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Synthesis of a disialylated hexasaccharide of Type VIII Group B *Streptococcus* capsular polysaccharide

Eva Eichler, Harold J. Jennings, Michel Gilbert, Dennis M. Whitfield *

National Research Council, 100 Sussex Drive, Ottawa, Ontario, K1A 0R6 Canada

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Abstract

As part of our program to design, develop and prepare protective vaccines against the bacterial pathogens Group B *Streptococcus*, we report the synthesis of a disialylated hexasaccharide. This hexasaccharide represents a portion of the serotype-specific capsular polysaccharide of Type VIII that has the tetrasaccharide repeat unit {β-L-Rha*p*-(1→4)-β-D-Glc*p*-(1→4)-[α-Neu5Ac-(2→3)]-β-D-Gal*p*-(1→4)}$_n$. A tetrasaccharide corresponding to this repeat unit has been synthesized by us [E. Eichler, H.J. Jennings, D.M. Whitfield, *J. Carbohydr. Chem.*, 16 (1997) 385–411]. Since the protective epitopes are believed to involve several repeat units, methods to extend this tetrasaccharide were examined. This objective requires a glycosylation of the unreactive OH-4 of the β-L-Rha*p*, which was accomplished by coupling a D-Gal*p* glycosyl trichloroacetimidate donor with a β-L-Rha*p*-(1→4)-D-Glc*p* acceptor. Subsequent coupling of this trisaccharide as a donor to an α-Neu5Ac-(2→3)-D-Gal*p* disaccharide acceptor gave a pentasaccharide. The pentasaccharide was deprotected and enzymatically sialylated using an α-(2→3)-sialyltransferase from *Campylobacter jejuni* to give the title hexasaccahride α-Neu5Ac-(2→3)-β-D-Gal*p*-(1→4)-β-L-Rha*p*-(1→4)-β-D-Glc*p*-(1→4)-[α-Neu5Ac-(2→3)]-β-D-Gal*p*-(1→O)-$(CH_2)_3N_3$.

Keywords: Oligosaccharide synthesis; Sialyltransferase; Chemoenzymatic; Group B *Streptococcus*

1. Introduction

As part of our institute's program to design, develop and prepare protective vaccines against bacterial pathogens, we report the synthesis of a disialylated hexasaccharide. This hexasaccharide represents a portion of the serotype-specific capsular polysaccharide of Group B *Streptococcus* (GBS) Type VIII that has the tetrasaccharide repeat unit {β-L-Rha*p*-(1→4)-β-D-Glc*p*-(1→4)-[α-Neu5Ac-(2→3)]-β-D-Gal*p*-(1→4)}$_n$ (Fig. 1) [1]. GBS has long been recognized as a major cause of neonatal sepsis and meningitis [2]. Type VIII has been identified among disease-causing isolates in Japan where it is now a prevalent strain [3]. From our experience with other serotypes, it is anticipated that the protective antigen will be a conformational epitope that consists of at least two repeating units [4]. Previously we have synthesized tetrasaccharides that correspond to the repeating unit [5]. In order to extend such tetrasaccharides, it was necessary to form the β-D-Gal*p*-(1→4)-β-L-Rha*p* bond. This objective requires the glycosylation of the unreactive OH-4 of the L-Rha*p*. The β-D-Gal*p*-(1→4)-α-L-Rha*p* bond has been successfully synthesized to form a disaccharide [6,7]. This work describes the successful achievement of this goal and the subsequent elaboration of the molecule into disialylated hexasaccharides **1** and **2**.

* Corresponding author. Tel.: + 1-613-993-5265; fax: + 1-613-952-9092.

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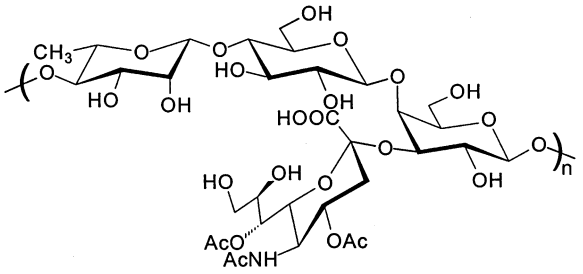


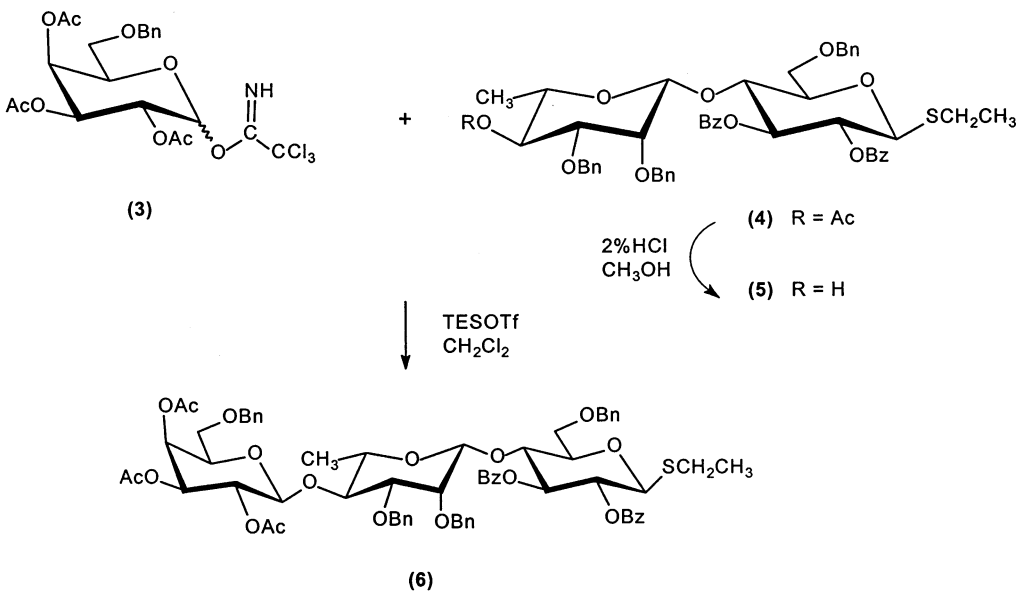
Fig. 1. Tetrasaccharide repeat unit of Group B *Streptococcus* (GBS) Type VIII.

2. Results and discussion

Our initial strategy was to use the tetrasaccharide donor we had developed in the previous synthesis to glycosylate the OH-4 of the L-Rha*p*. However, this could not be accomplished, so several alternate routes were examined. Thus, known trichloroacetimidate D-Gal*p* donor **3** [8] was reacted with β-L-Rha*p*-(1→4)-D-Glc*p* disaccharide **5** under promotion with triethylsilyl trifluoromethanesulfonate [9,10] to yield trisaccharide **6** in 67% isolated yield. The OH-4 of **5** was liberated from known disaccharide **4** by carefully controlled methanolysis with 2% HCl in methanol [11,12]. The protecting group pattern of **4**, which allows for chain extension at the L-Rha*p* OH-4, had been developed in the design stage of the synthesis (see Scheme 1). The good yield of this glycosylation contrasted greatly with the previously unsuccessful results with tetrasaccharide donors.

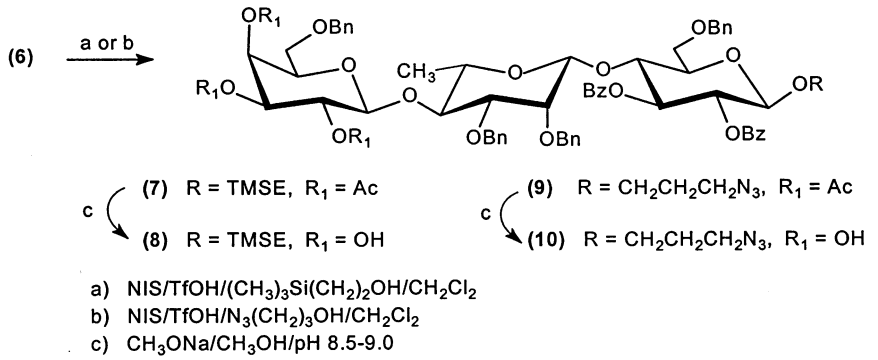
Since trisaccharide **6** has an alkylthio substituent at its reducing termini, it was easily activated under NIS/TfOH conditions [13,14]. Thus, **6** was glycosylated with the linkers 2-trimethylsilylethanol [15,16] or 3-azidopropan-1-ol [17] to give the trisaccharides **7** and **9** (see Scheme 2). The acetates were cleaved by mild treatment with sodium methoxide in methanol to yield the triols **8** and **10**. Subsequent attempts to sialylate these triols with the sialyl donor **11** [18,19] did lead to the expected O-3 regioselectivity, but the resulting tetrasaccharides **12** and **13** were isolated as inseparable mixtures of α,β anomers (see Scheme 3). This contrasts with the high stereoselectivity observed in the formation of disaccharide **14** (see below) under very similar conditions. The factors that control these differences are not known.

The direction of chain extension was therefore reversed, and trisaccharide **6** was reacted with known disaccharide diol **14** [5,20] to yield pentasaccharide **15** with high regioselectivity and stereoselectivity (see Scheme 4). An im-

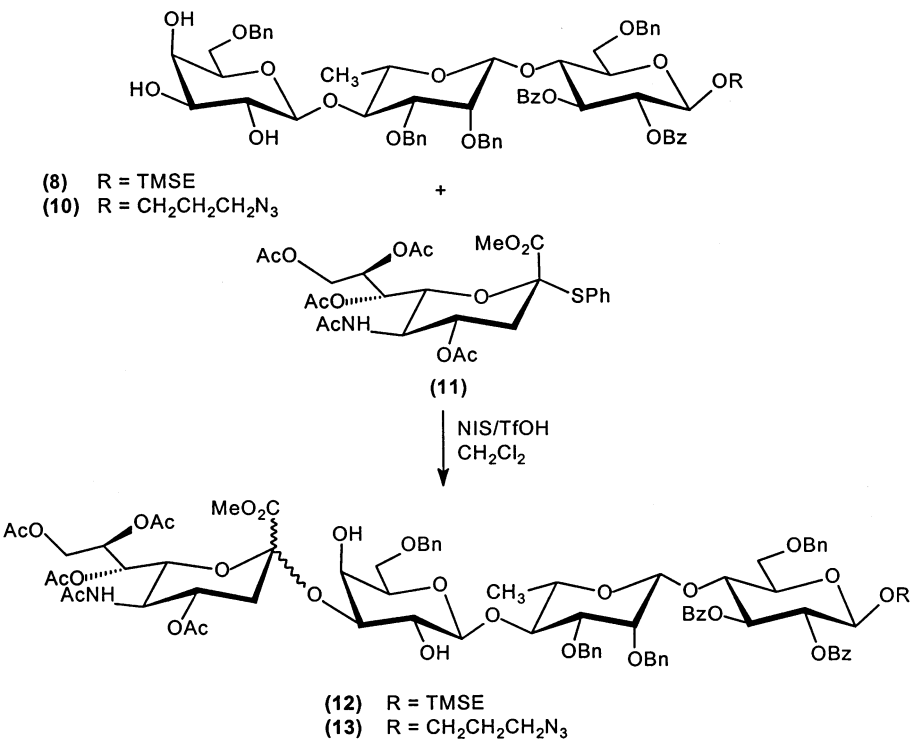


Scheme 1.

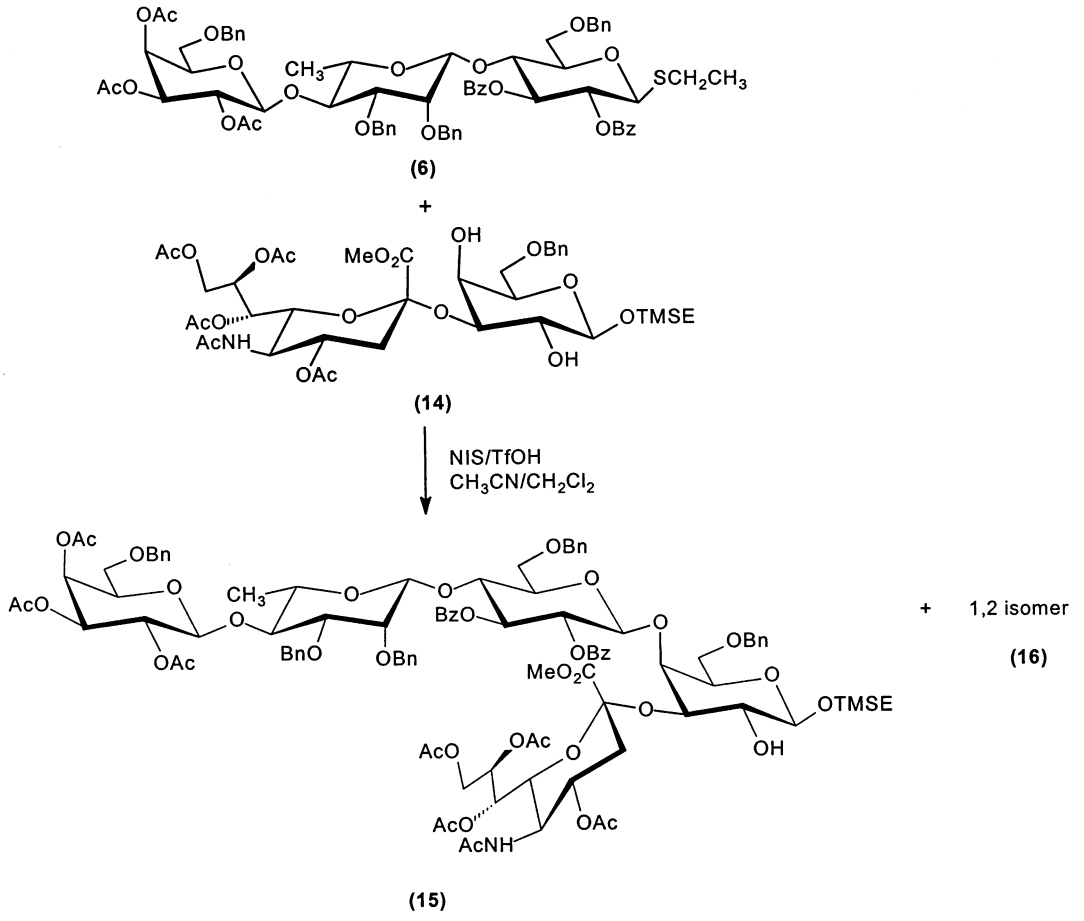
pure fraction with ^{1}H NMR resonances similar to those of **15** was isolated, which by analogy to previous results is likely to be the 1,2-isomer **16**. The 1,4 regiochemistry of **15** follows from observation of a COSY crosspeak between the OH-2^{II} and H-2^{II} at δ_H 2.25 and 3.55 ppm, respectively. After hydrogenation to cleave the *O*-benzyl protecting groups, cf. **17**, the *O*-acyl groups were cleaved under Zemplén conditions, followed by basic hydrolysis of the methyl ester, to give the deprotected pentasaccharide **18** (see Scheme 5). Alternatively **17** was reacetylated to give **19**. Then, the linker, 3-azido-propan-1-ol, was added after conversion to the hemiacetal **20** and activation as the trichloroacetimidate **21** to give **22** in an overall yield of 37% from **15**. Then, **22** was deprotected to yield **23** (see Scheme 6). Both **18** and **23** were completely characterized by ^{1}H and ^{13}C NMR spectroscopy in D_2O. The assignments of the 1D ^{1}H and ^{13}C spectra are based on 2D ^{1}H-^{1}H COSY, 2D ^{13}C-^{1}H COSY, 2D ^{1}H-^{1}H ROESY, 2D ^{1}H-^{1}H TOCSY and 1D-selTOCSY experiments at 600.1 and 150.9 MHz, respectively (see Tables 1 and 2). The resonances of **18** and **23** resembled those of the tetrasaccharide without D-Gal*p*^{V} except for the resonances of



Scheme 2.



Scheme 3.

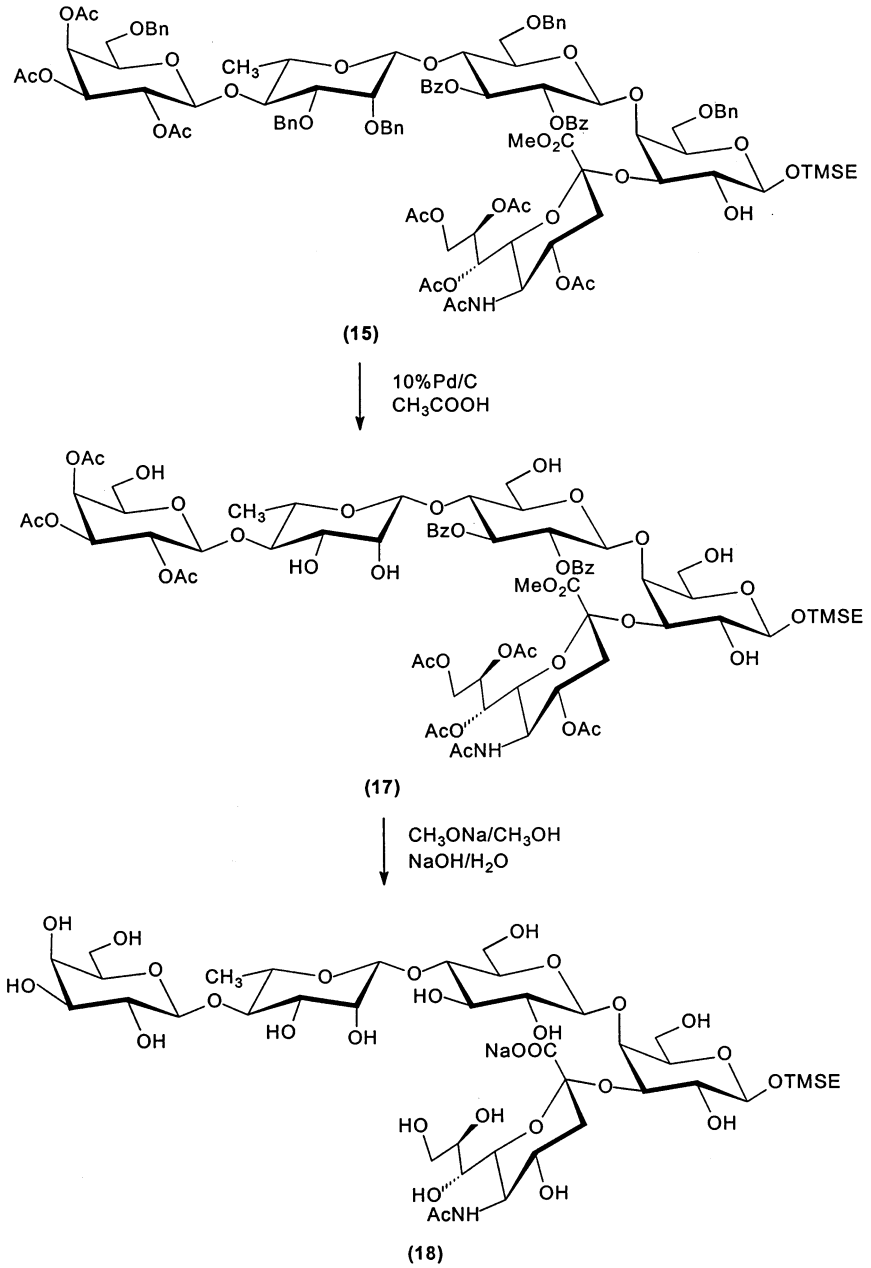


Scheme 4.

L-Rha*p* H-4^{IV}, H-5^{IV}, C-4^{IV} and C-3^{IV}. These changes reflect the successful glycosylation of L-Rha*p* O-4^{IV}. (For the I, II to VI nomenclature see Section 3 and Scheme 7.)

In order to install the terminal sialic acid of **1** and **2**, pentasaccharides **18** and **23** were enzymatically glycosylated using β-Neu5Ac-(2→CMP) as donor as catalyzed by a recently cloned and expressed α-Neu5Ac-(2→3)-transferase from *C. jejuni* (M. Gilbert, A.-M Cunningham, M.F. Karwaski, W.W. Wakarchuk, unpublished observations). As judged by TLC, the reactions went to completion to yield **1** and **2** (see Scheme 7). It should be noted that the α-Neu5Ac-(2→3)-transferase from *Neisseria meningitidis* was unable to catalyze this transformation. This transferase sialylates a number of Gal*p* derivatives [21]. In fact, to the best of our knowledge, this is the first observation of the transfer of Neu5Ac to a terminal D-Gal*p* linked to an L-Rha*p*. The products were purified by eluting with water on a Bio-Rad P-2 gel permeation column. Hexasaccharide **1** was repurified on a C_{18} reversed-phase Sep-Pak, and only enough pure compound to characterize by ^{1}H NMR spectroscopy was isolated. For hexasaccharide **2** the fractions from the P-2 column were nearly pure (see Fig. 2), and the yield from **23** was nearly quantitative. The ^{1}H and ^{13}C NMR spectra in D_2O of **2** could be completely assigned (see Table 1). A comparison of the resonances of the Neu5Ac^I attached to the reducing D-Gal*p*^{II} in **2** versus **23** (Neu5Ac^I) shows only small changes. The biggest changes are for H-4^I and C-4^I, possibly suggesting a small reorientation of this part of the molecule.

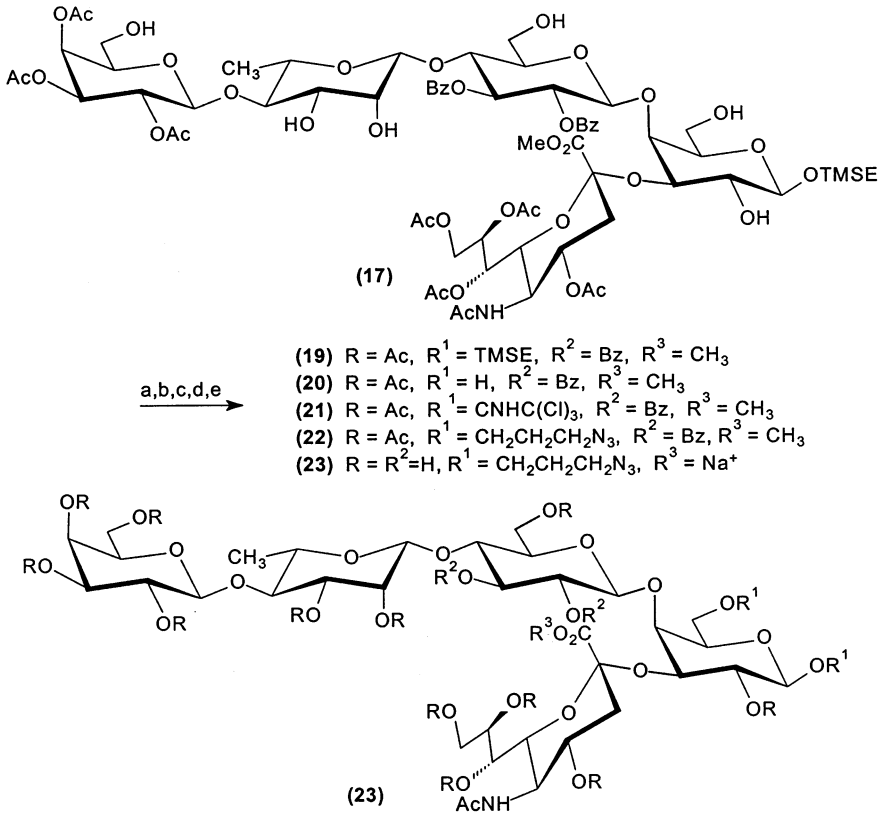
Thus, a hexasaccharide representing one-and-a-half repeat units of the capsular polysaccharide of Group B *Streptococcus* (GBS) Type VIII has been synthesized and characterized. The combined chemoenzymatic synthesis opens the way to efficiently prepare multiple repeat units of this polysaccharide.



Scheme 5.

3. Experimental

General methods.—Optical rotations were measured ($\lambda = 589$ nm) at room temperature (rt) using a Perkin–Elmer 243 polarimeter in a 10-cm 1-mL cell. The ^{1}H and ^{13}C NMR spectra were recorded at 500.1 and 125 MHz or 600.1 and 150.9 MHz on Bruker or Varian spectrometers, respectively, in either $CDCl_3$ or D_2O. ^{1}H NMR spectra in $CDCl_3$ were referenced to residual $CHCl_3$ at 7.24 ppm, and ^{13}C NMR spectra to the central peak of $CDCl_3$ 77.0 ppm. In D_2O spectra were referenced to internal acetone at 2.225 and 31.55 ppm, for ^{1}H and ^{13}C NMR spectra, respectively. Assignments were made by standard ^{1}H-^{1}H-COSY and ^{1}H-coupled ^{13}C-^{1}H-COSY experiments. For **1, 2, 18**, and **23**, additional 2D gradient-enhanced ^{1}H, decoupled ^{13}C-^{1}H-COSY, ^{1}H-^{1}H-TOCSY,^{1}H-^{1}H-ROESY and 1D gradient-enhanced ^{1}H sel TOCSY measurements were made at 298 K using standard



Scheme 6.

Varian pulse sequences. The results are presented in Table 1 (^{1}H) and 2 (^{13}C). For NMR assignments, all carbohydrate residues in products larger than monosaccharides have been designated as follows: rhamnose IV, glucose III, galactose II, nonreducing terminal galactose V, neuraminic acid I and non-reducing terminal neuraminic acid VI. The mass spectral analysis was done on a Jeol JMS-AX505-H forward mass spectrometer. Fast-atom bombardment mass spectrometry (FABMS) was performed using xenon atoms at 6 kV as the source. Thioglycerol or a mixture of glycerol and thioglycerol was used as the FAB matrix. Typically 10–15 full-range, low-resolution MS scans were averaged to yield a low-resolution mass spectrum. For high-resolution MS, the electric sector was scanned over the range of interest. Typically polyethylene glycol or polypropylene glycol was used as an internal mass standard, and between 75 and 150 scans were averaged. The electrospray-ionization mass spectra (ESIMS) were recorded on a Fisons VG-Quattro spectrometer in the loop injection mode using 1:1 acetonitrile–water with 0.4% acetic acid as matrix in the positive-ion mode. MALDIMS spectra were taken on a Voyager-De STR Biochemistry Workstation, from PerSeptive Biosystems, Framingham, MA, USA. TLC was performed on E. Merck Silica Gel 60 F_{254} plates. Preparative silica gel chromatography used E. Merck Silica Gel 60 (70–230 mesh), and MPLC used E. Merck Silica Gel 60 (230–

Table 1
^{1}H NMR spectral data of pentasaccharides **17** and **23** and hexasaccharides **1** and **2** [a]

1	IV	III	II	V	I	VI
H-1	4.87	4.80	4.52 (7.8)	4.73 (7.8)		
H-2	4.09	3.30 (8.3)	3.63	3.58		
H-3	3.83	3.67	4.17	4.12	2.72 (3.4)	2.76 (3.4)
H-3′					1.84 (12.2)	1.80 (12.2)
H-4	3.62	3.63	4.13	3.95	3.69	3.69
H-5	3.50	3.52	3.72	[b]	3.81	3.85
H-6	1.38 (5.8)	3.83	[b]	[b]	3.58	3.65
H-6′		3.92	[b]	[b]		
H-7					[b]	[b]
H-8					[b]	[b]
H-9					[b]	[b]
H-9′					[b]	[b]
Other resonances:						
$OCH_2CH_2CH_2Si(CH_3)_3$ 4.03, 3.76; 0.95; 1.05, −0.02 (9)						
CH_3CON 2.02 (3)						

2	IV	III	II	V	I	VI
H-1	4.88	4.82	4.50 (7.8)	4.73 (7.8)		
H-2	4.10	3.29 (8.3)	3.65	3.52		
H-3	3.82	3.66	4.18	4.12	2.72 (3.4)	2.76 (3.4)
H-3′					1.82 (12.2)	1.80 (12.2)
H-4	3.63	3.61	4.15	3.94	3.72	3.70
H-5	3.48	3.51	3.73	3.67	3.82	3.84
H-6	1.38 (5.8)	3.83	3.71	3.73	3.58	3.64
H-6′		3.91	3.76	3.77		
H-7					3.59	3.59
H-8					3.79	3.87
H-9					3.63	3.63
H-9′					3.84	3.86
Other resonances:						
$OCH_2CH_2CH_2N_3$ 3.98; 3.76; 3.46 (2); 1.92 (2)						
CH_3CON 2.02 (3)						

17	IV	III	II	V	I
H-1	4.86	4.80 (8.0)	4.49 (8.0)	4.64 (7.9)	
H-2	4.07 (3.2)	3.29 (8.4)	3.62 (2.6)	3.52	
H-3	3.82	3.65	4.16 (9.8)	3.64	2.71 (4.5)
H-3′					1.84 (12.2)
H-4	3.62	3.61	4.12	3.90	3.68
H-5	3.45	3.51	3.72	3.66	3.81
H-6	1.40 (6.1)	3.81	3.69 [c]	3.69 [c]	3.57
H-6′		3.90	3.75 [c]	3.75 [c]	
H-7					3.61
H-8					3.80
H-9					3.64
H-9′					3.87
Other resonances:					
$OCH_2CH_2Si(CH_3)_3$ 4.03; 3.76; 1.08; 0.97; −0.02 (9)					
CH_3CON 2.02; (3)					

23	IV	III	II	V	I
H-1	4.87	4.82 (8.4)	4.50 (8.0)	4.66 (7.7)	
H-2	4.09	3.30 (8.5)	3.66	3.52	
H-3	3.83	3.67	4.19 (9.8)	3.68	2.72 (4.4)
H-3′					1.84 (12.8)
H-4	3.63	3.62	4.14	3.91	3.70
H-5	3.49	3.52	3.71	3.67	3.83
H-6	1.40 (5.8)	3.83	3.70	3.72	3.57
H-6′		3.93	3.74	3.76	

Table 1 (Continued)

H-7	3.59
H-8	3.79
H-9	3.62
H-9′	3.84
Other resonances:	
$OCH_2CH_2CH_2N_3$ 3.86; 3.76; 3.45 (2); 1.93 (2)	
CH_3CON 2.02 (3)	

[a] Chemical shifts in ppm and (coupling constants) in Hz in D_2O.
[b] Assignments are uncertain.
[c] Assignments are tentative.

Table 2
^{13}C NMR spectral data of pentasaccharides **17** and **23** and hexasaccharide **2** [a]

2	IV	III	II	IV	I	VI
C-1	101.33	103.45	103.31	104.41	174.32	175.86
C-2	71.39	74.18	70.23	70.90	101.40	100.66
C-3	73.55	76.35	76.11	76.59	39.38	40.52
C-4	81.73	77.49	75.65	68.27	70.44	69.32
C-5	71.61	75.03	75.03	75.80	52.52	52.52
C-6	17.69	61.79	61.53	61.69	73.73	73.73
C-7					68.86	68.97
C-8					72.87	72.67
C-9					63.45	63.45
Other resonances:						
$OCH_2CH_2CH_2N_3$ 69.06; 48.76; 22.87						
CH_3CON 29.08, 173.8						

17	IV	III	II	V	I
C-1	101.31	103.50	102.57	104.56	178.0
C-2	71.51	74.15	70.19	72.48	101.43
C-3	73.48	76.35	76.24	73.58	39.29
C-4	81.54	77.47	75.70	69.35	69.40
C-5	71.64	74.96	74.97	75.99	52.48
C-6	17.36	61.77	61.72	61.42	73.74
C-7					68.89
C-8					72.92
C-9					63.49
Other resonances:					
$OCH_2CH_2Si(CH_3)_3$ 69.08; 18.40; −1.70					
CH_3CON 22.87; 176.2					

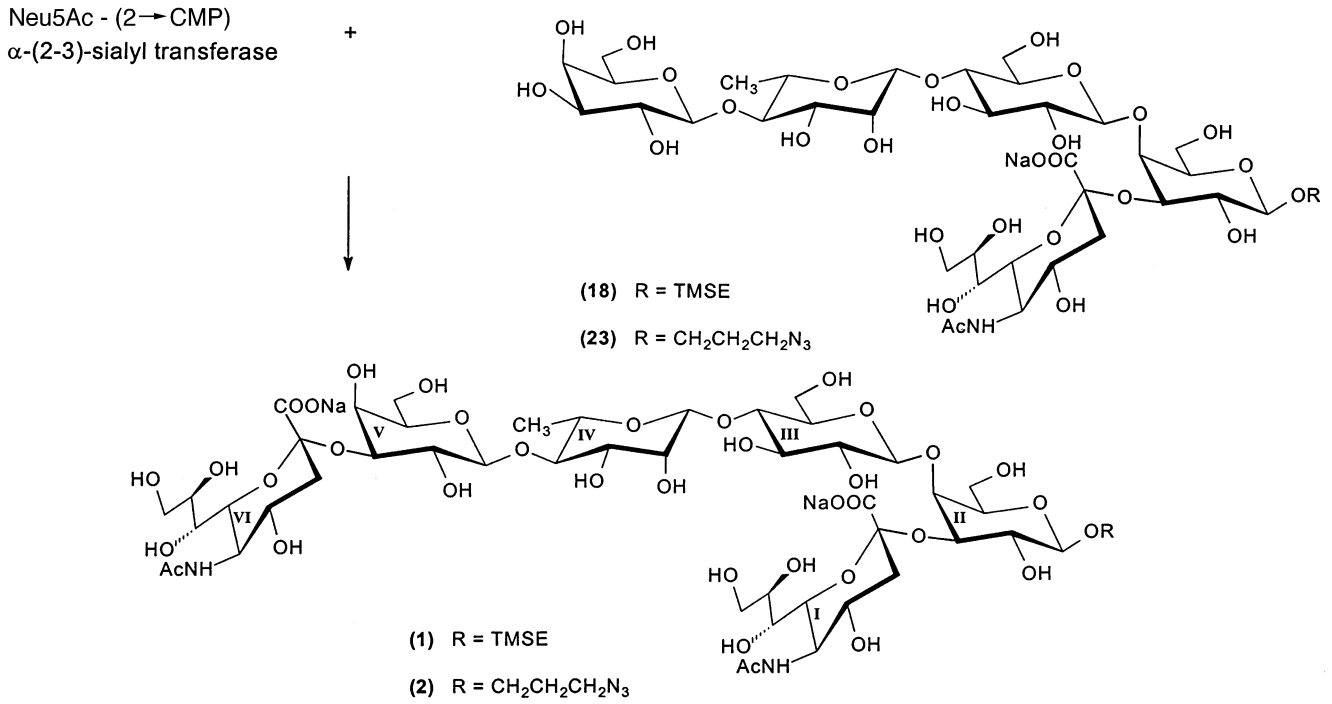
23	IV	III	II	V	I
C-1	101.31	103.45	103.30	104.57	175.40
C-2	71.51	74.05	70.10	72.48	101.42
C-3	73.48	76.30	76.05	73.58	39.40
C-4	81.50	77.40	75.60	69.40	69.39
C-5	71.64	74.97	74.97	75.99	52.40
C-6	17.36	61.72	61.71	61.51	73.65
C-7					68.90
C-8					72.90
C-9					63.35
Other resonances:					
$OCH_2CH_2CH_2N_3$ 68.08; 48.75; 29.09					
CH_3CON 22.81, 173.90					

[a] Chemical shifts in ppm in D_2O.

400 mesh). Detection was effected by examination under UV light and by charring with 5% sulfuric acid in water. Solutions were evaporated at or below 40 °C at aspirator pressure. Microanalyses were carried out by the analytical services of this department, and all samples submitted for elemental analyses were dried overnight under vacuum with

phosphorus pentoxide at 56 °C (refluxing acetone).

2,3,4-Tri-O-*acetyl-6*-O-*benzyl-α,β*-D-*galactopyranosyl trichloroacetimidate* (**3**). 2-(Trimethylsilyl)ethyl 6-*O*-benzyl-β-D-galactopyranoside [5] (1.3 g, 3.5 mmol) was dissolved in dry pyridine (20 mL) and chilled to 0 °C in an ice bath under an argon atmosphere. Acetic



Scheme 7.

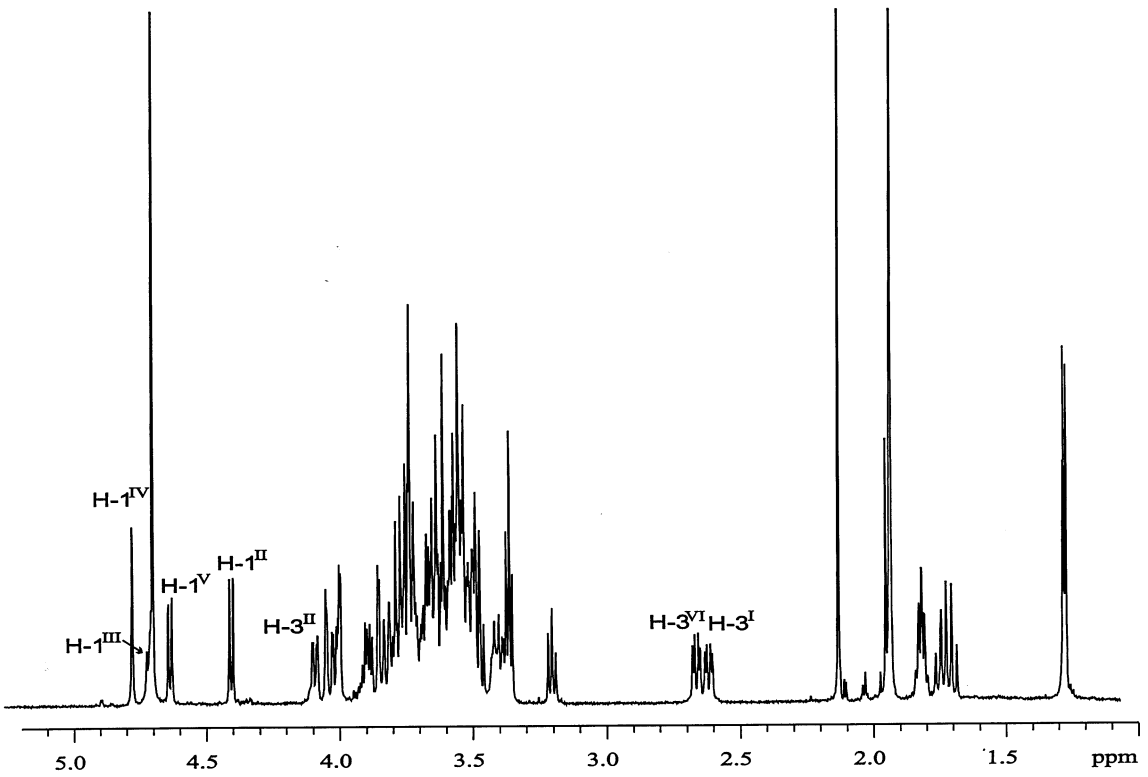


Fig. 2. ^{1}H NMR spectrum of hexasaccharide **2** in D_2O at 23 °C.

anhydride (8 mL) was added by syringe, and the mixture was stirred overnight. The temperature was allowed to rise to rt. The solution was concentrated in the morning, and co-concentrated three times with toluene. Crude 2-(trimethylsilyl)ethyl 2,3,4-tri-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranose (1.75 g, ~100%) was carried on as such to the next step. The syrup was dissolved in CH_2Cl_2 (72 mL), and trifluoroacetic acid (8 mL) was added. The mixture was stirred at rt for 5 h. Ethyl acetate (5 mL) was added to terminate the reaction. After stirring for a few min, the solvents were concentrated, then co-concentrated three times with ethyl acetate. The residue was purified on column chromatography using 3:7 ethyl acetate–hexanes to obtain a syrupy material (1.32 g, 97%). 2,3,4-Tri-*O*-acetyl-6-*O*-benzyl-D-galactopyranose was dissolved in dry CH_2Cl_2 (15 mL), and the solution was chilled under an argon atmosphere to 0 °C. While stirring, trichloroacetonitrile (4 mL) was added, followed by 50 μL of 1,8-diazabicylco[5.4.0]undec-7-ene (DBU). The mixture was stirred for 4 h, then co-concentrated. Purification of the residue by column chromatography using 4:1 hexanes–ethyl acetate as eluant afforded the α isomer (360 mg, 20%), a mixture of α and β isomers (535 mg, 29%), and the β isomer (662 mg, 37%) for a total yield of 86% of **3** as amorphous solids. Data for the α isomer: $[\alpha]_D + 68.7°$ (*c* 0.38, chloroform); ^{1}H NMR ($CDCl_3$): δ 8.62 (s, 1 H, NH), 7.34 (m, 5 H, Ph), 6.58 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 5.63 (d, 1 H, $J_{3,4}$ 3.1 Hz, H-4), 5.42 (dd, 1 H, $J_{2,3}$ 10.8 Hz, H-3), 5.34 (dd, 1 H, H-2), 4.53 (d, 1 H, *J* 11.9 Hz, CH_2Ph), 4.39 (m, 2 H, CH_2Ph and H-5), 3.54 (dd, 1 H, $J_{6,6'}$ 9.6 Hz and $J_{5,6}$ 5.8 Hz, H-6), 3.46 (dd, 1 H, $J_{5,6'}$ 7.2 Hz, H-6′), 2.06 (s, 3 H, OAc), 2.01 (s, 3 H, OAc), 2.00 (s, 3 H, OAc); ^{13}C NMR ($CDCl_3$): δ 93.94 (C-1), 161.27 (C=N), 91.07 (CCl_3). Anal. Calcd for $C_{21}H_{24}Cl_3NO_9$ (540.78): C, 46.64; H, 4.47; N, 2.59. Found: C, 46.27; H, 4.29; N, 3.09. Data for the β isomer: $[\alpha]_D - 0.6°$ (*c* 0.32, chloroform); ^{1}H NMR ($CDCl_3$): δ 8.70 (s, 1 H, NH), 7.26–7.35 (m, 5 H, Ph), 5.82 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1), 5.54 (dd, 1 H, $J_{3,4}$ 3.3 Hz and $J_{4,5}$ 0.8 Hz, H-4), 5.46 (dd, 1 H, $J_{2,3}$ 10.4 Hz, H-2), 5.12 (dd, 1 H, H-3), 4.56 (d, 1 H, *J* 12.0 Hz, CH_2Ph), 4.43 (d, 1 H, *J* 12.0 Hz, CH_2Ph), 4.05 (t, 1 H, H-5), 3.56 (dd, 1 H, $J_{6,6'}$ 9.5 Hz and $J_{5,6}$ 5.7 Hz, H-6), 3.50 (dd, 1 H, $J_{5,6'}$ 7.4 Hz, H-6′), 2.07 (s, 3 H, OAc), 2.01 (s, 3 H, OAc), 2.00 (s, 3 H, OAc); ^{13}C NMR ($CDCl_3$): δ 96.45 (C-1), 161.36 (C=N), 91.0 CCl_3. Anal. Calcd for $C_{21}H_{24}Cl_3NO_9$ (540.78): C, 46.64; H, 4.47; N, 2.59. Found: C, 46.63; H, 4.48; N, 2.62.

*Ethyl 2,3-di-*O*-benzyl-β-*L*-rhamnopyranosyl-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-1-thio-β-*D*-glucopyranoside* (**5**). Ethyl 4-*O*-acetyl-2,3-di-*O*-benzyl-β-L-rhamnopyranosyl-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-1-thio-β-D-glucopyranoside **(4)** [5] (850 mg, 0.95 mmol) was dissolved in dry MeOH (85 mL) and chilled to 0 °C in an ice bath. To this was added a 3% solution of HCl in MeOH (30 mL). The mixture was left stirring overnight and allowed to warm to rt. The mixture was chilled before neutralizing it with solid sodium hydrogencarbonate. Inorganic material was filtered off, and the filtrates were concentrated. The residue was purified by chromatography using 47:3 toluene–ethyl acetate to obtained an amorphous solid **5** (720 mg, 85%): $[\alpha]_D +$ 76.2° (*c* 0.45, chloroform); ^{1}H NMR ($CDCl_3$): δ 7.95–6.88 (m, 25 H, 5Ph), 5.75 (t, 1 H, $J_{3,4}$ 9.5 Hz, H-3^{III}), 5.40 (t, 1 H, $J_{2,3}$ 9.8 Hz, H-2^{III}), 4.82 (d, 1 H, *J* 12.3 Hz, CH_2Ph), 4.72 (d, 1 H, $J_{1,2}$ 9.9 Hz, H-1^{III}), 4.63 (d, 1 H, *J* 12.1 Hz, CH_2Ph), 4.57 (d, 1 H, *J* 12.1 Hz, CH_2Ph), 4.53 (d, 1 H, *J* 12.3 Hz, CH_2Ph), 4.42 (s, 1 H, H-1^{IV}), 4.05 (d, 1 H, *J* 10.5 Hz, CH_2Ph), 3.99 (t, 1 H, $J_{3,4}$ and $J_{4,5}$ 9.3 Hz, H-4^{III}), 3.83 (d, 1 H, *J* 11.6 Hz, CH_2Ph), 3.79–3.75 (m, 2 H, H-5^{III} and H-6^{III}), 3.52–3.48 (m, 3 H, H-2^{IV}, H-4^{IV} and H-6′^{III}), 3.09 (dt, 1 H, $J_{4,5}$ 6.0 and $J_{5,6}$ 6.1 Hz, H-5^{IV}), 2.78–2.73 (m, 2 H, CH_3CH_2S), 2.65 (d, 1 H, $J_{3,4}$ 9.3 Hz and $J_{2,3}$ 2.9 Hz, H-3^{IV}), 2.06 (d, 1 H, $J_{4,OH}$ 1.9 Hz, OH-4^{IV}), 1.28–1.24 (m, 6 H, CH_3CH_2S, and H-6^{IV}). Anal. Calcd for $C_{49}H_{52}O_{11}S$ (849.02): C, 69.32; H, 6.17; S, 3.77. Found: C, 69.67; H, 5.92.

*Ethyl 2,3,4-tri-*O*-acetyl-6-*O*-benzyl-β-*D*-galactopyranosyl-(1→4)-2,3-di-*O*-benzyl-β-*L*-rhamnopyranosyl-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-1-thio-β-*D*-glucopyranoside* (**6**). Alcohol **5** (485 mg, 0.57 mmol) was dissolved in CH_2Cl_2 (50 mL), and 2,3,4-tri-*O*-acetyl-6-*O*-benzyl-D-galactopyranosyl trichloroacetimid-

ate **3** (462 mg, 0.85 mmol) was added, followed by activated 4 Å molecular sieves (1.5 g). The suspension was stirred at rt for 1.5 h under an argon atmosphere, then chilled to −5 °C in a $CaCl_2$–ice bath, and triethylsilyl trifluoromethanesulfonate (13 μL in 1.3 mL CH_2Cl_2) was added. Triethylamine was added to terminate the reaction, and after further dilution with CH_2Cl_2, some solids were filtered off through Celite. The residue was purified by chromatography using 3:7 ethyl acetate–hexanes as eluant to give **6** as an amorphous solid (469 mg, 67%): $[\alpha]_D + 11.8°$ (*c* 0.44, chloroform); ^{1}H NMR ($CDCl_3$): δ 7.95–7.06 (m, 30 H, 6 Ph), 5.69 (t, 1 H, $J_{2,3}$ and $J_{3,4}$ 9.5 Hz, H-3^{III}), 5.40–5.37 (m, 2 H, H-2^{III} and H-4^{V}), 5.02 (dd, 1 H, $J_{2,3}$ 10.4 Hz, H-2^{V}), 4.9 (dd, 1 H, $J_{3,4}$ 3.4 Hz, H-3^{V}), 4.8 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1^{V}), 4.7 (m, 2 H, H-1^{III}, and CH_2Ph), 4.6 (d, 1 H, *J* 12.0, CH_2Ph), 4.56–4.48 (m, 3 H, $3CH_2Ph$), 4.37 (m, 2 H, H-1^{IV} and CH_2Ph), 4.02 (d, 1 H, *J* 9.8 Hz, CH_2Ph), 3.96 (t, 1 H, $J_{4,5}$ 9.4 Hz, H-4^{III}), 3.76–3.72 (m, 5 H, H-5^{V}, H-5^{III}, CH_2Ph, H-6^{III} and H-6′^{III}), 3.63 (t, 1 H, $J_{3,4}$ and $J_{4,5}$ 9.3 Hz, H-4^{IV}), 3.44 (m, 3 H, H-2^{IV}, H-6^{II} and H-6^{V}), 3.10 (dd, 1 H, $J_{4,5}$ 11.9 Hz and $J_{5,6}$ 5.8 Hz, H-5^{IV}), 2.77–2.71 (m, 3 H, SCH_2CH_3 and H-3^{IV}), 2.01 (s, 3 H, OAc), 1.92 (s, 3 H, OAc), 1.77 (s, 3 H, OAc), 1.29–1.22 (m, 6 H, SCH_2CH_3; and H-6^{IV}). Anal. Calcd for $C_{68}H_{74}O_{19}S$ (1227.39): C, 66.54; H, 6.07; S, 2.61. Found: C, 66.21; H, 5.83.

2-(Trimethylsilyl)ethyl 2,3,4-tri-O-*acetyl-6*-O-*benzyl-β*-D-*galactopyranosyl*-(1→4)-*2,3-di*-O-*benzyl-β*-L-*rhamnopyranosyl*-(1→4)-*2,3-di*-O-*benzoyl-6*-O-*benzyl-β*-D-*glucopyranoside* (**7**). To a solution of **6** (180 mg, 0.147 mmol) and 2-(trimethylsilyl)ethanol (50 μL, 0.37 mmol) in dry CH_2Cl_2 (30 mL) was added 4 Å molecular sieves (700 mg), and the mixture was stirred at rt for 2 h under an argon atmosphere, at the end of which time NIS (83 mg, 0.37 mmol) was added. After the reaction mixture was stirred for 15 min, a solution of TfOH (0.21 mL in 5 mL of dichloromethane) was added (0.15 mL). When a TLC was taken 5 min after the addition of acid, it showed total consumption of the thioglycoside **6**. Triethylamine (0.15 mL) was then added to neutralize the reaction, and after further dilution with dichloromethane (100 mL), some solids were filtered off through Celite. The residue was purified by chromatography using 1:3 ethyl acetate–hexanes as eluant to obtain a white amorphous solid **7** (170 mg, 90%): $[\alpha]_D + 7.17°$ (*c* 0.46, chloroform); ^{1}H NMR ($CDCl_3$): δ 7.95–6.88 (m, 30 H, 6Ph) 5.66 (t, 1 H, $J_{3,4}$ 9.6 Hz, H-3^{III}), 5.39 (d, 1 H, $J_{3,4}$ 3.2 Hz, H-4^{V}), 5.34 (dd, 1 H, $J_{2,3}$ 10.0 Hz, H-2^{III}), 5.02 (dd, 1 H, $J_{2,3}$ 10.3 Hz, H-2^{V}), 4.90 (dd, 1 H, H-3^{V}), 4.82 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^{V}), 4.73 (d, 1 H, *J* 12.3 Hz, CH_2Ph), 4.70 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{III}), 4.62 (d, 1 H, *J* 12.1 Hz, CH_2Ph), 4.57–4.48 (m, 3 H, $3CH_2Ph$), 4.38–4.36 (m, 2 H, H-1^{IV} and CH_2 Ph), 4.04–3.98 (m, 2 H, CH_2Ph and CH_2CH_2Si), 3.93 (t, 1 H, $J_{4,5}$ 9.2 Hz, H-4^{III}), 3.75–3.70 (m, 5 H, H-5^{III}, H-6^{III}, H-6$^{III\prime}$, H-5^{V} and CH_2Ph), 3.65–3.58 (m, 2 H, CH_2CH_2Si and H-4^{IV}), 3.44–3.39 (m, 3 H, H-6^{V}, H-6′^{II} and H-2^{IV}), 3.14–3.10 (m, 1 H, H-5^{IV}), 2.75 (dd, 1 H, $J_{3,4}$ 9.2 Hz and $J_{2,3}$ 2.8 Hz, H-3^{IV}), 2.02 (s, 3 H, OAc), 1.93 (s, 3 H, OAc), 1.77 (s, 3 H, OAc), 1.25 (d, 3 H, $J_{5,6}$ 6.0 Hz, H-6^{IV}), 0.91–0.84 (m, 2 H, CH_2CH_2Si), −0.08 (s, 9 H, $Si(CH_3)_3$); ^{13}C NMR ($CDCl_3$): δ 102.39 (*J* 156.3 Hz, C–H-1^{IV}), 100.49 (*J* 170.8, C–H-1^{V}), 100.14 (*J* 160.6, C–H-1^{III}). Anal. Calcd for $C_{17}H_{82}O_{20}Si$ (1283.52): C, 66.44; H, 6.44; Si, 2.19. Found: C, 66.25; H, 6.47.

2-(Trimethylsilyl)ethyl 6-O-*benzyl-β*-D-*galactopyranosyl*-(1→4)-*2,3-di*-O-*benzyl-β*-L-*rhamnopyranosyl*-(1→4)-*2,3-di*-O-*benzoyl-6*-O-*benzyl-β*-D-*glucopyranoside* (**8**). To a solution of **7** (190 mg, 0.148 mmol) in dry MeOH (25 mL), under an argon atmosphere, 1 M sodium methoxide was added dropwise until pH 9. The reaction was monitored by TLC. After 4 h, additional MeOH was added, and the mixture was neutralized with Rexyn 101 (H^+) ion-exchange resin, filtered, and the filtrate was concentrated. The residue was purified on a short column of silica gel using 3:1 ethyl acetate–hexanes as eluant to obtain syrupy **8** (165 mg, 96%): $[\alpha]_D + 30.4°$ (*c* 0.33, chloroform); ^{1}H NMR ($CDCl_3$): δ 7.94–6.95 (m, 30 H, 6 Ph), 5.69 (t, 1 H, $J_{3,4}$ 9.7 Hz, H-3^{III}), 5.34 (dd, 1 H, $J_{2,3}$ 9.8 Hz, H-2^{III}), 4.8 (d, 1 H, *J* 12.2 Hz, CH_2Ph), 4.71 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1^{III}), 4.6 (d, 1 H, *J* 12 Hz, CH_2Ph), 4.57–4.52 (m, 2 H, CH_2PH), 4.38 (brs, 1 H, H-1^{IV}), 4.28 (brd, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{V}),

4.06–4.02 (m, 2 H, CH_2Ph and CH_2CH_2Si), 3.98–3.92 (m, 2 H, H-4^{III} and H-4^{V}), 3.85 (d, 1 H, J 11.4 Hz, CH_2Ph), 3.76–3.67 (m, 4 H, CH_2Ph, H-5^{III}, H-6^{III} and H-6^{V}), 3.64–3.53 (m, 6 H, CH_2Ph, H-5^{V}, H-6′^{V}, H-4^{IV}, H-2^{IV} and CH_2CH_2Si), 3.44 (m, 3 H, H-2^{V}, H-3^{V} and H-6′^{III}), 3.14 (m, 1 H, H-5^{IV}), 2.91 (dd, 1 H, $J_{3,4}$ 9.7 Hz and $J_{2,3}$ 2.7 Hz, H-3^{IV}), 1.33 (d, 3 H, $J_{5,6}$ 6.1 Hz, H-6^{IV}), 0.90–0.84 (m, 2 H, CH_2CH_2Si), 0.08 (s, 9 H, $Si(CH_3)_3$); ^{13}C NMR ($CDCl_3$): δ 100.02 (J 159.6 Hz, C-H-1^{III}), 102.24 (J 156.0 Hz, C–H-1^{IV}), 104.85 (J 160.6 Hz, C–H-1^{V}). Anal. Calcd for $C_{65}H_{76}O_{17}Si$ (1157.39): C, 67.45; H, 5.52; Si, 2.42. Found: C, 66.97; H, 6.13.

3-Azidopropyl 2,3,4-tri-O-*acetyl-6*-O-*benzyl-β*-D-*galactopyranosyl*-(1→4)-*2,3-di*-O-*benzyl-β*-L-*rhamnopyranosyl*-(1→4)-*2,3-di*-O-*benzoyl-6*-O-*benzyl-β*-D-*glucopyranoside* (**9**). Trisaccharide **6** (450 mg, 0.36 mmol) was reacted with 3-azidopropan-1-ol (110 mg, 1.08 mmol) as for the conversion of **6** to **7**. The product was purified by silica gel chromatography using 7:3 hexanes–ethyl acetate as eluant to give a white foamy solid **9** (430 mg, 78%): $[\alpha]_D$ + 11.8° (*c* 0.6 chloroform); ^{1}H NMR ($CDCl_3$): δ 7.96–7.00 (m, 30 H, 6 Ph), 5.67 (t, 1 H, $J_{3,4}$ 9.7 Hz, H-3^{III}), 5.39 (d, 1 H, $J_{3,4}$ 3.3 Hz, H-4^{V}), 5.33 (dd, 1 H, $J_{2,3}$ 10.0 Hz, H-2^{III}), 5.03 (dd, 1 H, $J_{2,3}$ 10.4 Hz, H-2^{V}), 4.88 (dd, 1 H, $J_{3,4}$ 3.4 Hz, H-3^{V}), 4.82 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1^{V}), 4.72 (d, 1 H, J 12.3 Hz, CH_2Ph), 4.68 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{III}), 4.61 (d, 1 H, J 12.1 Hz, CH_2Ph), 4.56–4.48 (m, 3 H, 3CH_2Ph), 4.38 (s, 1 H, H-1^{IV}), 4.37 (d, 1 H, J 11.1 Hz, CH_2Ph), 4.03 (d, 1 H, J 9.8 Hz, CH_2Ph), 3.99–3.93 (m, 2 H, H-4^{III} and $OCH_2CH_2CH_2N_3$), 3.76–3.65 (m, 5 H, H-5^{III}, H-6^{III}, H-6$^{III\prime}$, H-5^{V} and CH_2Ph), 3.63 (t, 1 H, $J_{4,5}$ 9.3 Hz, H-4^{IV}), 3.61–3.57 (m, 1 H, $OCH_2CH_2CH_2N_3$), 3.48–3.39 (m, 3 H, H-2^{IV}, H-6^{V} and H-6′^{V}), 3.24–3.20 (m, 2 H, CH_2N_3), 3.14–3.11 (m, 1 H, H-5^{IV}), 2.77 (dd, 1 H, $J_{3,4}$ 9.4 Hz and $J_{2,3}$ 3.0 Hz, H-3^{IV}), 2.01 (s, 3 H, OAc), 1.93 (s, 3 H, OAc), 1.84–1.69 (m, 5 H, OAc and $CH_2CH_2CH_2$), 1.27 (d, 3 H, $J_{5,6}$ 6.1 Hz, H-6^{IV}); ^{13}C NMR ($CDCl_3$): δ 102.6 (J 156.7 Hz, C–H-1^{V}), 101.10, (J 161.0 Hz, C–H-1^{III}), 100.75 (J 163.7 Hz, C–H-1^{IV}). Anal. Calcd for $C_{69}H_{75}N_3O_{20}$ (1266.37): C, 65.44; H, 5.97; N, 3.31. Found: C, 65.72; H, 5.62; N, 2.73.

3-Azidopropyl 6-O-*benzyl-β*-D-*galactopyranosyl*-(1→4)-*2,3-di*-O-*benzyl-β*-L-*rhamnopyranosyl*-(1→4)-*2,3-di*-O-*benzoyl-6*-O-*benzyl-β*-D-*glucopyranoside* (**10**). Trisaccharide **9** (330 mg, 0.26 mmol) was treated as for the conversion of **7** to **8**. The product was purified by silica gel chromatography using 3:2 ethyl acetate–hexanes as an eluant to obtain an amorphous solid **10** (220 mg, 62%): $[\alpha]_D$ + 32.6° (*c* 0.17, chloroform); ^{1}H NMR ($CDCl_3$): δ 7.94–6.95 (m, 30 H, 6 Ph), 5.69 (t, 1 H, $J_{3,4}$ 9.6 Hz, H-3^{III}), 5.34 (dd, 1 H, $J_{2,3}$ 10.1 Hz, H-2^{III}), 4.80 (d, 1 H, J 12.2 Hz, CH_2Ph), 4.68 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1^{III}), 4.62 (d, 1 H, J 12.1 Hz, CH_2Ph), 4.56–4.52 (m, 4 H, 4CH_2Ph), 4.49 (s, 1 H, H-1^{IV}), 4.37 (m, 1 H, H-1^{V}), 4.02 (d, 1 H, J 9.7 Hz, CH_2Ph), 3.99–3.95 (m, 2 H, H-4^{III} and OCH_2–$CH_2CH_2N_3$), 3.92 (brs, 1 H, H-4^{V}), 3.86 (d, 1 H, J 11.4 Hz, CH_2Ph), 3.76–3.66 (m, 5 H, H-5^{V}, H-6^{III}, H-6′^{III}, H-6^{V} and H-6′^{II}), 3.63–3.53 (m, 4 H, H-5^{III}, H-2^{IV}, H-4^{IV}, and $OCH_2CH_2CH_2N_3$), 3.43–3.42 (m, 2 H, H-2^{V} and H-3^{V}), 3.25–3.19 (m, 2 H, CH_2N_3), 3.15–3.12 (m, 1 H, H-5^{IV}), 2.90 (dd, 1 H, $J_{3,4}$ 9.6 Hz and $J_{2,3}$ 2.9 Hz, H-3^{IV}), 2.57 (brs, 1 H, OH), 2.42 (brs, 1 H, OH), 1.80–1.73 (m, 2 H, $CH_2CH_2CH_2N_3$), 1.33 (d, 3 H, $J_{5,6}$ 6.1 Hz, H-6^{IV}); ^{13}C NMR ($CDCl_3$): δ 104.86 (J 160.1 Hz, C–H-1^{V}), 102.18 (J 155.0, C–H-1^{IV}), 100.65 (J 161.2 Hz, C–H-1^{III}). Anal. Calcd for $C_{63}H_{69}N_3O_{17}$ (1140.262): C, 66.36; H, 6.09; N, 3.68. Found: C, 66.50; H, 6.26; N, 3.60.

2-(Trimethylsilyl)ethyl [methyl 5-acetamido-4,7,8,9-tetra-O-*acetyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonate*-(2→3)*]-6*-O-*benzyl-β*-D-*galactopyranosyl*-(1→4)-*2,3-di*-O-*benzyl-β*-L-*rhamnopyranosyl*-(1→4)-*2,3-di*-O-*benzoyl-6*-O-*benzyl-β*-D-*glucopyranoside* (**12**). A suspension of **8** (120 mg, 0.103 mmol), phenyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranoside)onate (**11**) [18,19] (121 mg, 0.21 mmol), and activated 3 Å molecular sieves (600 mg) in CH_3CN (3 mL) was stirred at rt for 3 h under an argon atmosphere, at the end of which time NIS (140 mg, 0.62 mmol) was added. The reaction mixture was stirred for 15 min and chilled to −40 °C, then a solution of TfOH (0.4 mL in 10 mL of dichloromethane) (0.45 mL) was added. The mixture was stirred at −40 °C for

2 h, then overnight at +4 °C. Triethylamine (0.5 mL) was added in the morning to neutralize the reaction, and after further dilution with CH_2Cl_2 (60 mL), some solids were filtered off through Celite. The residue was purified first on a silica gel column, then on a Sephadex LH 20 column using 4:3 $CHCl_3$–MeOH as eluant to give **12** (26 mg, 19%) as a 5:3 α:β mixture: ^{1}H NMR ($CDCl_3$): δ 2.55 (dd, $J_{3,3'}$ 13.7 Hz and $J_{3,4}$ 4.7 Hz, H-3^{I}α), 2.15 (m, H-3$^{I'}$β); FABMS (positive-ion) Anal. Calcd for $C_{85}H_{102}NNaO_{29}Si$: 1652.81. Found: *m*/*z* 1652.62 [M + Na].

3-Azidopropyl [5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-*2-nonulopyranosylonate*-(2→3)*]-6-O-benzyl-β-D-galactopyranosyl*-(1→4)-*2,3-di*-O-*benzyl-β*-L-*rhamnopyranosyl*-(1→4)-*2,3-di*-O-*benzoyl-6*-O-*benzyl-β*-D-*glucopyranoside* (**13**). A suspension of **10** (100 mg, 0.088 mmol) was treated as above for **12**. After dry silica gel column chromatography using 4:1 ethyl acetate–toluene as the eluant, the product was further purified on a Sephadex LH 20 column, using 4:3 $CHCl_3$–MeOH as eluant, and again on silica gel using 11:11:1 ethyl acetate–hexanes–EtOH to yield still impure fractions that were not further characterized.

2-(Trimethylsilyl)ethyl 2,3,4-tri-O-*acetyl-6*-O-*benzyl-β*-D-*galactopyranosyl*-(1→4)-*2,3-di*-O-*benzyl-β*-L-*rhamnopyranosyl*-(1→4)-*2,3-di*-O-*benzoyl-6*-O-*benzyl-β*-D-*glucopyranosyl*-(1→2)-*[methyl-5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonate*-(2→3)*]-6*-O-*benzyl-β*-D-*galactopyranoside* (**16**) *and 2-(Trimethylsilyl)ethyl 2,3,4-tri*-O-*acetyl-6*-O-*benzyl-β*-D-*galactopyranosyl*-(1→4)-*2,3-di*-O-*benzyl-β*-L-*rhamnopyranosyl*-(1→4)-*2,3-di*-O-*benzyl-6*-O-*benzyl-β*-D-*glucopyranosyl*-(1→4)-*[methyl 5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonate*-(2→3)*]-6*-O-*benzyl-β*-D-*galactopyranoside* (**15**). Trisaccharide **6** (450 mg, 0.37 mmol) and 2-(trimethylsilyl)ethyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-6-*O*-benzyl-β-D-galactopyranoside (**14**) [5,20] (325 mg, 0.385 mmol) were dissolved in CH_2Cl_2 (40 mL), and powdered 4 Å molecular sieves (1.1 g) were added. The mixture was maintained under an argon atmosphere and stirred for 2 h, at the end of which time NIS (206 mg, 0.915 mmol) was added. After the reaction mixture was stirred for 15 min, a solution of TfOH (0.38 mL in 10 mL of CH_2Cl_2) was added (0.7 mL). TLC analysis after 5 min showed total consumption of the thioglycoside **6**. Triethylamine (1 mL) was added to neutralize the reaction, and after further dilution with CH_2Cl_2 (100 mL), some solids were filtered off through Celite. The filtrate was washed (2 × 10% $Na_2S_2O_4$, 3 × 0.05 M HCl, 2 × $NaHCO_3$, water, and brine), dried ($MgSO_4$), filtered and concentrated. The residue was purified by silica gel chromatography, using a gradient of 65→75 ethyl acetate–35→25 hexanes as eluent, to give a foamy solid **15** (251 mg, 34%) and impure **16** (178 mg, 24%) that was not further characterized. Data for **15**: $[\alpha]_D$ +4.6° (*c* 0.24, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.9–6.9 (m, 35 H, 7 Ph), 5.64 (t, 1 H, $J_{3,4}$ and $J_{2,3}$ 9.6 Hz, H-3^{III}), 5.38 (m, 2 H, H-4^{V}, and H-8^{I}), 5.27–5.20 (m, 3 H, H-2^{III}, H-7^{I} and NH), 5.08 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1^{III}), 5.02 (m, 1 H, H-2^{V}), 4.96 (m, 1 H, H-4^{I}), 4.89 (dd, 1 H, $J_{2,3}$ 10.3 Hz and $J_{3,4}$ 3.4 Hz, H-3^{V}), 4.82 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^{V}), 4.75 (d, 1 H, *J* 12.2 Hz, CH_2Ph), 4.58–4.41 (m, 8 H, CH_2Ph), 4.39–4.34 (m, 2 H, H-1^{IV} and CH_2Ph), 4.28 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1^{II}), 4.17 (dd, 1 H, $J_{9,9'}$ 12.6 Hz and $J_{8,9}$ 2.6 Hz, H-9^{I}), 4.02 (dd, 1 H, $J_{8,9'}$ 5.1 Hz, H-9′^{I}), 3.98–3.90 (m, 6 H, H-5^{V}, H-4^{III}, H-5^{I}, N-6^{I}, H-3^{V} and CH_2CH_2Si), 3.86–3.83 (m, 3 H, H-4^{II}, H-6^{II} and H-6^{V}), 3.75–3.66 (m, 5 H, OMe, H-5^{III} and H-5^{II}), 3.63–3.56 (m, 4 H, H-4^{IV}, H-6′^{II}, H-6′$^{II'}$ and CH_2CH_2Si), 3.48–3.35 (m, 4 H, H-6^{III}, H-6$^{III'}$, H-2^{IV}, and H-2^{II}), 3.12 (dd, 1 H, $J_{4,5}$ 9.1 Hz and $J_{5,6}$ 6.1 Hz, H-5^{IV}), 2.77 (dd, 1 H, $J_{3,4}$ 9.5 Hz and $J_{2,3}$ 3.0 Hz, H-3^{IV}), 2.69 (dd, 1 H, $J_{3,3'}$ 13.2 Hz and $J_{3,4}$ 4.6 Hz, H-3^{I}), 2.25 (brs, 1 H, OH-2^{II}), 2.06 (s, 3 H, OAc), 2.05 (s, 3 H, OAc), 2.04 (s, 3 H, OAc), 2.01 (s, 3 H, OAc), 1.98 (s, 3 H, OAc), 1.92 (s, 3 H, OAc), 1.88 (s, 3 H, OAc), 1.85 (t, 1 H, $J_{3',4}$ 13.3 Hz, H-3′^{I}), 1.76 (s, 3 H, NAc), 1.27 (d, 3 H, H-6^{IV}), 1.03–0.85 (m, 2 H, CH_2CH_2Si), −0.05 (s, 9 H, $Si(CH_3)_3$); ^{13}C NMR ($CDCl_3$): δ 100.70 (*J* 167.5 Hz, C–H-1^{III}), 100.73 (*J* 169.0 Hz, C–H-1^{V}), 102.22 (*J* 159.8 Hz, C–H-1^{II}), 102.76 (*J* 157.1 Hz, C–H-1^{IV}); FABMS (positive-ion): Anal. Calcd for $C_{104}H_{125}ONNaSi$: 2032.19. Found: *m*/*z* 2032.20 [M + Na].

2-(Trimethylsilyl)ethyl 2,3,4-tri-O-*acetyl*-β-D-*galactopyranosyl*-(1→4)-β-L-*rhamnopyranosyl*-(1→4)-*2,3-di*-O-*benzoyl*-β-D-*glucopyranosyl*-(1→4)-*[methyl 5-acetamido-4,7,8,9-tetra*-O-*acetyl*-*3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonate*-(2→3)*]*-β-D-*galactopyranoside* (**17**). Pentasaccharide **15** (165 mg, 0.082 mmol) in glacial acetic acid (15 mL) was hydrogenated over 10% Pd/C (200 mg). After filtration through Celite and evaporation of the filtrates, the residue was co-concentrated with toluene (2×) to yield a sandy-looking amorphous solid **17** (130 mg, 100%) that was used as such for the next step.

2-(Trimethylsilyl)ethyl β-D-*galactopyranosyl*-(1→4)-β-L-*rhamnopyranosyl*-(1→4)-β-D-*glucopyranosyl*-(1→4)-*[5-acetamido-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonic acid*-(2→3)*]*-β-D-*galactopyranoside* (**18**). To a solution of **17** (21 mg, 0.014 mmol) in MeOH (6 mL) was added 1 M sodium methoxide (1 mL). This solution was stirred at rt for 6 h, then chilled to 0 °C, at which point water (0.5 mL) and 1 M sodium hydroxide (0.1 mL) were added. The mixture was stirred and allowed to come to rt overnight. Additional methanol was added, the mixture was neutralized with Rexyn 101 (H^+) ion-exchange resin and filtered, and the filtrate was concentrated. The residue was placed on a Bio-Gel P-2 column and eluted with water. The fractions were monitored by RI detection. Freeze-drying of the pure fractions gave **18** (11 mg, 78%) as its sodium salt: $[\alpha]_D + 1.9°$ (*c* 0.09, water); MALDIMS: Anal. Calcd for $C_{40}H_{71}O_{28}NNaSi$: 1065.07. Found: *m/z* 1065.07 [M + Na].

3-Azidopropyl 2,3,4,6-tetra-O-*acetyl*-β-D-*galactopyranosyl*-(1→4)-*2,3-di*-O-*acetyl*-β-L-*rhamnopyranosyl*-(1→4)-*6*-O-*acetyl-2,3-di*-O-*benzoyl*-β-D-*glucopyranosyl*-(1→4)-*[methyl 5-acetamido-4,7,8,9-tetra*-O-*acetyl*-*3,5-dideoxy*-D-glycero-α-D-galacto-*nonulopyranosylonate*-(2→3)*]-2,6-di*-O-*acetyl*-β-D-*galactopyranoside* (**22**). To a solution of **17** (130 mg, 0.08 mmol) in pyridine (10 mL) at 0 °C under argon was added dropwise acetic anhydride (5 mL). This mixture was stirred at 0 °C for 3 h, then allowed to come to rt overnight. The mixture was stirred for 40 h, then concentrated and co-concentrated with toluene (2×). After further drying in vacuo a sandy-looking solid was obtained, 2-(trimethylsilyl)ethyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3-di-*O*-acetyl-β-L-rhamnopyranosyl-(1→4)-6-*O*-acetyl-2,3-di-*O*-benzoyl-β-D-glucopyranosyl-(1→4)-[methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)]-β-D-galactopyranoside (**19**, 145 mg, 96%). A solution of **19** (140 mg, 0.077 mmol) in dry CH_2Cl_2 (4.5 mL) was cooled to 0 °C under an argon atmosphere, and trifluoroacetic acid (0.5 mL) was added. TLC analysis indicated that the reaction was complete after stirring for 3 h at 0 °C. Ethyl acetate (5 mL) was added, and the reaction mixture was concentrated, then co-concentrated twice more with ethyl acetate. Purification by silica gel column chromatography of the residue with ethyl acetate as an eluent gave 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3-di-*O*-acetyl-β-L-rhamnopyranosyl-(1→4)-6-*O*-acetyl-2,3-di-*O*-benzoyl-β-D-glucopyranosyl-(1→4)-[methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)]-2,6-di-*O*-acetyl-D-galactopyranose (**20**, 119 mg, 89%) as an amorphous solid. To a solution of **20** (60 mg, 0.035 mmol) in CH_2Cl_2 (15 mL) and trichloroacetonitrile (0.2 mL) under argon atmosphere was added DBU (5 mg) at −5 °C, and the mixture was stirred for 3 h. It was then concentrated, and the residue was purified by silica gel column chromatography using 9:1 ethyl acetate–hexanes as eluant to obtain an amorphous solid, 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3-di-*O*-acetyl-β-L-rhamnopyranosyl-(1→4)-6-*O*-acetyl-2,3-di-*O*-benzoyl-β-D-glucopyranosyl-(1→4)-[methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)]-2,6-di-*O*-acetyl-D-galactopyranosyl trichloroacetimidate (**21**) (57 mg, 87%). To a solution of **21** (57 mg, 0.03 mmol) and 3-azidopropan-1-ol [17] (10 mg, 0.1 mmol), in dry CH_2Cl_2 (4 mL) were added 4 Å molecular sieves (170 mg), and the mixture was stirred at rt for 1.5 h, then cooled to 0 °C under an argon atmosphere. Boron trifluoride etherate (50 μL) was added, and after the mixture had been stirred for 1 h at 0 °C, the reaction was complete. Triethylamine (0.1 mL) was then added, and the suspension was further diluted with

CH_2Cl_2, then filtered through Celite. The filtrate was concentrated, and the residue was purified by silica gel chromatography using 9:1 ethyl acetate–hexanes as an eluant to obtain **22** (42 mg, 77%): $[\alpha]_D + 18.9°$ (*c* 1.1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.93–7.29 (m, 10 H, 2 Ph), 5.50 (t, 1 H, $J_{3,4}$ 9.6 Hz, H-3^{III}), 5.45 (m, 1 H, H-8^{I}), 5.30 (m, 2 H, H–Nu 7 and H-4^{V}), 5.21 (t, 1 H, $J_{2,3}$ 9.8 Hz, H-2^{III}), 5.04–5.01 (m, 3 H, H-2^{V}, H-2^{IV} and NH), 4.89–4.83 (m, 4 H, H-1^{III}, H-4^{I}, H-2^{II} and H-3^{V}), 4.56–4.53 (m, 2 H, H-3^{IV} and H-1^{IV}), 4.50–4.47 (m, 2 H, H-1^{V} and H-4^{II}), 4.38 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^{II}), 4.34 (bdd, 1 H, H-9^{I}), 4.23–4.02 (m, 8 H, H-3^{II}, H-6^{III}, H-4^{III}, H-6^{II}, H-$6'^{III}$, H-$6'^{II}$, H-6^{V} and H-$6'^{II}$), 3.98–3.92 (m, 2 H, H-5^{I} and H-$9'^{I}$), 3.85–3.82 (m, 4 H, OMe, and H-5^{V}), 3.73–3.70 (m, 2 H, H-6^{I} and $OCH_2CH_2CH_2N$), 3.59 (m, 2 H, H-5^{III} and H-5^{V}), 3.46 (m, 1 H, $CH_2CH_2CH_2N$), 3.42 (t, 1 H, $J_{3,4}$ and $J_{4,5}$ 9.3 Hz, H-4^{IV}), 3.28–3.21 (m, 3 H, H-5^{IV} and $CH_2CH_2CH_2N$), 2.57 (dd, 1 H, $J_{3,3'}$ 12.6 Hz and $J_{3,4}$ 4.8 Hz, H-3^{I}), 2.12 (brs, 9 H, 3OAc), 2.09 (s, 3 H, OAc), 2.06 (s, 3 H, OAc), 2.04 (s, 3 H, OAc), 2.03 (s, 3 H, OAc), 2.02 (s, 3 H, OAc), 2.00 (brs, 6 H, 2OAc), 1.93 (s, 3 H, OAc), 1.92 (s, 3 H, OAc), 1.87 (s, 3 H, OAc), 1.85 (s, 3 H, NAc), 1.79–1.63 (m, 2 H, $CH_2CH_2CH_2$), 1.59 (t, 1 H, $J_{3',4}$ 12.4, H-$3'^{I}$), 1.31 (d, 3 H, $J_{5,6}$ 6.1 Hz, H-6^{IV}); ^{13}C NMR ($CDCl_3$): δ 101.09 (*J* 162.0 Hz, C–H-1^{V}), 100.63, (*J* 165.1 Hz, C–H-1^{II}) 100.42, (*J* 165.2 Hz, C–H-1^{III}), 97.92 (*J* 157.8 Hz, C–H-1^{IV}); ESIMS (positive-ion) Anal. Calcd for $C_{79}H_{100}N_4NaO_{43}$: 1815.6. Found: *m/z* 1815.5 [M + Na].

3-Azidopropyl β-D-*galactopyranosyl*-(1→4)-β-L-*rhamnopyranosyl*-(1→4)-β-D-*glucopyranosyl*-(1→4)-*[5-acetamido-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonic acid*-(2→3)*]*-β-D-*galactopyranoside* (**23**). To a solution of **22** (26 mg, 0.0145 mmol) in dry MeOH (5 mL) was added 1 M sodium methoxide in MeOH (1 mL). This solution was stirred at rt for 6 h, then chilled to 0 °C, and water (1 mL) was added, followed by 1 M NaOH (0.2 mL). This solution was stirred overnight, then further diluted with MeOH, and the reaction was neutralized with Rexyn 101 (H^+) ion-exchange resin and concentrated. The residue was placed on a Bio-Gel P2 column and eluted with water (RI monitoring). Freeze-drying of the pure fractions gave **23** (12 mg, 81%) as its sodium salt: $[\alpha]_D + 17.1°$ (*c* 0.33, water); MALDIMS Anal. Calcd for $C_{38}H_{64}N_4NaO_{28}$: 1047.94. Found: *m/z* 1047.17 [M + Na].

3-Azidopropyl (5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-*2-nonulopyranosylonic acid)*-(2→3)-β-D-*galactopyranosyl*-(1→4)-β-L-*rhamnopyranosyl*-(1→4)-β-D-*glucopyranosyl*-(1→4)-*[5-acetamido-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonic acid*-(2→3)*]*-β-D-*galactopyranoside* (**2**). Pentasaccharide **23** (2.0 mg; μmol) was sialylated using 0.45 U of an α-2,3-sialyltransferase (CST-06) from *C. jejuni*. The reaction mixture included 100 mM of Tris pH 7, 10 mM of $MgCl_2$, 8 mM of β-Neu5AcCMP, and 10 units of alkaline phosphatase. The reaction was performed at 37 °C for 3 h in a final volume of 0.3 mL. Product formation was followed by TLC on silica using 2-propanol–butanol–0.1 M HCl (2:1:1) as the developing solvent (R_f 0.2). The residue was placed on a Bio-Gel P2 column and eluted with water (RI detection). Freeze-drying of the pure fractions gave **2** (2.3 mg, 85%) as its disodium salt: $[\alpha]_D + 22.1°$ (*c* 0.1, water); ESIMS (positive-ion) Anal. Calcd for $C_{49}H_{81}N_5Na_3O_{36}$: 1382.20. Found: *m/z* 1382.29 [M + 3Na].

2-(Trimethylsilyl)ethyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-*2-nonulopyranosylonic acid*-(2→3)-β-D-*galactopyranosyl*-(1→4)-β-L-*rhamnopyranosyl*-(1→4)-β-D-*glucopyranosyl*-(1→4)-*[5-acetamido-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonic acid*-(2→3)*]*-β-D-*galactopyranoside* (**1**). Pentasaccharide **18** (2.0 mg; 1.9 μmol) was treated as above for **23** to **2**. ^1H NMR analysis of the fractions from Bio-Gel P2 indicated some CMP-derived impurities that were removed by passing through a C_{18} Sep-Pak cartridge loading in water and eluting successively with 10% CH_3OH_{aq}, 50% CH_3OH_{aq} and 100% CH_3OH to yield **1** (0.7 mg, 26%) as its disodium salt: $[\alpha]_D + 17.1°$ (*c* 0.06, water); ESIMS (positive-ion) Anal. Calcd for $C_{51}H_{87}N_2NaO_{36}Si$: 1355.34. Found: *m/z* 1355.36 [M + Na], 1377.13 [M + 2Na], 1398.92 [M + 3Na].

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