1^c), 160.49 (C=NH), 169.06, 169.40, 169.52, 169.78, 170.02, 170.23 (2), 170.38, 170.62 (9 × CH_3C=O); HRFABMS: Calcd for $C_{46}H_{53}Cl_3LiN_2O_{26}$ (M + Li): 1161.2112. Found: 1161.2104.

2,3,4,6-Tetra-O-*acetyl*-β-D-*galactopyranosyl*-(1 → 4)-3-O-*acetyl*-6-O-*chloroacetyl*-2-*deoxy*-2-*phthalimido*-β-D-*glucopyranose* (**12**).—To a solution of **7** (1.1 g, 1.3 mmol) in CH_2Cl_2 (20 mL) was added CF_3CO_2H (10 mL). The mixture was stirred at room temperature for 2 h until TLC showed that the reaction was complete. EtOAc (50 mL) was added, and the solution was subsequently washed with water, aq $NaHCO_3$, and water, dried and concentrated. Purification by chromatography (2:1 EtOAc–hexane) gave **12** as a glassy solid (0.9 g, 93%): $[\alpha]_D$ +47.9° (*c* 0.9, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.889, 1.941, 2.024, 2.047, 2.111 (5s, 3 H each, 5 × OAc), 3.283 (bs, 1 H, 1^a-OH), 4.583 (d, 1 H, H-1^b, $J_{1,2}$ 8.0 Hz), 4.946 (dd, 1 H, H-3^b, $J_{2,3}$ 10.4 Hz, $J_{3,4}$ 3.4 Hz), 5.097 (dd, 1 H, H-2^b, $J_{1,2}$ 8.0 Hz), 5.316 (d, 1 H, H-4^b), 5.633 (d, 1 H, H-1^a, $J_{1,2}$ 6.5 Hz), 5.766 (dd, 1 H, H-3^a, $J_{2,3}$ 8.5 Hz, $J_{3,4}$ 10.4 Hz), 7.701–7.864 (m, 4 H, phth).

2,3,4,6-Tetra-O-*acetyl*-β-D-*galactopyranosyl*-(1 → 4)-3-O-*acetyl*-6-O-*chloroacetyl*-2-*deoxy*-2-*phthalimido*-β-D-*glucopyranosyl trichloroacetimidate* (**13**).—To a solution of **12** (0.45 g, 0.59 mmol) and Cl_3CCN (2 mL) in CH_2Cl_2 (20 mL) anhydrous K_2CO_3 (0.5 g) was added, and the mixture was stirred at room temperature for 4 h. The filtrate was concentrated, and purification by column chromatography (1:1 EtOAc–hexane) gave amorphous **13** (0.42 g, 78%): $[\alpha]_D$ +31.6° (*c* 1.4, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.913, 1.944, 2.046, 2.050, 2.119 (5s, 3 H each, 5 × OAc), 4.505 (dd, 1 H, H-2^a, $J_{2,3}$ 9.5 Hz), 4.588 (d, 1 H, H-1^b, $J_{1,2}$ 8.0 Hz), 4.952 (dd, 1 H, H-3^b, $J_{2,3}$ 10.3 Hz, $J_{3,4}$ 3.4 Hz), 5.855 (dd, 1 H, H-3^a, $J_{3,4}$ 8.3 Hz), 6.590 (d, 1 H, H-1^a, $J_{1,2}$ 9.0 Hz), 7.689–7.975 (m, 4 H, phth), 8.624 (s, 1 H, C=NH); ^{13}C NMR ($CDCl_3$) δ 20.51–20.63 (5 × CH_3C=O), 40.68 ($ClCH_2$C=O), 53.88 (C-2^a), 60.96, 63.31, 66.72, 69.18, 70.47, 70.85, 71.01, 73.30, 76.05, 93.48 (C-1^a), 100.69 (C-1^b), 123.68, 131.29, 134.41 (phth), 160.51 (C=NH), 166.66 ($ClCH_2$C=O), 167.46 (phth), 169.01, 169.10, 170.01, 170.12, 170.37 (5 × CH_3C=O); HRFABMS: Calcd for $C_{34}H_{36}Cl_4LiN_2O_{18}$ (M + Li): 907.0877. Found: 907.0886.

Methyl 2,3,4,6-tetra-O-*acetyl*-β-D-*galactopyranosyl*-(1 → 4)-3-O-*acetyl*-6-O-*chloroacetyl*-2-*deoxy*-2-*phthalimido*-β-D-*glucopyranosyl*-(1 → 3)-2,4,6-*tri*-O-*benzyl*-β-D-*galactopyranoside* (**15**).—To a solution of **13** (0.39 g, 0.43 mmol) and **14** (0.40, 0.86 mmol) in CH_2Cl_2 (20 mL) powdered 4 Å molecular sieves (1.5 g) were added. The mixture was stirred at room temperature for 1 h. $CF_3SO_3SiMe_3$ triflate (40 μL, 0.21 mmol) was then added at −45 °C, and the mixture was stirred at that temperature for 2 h. The mixture was neutralized by the addition of a solution of 2,6-lutidine (5 mL) in CH_2Cl_2 (50 mL), washed with water, N HCl, and water, dried and concentrated. Purification by column chromatography (1:1 EtOAc–hexane) gave amorphous **15** (0.48

g, 94%): $[\alpha]_D$ 0.0° (*c* 0.94, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.881, 1.957, 2.018, 2.057, 2.126 (5s, 3 H each, 5 × OAc), 3.347 (s, 3 H, OMe), 3.939 (s, 2 H, $ClCH_2C$=O), 4.129 (d, 1 H, H-1^a, $J_{1,2}$ 7.6 Hz), 4.257 (dd, 1 H, H-2^b, $J_{2,3}$ 10.4 Hz), 4.551 (d, 1 H, H-1^c, $J_{1,2}$ 8.0 Hz), 4.952 (dd, 1 H, H-3^c, $J_{3,4}$ 3.4 Hz), 5.111 (dd, 1 H, H-2^c, $J_{2,3}$ 10.4 Hz), 5.321 (d, 1 H, H-4^c), 5.700 (d, 1 H, H-1^b, $J_{1,2}$ 8.4 Hz), 5.780 (dd, 1 H, H-3^b, $J_{3,4}$ 8.8 Hz), 7.065–7.333 (m, 15 H, 3 × Ph), 7.539–7.668 (m, 4 H, phth); ^{13}C NMR ($CDCl_3$) δ 20.53–20.69 (5 × CH_3C=O), 40.49 ($ClCH_2C$=O), 55.44 (C-2^b), 56.91 (OMe), 98.81 (C-1^a), 100.98 (C-1^c), 104.96 (C-1^b), 123.35-134.22, 137.96, 138.76, 138.91 (3 × Ph, phth), 166.68 ($ClCH_2C$=O), 167.60 (phth), 169.03, 169.64, 170.07, 170.15, 170.32 (5 × CH_3C=O); HRFABMS: Calcd for $C_{60}H_{66}ClLiNO_{23}$ (M + Li): 1210.3874. Found: 1210.3877.

Methyl 2,3,4,6-tetra-O-*acetyl*-β-D-*galactopyranosyl*-(*1* → *4*)-*3*-O-*acetyl-2-deoxy-2-phthalimido*-β-D-*glucopyranosyl*-(*1* → *3*)-*2,4,6-tri*-O-*benzyl*-β-D-*galactopyranoside* (**16**). —To a solution of **15** (0.42 g, 0.35 mmol) in 1:1 CH_2Cl_2–MeOH (20 mL) thiourea (0.30 g, 3.95 mmol) and 2,6-lutidine (2 mL) were added. The mixture was stirred at room temperature for 16 h. EtOAc (100 mL) was added, and the solution was subsequently washed with water, N HCl, and water, dried and concentrated to give **16** as a solid (0.40 g, 100%): $[\alpha]_D$ − 6.3° (*c* 0.3, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.870, 1.949, 2.011, 2.038, 2.116 (5s, 3 H each, 5 × OAc), 3.339 (s, 3 H, OMe), 3.987 (t, 1 H, H-4^b, $J_{4,5}$ 9.4 Hz), 4.125 (d, 1 H, H-1^a, $J_{1,2}$ 7.6 Hz), 4.217 (dd, 1 H, H-2^b, $J_{2,3}$ 10.4 Hz), 4.398 and 4.447 (2d, 1 H each, CH_2Ph, *J* 11.8 Hz), 4.491 and 4.211 (2d, 1 H each, CH_2Ph, *J* 11.1 Hz), 4.537 and 4.780 (2d, 1 H each, CH_2Ph, *J* 11.7 Hz), 4.633 (d, 1 H, H-1^c, $J_{1,2}$ 7.9 Hz), 4.974 (dd, 1 H, H-3^c, $J_{3,4}$ 3.4 Hz), 5.099 (dd, 1 H, H-2^c, $J_{2,3}$ 10.3 Hz), 5.311 (d, 1 H, H-4^c), 5.726 (d, 1 H, H-1^b, $J_{1,2}$ 8.4 Hz), 5.770 (dd, 1 H, H-3^b, $J_{3,4}$ 9.4 Hz), 7.135–7.304 (m, 15 H, 3 × Ph), 7.564–7.678 (m, 4 H, phth).

Methyl 2,6-di-O-*acetyl-3,4-di*-O-*isopropylidene*-β-D-*galactopyranosyl*-(*1* → *4*)-*2,3,6-tri*-O-*acetyl*-β-D-*glucopyranosyl*-(*1* → *6*)-[*2,3,4,6-tetra*-O-*acetyl*-β-D-*galactopyranosyl*-(*1* → *4*)]-*3*-O-*acetyl-2-deoxy-2-phthalimido*-β-D-*glucopyranosyl*-(*1* → *3*)-*2,4,6-tri*-O-*benzyl*-β-D-*galactopyranoside* (**18**).—To a solution of **16** (0.38 g, 0.34 mmol) and **17** (0.40, 0.54 mmol) in CH_2Cl_2 (15 mL) powdered 4 Å molecular sieves (1.1 g) were added. The mixture was stirred at room temperature for 1 h. $CF_3SO_3SiMe_3$ (50 μL, 0.26 mmol) was then added at −45 °C, and the mixture was stirred at that temperature for 2 h. The mixture was neutralized by the addition of a solution of 2,6-lutidine (5 mL) in CH_2Cl_2 (50 mL), washed with water, N HCl, and water, dried and concentrated. Purification by column chromatography (2:1 EtOAc–hexane) gave amorphous **18** (0.28 g, 49%): $[\alpha]_D$ − 1.0° (*c* 0.8, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.288, 1.487 (2s, 3 H each, CMe_2), 1.852, 1.949, 1.987 (2), 2.011, 2.016, 2.036, 2.095, 2.102, 2.121 (9s, 10 × OAc), 3.348 (s, 3 H, OMe), 4.151 (d, 1 H, H-1^a, $J_{1,2}$ 8.8 Hz), 4.495 (d, 1 H, H-1^c, $J_{1,2}$ 8.2 Hz), 4.578 (d, 1 H, H-1^d, $J_{1,2}$ 8.0 Hz), 4.908 (dd, 1 H, H-2^d, $J_{2,3}$ 9.2 Hz), 4.992 (dd, 1 H, H-3^c, $J_{3,4}$ 3.2 Hz), 5.080 (dd, 1 H, H-2^c, $J_{2,3}$ 10.4 Hz), 5.199 (t, 1 H, H-3^d, $J_{3,4}$ 9.2 Hz), 5.317 (d, 1 H, H-4^c), 5.633 (d, 1 H, H-1^b, $J_{1,2}$ 8.3 Hz), 5.774 (t, 1 H, H-3^b, $J_{2,3}$ 9.3 Hz, $J_{3,4}$ 9.3 Hz), 7.028–7.285 (m, 15 H, 3 × Ph), 7.357–7.720 (m, 4 H, phth); ^{13}C NMR ($CDCl_3$) δ 20.55–20.85 (10 × CH_3C=O), 26.14, 27.33 (CMe_2), 55.54 (C-2^b), 56.86 (OMe), 60.83, 62.26, 63.11, 66.75, 67.94, 68.26, 69.29, 70.67, 70.76, 70.80, 70.89, 71.99, 72.53, 72.79, 72.86, 72.95, 73.07, 73.41, 73.80, 74.77, 74.83, 76.08, 77.01, 78.92,

79.87, 98.39 (C-1^a), 100.64, 100.83, 101.20 (C-1^c, C-1^d, and C-1^e), 104.96 (C-1^b), 110.84 ($OCMe_2$), 123.42–134.15, 138.08, 138.85, 138.93 (3 × Ph, phth), 167.50 (phth), 169.00, 169.17, 169.45, 169.68, 169.92, 170.06, 170.19, 170.32, 170.37, 171.36 (10 × CH_3C=O); FABMS: Calcd for $C_{83}H_{99}LiNO_{37}$ (M + Li): 1708.61. Found: 1708.34.

*Methyl 2,6-di-*O*-acetyl-β-*D*-galactopyranosyl-(1 → 4)-2,3,6-tri-*O*-acetyl-β-*D*-glucopyranosyl-(1 → 6)-[2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl-(1 → 4)]-3-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl-(1 → 3)-2,4,6-tri-*O*-benzyl-β-*D*-galactopyranoside* (**19**).—To a solution of **18** (0.26 g, 0.15 mmol) in CH_2Cl_2 (50 mL) was added 90% CF_3CO_2H (5 mL) at 0 °C. The mixture was stirred at 0 °C for 2 h, washed subsequently with cold water, aq $NaHCO_3$, and water, dried, and concentrated to give amorphous **19** (0.225 g, 89%): $[\alpha]_D$ −6.2° (*c* 0.39, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.856, 1.953, 1.993, 2.015, 2.021 (2), 2.041, 2.087, 2.118, 2.125 (9s, 10 × OAc), 2.634 (d, 1 H, 4^e-OH, *J* 4.0 Hz), 3.134 (d, 1 H, 3^e-OH, *J* 6.9 Hz), 3.350 (s, 3H, OMe), 4.498 (d, 1 H, H-1^c, $J_{1,2}$ 8.2 Hz), 4.610 (d, 1 H, H-1^d, $J_{1,2}$ 7.8 Hz), 4.913 (dd, 1 H, H-2^d, $J_{2,3}$ 9.0 Hz), 4.993 (dd, 1 H, H-3^c, $J_{3,4}$ 3.2 Hz), 5.087 (dd, 1 H, H-2^c, $J_{2,3}$ 10.4 Hz), 5.213 (t, 1 H, H-3^d, $J_{3,4}$ 9.2 Hz), 5.318 (d, 1 H, H-4^c), 5.636 (d, 1 H, H-1^b, $J_{1,2}$ 8.3 Hz), 5.779 (dd, 1 H, H-3^b, $J_{2,3}$ 10.4 Hz, $J_{3,4}$ 8.9 Hz), 7.126–7.302 (m, 15 H, 3 × Ph), 7.447–7.564 (m, 4 H, phth).

*Methyl 2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl-(1 → 6)-[2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl-(1 → 4)]-3-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl-(1 → 3)-2,6-di-*O*-acetyl-β-*D*-galactopyranosyl-(1 → 4)-2,3,6-tri-*O*-acetyl-β-*D*-glucopyranosyl-(1 → 6)-[2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl-(1 → 4)]-3-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl-(1 → 3)-2,4,6-tri-*O*-benzyl-β-*D*-galactopyranoside* (**20**).—To a solution of **11** (0.20 g, 0.12 mmol) and **19** (0.20, 0.17 mmol) in CH_2Cl_2 (15 mL) were added powdered 4 Å molecular sieves (0.9 g). The mixture was stirred at room temperature for 1 h. $CF_3SO_3SiMe_3$ (33 μL, 0.17 mmol) was then added at −45 °C, and the mixture was stirred at that temperature for 2 h. The mixture was neutralized by the addition of a solution of 2,6-lutidine (5 mL) in CH_2Cl_2 (50 mL), washed with water, N HCl, and water, dried and concentrated. Purification by column chromatography (4:1 EtOAc–hexane) gave amorphous **20** (0.22 g, 69%): $[\alpha]_D$ −8.2° (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.389, 1.839, 1.848, 1.938 (2), 1.953, 1.975, 2.003 (3), 2.015, 2.029, 2.032, 2.054, 2.087, 2.091, 2.104 (2), 2.109 (15s, 19 × OAc), 2.811 (s, 1 H, 4$^{a'}$-OH), 3.337 (s, 3, OMe), 5.306 (2d, 1 H each, H-4^c, H-4$^{c'}$), 5.429 (d, 1 H, H-1$^{b'}$, $J_{1,2}$ 8.4 Hz), 5.613 (d, 1 H, H-1^b, $J_{1,2}$ 8.4 Hz), 5.629 (dd, 1 H, H-3$^{b'}$, $J_{2,3}$ 9.3 Hz, $J_{3,4}$ 10.6 Hz), 5.749 (dd, 1 H, H-3^b, $J_{2,3}$ 9.0 Hz, $J_{3,4}$ 10.3 Hz), 7.029–7.265 (m, 15 H, 3 × Ph), 7.548–7.807 (m, 4 H, phth); ^{13}C NMR ($CDCl_3$) δ 19.87–20.95 (19 × CH_3C=O), 54.60, 55.53 (C-2^b, C-2$^{b'}$), 56.86 (OMe), 60.82 (2), 62.08, 62.24, 63.27, 66.74, 66.81, 67.96 (4), 68.27, 69.26, 69.50, 70.21, 70.51, 70.64, 70.75 (4), 71.82 (2), 72.07, 72.45 (2), 72.58, 72.84, 72.92, 73.36, 73.80, 74.41, 74.73 (2), 75.51, 75.88, 76.07, 77.03, 78.93, 79.82, 80.26, 98.11, 98.33, 100.51, 100.63 (2), 101.12, 101.48, 104.96 (8 × C-1), 123.62–138.95 (3 × Ph, phth), 167.49–170.78 (*C*=O); FABMS: Calcd for $C_{124}H_{146}LiN_2O_{62}$ (M + Li): 2661.85. Found: 2662.25.

*Methyl β-*D*-glucopyranosyl-(1 → 6)-[β-*D*-galactopyranosyl-(1 → 4)]-2-acetamido-2-deoxy-β-*D*-glucopyranosyl-(1 → 3)-β-*D*-galactopyranosyl-(1 → 4)-β-*D*-glucopyranosyl-(1 → 6)-[β-*D*-galactopyranosyl-(1 → 4)]-2-acetamido-2-deoxy-β-*D*-glucopyranosyl-(1 →*

*3)-β-*D*-galactopyranoside* (**21**).—A solution of **20** (0.20 g, 75 μmol) in 95% ethanol (15 mL) was treated with hydrazine hydrate (2 mL). The mixture was refluxed for 16 h. The solvent was evaporated by the addition of ethanol and toluene. The residue was dissolved in 10:1 MeOH–water (10 mL) and treated with acetic anhydride (1 mL) at room temperature for 3 h. The solvent was removed by evaporation, and the residue was purified through a Sephadex G-10 column (water as eluent). The carbohydrate-containing fractions were collected and lyophilized (ca. 100 mg). A mixture of the above product and 10% Pd/C (50% water, 500 mg) in 20% HOAc (20 mL) was subjected to a hydrogen pressure (40 psi) for 16 h. The filtrate was lyophilized, and the crude product was purified again through a Sephadex G-10 column. The fractions were collected and lyophilized to give **21** (36 mg, 35%) as an amorphous solid: $[\alpha]_D$ +10.2° (*c* 0.2, H_2O); FABMS: Calcd for $C_{53}H_{90}N_2O_{41}$: 1411.29. Found: 1411.90; for NMR data see Tables 1 and 2.

*Methyl β-*D*-glucopyranosyl-(1 → 6)-[α-*N*-acetylneuraminyl-(2 → 3)-β-*D*-galactopyranosyl-(1 → 4)]-2-acetamido-2-deoxy-β-*D*-glucopyranosyl-(1 → 3)-β-*D*-galactopyranosyl-(1 → 4)-β-*D*-glucopyranosyl-(1 → 6)-[α-*N*-acetylneuraminyl-(2 → 3)-β-*D*-galactopyranosyl-(1 → 4)]-2-acetamido-2-deoxy-β-*D*-glucopyranosyl-(1 → 3)-β-*D*-galactopyranoside* (**22**).—A solution of **21** (7.0 mg, 5.0 μmol), CMP-NeuAc (5 mg) in water (0.75 mL) was adjusted to pH 7.5 by the addition of N sodium cacodylate. To the above solution was added 1% HSA (15 μL), alkaline phosphatase (5 U) and α-(2 → 3)-sialyltransferase (35 mU). Again the pH was adjusted to 7.5 using N sodium cacodylate solution. After 24 h at room temperature additional CMP-NeuAc (2 mg) was added, and the mixture was incubated for another 16 h. The mixture was purified on a Bio-Gel P-2 column using water as eluent. The fractions were collected and lyophilized to give **22** (8.0 mg, 80%); $[\alpha]_D$ +15° (*c* 0.2, H_2O); FABMS: Calcd for $C_{75}H_{124}N_4O_{57}$ (1993.80): 1994.73 $[M + H]^+$, 2016.61 $[M + Na]^+$, 2038.39 $[M + 2Na]^+$, 2060.35 $[M + 3Na]^+$ (positive mode); 1993.03 $[M-H]^-$ (negative mode); For NMR data see Tables 1 and 2.

*Methyl β-*D*-glucopyranosyl-(1 → 6)-{α-*N*-[C^{13}]acetylneuraminyl-(2 → 3)-β-*D*-galactopyranosyl-(1 → 4)}-2-acetamido-2-deoxy-β-*D*-glucopyranosyl-(1 → 3)-β-*D*-galactopyranosyl-(1 → 4)-β-*D*-glucopyranosyl-(1 → 6)-{α-*N*-[C^{13}]acetylneuraminyl-(2 → 3)-β-*D*-galactopyranosyl-(1 → 4)}-2-acetamido-2-deoxy-β-*D*-glucopyranosyl-(1 → 3)-β-*D*-galactopyranoside* (**23**).—A solution of **21** (5.0 mg, 3.5 μmol), 8% ^{13}C-labeled NeuAc (5 mg), and CTP (10 mg) in water (0.50 mL) was adjusted to pH 7.5 by the addition of N sodium cacodylate. To the above solution were added 1% HSA (10 μL), alkaline phosphatase (5 U), sialic acid-CMP synthetase (1 U), and α-(2 → 3)-sialyltransferase (30 mU). Again the pH was adjusted to 7.5 using N sodium cacodylate solution. After 24 h at 37 °C additional [^{13}C]NeuAc (2 mg) and CTP (3 mg) were added, and the mixture was incubated for another 16 h. The mixture was purified on a Bio-Gel P-2 column using water as eluent. The fractions were collected and lyophilized to give **23** (5.0 mg, 71%); For ^{1}H NMR data see Table 1 and for the ^{13}C NMR spectrum see Fig. 3.

*Methyl β-*D*-glucopyranosyl-(1 → 6)-[α-*N*-propionylneuraminyl-(2 → 3)-β-*D*-galactopyranosyl-(1 → 4)]-2-acetamido-2-deoxy-β-*D*-glucopyranosyl-(1 → 3)-β-*D*-galactopyranosyl-(1 → 4)-β-*D*-glucopyranosyl-(1 → 6)-[α-*N*-propionylneuraminyl-(2 → 3)-β-*D*-galactopyranosyl-(1 → 4)]-2-acetamido-2-deoxy-β-*D*-glucopyranosyl-(1 → 3)-β-*D*-galactopyranoside* (24).—A solution of **21** (6.5 mg, 4.6 μmol), NeuPr (7 mg), and CTP

(13 mg) in water (0.75 mL) was adjusted to pH 7.5 by the addition of N sodium cacodylate. To the above solution were added 1% HSA (20 μL), alkaline phosphatase (5 U), sialic acid-CMP synthetase (1 U), and α-(2 → 3)-sialyltransferase (45 mU). Again the pH was adjusted to 7.5 using N sodium cacodylate solution. After 24 h at 37 °C additional NeuPr (5 mg) and CTP (5 mg) were added, and the mixture was incubated for another 16 h. The mixture was purified on a Bio-Gel P-2 column using water as eluent. The fractions were collected and lyophilized to give **24** (7.0 mg, 75%): $[\alpha]_D$ +8° (*c* 0.2, H_2O); FABMS: Calcd for $C_{77}H_{128}N_4O_{57}$ (2021.9): 2021.3 $[M]^+$, 2022.3 $[M + H]^+$, 2043.8 $[M + Na]^+$, 2065.3 $[M + 2Na]^+$; For NMR data see Tables 1 and 2.

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