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Polymer-supported and chemoenzymatic synthesis of the *Neisseria meningitidis* pentasaccharide: a methodological comparison

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Abstract

Neisseria meningitidis trisaccharide [GlcNAcβ(1→3)Galβ(1→4)Glc-R], tetrasaccharide [Galβ(1→4)GlcNAcβ(1→3)Galβ(1→4)Glc-R], and a pentasaccharide [Neu5Acα(2→3)Galβ(1→4)GlcNAcβ(1→3)Galβ(1→4)Glc-SPh] were prepared via conventional chemical synthesis, polymer-supported synthesis, and chemoenzymatic methods, starting from D-lactose. The polymer polyethyleneglycol monomethylether (MPEG) and the linker dioxyxylene (DOX) were used with a lactose-bound acceptor to improve the purification process. Several enzymes (*LgtA*, *GalE-LgtB* fusion, and CMP-Neu5Ac synthetase/sialyltransferase fusion) were used for syntheses of these oligosaccharides. Excellent stereo- and regioselectivities as well as high yield (>90% from Galβ(1→4)Glc-SPh) of the pentasaccharide were obtained. Both of the convenient processes are suitable for efficient preparation of target oligosaccharides.

Keywords: Polymer-supported synthesis; Chemoenzymatic synthesis; Glycosyltransferase; *Neisseria meningitidis* LOS

1. Introduction

The emerging understanding of the critical role of oligosaccharides in cellular biological recognition events and the promise of therapeutics based on oligosaccharides creates an urgent need for an efficient synthetic methodology for oligosaccharides [1]. Although the progress made in chemical oligosaccharide synthesis in recent years has been remarkable [2], oligosaccharide synthesis is still difficult and time-consuming. These problems are mainly due to the requirement of multistep transformations involving iterative protection–glycosylation–deprotection reaction sequences with chromatographic purification of intermediates at each stage of the synthesis. Such preparations would greatly benefit from developments in polymer-supported and chemoenzymatic oligosaccharide synthetic strategies. Polymer-supported syntheses offer many advantages over solution-phase reactions including increased speed of synthesis, due to the minimization of purification processes [3]. Polymer-supported methods based on a soluble polymer polyethyleneglycol monomethylether [MeO–$(CH_2CH_2O)_n$H, average M_w 5000], MPEG, have been studied in our group in order to synthesize oligosaccharide fragments of the serotype specific capsu-

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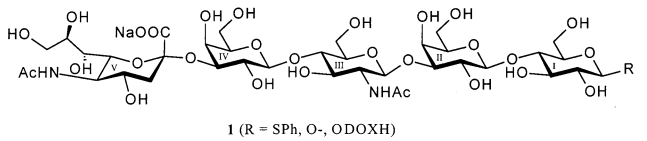
lar polysaccharide as a part of the process towards developing vaccines against Group B *Streptococcus* bacteria [4]. On the other hand, as more glycosyltransferases become commercially available, enzymatic synthesis is becoming an alternative for the preparation of oligosaccharides [5]. By using enzymes, glycosylations can be performed under mild reaction conditions with high stereo- and regioselectivity, without the need to protect the functional groups of the sugars. Both methods can facilitate large-scale synthesis of oligosaccharides.

Our interest is directed toward an efficient approach to the synthesis of *Neisseria meningitidis* pentasaccharide [Neu5Acα(2→3)-Galβ(1→4)GlcNAcβ(1→3)Galβ(1→4)Glc-R] (**1**) (Fig. 1) [6]. This pentasaccharide is the upstream terminus of the *N. meningitidis* lipooligosaccharide (LOS), and it has been shown to play an important role in the pathogenesis of disease caused by these organisms [7]. A process for synthesizing these oligosaccharides is clearly needed to enable widespread use. Several papers have been published describing the chemical syntheses of lacto-*N*-neotetraose and similar structures [8]. Also, both glycosyltransferases and glycosylases have been used, but the yields of the enzymatic reaction were low (10–30%) [9,10]. In this communication, polymer-supported and chemoenzymatic methods for the preparation of the *N. meningitidis* pentasaccharide **1** are compared with classical chemical methods.

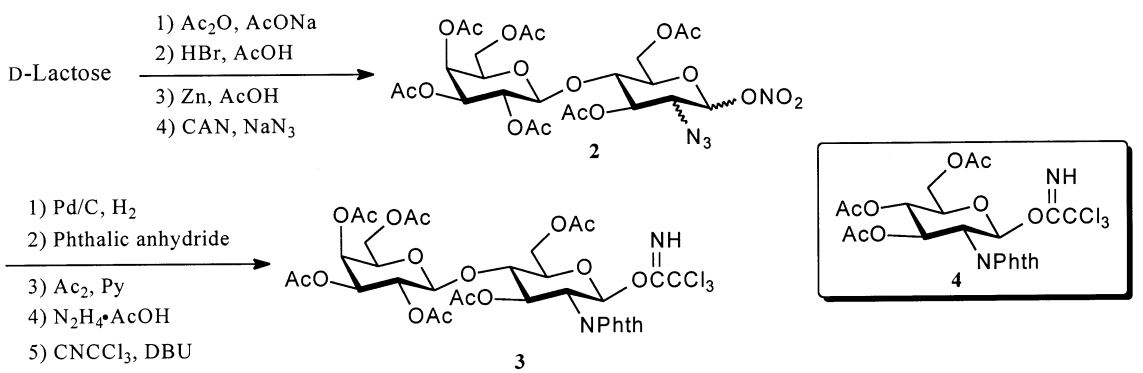


1 (R = SPh, O-, ODOXH)

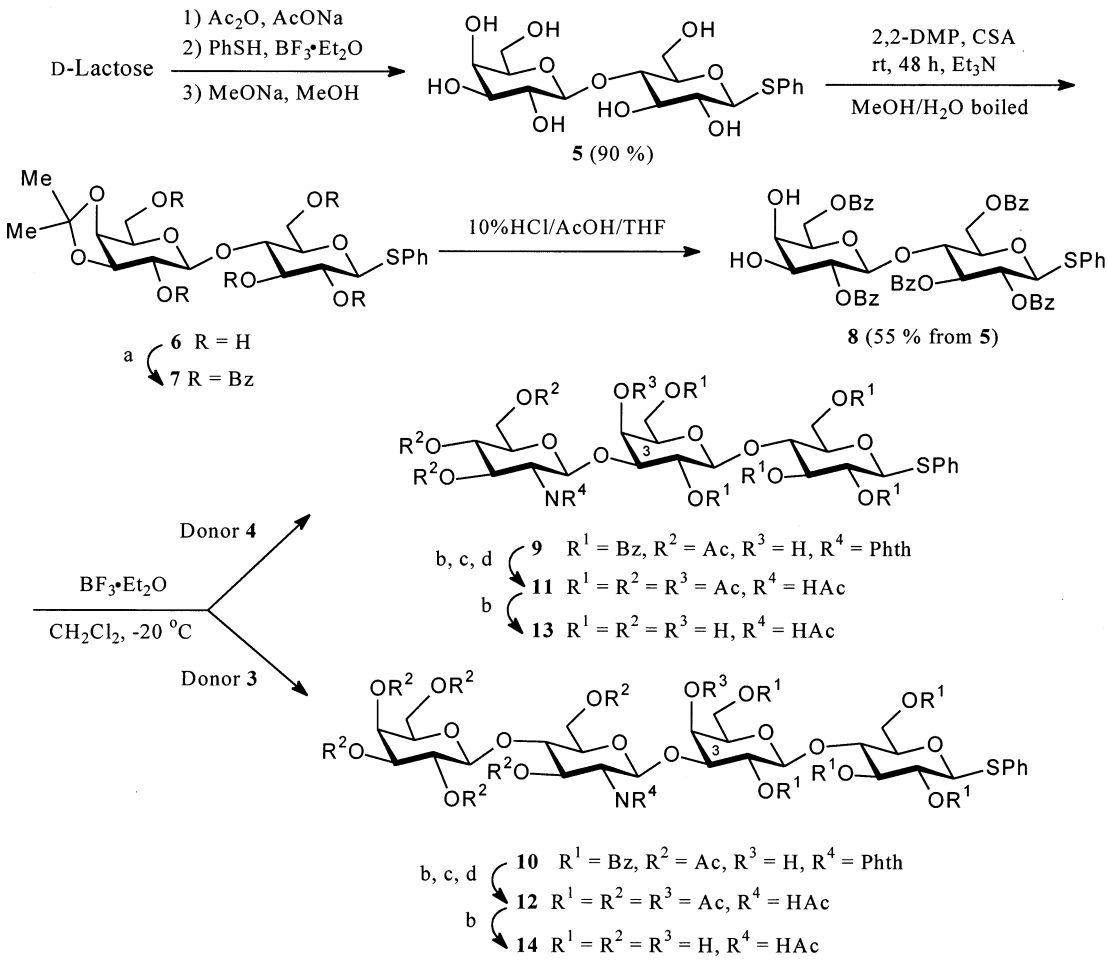
Fig. 1. *Neisseria meningitidis* pentasaccharide **1**.

2. Results and discussion

For comparison, trisaccharide **13** and tetrasaccharide **14** were prepared by conventional chemical methods (Scheme 2). The key step was the formation of the GlcNAcβ(1→3)Gal glycosidic linkage. For this purpose, trichloroacetimidate **3** [11–13] (Scheme 1) containing a 2-*N*-phthalimido group, was selected as a glycosyl donor. The phthalimido group aids the stereoselectivity, both by neighboring group participation and by its bulkiness. Monosaccharide glycosyl donor **4** was prepared similarly [14]. The diol acceptor **8** [15] (50%) was prepared from D-lactose via its phenylthio derivative **5** [16a] and the 3,4-*O*-isopropylidene derivative **6** [16b]. Benzoylation of the free OH groups in **6**, followed by deprotection of the isopropylidene group (1 M HCl–AcOH–THF) gave the diol acceptor **8**. The donor (**4** or **3**) and the diol acceptor **8** were then coupled through a regioselective Schmidt glycosylation [9] with $BF_3{\cdot}Et_2O$ to give only β(1→3) trisaccharide **9** or β(1→3) tetrasaccharide **10** in 40–45% isolated yields. These low yields are the result of the losses during repeated chromatography. The β(1→3) glycosidic linkage was determined first by 1D and 2D NMR spectroscopy (gCOSY and



Scheme 1.



Scheme 2. Reagents: (a) BzCl, Py; (b) MeONa, MeOH; (c) NH_2NH_2; (d) Ac_2O, Py.

gHSQC) (**9**: $\delta^{III}_{H\text{-}1}$, 5.48 ppm, $J_{1,2}$ 8.5 Hz and $\delta^{III}_{C\text{-}1}$, 98.58 ppm; **10**: $\delta^{III}_{H\text{-}1}$, 5.48 ppm, $J_{1,2}$ 8.5 Hz and $\delta^{III}_{C\text{-}1}$, 98.15 ppm) and confirmed by $^1H-^{13}C$ gHMBC NMR spectroscopy, which clearly showed that GlcNAc C-1^{III} is connected to Gal H-3^{II} in both **9** and **10**. The sequence deprotection–acetylation–deprotection of **9** and **10** afforded trisaccharide **13** (75%) and tetrasaccharide **14** (66%). The ^1H and ^{13}C NMR spectra in D_2O of **13** and **14** could be completely assigned (Tables 1 and 2). Evidently, this is a quite laborious and time consuming protocol involving extensive purification by chromatography and is not suitable for a large-scale synthesis.

As a solution to this problem, MPEG polymer-supported strategy was examined. The MPEG strategy is based on the carbohydrate-linker–MPEG synthon that is soluble under reaction conditions, but insoluble during work up, while all impurities are soluble [17]. The dioxyxylene $-CH_2C_6H_4CH_2O-$ (DOX), introduced by Krepinsky et al. [18], is a superior linker because it can be bound via an ether linkage, it is stable under most reaction conditions, and is removable from final oligosaccharides. Thus, the phenylthio derivative **7** was glycosylated with alcohol MPEG-DOX-OH under NIS–AgOTf conditions, then the 3,4-*O*-isopropylidene group was cleaved (60% AcOH) to give a nearly quantitative yield of the polymer-bound diol acceptor **15** (Scheme 3). Glycosylation of **15** with **4** or **3**, under the same reaction conditions as the solution method ($BF_3{\cdot}Et_2O$, -20 °C), led to the β(1 → 3) linked **16** or **17** polymer-bound tri- or tetrasaccharide in a high yield (based on the acceptor). Unreacted diol acceptor **15** (about 10%) was also present in each of the glycosylation reactions, and it was isolated as peracetylated compound **21** after the cleavage step (Scheme 3).

In order to characterize these compounds, the products were cleaved from the polymer using the recently developed protocol involving $Sc(OTf)_3$–Ac_2O [19]. The cleavage occurs at the C–O bond between the benzylic carbon of the DOX and the terminal oxygen of the

Table 1
^{1}H NMR chemical shifts [a] in ppm for tri-, tetra-, and pentasaccharides **13**, **14**, **20**, and **1**

13	I	II	III		
H-1	4.86 (10.0)	4.47 (8.0)	4.71 (8.0)		
H-2	3.45	3.61	3.78		
H-3	3.70	3.75	3.60		
H-4	3.72	4.18 (2.5)	3.50		
H-5	3.67	3.76	3.48		
H-6	4.00	3.81	3.93		
H-6′	3.83	3.77	3.79		
Other resonances:					
SPh 7.62 (d, 2 H, Ph), 7.48–7.41 (m, 3 H, Ph)					
2-NHAc 2.06					
14	I	II	III	IV	
H-1	4.86 (10.0)	4.47 (7.5)	4.73 (8.5)	4.51 (7.5)	
H-2	3.44	3.61	3.83	3.57	
H-3	3.71	3.75	3.75	3.70	
H-4	3.69	4.18	3.77	3.96	
H-5	3.66	3.74	3.61	3.73	
H-6	3.99	3.83	3.98	3.83	
H-6′	3.82	3.76	3.90	3.76	
Other resonances:					
SPh 7.62 (d, 2 H, Ph), 7.48–7.41 (m, 3 H, Ph)					
2-NHAc 2.06					
20	I	II	III	IV	
H-1	4.58 (7.5)	4.47 (8.0)	4.74 (8.0)	4.51 (8.0)	
H-2	3.38	3.61	3.84	3.57	
H-3	3.65	3.75	3.76	3.70	
H-4	3.66	4.18	3.77	3.96	
H-5	3.61	3.75	3.62	3.73	
H-6	4.01	3.84	3.99	3.84	
H-6′	3.83	3.76	3.88	3.76	
Other resonances:					
CH_2PhOCH_2OH 7.50, 7.46, 4.97 (d, CH_2Ph), 4.80 (d, CH_2Ph), 4.68 (s, CH_2OH)					
2-NHAc 2.07					
1	I	II	III	IV	V
H-1	4.84 (10.0)	4.45 (7.5)	4.71 (8.0)	4.57 (7.5)	
H-2	3.42	3.60.	3.82	3.59	
H-3	3.70	3.74	3.77	4.14	1.82 (13.0)
H-3′					2.78 (12.5, 5.0)
H-4	3.68	4.17	3.74	3.98	3.71
H-5	3.65	3.74	3.59	3.79	3.87
H-6	3.98	3.76	3.98	3.65	3.65
H-6′	3.81	3.65	3.89	3.76	
H-7					3.60
H-8					3.90
H-9					3.89
H-9′					3.65
Other resonances:					
SPh 7.61 (d, 2 H, Ph), 7.46–7.41 (m, 3 H, Ph)					
2-NHAc 2.06; 5-NHAc 2.05					

[a] Recorded at 500 MHz in D_2O at 300 K.

Table 2
^{13}C NMR chemical shifts [a] (δ) in ppm for tri-, tetra-, and pentasaccharides **13**, **14**, **20**, **1**

13	I	II	III
C-1	88.14	103.86	103.86
C-2	72.45	71.03	56.70
C-3	78.91	75.92	74.59
C-4	76.80	69.38	70.71
C-5	79.73	82.95	76.68
C-6	61.14	62.00	61.53
Other resonances:			
SPh 132.84, 132.62, 130.30, 129.11			
2-NHAc 175.88, 23.24			

14	I	II	III	IV
C-1	88.12	103.86	103.74	103.86
C-2	72.42	70.95	56.18	71.96
C-3	73.50	76.34	73.17	76.76
C-4	78.88	69.34	79.54	69.54
C-5	79.70	83.02	75.54	75.87
C-6	61.08	61.96	60.86	61.96
Other resonances:				
SPh 132.87, 132.65, 130.33, 129.14				
2-NHAc 175.90, 23.16				

20	I	II	III	IV
C-1	101.70	103.57	103.44	103.56
C-2	73.50	70.65	55.89	71.66
C-3	73.10	76.04	72.88	73.20
C-4	79.06	69.02	78.87	69.24
C-5	75.48	82.73	75.25	75.57
C-6	61.43	61.72	61.63	61.72
Other resonances:				
CH_2PhOCH_2OH 141.05, 136.66, 129.72, 128.35, 71.94 (C, CH_2Ph), 64.29 (C, OCH_2OH)				
2-NHAc 175.61, 22.87				

1	I	II	III	IV	V
C-1	88.24	104.00	103.93	103.69	174.98
C-2	72.55	71.09	56.30	70.51	100.93
C-3	76.90	76.22	73.26	76.62	40.78
C-4	79.03	69.74	76.30	68.60	69.74
C-5	79.84	83.14	75.68	79.15	52.82
C-6	61.23	62.13	60.97	62.13	74.02
C-7					69.22
C-8					72.89
C-9					63.70
Other resonances:					
SPh 133.00, 132.77, 130.44, 129.25					
2-NHAc 175.98, 23.14; 5-NHAc 176.13, 23.28					

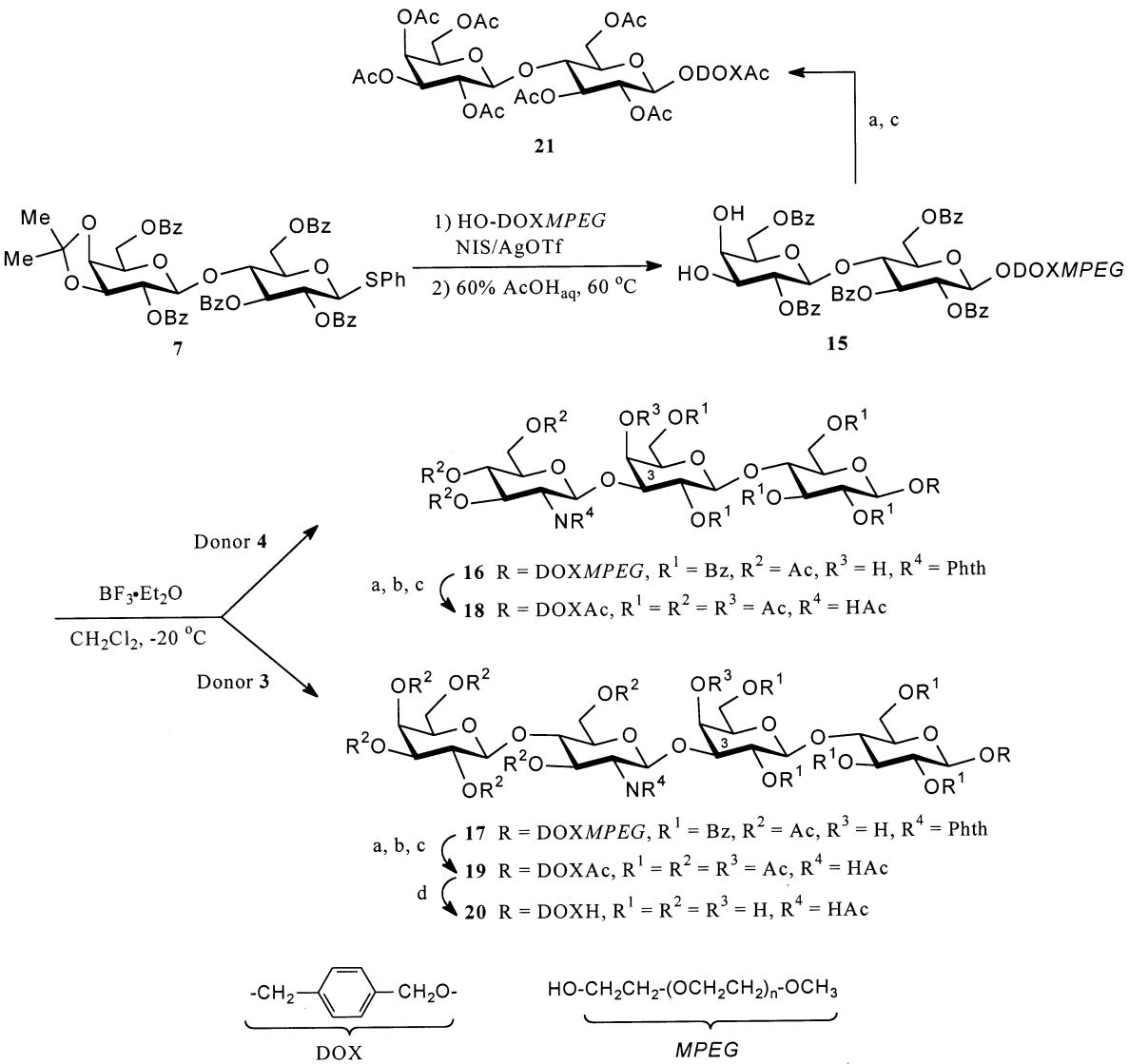
[a] Recorded at 75 MHz in D_2O at 300 K.

MPEG while all hydroxyls are acetylated. First, the *O*- and *N*-acyl groups in **16** and **17** were removed by treatment with 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU), followed by ethylenediamine. The product was cleaved from the polymer with $Sc(OTf)_3$–Ac_2O to give peracetylated trisaccharide **18** (52%) and tetrasaccharide **19** (53%). The stereo- and regiochemistry of β(1→3) linked **18** and **19** was confirmed by 1D and 2D NMR spectroscopy (gCOSY, gHSQC, and gHMBC) (**18**: $\delta^{III}_{H\text{-}1}$, 5.01 ppm, $J_{1,2}$ 8.0 Hz and $\delta^{III}_{C\text{-}1}$, 99.49 ppm; **19**: $\delta^{III}_{H\text{-}1}$, 5.19 ppm, $J_{1,2}$ 8.0 Hz and $\delta^{III}_{C\text{-}1}$, 100.19 ppm). Then, **19** was converted to the final tetrasaccharide **20** with NaOMe–MeOH in an 87% yield. The assignment of the ^1H and ^{13}C NMR spectra of **20** is shown in Tables 1 and 2. Since elaborate and extensive chromatography was avoided in the purification process, this polymer-supported method is suitable for synthesis of large quantities of oligosaccharides. Typical laboratory scales are 50 mg–50 g of MPEG. Larger scales are mainly limited by apparatus size and not the chemistry [20].

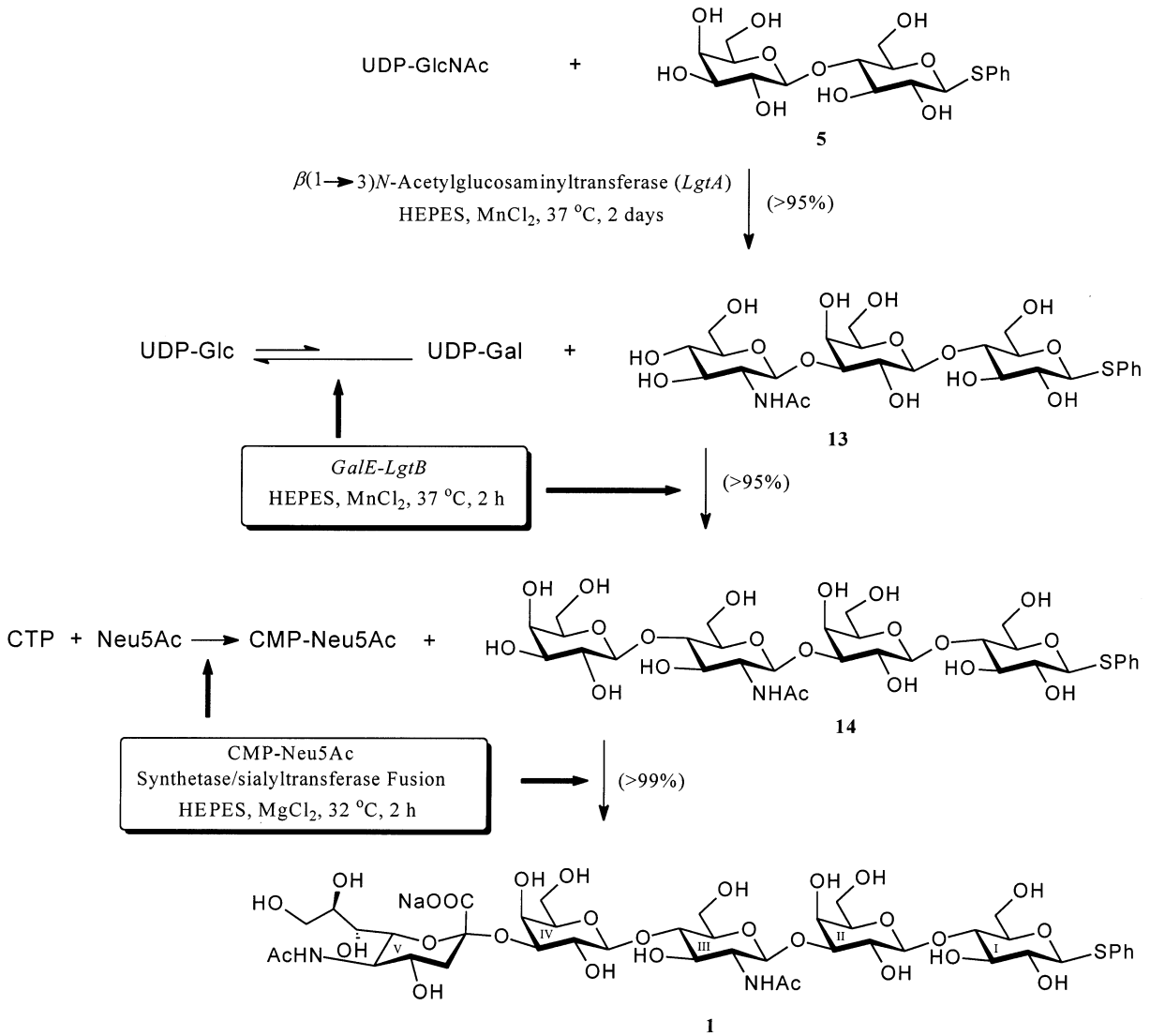
The enzymatic method is a powerful tool for oligosaccharide synthesis. Several *Neisserial* glycosyltransferases such as β(1→3)-*N*-acetylglucosaminyl transferase (*LgtA*), β(1→4)-galactosyltransferase (*LgtB*), UDP-galactose-4-epimerase (*GalE*), CMP-Neu5Ac-synthetase, and α(2→3)-sialyltransferase from *N. meningitidis* have been cloned and overexpressed in *Escherichia coli* in our institute [7]. These enzymes can catalyze the transfer of an oligosaccharide from a sugar nucleotide donor (e.g., UDP-GlcNAc, UDP-Glc, and CMP-Neu5Ac) to an acceptor. The lactose derivative phenyl β-D-galactopyranosyl-(1→4)-1-thio-β-D-glucopyranoside (**5**) was chosen as an initial acceptor substrate for enzymatic synthesis of trisaccharide **13**. The phenylthio group was purposefully introduced to aid in monitoring of the separation of the target product by C_{18} reversed-phase chromatography and for further chemical or enzymatic manipulation. The β(1→3)-*N*-acetylglucosaminyltransferase (*LgtA*) catalyzed glycosylation used UDP-GlcNAc as a donor and **5** as an acceptor to give stereo- and regioselectively the β(1→3) linked trisaccharide **13** in an 95% yield (see Scheme 4). The pure compound **13** was obtained by a single C_{18} reversed-phase chromatography using 17:3–4:1 water–MeOH as eluent, followed by freeze-drying. Subsequently, the tetrasaccharide Galβ(1→4)GlcNAcβ(1→3)Galβ(1→4)Glc-SPh (**14**) was constructed. A fusion protein *GalE-LgtB* [21] was used as a catalyst with UDP-glucose (UDP-Glc) as a glycosyl donor. This fusion

enzyme allows the relatively inexpensive UDP-Glc to be converted to the more expensive UDP-Gal under equilibrium conditions, with the enzyme *GalE* as the catalyst. The equilibrium is in favor of the formation of UDP-Glc (7:1), but the equilibrium is driven by the UDP-Gal being consumed by the transferase *LgtB* to give the β(1→4) linked tetrasaccharide **14**. The enzymes showed excellent stereo- and regioselectivity (see Scheme 4). The desired compound **14** was isolated and purified in 96% yield using a C_{18} reversed-phase column (9:1–17:3 water–MeOH).

Since the chemical sialylation is one of the most difficult reactions in synthetic carbohydrate chemistry, sialylation with sialyltransferases is a valuable alternative [22]. Sialyltransferases transfer sialic acid from cytidine 5′-monophospho *N*-acetylneuraminic acid (CMP-Neu5Ac) onto the specific hydroxyl group of the acceptor sugar. CMP-Neu5Ac is costly and unstable, and therefore CMP-Neu5Ac was generated in situ by CMP-Neu5Ac synthetase. Also, the α(2→3)-sialyltransferase from *N. meningitidis* as expressed from *E. coli* is relatively insoluble and has a tendency to precipitate with the loss of activity during storage. Thus, a fused form of these two enzymes has been constructed and expressed in *E. coli* [23]. tetrasaccharide **14** was enzymatically glycosylated using in situ generation of CMP-Neu5Ac from CTP and sialic acid by this fusion enzyme. The reaction was very fast and complete in 2 h at 32 °C. The



Scheme 3. Reagents: (a) DBU, MeOH; (b) ethylenediamine; (c) $Sc(OTf)_3$, Ac_2O; (d) MeONa, MeOH.



Scheme 4. Enzymatic synthesis of *N. meningitidis* pentasaccharide **1**.

final pentasaccharide **1** was successfully isolated using C_{18} reversed-phase chromatography by controlled elution with water and then 99:1 water–MeOH. The yield of **1** was nearly quantitative. Complete ^{1}H and ^{13}C NMR assignments for **1** were achieved based on various 1D and 2D NMR experiments (1D TOCSY, gCOSY, gHSQC, gHMBC) (Tables 1 and 2). The H-3 resonance of Gal^{IV} in **1** (4.14 ppm) versus **14** (3.79 ppm) shows a large downfield shift confirming the $\alpha(2\rightarrow3)$ linkage in compound **1**.

Thus, we developed three methodologies for the syntheses of tri-, tetrasaccharides, and sialylated pentasaccharide (**13**, **14**, **20** and **1**). Polymer-supported and chemoenzymatic methodologies are powerful tools for these oligosaccharide syntheses. Notable a yield of nearly 80% of *N. meningitidis* pentasaccharide **1** from D-lactose was obtained via the chemoenzymatic method. Similar chemoenzymatic syntheses have been easily performed on gram scales in our laboratory [24]. The synthesized oligosaccharides are useful as starting substances for more complex molecules and will be used in the biophysical studies of the transferases used for their synthesis.

3. Experimental

General methods.—Melting points were measured on a Buchi 535 melting point apparatus and were not corrected. Optical rotations were obtained ($\lambda = 589$ nm) at 20 °C in a

10-cm, 1-mL cell using a Perkin–Elmer 243 polarimeter. NMR spectra were recorded on INOVA-500 or INOVA-200 instrument at 300 K. Chemical shifts were given in ppm relative to the signal of internal TMS or indirectly to solvent signals 7.26 ($CDCl_3$) or 4.81 (D_2O) for ^1H NMR spectra, and to the solvent signals 77.0 ($CDCl_3$) or 49.15 (internal methyl alcohol) for ^{13}C NMR spectra. All signal assignments were made by standard ^1H–^1H COSY and ^1H-decoupled ^{13}C–^1H COSY experiments. MALDIMS spectra were taken on a Voyager-De STR Biochemistry Workstation (PerSeptive Biosystems, Framingham, MA, USA). Thin-layer chromatography (TLC) was performed on E. Merck Silica Gel 60 F_{254} plates. Silica gel (230–400 mesh) was used for flash chromatography. C_{18} Silica gel (10% capped with TMS, 35–70 mesh) was used for reversed-phase chromatography.

Phenyl 2,6-di-O-*benzoyl-β*-D-*galactopyranosyl-(1→4)-2,3,6-tri*-O-*benzoyl-1-thio-β*-D-*glucopyranoside* (**8**).—The acceptor **8** was prepared in six steps starting from D-lactose [9,15,16]. Mp 117–119 °C; $[\alpha]_D$ + 39.0° (*c* 0.4, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.10–7.10 (m 30 H, 6Ph), 5.63 (t, 1 H, *J* 9.5 Hz, H-3^I), 5.39 (t, 1 H, *J* 9.5 Hz, H-2^I), 5.34 (t, 1 H, *J* 7.5 Hz, H-2^{II}), 4.83 (d, 1 H, *J* 10.0 Hz, H-1^I), 4.60 (d, 1 H, *J* 11.5 Hz, H-6^I), 4.59 (d, 1 H, *J* 8.0 Hz, H-1^{II}), 4.50 (dd, 1 H, *J* 11.5 Hz, 5.0 Hz, H-6^I), 4.04 (t, 1 H, *J* 9.5 Hz, H-4^I), 3.98 (dd, 1 H, *J* 11.0 Hz, 5.0 Hz, H-6^{II}), 3.88–3.80 (m, 1 H, H-5^I), 3.83 (d, 1 H, *J* 4.0 Hz, H-4^{II}), 3.71 (dd, 1 H, *J* 9.5 Hz, 3.0 Hz, H-3^{II}), 3.58–3.48 (m, 2 H, H-5^{II} and H-6^{II}); ^{13}C NMR ($CDCl_3$): δ 166.36 (Bz), 166.03 (Bz), 165.95 (Bz), 165.87 (Bz), 165.15 (Bz), 100.96 (C-1^{II}), 85.78 (C-1^I), 77.00 (C-5^I), 76.20 (C-4^I), 74.20 (C-3^I), 73.65 (C-2^{II}), 72.58 (C-5^{II}) 72.58 (C-3^{II}), 70.17 (C-2^I), 68.56 (C-4^{II}), 62.85 (C-6^I), 61.75 (C-6^{II}); MALDIMS: Anal. Calcd for $C_{53}H_{46}O_{15}SNa$: 977.24. Found: *m*/*z* 977.16 [M + Na].

Phenyl 3,4,6-tri-O-*acetyl-2-deoxy-2-phthalimido-β*-D-*glucopyranosyl-(1→3)-2,6-di*-O-*benzoyl-β*-D-*galactopyranosyl-(1→4)-2,3,6-tri*-O-*benzoyl-1-thio-β*-D-*glucopyranoside* (**9**).—The mixture of monosaccharide donor **4** (0.25 g, 0.26 mmol) and acceptor **8** (0.18 g, 0.31 mmol) was dried under high vacuum overnight. After cooling to −20 °C under an atmosphere of argon, CH_2Cl_2 (10 mL) was added, followed by $BF_3{\cdot}Et_2O$ (33 μL), and the mixture was stirred for 4 h. The reaction was quenched with several drops of Et_3N, and the mixture was concentrated under reduced pressure. Chromatography thrice afforded **9** (0.16 g, 42%). Mp 104–106 °C; $[\alpha]_D$ + 56.6° (*c* 0.6, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.10–7.02 (m, 34 H), 5.62 (t, 1 H, *J* 8.0 Hz, H-3^I), 5.60 (t, 1 H, *J* 9.0 Hz, H-3^{III}), 5.48 (d, 1 H, *J* 8.5 Hz, H-1^{III}), 5.33 (t, 1 H, *J* 10.0 Hz, H-2^I), 5.25 (t, 1 H, *J* 9.5 Hz, H-2^{II}), 5.06 (t, 1 H, *J* 9.5 Hz, H-4^{III}), 4.78 (d, 1 H, *J* 10.0 Hz, H-1^I), 4.46 (d, 1 H, *J* 8.0 Hz, H-1^{II}), 4.37 (d, 1 H, *J* 12.0 Hz, H-6^I), 4.28 (dd, 1 H, *J* 11.5 Hz, 5.5 Hz, H-6^I), 4.25 (dd, 1 H, *J* 11.0 Hz, 9.5 Hz, H-2^{III}), 4.20–4.13 (m, 2 H, H-6^{II} and H-6^{III}), 4.10 (d, 1 H, *J* 10.5 Hz, H-6^{III}), 4.00 (s, 1 H, H-4^{II}), 3.97 (t, 1 H, *J* 9.5 Hz, H-4^I), 3.84–3.78 (m, 1 H, H-5^{III}), 3.71 (dd, 1 H, *J* 10.0 Hz, 3.0 Hz, H-3^{II}), 3.68–3.63 (m, 1 H, H-5^I), 3.63–3.58 (m, 1 H, H-6^{II}), 3.56–3.49 (m, 1 H, H-5^{II}), 2.00 (s, 3 H, CH_3), 1.98 (s, 3 H, CH_3), 1.74 (s, 3 H, CH_3); ^{13}C NMR ($CDCl_3$): δ 170.47 (Ac), 169.89 (Ac), 169.26 (Ac), 167.60 (Phth), 166.50 (Phth), 165.98 (Bz), 165.71 (Bz), 165.39 (Bz), 165.11 (Bz), 163.95 (Bz), 100.52 (C-1^{II}), 98.58 (C-1^{III}), 85.73 (C-1^I), 81.20 (C-3^{II}), 76.86 (C-5^I), 75.37 (C-4^I), 73.72 (C-3^I), 72.19 (C-5^{II}), 72.02 (C-5^{III}), 70.46 (C-2^{II}), 70.32 (C-2^I), 70.22 (C-3^{III}), 68.60 (C-4^{III}), 67.81 (C-4^{II}), 62.64 (C-6^I), 62.63 (C-6^{II}), 61.78 (C-6^{III}), 54.17 (C-2^{III}); Anal. Calcd for $C_{73}H_{65}NO_{24}S$ (1372.38): C, 63.38; H, 4.77; N, 1.02. Found: C, 63.22; H, 4.43; N, 1.01.

Phenyl 2,3,4,6-tetra-O-*acetyl-β*-D-*galactopyranosyl)-(1→4)-3,6-di*-O-*acetyl-2-deoxy-2-phthalimido-β*-D-*glucopyranosyl-(1→3)-2,6-di*-O-*benzoyl-β*-D-*galactopyranosyl-(1→4)-2,3,6-tri*-O-*benzoyl-1-thio-β*-D-*glucopyranoside* (**10**).—Disaccharide donor **3** and receptor **8** were treated with $BF_3{\cdot}Et_2O$, as described above for **9**, to yield tetrasaccharide **10** (0.26 g, 40%). Mp 149–151 °C; $[\alpha]_D$ + 48.5° (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.09–6.99 (m, 34 H, Ph), 5.62 (t, 1 H, *J* 9.0 Hz, H-3^I), 5.55 (dd, 1 H, *J* 10.0 Hz, 8.0 Hz, H-3^{III}), 5.48 (d, 1 H, *J* 8.5 Hz, H-1^{III}), 5.32 (t, 1 H, *J* 9.5 Hz, H-2^I), 5.30 (d, 1 H, *J* 3.0 Hz, H-4^{IV}), 5.25 (t, 1 H, *J* 8.0 Hz, H-2^{II}), 5.08 (dd, 1 H, *J* 10.5 Hz, 8.5 Hz, H-2^{IV}), 4.94 (dd, 1 H, *J* 10.0 Hz, 3.0

Hz, H-3^{IV}), 4.78 (d, 1 H, *J* 10.0 Hz, H-1^{I}), 4.51 (d, 1 H, *J* 8.0 Hz, H-1^{IV}), 4.50 (d, 1 H, *J* 12.0 Hz, H-6^{III}), 4.47 (d, 1 H, *J* 8.0 Hz, H-1^{II}), 4.39 (d, 1 H, *J* 11.0 Hz, H-6^{I}), 4.26 (dd, 1 H, *J* 11.5 Hz, 5.0 Hz, H-6^{I}), 4.19 (dd, 1 H, *J* 11.0 Hz, 4.5 Hz, H-2^{II}), 4.16 (t, 1 H, *J* 10.0 Hz, H-2^{III}), 4.07–3.95 (m, 5 H, H-4^{I}, H-4^{II}, H-6^{III}, and 2 × H-6^{IV}), 3.82 (t, 1 H, *J* 6.5 Hz, H-5^{IV}), 3.78–3.72 (m, 2 H, H-4^{III} and H-5^{III}), 3.70–3.64 (m, 2 H, H-3^{II} and H-5^{I}), 3.62 (dd, 1 H, *J* 11.5, 7.5 Hz, H-6^{II}), 3.52 (m, 1 H, H-5^{II}), 2.10 (s, 3 H, CH_3), 2.04 (s, 3 H, CH_3), 2.01 (s, 3 H, CH_3), 1.99 (s, 3 H, CH_3), 1.94 (s, 3 H, CH_3), 1.78 (s, 3 H, CH_3); ^{13}C NMR ($CDCl_3$): *δ* 170.24 (Ac), 170.19 (Ac), 170.00 (Ac), 169.95 (Ac), 169.43 (Ac), 196.04 (Ac), 167.75 (Phth), 166.71 (Phth), 165.94 (Bz), 165.65 (Bz), 165.35 (Bz), 165.08 (Bz), 163.92 (Bz), 100.98 (C-1^{IV}), 100.47 (C-1^{II}), 98.15 (C-1^{III}), 85.66 (C-1^{I}), 81.35 (C-5^{I}), 76.85 (C-3^{II}), 76.68 (C-5^{III}), 75.39 (C-4^{I}), 73.72 (C-3^{I}), 72.74 (C-4^{III}), 72.24 (C-5^{II}), 70.90 (C-3^{III}) 70.80 (C-3^{IV}), 70.53 (C-5^{IV}), 70.35 (C-2^{II}), 70.35 (C-2^{I}), 69.02 (C-2^{IV}), 67.53 (C-4^{II}), 66.44 (C-4^{IV}), 62.74 (C-6^{II}), 62.58 (C-6^{I}), 61.69 (C-6^{III}), 60.55 (C-6^{IV}), 54.38 (C-2^{III}), 20.58 (Ac), 20.51 (Ac), 20.49 (Ac), 20.42 (Ac), 20.28 (Ac); Anal. Calcd for $C_{85}H_{81}NO_{32}S$ (1660.63): C, 61.47; H, 4.92; N, 0.84. Found: C, 61.73; H, 4.99; N, 0.83.

*Phenyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-*D*-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-1-thio-β-*D*-glucopyranoside* (**11**).—To a solution of **9** (100 mg, 0.07 mmol) in MeOH (10 mL) was added 1 M CH_3ONa (15 mL). After 4 h, the reaction mixture was neutralized with Rexyn 101 (H^+) resin and filtered, and the filtrate was concentrated. A mixture of the residue and hydrazine hydrate (1 mL) in EtOH (30 mL) was refluxed for 5 h. Upon cooling and concentration, the residue was treated with 1:1 Ac_2O–pyridine (10 mL) overnight. The reaction was quenched with MeOH (0 °C), and the mixture was extracted with EtOAc (3 × 30 mL). The organic layers were combined and dried over Na_2SO_4. The solvent was removed and the residue was chromatographed (10:1 EtOAc–MeOH) to give 64 mg (86%) of **11**. Mp 89–92 °C; $[\alpha]_D$ + 14.0° (*c* 0.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$): *δ* 7.50–7.27 (m 5 H, Ph), 5.47 (d, 1 H, *J* 8.5 Hz, NH), 5.46 (t, 1 H, *J* 11.5 Hz, H-3^{III}), 5.31 (d, 1 H, *J* 3.5 Hz, H-4^{II}), 5.18 (t, 1 H, *J* 9.0 Hz, H-3^{I}), 5.02 (t, 1 H, *J* 10.0 Hz, H-4^{III}), 5.00 (d, 1 H, *J* 8.0 Hz, H-1^{III}), 4.99 (t, 1 H, *J* 10.5 Hz, H-2^{II}), 4.89 (t, 1 H, *J* 9.5 Hz, H-2^{I}), 4.66 (d, 1 H, *J* 10.5 Hz, H-1^{I}), 4.48 (dd, 1 H, *J* 12.0, 2.0 Hz, H-6^{I}), 4.36 (dd, 1 H, *J* 12.0, 2.5 Hz, H-6^{III}), 4.33 (d, 1 H, *J* 8.0 Hz, H-1^{II}), 4.19 (dd, 1 H, *J* 11.5, 6.0 Hz, H-6^{I}), 4.04 (dd, 1 H, *J* 11.0, 4.0 Hz, H-6^{III}), 4.02 (m, 2 H, 2 × H-6^{II}), 3.82–3.73 (m, 2 H, H-3^{II} and H-5^{II}), 3.69 (t, 1 H, *J* 12.0 Hz, H-4^{I}), 3.66 (m, 1 H, H-5^{III}), 3.63 (m, 1 H, H-5^{I}), 3.32 (m, 1 H, H-2^{III}), 2.10 (s, 6 H, 2 × CH_3), 2.09 (s, 3 H, CH_3), 2.07 (s, 3 H, CH_3), 2.06 (s, 3 H, CH_3), 2.05 (s, 3 H, CH_3), 2.04 (s, 3 H, CH_3), 1.99 (s, 6 H, 2 × CH_3), 1.89 (s, 3 H, CH_3); ^{13}C NMR ($CDCl_3$): *δ* 170.69 (Ac), 170.49 (Ac), 169.74 (Ac), 169.56 (Ac), 169.50 (Ac), 169.43 (Ac), 169.07 (Ac), 132.84 (Ph), 131.95 (Ph), 128.85 (Ph), 128.21 (Ph), 100.62 (C-1^{II}), 99.52 (C-1^{III}), 85.57 (C-1^{I}), 76.74 (C-5^{I}), 75.92 (C-3^{II}), 75.57 (C-4^{I}), 73.57 (C-3^{I}), 71.68 (C-5^{III}), 71.18 (C-5^{II}), 71.13 (C-3^{III}), 70.92 (C-2^{II}), 70.20 (C-2^{I}), 68.83 (C-4^{II}), 68.66 (C-4^{III}), 62.27 (C-6^{I}), 61.51 (C-6^{II}), 61.09 (C-6^{III}), 56.12 (C-2^{III}), 20.83 (Ac), 20.79 (Ac), 20.73 (Ac), 20.71 (Ac), 20.68 (Ac), 20.60 (Ac); Anal. Calcd for $C_{44}H_{57}NO_{24}S$ (1015.96): C, 52.02; H, 5.66; N, 1.38. Found: C, 51.94; H, 5.70; N, 1.38.

*Phenyl 2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-*D*-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-1-thio-β-*D*-glucopyranoside* (**12**).—The protected compound **10** (165 mg, 0.10 mmol) was treated with CH_3ONa, hydrazine hydrate, and Ac_2O, as described for **11**, to yield the peracylated tetrasaccharide **12** (110 mg, 86%). Mp 129–130 °C; $[\alpha]_D$ + 3.5° (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$): *δ* 7.48–7.28 (m 5 H, Ph), 5.34 (d, 1 H, *J* 3.0 Hz, H-4^{IV}), 5.32 (d, 1 H, *J* 8.5 Hz, NH), 5.29 (d, 1 H, *J* 3.0 Hz, H-4^{II}), 5.18 (t, 2 H, *J* 9.0 Hz, H-3^{III} and H-3^{I}), 5.10 (dd, 1 H, *J* 11.0, 7.5 Hz, H-2^{IV}), 4.99–4.94 (m, 2 H, H-2^{II} and H-3^{IV}), 4.90 (t, 1 H, *J* 9.5 Hz, H-2^{I}), 4.77 (dd, 1 H, *J* 11.5, 2.0 Hz, H-6^{III}), 4.66 (d, 2 H, *J* 9.0 Hz, H-1^{I} and H-1^{III}), 4.54 (d, 1 H, *J* 8.0 Hz, H-1^{IV}), 4.48 (d, 1 H, *J* 10.5 Hz, H-6^{I}), 4.33 (d, 1 H, *J* 8.0 Hz, H-1^{II}), 4.14–4.08 (m, 3 H, H-6^{I}

and 2 × H-6^{IV}), 4.08–3.99 (m, 2 H, 2 × H-6^{II}), 3.96 (dd, 1 H, *J* 11.5, 3.0 Hz, H-6^{III}), 3.86 (t, 1 H, *J* 7.0 Hz, H-5^{IV}), 3.78 (t, 1 H, *J* 9.5 Hz, H-4^{III}), 3.76 (t, 1 H, *J*, 6.0 Hz, H-5^{II}), 3.72 (m, 1 H, H-3^{II}), 3.70 (t, 1 H, *J* 10.0 Hz, H-4^{I}), 3.66–3.60 (m, 1 H, H-5^{I}), 3.55–3.48 (m, 2 H, H-2^{III} and H-5^{III}), 2.14 (s, 6 H, 2 × CH_3), 2.10 (s, 6 H, 2 × CH_3), 2.07 (s, 6 H, 2 × CH_3), 2.06 (s, 3 H, CH_3), 2.05 (s, 6 H, 2 × CH_3), 2.04 (s, 3 H, CH_3), 2.00 (s, 3 H, CH_3), 1.96 (s, 3 H, CH_3), 1.89 (s, 3 H, CH_3); ^{13}C NMR ($CDCl_3$): δ 170.56 (Ac), 170.42 (Ac), 170.38 (Ac), 170.35 (Ac), 170.25 (Ac), 170.08 (Ac), 170.03 (Ac), 169.75 (Ac), 169.72 (Ac), 169.50 (Ac), 169.06 (Ac), 168.82 (Ac), 132.83 (Ph), 131.97 (Ph), 128.85 (Ph), 128.21 (Ph), 101.06 (C-1^{IV}), 100.61 (C-1^{II}), 100.20 (C-1^{III}), 85.58 (C-1^{I}), 76.75 (C-5^{I}), 75.74 (C-3^{II}), 75.69 (C-4^{III}), 75.60 (C-4^{I}), 73.59 (C-3^{I}), 72.66 (C-5^{III}), 71.82 (C-3^{III}), 71.08 (C-5^{II}), 70.99 (C-2^{II}), 70.85 (C-3^{IV}), 70.69 (C-5^{IV}), 70.22 (C-2^{I}), 69.15 (C-2^{IV}), 68.86 (C-4^{II}), 66.60 (C-4^{IV}), 62.28 (C-6^{I}), 61.51 (C-6^{II}), 60.75 (C-6^{IV}), 60.03 (C-6^{III}), 55.04 (C-2^{III}), 20.83 (Ac), 20.82 (Ac), 20.80 (Ac), 20.73 (Ac), 20.71 (Ac), 20.68 (Ac), 20.63 (Ac), 20.62 (Ac), 20.59 (Ac), 20.48 (Ac); Anal. Calcd for $C_{56}H_{73}NO_{32}S$ (1304.25): C, 51.57; H, 5.64; N, 1.07. Found: C, 51.30; H, 5.97; N, 1.10.

*Phenyl 2-acetamido-2-deoxy-β-*D*-glucopyranosyl-(1→3)-β-*D*-galactopyranosyl-(1→4)-1-thio-β-*D*-glucopyranoside* (**13**).—Compound **11** (50 mg, 0.05 mmol) was deacetylated (Zempen), and the reaction mixture was neutralized with Rexyn 101 (H^+) resin and filtered. The filtrate was concentrated, and the residue was dissolved in water and freeze-dried to give the final trisaccharide **13** (27 mg, 87%). $[\alpha]_D$ + 26.7° (*c* 0.2, MeOH); Anal. Calcd for $C_{26}H_{39}NO_{15}S$ (637.66): C, 48.97; H, 6.16; N, 2.20. Found: C, 48.96; H, 6.05; N, 2.09; MALDIMS: Anal. Calcd for $C_{26}H_{39}NO_{15}SNa$: 660.1938. Found: *m/z* 660.295 [M + Na].

*Phenyl β-*D*-galactopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-(1→3)-β-*D*-galactopyranosyl-(1→4)-1-thio-β-*D*-glucopyranoside* (**14**).—Protected tetrasaccharide **12** (60 mg, 0.05 mmol) was treated as above for **13** to give **14** (30 mg, 77%). $[\alpha]_D$ − 138.26° (*c* 0.07, H_2O); Anal. Calcd for $C_{26}H_{39}NO_{15}S{\cdot}3\ H_2O$ (853.84): C, 45.06; H, 6.49; N, 1.64. Found: C, 44.66; H, 6.33; N, 1.74.

*MPEGDOXyl 2,6-di-*O*-benzoyl-β-*D*-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-*D*-glucopyranoside* (**15**).—Compound **7** (300 mg, 0.30 mmol), HODOXMPEG (772 mg, 0.15 mmol) and activated 4 Å molecular sieves (500 mg) were dried overnight at high vacuum in a flask covered with aluminum foil. Dichloromethane (5 mL) was added and the mixture was cooled in an ice bath. After stirring for 0.5 h, *N*-iodosuccinimide (NIS, 136 mg, 0.60 mmol) was added, followed by silver trifluoromethanesulfonate (77.5 mg, 0.30 mmol), and the reaction was maintained at 0 °C for 3 h. The mixture was cooled to 0 °C, neutralized with several drops of diisopropylethylamine, and the polymer was precipitated with TBME (260 mL). The solids were recovered by filtration and washed several times with diethyl ether. The solids were dissolved with a hot solution of 0.5% ethanolic imidazole (175 mL), and the resulting solution was kept at − 20 °C for 1 h to complete precipitation. The precipitate was collected by filtration and, after washing with cold EtOH and diethyl ether, it was taken up in CH_2Cl_2 and filtered, and the filtrate was evaporated to give a polymer-bound product. A solution of the foregoing product in 60% AcOH (120 mL) was stirred at 60 °C for 2 h. The solvent was evaporated under reduced pressure, the residue was dissolved in CH_2Cl_2 (3 mL), and TBME (250 mL) was added to precipitate the polymer. The precipitate was recovered by filtration and, after rinsing with TBME, reprecipitated from absolute EtOH (150 mL). The resulting solid was collected by filtration, and after washing with cold EtOH and diethyl ether it was dissolved in CH_2Cl_2, filtered, and evaporated to yield the polymer-bound acceptor **15** (840 mg, 92%).

*MPEGDOXyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl-(1→3)-2,6-di-*O*-benzoyl-β-*D*-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-*D*-glucopyranoside* (**16**).—A mixture of monosaccharide donor **4** (50 mg, 0.09 mmol) and polymer-bound acceptor **15** (430 mg, 0.07 mmol) was dried under high vacuum overnight. After cooling (− 20 °C) in an argon atmosphere CH_2Cl_2 (10 mL) was added, followed by $BF_3{\cdot}Et_2O$ (20 μL), and the

mixture was stirred for 4 h. The reaction was quenched with several drops of diisopropylethylamine, and the polymer was precipitated with TBME (150 mL). The solid was collected by filtration and re-precipitated from absolute EtOH (90 mL). The precipitate was filtered and washed with cold EtOH and diethyl ether. Then it was dissolved in CH_2Cl_2, filtered and evaporated to yield polymer-bound trisaccharide **16** (420 mg, 91%).

MPEGDOXyl 2,3,4,6-tetra-O-*acetyl*-β-D-*galactopyranosyl*-(1→4)-*3,6-di*-O-*acetyl*-*2-deoxy*-*2*-*phthalimido*-β-D-*glucopyranosyl*-(1→3)-*2,6-di*-O-*benzoyl*-β-D-*galactopyranosyl*(1→4)-*2,3,6-tri*-O-*benzoyl*-β-D-*glucopyranoside* (**17**).—The mixture of disaccharide donor **3** (174 mg, 0.20 mmol) and polymer-bound acceptor **15** (600 mg, 0.10 mmol) was treated with $BF_3{\cdot}Et_2O$ (30 μL), as described above for **16** to give **17** (620 mg, 92%).

4-Acetoxymethylbenzyl 2-acetamido-3,4,6-tri-O-*acetyl*-*2*-*deoxy*-β-D-*glucopyranosyl*-(1→3)-*2,4,6-tri*-O-*acetyl*-β-D-*galactopyranosyl*-(1→4)-*2,3,6-tri*-O-*acetyl*-β-D-*glucopyranoside* (**18**).—The polymer-bound trisaccharide **16** (400 mg, 0.06 mmol) was dissolved in MeOH (10 mL), 1,8-diazabicyclo[5,4,0]undec-7-ene (five drops) was added, and the mixture was stirred under an argon atmosphere overnight. After cooling in ice, the polymer was precipitated with TBME (140 mL). The solid was collected by filtration and reprecipitated from absolute EtOH (80 mL), and the solid was dissolved in CH_2Cl_2, filtered and evaporated to yield *O*-de-acylated polymer-bound trisaccharide. To this solid was added absolute EtOH (6 mL) and ethylenediamine (0.30 mL, 4.5 mmol), and the mixture was heated at 70 °C under an atmosphere of argon for 2 h. The reaction mixture was diluted with absolute EtOH (10 mL) and tightly stoppered and put in a freezer for about 1 h. The resulting precipitate was recovered by filtration, washed with cold EtOH and diethyl ether, and then dissolved in CH_2Cl_2, filtered and evaporated to obtain the crude amine. After drying under high vacuum, this solid was dissolved in CH_2Cl_2 (3 mL), and Ac_2O (3 mL) was added under an atmosphere of argon. After 15 min scandium(III) trifluoromethanesulfonate (15 mg, 0.03 mmol) was added, and the mixture was left to stir for 5 h. Ammonium bicarbonate (15 mg) was added, and the polymer was precipitated with TBME (100 mL) and, after filtration, the residue was reprecipitated from absolute EtOH (50 mL). The combined filtrates were evaporated to dryness, followed by co-evaporation with toluene (2 × 20 mL). The residue was purified by chromatography (19:1 EtOAc–MeOH) to yield **18** (35 mg, 52%) and the by-product **21** (4.6 mg, 10%). **18**: $[\alpha]_D$ −1.2° (*c* 0.8 $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.33 (d, 2 H, *J* 7.5 Hz, Ph), 7.27 (d, 2 H, *J* 7.0 Hz, Ph), 5.48 (t, 1 H, *J* 10.0 Hz, H-3^{III}), 5.44 (d, 1 H, *J* 8.0 Hz, NH), 5.32 (d, 1 H, *J* 3.0 Hz, H-4^{II}), 5.13 (t, 1 H, *J* 9.0 Hz, H-3^{I}), 5.09 (s, 2 H, CH_2OAc), 5.04 (t, 1 H, *J* 9.5 Hz, H-4^{III}), 5.01 (d, 1 H, *J* 8.0 Hz, H-1^{III}), 4.98 (t, 1 H, *J* 7.5 Hz, H-2^{II}), 4.96 (t, 1 H, *J* 9.5 Hz, H-2^{I}), 4.85 (d, 1 H, *J* 12.0 Hz, CH_2Ph), 4.59 (d, 1 H, *J* 12.0 Hz, CH_2Ph), 4.52–4.46 (m, 2 H, H-1^{I} and H-6^{I}), 4.38 (dd, 1 H, *J* 12.0, 1.5 Hz, H-6^{III}), 4.35 (d, 1 H, *J* 7.5 Hz, H-1^{II}), 4.13 (dd, 1 H, *J* 12.0, 5.5 Hz, H-6^{I}), 4.09–4.01 (m, 3 H, H-6^{III} and 2 × H-6^{II}), 3.81–3.74 (m, 3 H, H-3^{II}, H-5^{II}, and H-4^{I}), 3.66 (m, 1 H, H-5^{III}), 3.60–3.56 (m, 1 H, H-5^{I}), 3.28 (m, 1 H, H-2^{III}), 2.14 (s, 3 H, CH_3), 2.10 (s, 9 H, 3 × CH_3), 2.08 (s, 6 H, 2 × CH_3), 2.02 (s, 3 H, CH_3), 2.01 (s, 3 H, CH_3), 2.00 (s, 6 H, 2 × CH_3), 1.90 (s, 3 H, CH_3); ^{13}C NMR ($CDCl_3$): δ 170.76 (Ac), 170.65 (Ac), 170.43 (Ac), 170.42 (2 × Ac), 170.31 (Ac), 169.76 (Ac), 169.74 (2 × Ac), 169.38 (Ac), 169.05 (Ac), 136.70 (Ph), 135.70 (Ph), 128.36 (Ph), 127.82 (Ph), 100.62 (C-1^{II}), 99.49 (C-1^{III}), 99.05 (C-1^{I}), 75.64 (C-3^{II}), 75.64 (C-4^{I}), 72.79 (C-5^{I}), 72.51 (C-3^{I}), 71.72 (C-2^{I}), 71.58 (C-5^{III}), 71.19 (C-5^{II}), 71.18 (C-3^{III}), 70.99 (C-2^{II}), 70.31 (C-CH_2Ph), 68.87 (C-4^{II}), 68.63 (C-4^{III}), 65.94 (C–CH_2OAc), 62.08 (C-6^{I}), 61.59 (C-6^{II}), 61.12 (C-6^{III}), 56.23 (C-2^{III}), 23.36 (Ac), 21.06 (Ac), 20.95 (Ac), 20.78 (Ac), 20.72 (Ac); MALDIMS: Anal. Calcd for $C_{48}H_{63}NO_{27}Na$: 1108.35. Found: *m*/*z* 1108.12 [M + Na].

4-Acetoxymethylbenzyl 2,3,4,6-tetra-O-*acetyl*-β-D-*galactopyranosyl*-(1→4)-*2,3,6-tri*-O-*acetyl*-β-D-*glucopyranoside* (**21**).—$[\alpha]_D$ −26.9° (*c* 0.4 $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.33 (d, 2 H, *J* 8.0 Hz, Ph), 7.27 (d, 2 H, *J* 8.0 Hz, Ph), 5.34 (d, 1 H, *J* 3.0 Hz, H-4^{II}), 5.16 (t, 1 H, *J* 10.0 Hz, H-3^{I}), 5.10 (s, 2 H, CH_2OAc),

5.10 (t, 1 H, *J* 7.5 Hz, H-2^{II}), 4.96 (t, 1 H, *J* 7.5 Hz, H-2^{I}), 4.95 (dd, 1 H, *J* 10.0, 4.0 Hz, H-3^{II}), 4.85 (d, 1 H, *J* 12.5 Hz, CH_2Ph), 4.59 (d, 1 H, *J* 12.5 Hz, CH_2Ph), 4.52 (d, 1 H, *J* 8.0 Hz, H-6^{I}), 4.50 (d, 1 H, *J* 7.5 Hz, H-1^{I}), 4.48 (d, 1 H, *J* 8.0 Hz, H-1^{II}), 4.16–4.04 (m, 3 H, H-6^{I} and 2 × H-6^{II}), 3.86 (t, 1 H, *J* 7.0 Hz, H-5^{II}), 3.82 (t, 1 H, *J* 10.0 Hz, H-4^{I}), 3.60–3.55 (m, 1 H, H-5^{I}), 2.14 (s, 3 H, CH_3), 2.13 (s, 3 H, CH_3), 2.10 (s, 3 H, CH_3), 2.05 (s, 3 H, CH_3), 2.04 (s, 3 H, CH_3), 2.03 (s, 3 H, CH_3), 2.01 (s, 3 H, CH_3), 1.96 (s, 3 H, CH_3); ^{13}C NMR ($CDCl_3$): δ 170.84 (Ac), 170.35 (2 × Ac), 170.14 (Ac), 170.06 (Ac), 169.78 (Ac), 169.59 (Ac), 169.05 (Ac), 136.80 (Ph), 135.82 (Ph), 128.44 (Ph), 127.91 (Ph), 101.08 (C-1^{II}), 99.09 (C-1^{I}), 76.25 (C-4^{I}), 72.82 (C-5^{I}), 72.74 (C-3^{I}), 71.69 (C-2^{I}), 71.07 (C-3^{II}), 70.73 (C-5^{II}), 70.33 (C–CH_2Ph), 69.16 (C-CH_2OAc), 66.64 (C-4^{II}), 65.94 (C-2^{II}), 61.98 (C-6^{I}), 60.83 (C-6^{II}), 21.01 (Ac), 20.88 (Ac), 20.81 (Ac), 20.70 (Ac), 20.63 (Ac), 20.51 (Ac); MALDIMS: Anal. Calcd for $C_{36}H_{46}O_{20}Na$: 821.25. Found: *m*/*z* 821.43 [M + Na].

4-Acetoxymethylbenzyl 2,3,4,6-tetra-O-*acetyl-β*-D-*galactopyranosyl-(1→4)-2-acetamido-3,6-di*-O-*acetyl-2-deoxy-β*-D-*glucopyranosyl-(1→3)-2,4,6-tri*-O-*acetyl-β*-D-*galactopyranosyl-(1→4)-2,3,6-tri*-O-*acetyl-β*-D-*glucopyranoside* (**19**).—tetrasaccharide **19** (65 mg, 53%) was obtained from **17** as described above for **18**. $[\alpha]_D$ −4.8° (*c* 2.6 $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.33 (d, 2 H, *J* 7.5 Hz, Ph), 7.26 (d, 2 H, *J* 8.0 Hz, Ph), 5.39 (d, 1 H, *J* 9.0 Hz, NH), 5.34 (d, 1 H, *J* 3.0 Hz, H-4^{IV}), 5.29 (d, 1 H, *J* 3.0 Hz, H-4^{II}), 5.19 (t, 1 H, *J* 9.5 Hz, H-3^{III}), 5.13 (t, 1 H, *J* 9.0 Hz, H-3^{I}), 5.11 (t, 1 H, *J* 9.5 Hz, H-2^{IV}), 5.09 (s, 2 H, CH_2OAc), 5.00–4.93 (m, 3 H, H-2^{I}, H-3^{IV}, and H-2^{II}), 4.85 (d, 1 H, *J* 11.5 Hz, CH_2Ph), 4.77 (d, 1 H, *J* 10.5 Hz, H-6^{III}), 4.68 (d, 1 H, *J* 8.0 Hz, H-1^{III}), 4.59 (d, 1 H, *J* 12.0 Hz, CH_2Ph), 4.54 (d, 1 H, *J* 8.0 Hz, H-1^{IV}), 4.50 (d, 1 H, *J* 8.0 Hz, H-1^{I}), 4.48 (d, 1 H, *J* 10.5 Hz, H-6^{I}), 4.34 (d, 1 H, *J* 8.0 Hz, H-1^{II}), 4.16–4.00 (m, 5 H, H-6^{I}, 2 × H-6^{IV}, and 2 × H-6^{II}), 3.96 (dd, 1 H, *J* 12.05, 2.5 Hz, H-6^{III}), 3.87 (t, 1 H, *J* 7.0 Hz, H-5^{IV}), 3.82–3.70 (m, 4 H, H-4^{III}, H-5^{II}, H-4^{I}, and H-3^{II}), 3.59–3.53 (m, 1 H, H-5^{I}), 3.53–3.48 (m, 2 H, H-2^{III} and H-5^{III}), 2.14 (s, 9 H, 3 × CH_3), 2.10 (s, 6 H, 2 × CH_3), 2.09 (s, 3 H, CH_3), 2.06 (s, 6 H, 2 × CH_3), 2.05 (s, 3 H, CH_3), 2.04 (s, 3 H, CH_3), 2.01 (s, 3 H, CH_3), 2.00 (s, 3 H, CH_3), 1.97 (s, 3 H, CH_3), 1.90 (s, 3 H, CH_3); ^{13}C NMR ($CDCl_3$): δ 170.82 (Ac), 170.58 (Ac), 170.49 (Ac), 170.44 (Ac), 170.38 (Ac), 170.33 (Ac), 170.32 (Ac), 170.09 (Ac), 170.04 (Ac), 169.80 (Ac), 169.78 (Ac), 169.55 (Ac), 169.08 (Ac), 168.85 (Ac), 136.75 (Ph), 135.73 (Ph), 128.38 (Ph), 127.83 (Ph), 101.05 (C-1^{IV}), 100.64 (C-1^{II}), 100.19 (C-1^{III}), 99.05 (C-1^{I}), 76.76 (C-3^{II}), 75.67 (C-4^{III}), 75.63 (C-4^{I}), 72.74 (C-5^{I}), 72.66 (C-5^{III}), 72.51 (C-3^{I}), 71.83 (C-3^{III}), 71.51 (C-2^{I}), 71.05 (C-5^{II}), 71.01 (C-3^{IV}), 70.83 (C-2^{II}), 70.67 (C-5^{IV}), 70.27 (C-CH_2Ph), 69.13 (C-2^{IV}), 68.85 (C-4^{II}), 66.59 (C-4^{IV}), 65.89 (C-CH_2OAc), 62.05 (C-6^{I}), 61.50 (C-6^{II}), 60.73 (C-6^{IV}), 60.28 (C-6^{III}), 55.02 (C-2^{III}), 23.13 (Ac), 20.96 (Ac), 20.86 (Ac), 20.83 (Ac), 20.77 (Ac), 20.71 (Ac), 20.69 (Ac), 20.65 (Ac), 20.60 (Ac), 20.48 (Ac); MALDIMS: Anal. Calcd for $C_{60}H_{79}NO_{35}Na$: 1396.43. Found: *m*/*z* 1396.32 [M + Na].

4-Hydroxymethylbenzyl β-D-*galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β*-D-*glucopyranosyl-(1→3)-β*-D-*galactopyranosyl-(1→4)-β*-D-*glucopyranoside* (**20**).—To a solution of **19** (60 mg, 0.05 mmol) in dry MeOH (5 mL) was added 1 M CH_3ONa in MeOH (5 mL). The mixture was stirred at room temperature for 5 h, then diluted with MeOH, the mixture was neutralized with Rexyn 101 (H^+) resin and filtered. The combined filtrates were concentrated and dissolved in water for freeze-drying to give tetrasaccharide **20** (27 mg, 87%). $[\alpha]_D$ +15.4° (*c* 0.3, H_2O); Anal. Calcd for $C_{34}H_{53}NO_{22}$ (827.79): C, 49.33; H, 6.45; N, 1.69. Found: C, 49.33; H, 6.81; N, 1.87.

Enzymatic synthesis of phenyl 2-acetamido-2-deoxy-β-D-*glucopyranosyl-(1→3)-β*-D-*galactopyranosyl-(1→4)-1-thio-β*-D-*glucopyranoside* (**13**).—β(1→3)*N*-Acetylglucosaminyltransferase (*LgtA*, 0.5 units, 9.2 mL) was added to a solution of 2 mM acceptor **5** (20 mg, 460 μL of 100 mM), 10 mM $MnCl_2$ (2300 μL of 100 mM), and 4 mM UDP-GlcNAc donor (4600 μL of 20 mM) in 50 mM HEPES buffer (1150 μL of 1 M, pH 7.4). The reaction was performed at 37 °C for 2 days. The product formation (R_f 0.55) was followed by TLC on silica (9:9:2 MeOH–$CHCl_3$–0.5%

$CaCl_2$). The total volume was adjusted to 23 mL with water and applied to a C_{18} reversed-phase column, eluting with 17:3–4:1 water–MeOH, which afforded pure **13** (28.2 mg, 96%).

Enzymatic synthesis of phenyl β-D-galacto-pyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-1-thio-β-D-glucopyranoside (**14**).—A fusion enzyme UDP-galactose 4-epimerase/β(1→4)galactosyltransferase (*GalE-LgtB*, 1.2 units, 6803 μL) was added to a solution of 2 mM acceptor **13** (20 mg, 313 μL of 20 mM), 10 mM $MnCl_2$ (1565 μL of 100 mM), and 1 mM UDP-Glc (940 μL of 20 mM) in 50 mM HEPES buffer (782 μL of 1 M, pH 7.4). The reaction was performed at 37 °C for a total of 2 h, while two additional portions of 1 mM UDP-Glc (940 μL of 20 mM) were added in 30 min intervals. The formation of **14** (R_f 0.40) was monitored by TLC (9:9:2 MeOH–$CHCl_3$–0.5% $CaCl_2$). Chromatography of the crude reaction mixture on a C_{18} reversed-phase column (9:1–17:3 water–MeOH) afforded pure **14** (24 mg, 96%).

Phenyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-*2-nonulopyranosylonic acid-(2→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-β-D-galactopyranosyl-(1→4)-1-thio-β-D-glucopyranoside* (**1**).—The reaction was performed in a total volume of 5.0 mL, and the following reagents were added sequentially: acceptor **14** (23 mg, 0.028 mmol), CTP (26.4 mg), sialic acid (15.4 mg). The pH was adjusted to 7.0 with NaOH, and 100 mM HEPES (500 μL of 1 M, pH 7.4), 20 mM $MgCl_2$ (100 μL of 1 M), and 0.2 mM DTT (100 μL of 10 mM) were added. The reaction was allowed to proceed at 32 °C after the addition of the CMP-Neu5Ac synthetase/sialyltransferase fusion enzyme (3.0 units, 500 μL). The reaction progress, i.e., the formation of material having R_f 0.2, was monitored by TLC (9:9:2 MeOH–$CHCl_3$–0.5% $CaCl_2$). After a total reaction time of 2 h, the crude product was chromatographed (C_{18} reversed-phase column; elution with water and then 99:1 water–MeOH) to yield pure pentasaccharide **1** (30 mg, 97%). $[\alpha]_D$ −36.0° (*c* 0.2, H_2O); Anal. Calcd for $C_{43}H_{65}N_2O_{28}SNa$ (1113.04): C, 46.40; H, 5.89; N, 2.52. Found: C, 46.23; H, 5.86; N, 2.70.

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