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A Convergent Synthesis of Core 2 Branched Sialylated and Sulfated Oligosaccharides

Jie Xia, James L. Alderfer, Thamarapu Srikrishnan, E. V. Chandrasekaran and Khushi L. Matta*

Molecular & Cellular Biophysics, Roswell Park Cancer Institute, Elm & Carlton Streets, Buffalo, NY 14263, USA

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Abstract—A convergent pathway for the syntheses of core 2 oligosaccharide analogues **1** and **2**, and a natural form sialylated and sulfated hexasaccharide **3** was developed. Construction of pentasaccharides **24**, **27** and hexasaccharide **28** was achieved by complete regioselective glycosylation of the 6-OH in the acceptors **5**, **7** and **8**, respectively, owing to the much higher reactivity of the primary hydroxyl group over the secondary axial hydroxyl group in these structures. *Stereoselective sialylation was accomplished using donor **10** with defined configuration established through X-ray crystallographic analysis*. Target oligosaccharides **1**–**3** were then obtained by the systematic deprotection of intermediates **24**, **27** and **29**. With these target oligosaccharides **1**–**3** obtained, biological evaluations of these molecules as enzyme substrates was undertaken and selectin binding studies are planned.

Introduction

Great advancement in synthetic carbohydrate chemistry has been realized.[1,2] The complex oligosaccharide molecules resulting from these efforts can provide for a wealth of information and offer insights into important biological processes.[3] The natural mucin ligands for cell adhesion proteins and various tumor-associated O-linked glycoproteins are known to contain the Galβ1→4GlcNAcβ1→6(Galβ1→3) GalNAcα sequence as their inner core structure. However, relatively little is known about the precise substrate specificities of the enzymes that can further modify this tetrasaccharide moiety. From structural studies conducted on natural ligands for selectins (such as GlyCAM-1),[4] it has been shown that the sulfate ester (designated by SE- in formulae) is generally located at the C-6 position of GlcNAc or galactose, whereas, sulfate is present at the C-3 position of galactose in sulfated mucin from colon cancer.[5] Interestingly, the NeuAcα2→3(SE-6Gal)β1→4GlcNAc sequence occurs as a part of GlyCAM-1, whereas, NeuAcα2→3(SE-6Gal)β1→3GalNAc has been found to be a part of certain sulfated mucins from colon cancer.[6] We have examined the specificity of sulfotransferases present in human breast and colon tissues and have made some very important observations. Ours was the first report wherein a well-defined core 2 tetrasaccharide structure was used to distinguish the sulfotransferase activities in these different human tissues.[7] Two distinct types of Gal:3-*O*-sulfotransferases were revealed. One was highly specific for the Galβ1→4GlcNAc branch of this tetrasaccharide and the other showed preference for the Galβ1→3GalNAc arm. We have also examined 3-*O*-sulfotransferase activities[7b] in various human tumor cell lines, for example colon LS180, SW1116 and breast line 435/LCC6. In fact, the availability of the modified analogues **A** and **B**, shown in Figure 1, enabled us to distinguish and therefore examine the status of these two different sulfotransferases in these sources.

We hereby describe the synthesis of modified pentasaccharides **1** and **2** as potential acceptors for 6-*O*-sulfotransferase capable of synthesizing the NeuAcα2→3(SE-6Gal)β1→3GalNAc and NeuAcα2→3(SE-6Gal)β1→4GlcNAc sequences, respectively. We argue that these branched compounds will be useful as preferred acceptors even in the presence of 3-*O*-sulfotransferase

*Corresponding author. Tel.: +1-716-845-2300; fax: +1-716-845-3458; e-mail: khushi.matta@roswellpark.org

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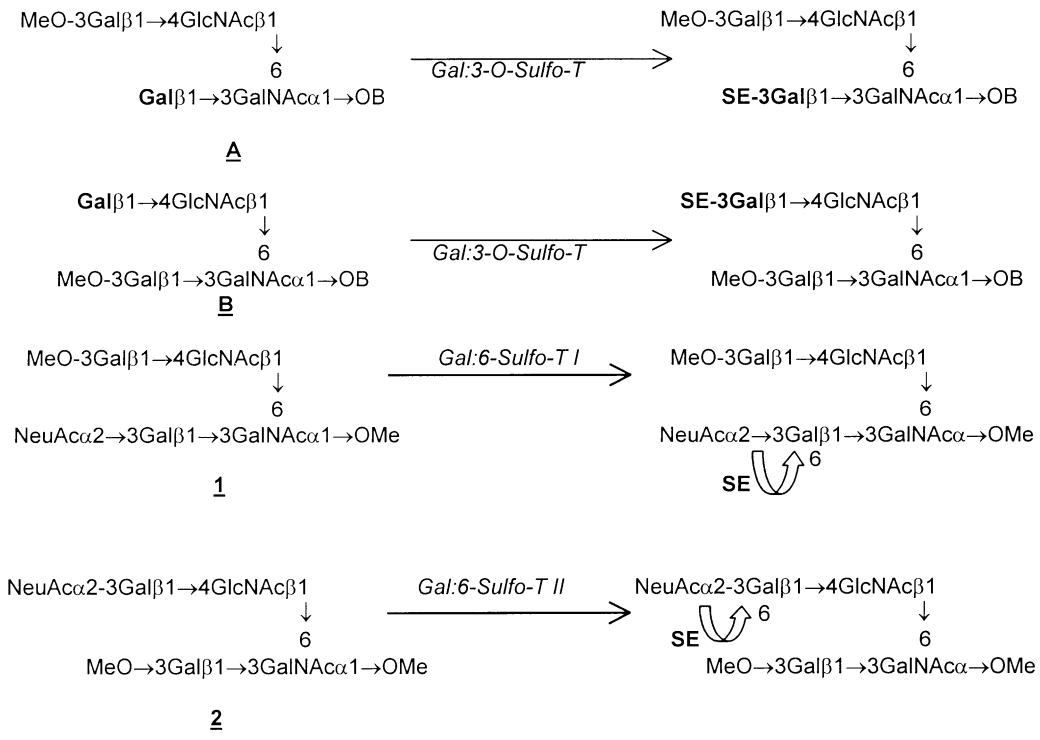


Figure 1.

capable of acting upon the Galβ1→4GlcNAc or Galβ1→3GalNAc sequences of *O*-linked glycoproteins. We also describe the synthesis of a sialylated and sulfated hexasaccharide, NeuAcα2→3Galβ1→4[Fucα1→3]SE-6GlcNAcβ1→6(Galβ1→3)GalNAcα1→OMe **3**, which is a part of GlyCAM-1, one of the naturally occurring ligands for 1-selectin.

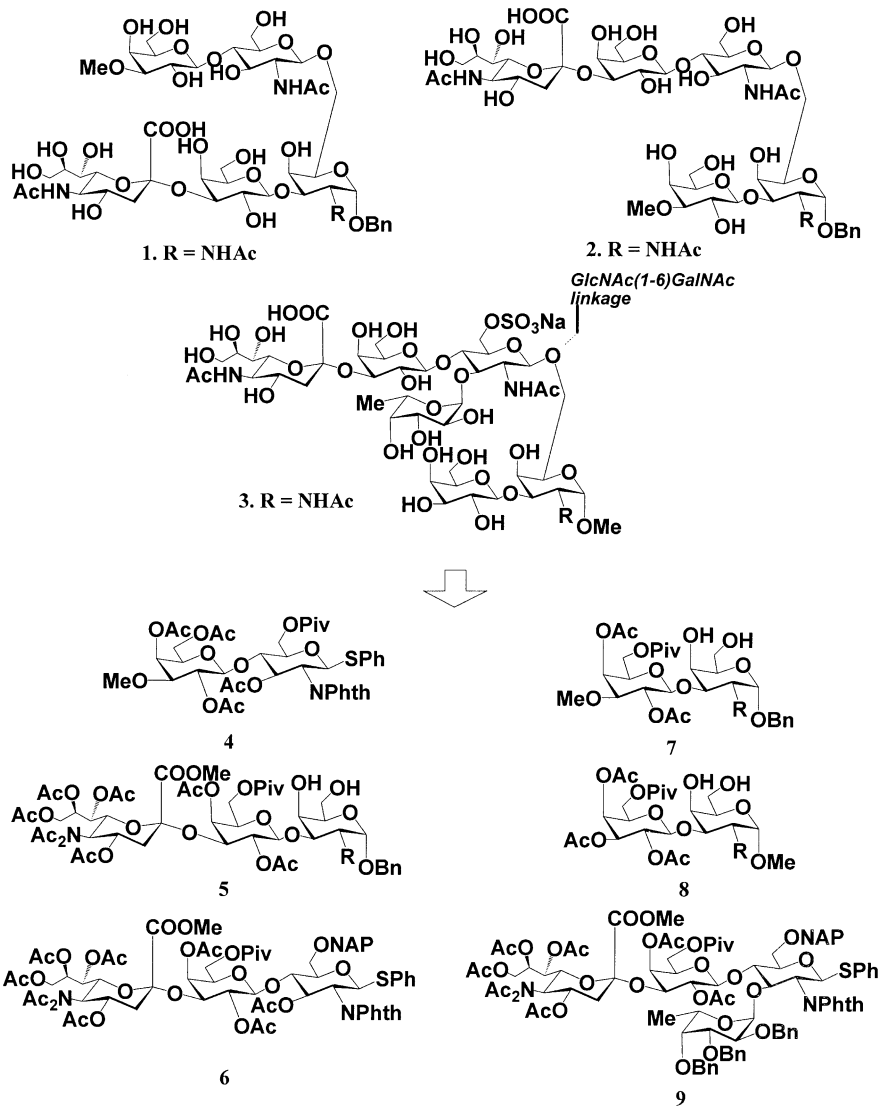
Results and Discussion

Our strategies for the assembly of oligosaccharides **1**, **2** and **3** is based on the observation of the much higher reactivity of a primary hydroxyl group over a secondary axial hydroxyl group in glycosylation reactions.[8a–e] This fact provides for a general route to the construction of core 2 branched oligosaccharide structures and their analogues. Therefore, pentasaccharides **1** and **2** were, respectively, synthesized from the MeO-3Galβ(1→4)GlcNphth disaccharide fragment **4** and the Neu5Acα(2→3)Galβ(1→3)GalNAc trisaccharide fragment **5**, and the Neu5Acα(2→3)Galβ(1→4)GlcNphth trisaccharide donor **6** with MeO-3Galβ(1→3)GalNAc disaccharide acceptor **7**. Similarly, hexasaccharide **3** was constructed from the Galβ(1→3)GalNAc fragment **8** and the sialylated Lewis^{x} donor **9** (Scheme 1). The key monosaccharide building blocks, **10**–**18**, required for the construction of these advanced intermediates are illustrated in Scheme 2.

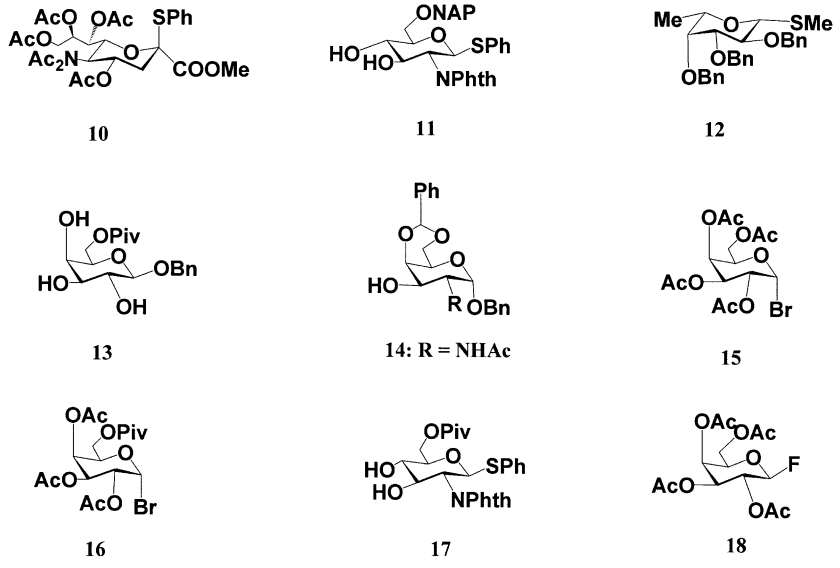
Since we needed to introduce a sulfate group at the C-6 position of GlcNAc in the sialyl Le^{x} moiety in hexasaccharide **3**, the sialylated Lewis^{x} donor **9** which carries the 2-naphthylmethyl (NAP) group at the C-6 position of GlcNphth was designed to enable the use of methyl-tri-*O*-benzyl-1-thio-β-L-fucoside **12** as a donor. The 2-naphthylmethyl (NAP) group was recently introduced for hydroxyl protection and this group is stable under a variety of strong Lewis acid and base conditions, or even in inorganic acid and base conditions. The most important factor is that the NAP group can be selectively removed in the presence of benzyl groups by DDQ.[9] Therefore, the complete syntheses of targets **1**–**3** are highly dependent upon the construction of disaccharide donor **4**, trisaccharide acceptor **5**, trisaccharide donor **6**, disaccharide acceptor **7**, sialylated Lewis^{x} tetrasaccharide donor **9** and disaccharide acceptor **8**. The novel sialyl Le^{x} donor **9** and disaccharide acceptor **8** were prepared according to our previously reported protocol.[8e] Preparations of the key intermediate building blocks **4**, **5** and **7** are illustrated in Schemes 3–5.

Construction of target **1** is shown in Schemes 3 and 4. Regio-selective and stereo-selective sialylation of HO-3 of the galactose moiety in acceptor **20** with donor **10** was successfully accomplished because of the higher nucleophilicity of HO-3 as compared to HO-2 and -4 of this same moiety. The reaction entailed employment of a defined configuration of the sialyl donor **10**.

Trisaccharide acceptor **5** was then obtained by a one-pot, two-step procedure in which compound **21** was treated with Ac_2O-pyridine in the presence of DMAP, followed by treatment with 60% acetic acid. The (2→3) linkage of **5** was confirmed by observation of a cross peak between H′-3 and C″-2 in the HMBC spectrum. The configuration of the glycoside of **5** was assigned according to literature methods.[11,12] and further con-



Scheme 1. Target oligosaccharide structures **1**–**3** and retrosynthetic analyses.



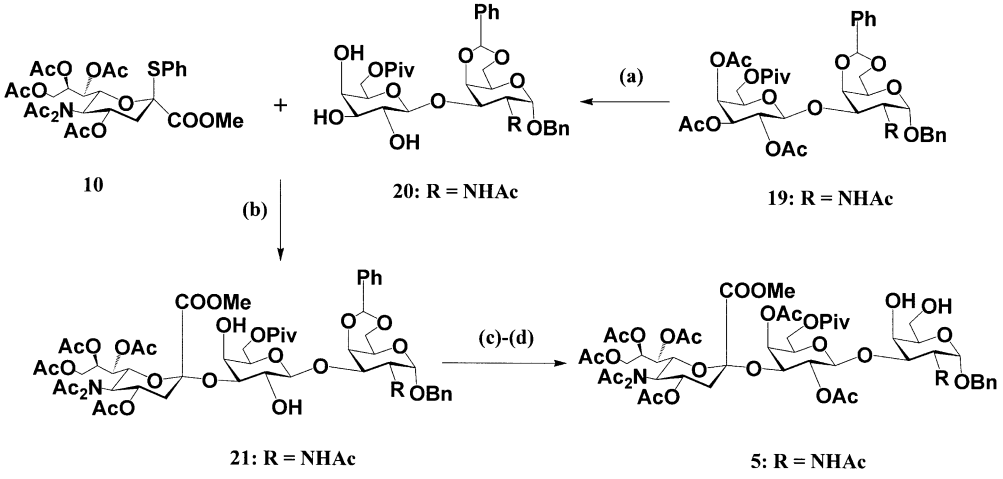
Scheme 2. The intermediate monosaccharide building blocks for target oligosaccharides **1**–**3**.

firmed by observation of a strong cross peak between H″-3a and C″-l in the HMBC spectrum of **5** because the α-sialoside is expected to have a large heteronuclear coupling constant, $J_{\text{c-1, H-3a.}}$[8b,12]

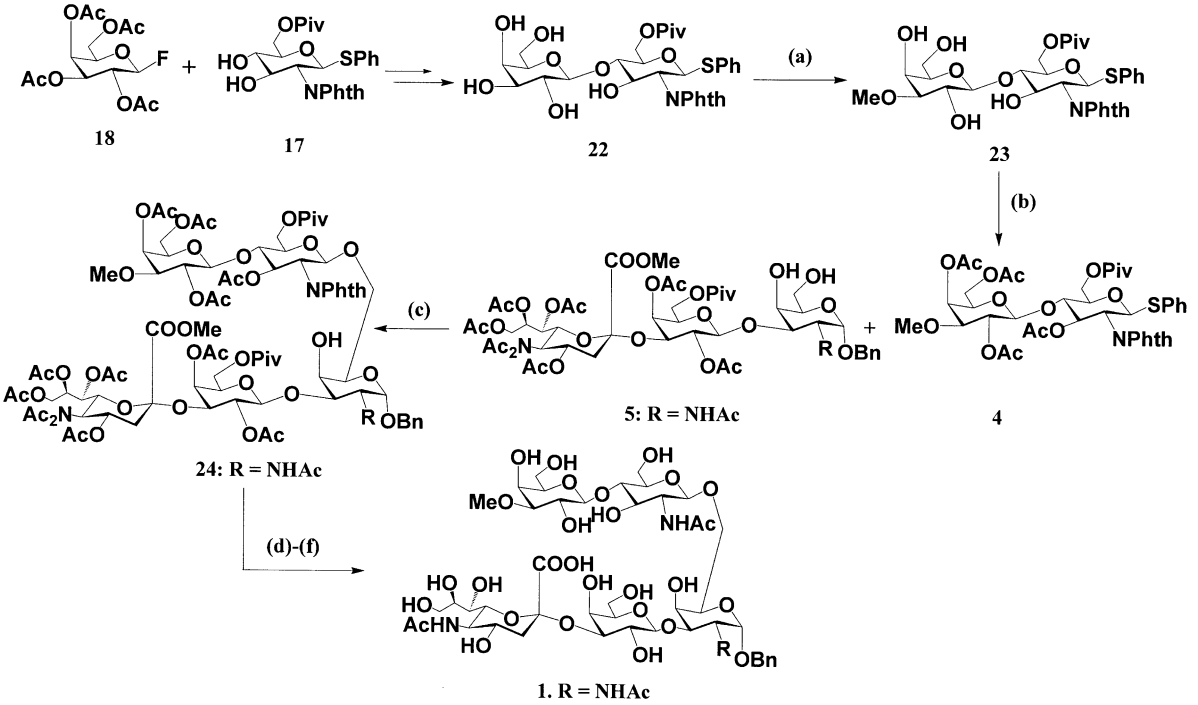
Selective methylation of the 3′-OH of disaccharide **22** was accomplished by a modified Hanessian procedure[13] using methyl iodide and dibutyltin oxide. The disaccharide donor **4** was then obtained after complete acetylation of **23** with acetic anhydride and pyridine. The NIS-TfOH promoted glycosylation of trisaccharide acceptor **5** with disaccharide donor **4** provided **24** as a single glycosylation product in good yield (65%) because of the much higher reactivity of the primary hydroxyl group in acceptor **5**. Compound **1** was obtained after global deprotection of **24** (Scheme 4).

X-ray crystallography

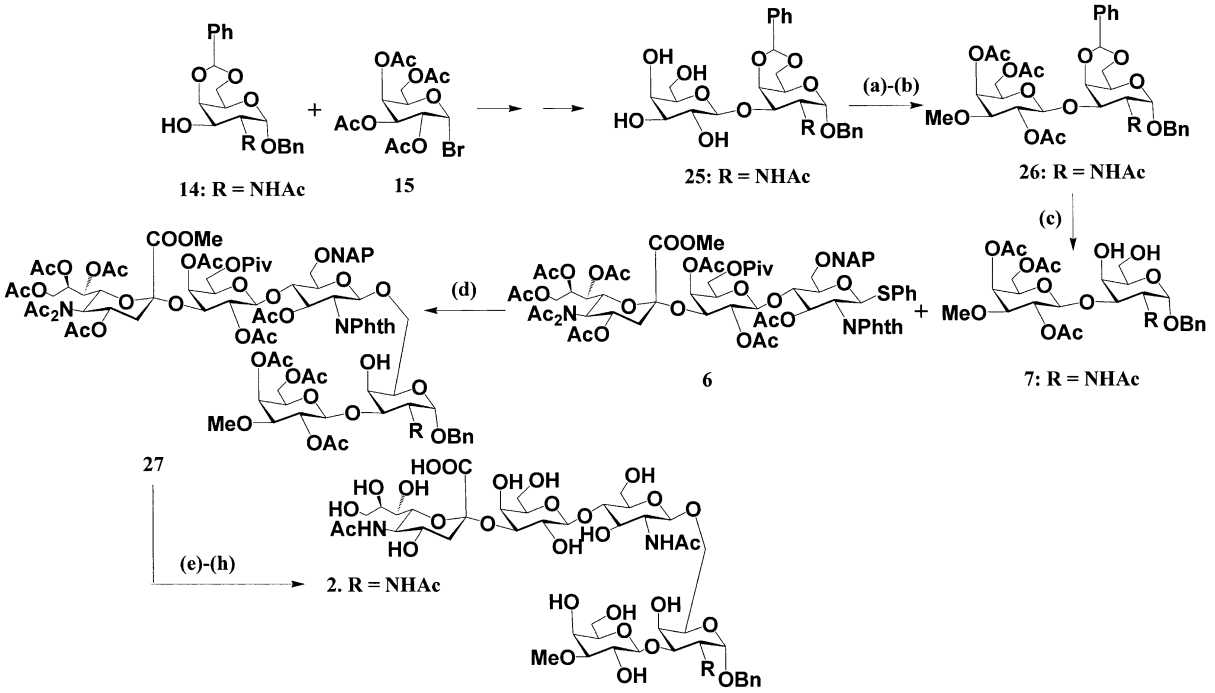
Crystals of **10** were obtained by a slow evaporation of the compound dissolved in anhydrous ethyl ether mixture. The crystals are rectangular needle shaped. A crystal of dimensions 0.25×0.35×0.15 mm was used for the present crystal structural investigation. The crystals belong to the Orthorhombic system, space group $P2_12_12_1$. Table 1 gives the crystal data and structure refinement parameters. Complete three-dimensional data was collected on a CAD-4 computer-controlled



Scheme 3. (a) 1 M CH_3ONa–CH_3OH/CH_2Cl_2–CH_3OH (1:1), −20 to −15 °C, 20 min, 85%; (b) NIS-TfOH/CH_3CN–CH_2Cl_2 (1:1), 3A-MS, −45 to −40 °C, 16 h, 45%; (c) Ac_2O–pyridine (1:1), DMAP, rt, 12 h; (d) 60% HOAc, 65 °C, 1.5 h, 72% in two steps.



Scheme 4. (a) (i) Bu_3SnO/benzene, reflux, 4 h; (ii) CH_3I-Bu_4NI/benzene-DMF, 40–50 °C, 4 days, 68%; (b) Ac_2O–pyridine (1:1), DMAP, rt, 12 h, 90%; (c) NIS-TfOH/CH_2Cl_2, 4A-MS, −65 to −60 °C, 65%; (d) LiI/pyridine, 120–125 °C, 8 h, N_2; (e) CH_3OH–$NH_2NH_2{\cdot}H_2O$, 80–85 °C, 6 h, then Ac_2O–pyridine (1:1), DMAP, rt, 12 h; (f) 1 M CH_3ONa–CH_3OH/CH_3OH–H_2O, rt, 12 h, 37% in three steps.



Scheme 5. (a) (i) Bu_3SnO/benzene, reflux, 4 h; (ii) CH_3I-Bu_4NI/benzene-DMF, 40–50 °C, 4 days, 65%; (b) Ac_2O–pyridine (1:1), DMAP, rt, 12 h, 85%; (c) 60% HOAc, 65 °C, 1 h; (d) NIS-TfOH/CH_2Cl_2, 4A-MS, −65 to −60 °C, 49%; (e) DDQ/CH_2Cl_2–CH_3OH (4:1), rt, 20 h, 70%; (f) LiI/pyridine, 120–125 °C, 8 h, N_2; (g) CH_3OH–$NH_2NH_2{\cdot}H_2O$, 80–85 °C, 6 h, then Ac_2O–pyridine (1:1), DMAP, rt, 12 h; (h) 1 M CH_3ONa–CH_3OH/CH_3OH–H_2O, rt, 12 h, 33% in three steps.

diffractometer. 6192 reflections ($2\theta_{max} = 150°$) were collected by the $\omega/2\theta$ method, out of which 5264 were considered significant ($1 \geq 2\sigma$). The crystal structure was solved by a routine and straightforward application of SHELX-96 program on a silicon graphics R10,000 computer. The structure was refined using SHELX-96 package[14] of programs. All the hydrogen atoms were located on a difference Fourier map. Refinements were carried out with anisotropic thermal parameters for the non-hydrogen atoms and isotropic thermal parameter for the hydrogen atoms. The final reliability index (*R*-factor) was 0.0712 for all the observed 5264 reflections and 0.0959 for all 6192 reflections with a goodness of fit parameter $S = 1.072$. Table 2 gives the final positional parameters for the non-hydrogen atoms in the structure. Figure 2 gives an ORTEP[10] diagram of the molecule.

The preparation of pentasaccharide **2** is outlined in Scheme 5. Starting with known compound **25** (synthesized from our protocol), regioselective methylation of the 3-hydroxyl group of galactose in **25** with methyl iodide in presence of dibutyltin oxide and tetrabutylammonium iodide in a mixture of benzene and DMF, followed by complete acetylation in dry pyridine with anhydrous acetic anhydride in the presence of a catalytic amount of DMAP, provided the disaccharide compound **26**. Disaccharide acceptor **7** was obtained in good yield (70%) by treatment of **26** with 60% HOAc at 65 °C for 1.5 h. Complete regioselective glycosylation of 6-OH of acceptor **6** with trisaccharide donor **7** was accomplished by an NIS–TfOH promoted system, providing pentasaccharide **27** as the only glycosylation product in modes yield (49%). Complete deprotection of pentasaccharide **27** to target **2** was accomplished in a five-step sequence — (1) removal of the NAP group by DDQ methodology, (2) removal of methyl from the carboxyl group in sialic acid, (3) removal of Phth, (4)

Table 1. Crystal data and structure refinement for **10**

Empirical formula	$C_{28}H_{35}O_{13}NS$
Color/shape	rectangular needle
Formula weight	625.6
Temperature	22±3 °C
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
Unit cell dimensions	a (Å) 10.120 (3) α = 90.0°
	b (Å) 16.684 (4) β = 90.0°
	c (Å) 18.746 (4) γ = 90.0°
Volume	3165.1 (14) (Å^3)
Z	4
Density calculated	1.313 mg/m^3
Absorption coefficient	1.443 mm^{-1}
Difftractometer	CAD-4
Radiation/wavelength	CuK_α/1.5418 Å
F(000)	1320
Crystal size	0.25×0.35×0.15 mm
Range for data collection	0–150°
Reflections collected	6192
Independent/observed reflections	5264 ($I \geq 2\sigma$)
Absorption correction	Semi-empirical from psi scans
Range of relat. trasm. factors	0.99 and 0.90
Refinement method	Full-matrix least squares on F^2
Computing	SHELXS-96
Data/restraints/parameters	5264/0/517
Goodness of fit on F^2	1.072
Function minimized	$\Sigma\left[\lvert F_o^2\rvert - (1/k)\lvert F_c^2\rvert\right]$
Final *R* indices	0.0959 for all 6192 reflections
	0.0712 for 5264 [$I \geq 2\sigma I$]
Final extinction coefficient	2.68×10^{-7}
Large diff. peaks and hole	±0.10 e/Å^3

complete acetylation with pyridine–Ac_2O, and, (5) complete deacetylation with 1 M sodium methoxide to give **2** in 25% yield.

Construction of hexasaccharide **3** is displayed in Scheme 6. The NIS-TfOH[15] promoted glycosylation of the primary hydroxyl group of disaccharide acceptor **8** with novel donor **9** gave **28** as a single glycosylation product in good yield (65%) due to much higher reactivity of the primary hydroxyl over the secondary axial hydroxyl group in acceptor **8**. Transformation of **28** into sulfated compound **29** was successfully achieved in five successive steps — (1) acetylation of **28** with Ac_2O–pyridine, (2) careful removal of NAP protection with our methodology,[9] (3) sulfation, (4) removal of benzyl under hydrogen atmosphere catalyzed by Pd/C (10%), followed by (5) treatment with acetic anhydride in pyridine. Target **3** was obtained from **29** by a global deprotection procedure in three steps: (1) removal of methyl with LiI–pyridine, (2) removal of phthalimido with CH_3OH–$NH_2{\cdot}NH_2{\cdot}H_2O$ (5:1), then, complete acetylation with Ac_2O–pyridine (1:1) in the presence of DMAP; (3) de-acetylation with 1 M sodium methoxide–methanol solution.

Table 2. Final fractional positional parameters for nonhydrogen atoms with estimated standard deviations given in parentheses for **10**

Atom	*x*	*y*	*z*	U_{eq}[a]
S	0.91206(10)	0.43862(6)	0.23055(4)	0.0534(3)
O1	0.9129(2)	0.43154(12)	0.37678(11)	0.0400(5)
O2	0.6807(4)	0.3206(2)	0.2832(3)	0.0866(12)
O3	0.5850(4)	0.6219(2)	0.2384(2)	0.0741(9)
O4	0.6593(2)	0.6198(2)	0.35010(14)	0.0492(6)
O5	1.0262(4)	0.6506(2)	0.2985(2)	0.0639(8)
O6	0.7845(5)	0.6670(2)	0.4999(2)	0.0756(10)
O7	0.2682(3)	0.4550(2)	0.49579(14)	0.0511(6)
O8	0.9846(2)	0.49965(14)	0.51308(11)	0.0396(5)
O9	1.0738(4)	0.6128(2)	0.5567(2)	0.0810(11)
O10	1.3652(6)	0.5325(4)	0.4162(3)	0.108(2)
O11	0.8401(3)	0.2882(2)	0.3590(2)	0.0599(7)
O12	1.0765(6)	0.2036(3)	0.4911(6)	0.133(3)
O13	1.2103(3)	0.3006(2)	0.4542(2)	0.0603(7)
N1	0.9135(3)	0.6532(2)	0.40243(13)	0.0413(6)
C1	0.9709(3)	0.5090(2)	0.38502(15)	0.0361(6)
C2	0.7145(4)	0.4822(2)	0.3212(2)	0.0480(7)
C4	0.8277(3)	0.4234(2)	0.3186(2)	0.0419(6)
C5	0.5742(4)	0.6427(2)	0.2993(2)	0.0529(8)
C6	0.7729(4)	0.3375(2)	0.3184(2)	0.0521(8)
C7	0.8497(5)	0.6995(2)	0.4547(2)	0.0551(9)
C8	1.1102(4)	0.3573(2)	0.4692(2)	0.0503(8)
C9	1.1627(3)	0.4380(2)	0.4467(2)	0.0407(6)
C10	1.1084(5)	0.3302(3)	0.2637(2)	0.0635(10)
C11	1.0623(3)	0.5060(2)	0.44932(15)	0.0367(6)
C12	0.9968(4)	0.5588(2)	0.5624(2)	0.0500(8)
C13	1.3682(4)	0.4992(3)	0.4725(3)	0.0605(10)
C14	0.7652(3)	0.5681(2)	0.3299(2)	0.0423(6)
C15	1.0767(5)	0.4083(3)	0.2456(2)	0.0557(9)
C16	0.8619(3)	0.5713(2)	0.39177(15)	0.0378(6)
C17	1.0135(3)	0.6806(2)	0.3563(2)	0.0463(7)
C18	0.9026(5)	0.5460(3)	0.6218(2)	0.0684(11)
C19	0.7986(8)	0.2057(3)	0.3593(3)	0.082(2)
C20	1.2415(8)	0.3112(5)	0.2721(3)	0.097(2)
C21	1.1109(6)	0.7406(3)	0.3833(4)	0.0778(14)
C22	1.2892(10)	0.1696(4)	0.4426(5)	0.104(2)
C23	1.3088(8)	0.4432(9)	0.2460(5)	0.121(4)
C24	0.4712(5)	0.6966(4)	0.3290(4)	0.0788(15)
C25	0.8560(8)	0.7891(3)	0.4500(4)	0.088(2)
C26	1.1725(7)	0.4670(4)	0.2370(3)	0.076(2)
C27	1.4747(11)	0.5000(7)	0.5256(10)	0.100(5)
C29	1.3369(8)	0.3663(8)	0.2642(5)	0.113(3)
C30	1.1806(6)	0.2235(3)	0.4651(3)	0.0743(13)

[a] U_{eq} is defined as the trace of the orthogonalized U_{ij} tensor.

The structure and purity of target oligosaccharides **1**, **2** and **3** were fully characterized by a combination of 1D (Fig. 3), and 2D-NMR techniques (2D ^1H–^1H DQF-COSY, 2D ROESY) and ESIMS spectroscopy.

Our earlier studies show that core 2 branched structures having 6-*O*-sulfated galactose in their Galβ(1→3)Gal NAc arm bind with selectins (L- and P-selectins). Our preliminary binding studies with natural form hexasaccharide **3** indicate that this molecule can weakly bind with L- and P-selectins. Examination of core 2 branched oligosaccharide analogues **1** and **2** as substrates for Gal:6-*O*-sulfotransferase are underway. Detailed biological studies of these oligosaccharides **1**–**3** will be reported elsewhere.

In summary, we have described a very effective protocol for the construction of complex, core 2 branched, sialylated and sulfated oligosaccharide structures **1** and **2** utilizing a strategy of regio- and stereoselective glycosylation of unprotected or partially protected acceptors. Additionally, a convergent method for the synthesis of complex oligosaccharides through employment of a versatile sialylated Lewis^x tetrasaccharide donor **9** has been developed in our laboratory.

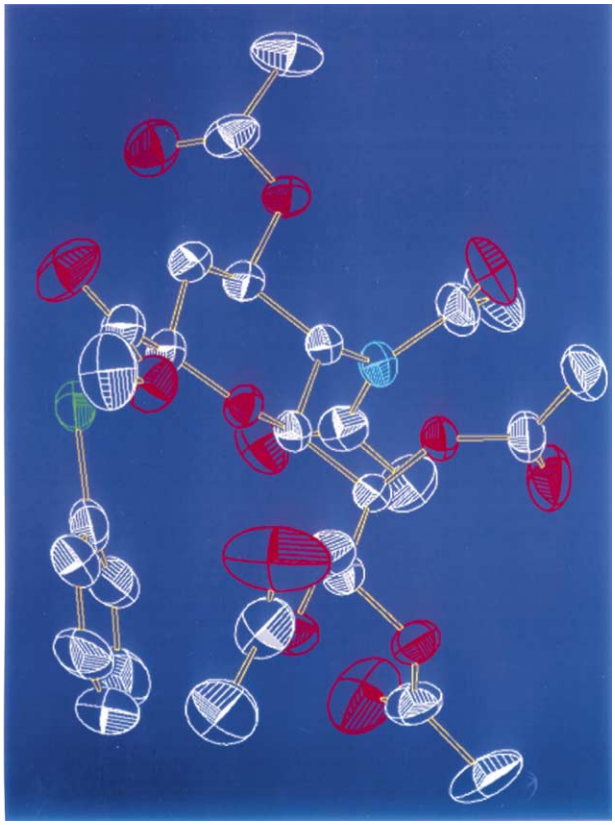
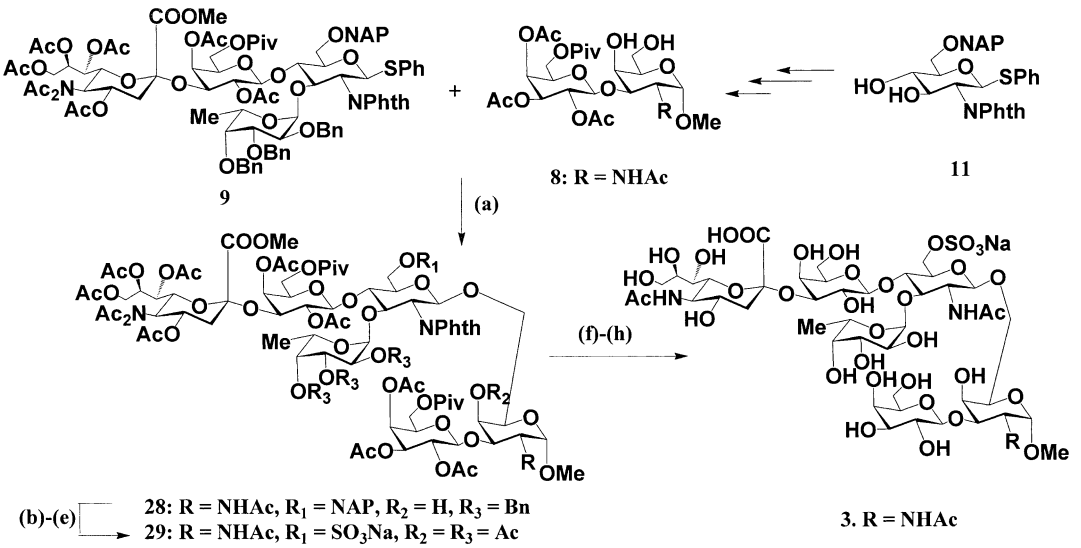


Figure 2. An ORTEP[10] diagram showing the molecular structure of compound **10**. The thermal ellipsoids are drawn with 50% probability. The coloring scheme followed is green (sulfur), red (oxygen), blue (nitrogen), and white (carbon).



Scheme 6. (a) NIS-TfOH/CH_2Cl_2, 4A-MS, −65 to −60 °C, 67%; (b) Ac_2O–pyridine (1:1), DMAP, rt, 85%; (c) DDQ/CH_2Cl_2–CH_3OH (4:1), rt, 22 h, 75%; (d) SO_3–pyridine/pyridine, 0–5 °C, 8 h, 85%; (e) Pd/C (10%), H_2, CH_2Cl_2–CH_3OH, rt, 6 h, then, Ac_2O–pyridine (1:1), DMAP, rt, 12 h; (f) LiI/pyridine, 120–125 °C, 8 h, N_2; (g) CH_3OH–$NH_2NH_2{\cdot}H_2O$, 80–85 °C, 6 h, then Ac_2O–pyridine (1:1), DMAP, rt, 12 h; (h) 1 M CH_3ONa–CH_3OH/CH_3OH–H_2O, rt, 12 h, 37% in three steps.

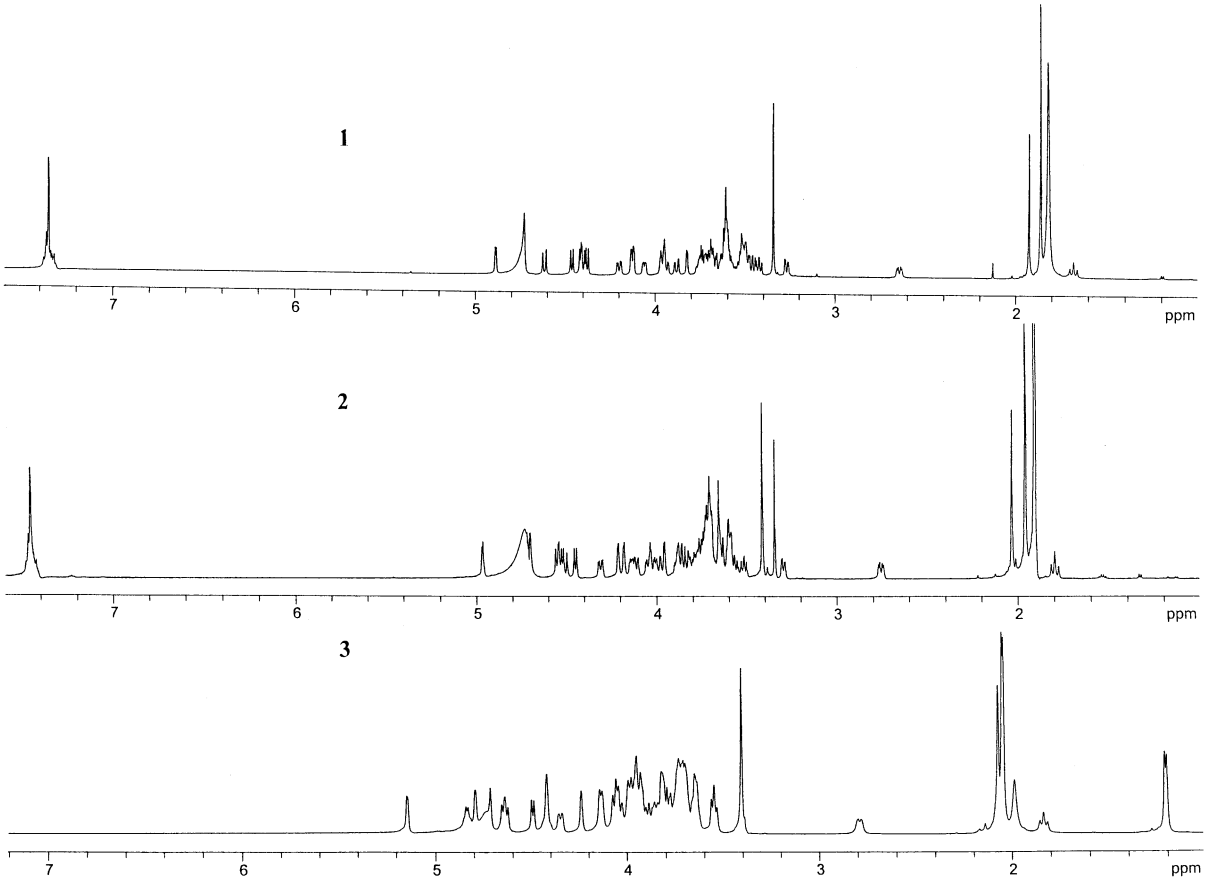


Figure 3. 600 MHz 1D ^1H NMR of oligosaccharides: **1** (D_2O), **2** ($D_2O + CD_3OD$) and **3** (D_2O) recorded at 303.0 K.

Experimental

General procedures

TLC was conducted on glass plates precoated with a 0.25-mm layer of silica gel 60 F-254 (Analtech GHLF uniplates). The components were visualized either by exposure to UV light or by spraying with 10% H_2SO_4, and 0.2% *p*-anisaldehyde in a solution of ethanol and heating or both. Solutions were concentrated under reduced pressure at <40 °C. The silica gel used for column chromatography was Baker Analyzed (60–200 mesh). ^1H NMR spectra were recorded at 30 °C with

either a Bruker AMX 400 (400 MHz) or AMX 600 (600 MHz) spectrometer. The values of δ (ppm) are given relative to internal Me_4Si (δ = 0) for solutions in $CDCl_3$, CD_2Cl_2, CD_3OD. ^{13}C NMR spectra were recorded at 303 K with a Bruker AMX 400 (100.6 MHz) spectrometer using $CDCl_3$ (77.0 ppm), CD_2Cl_2 (54.15 ppm), CD_3OD (49.15 ppm), acetone-d_6 (206.0 or 29.8 ppm) as reference. First-order chemical shifts and coupling constants (*J*/Hz) were obtained from 1D spectra and assignments of protons resonance were based on 2D DQF ^1H–^1H COSY and 2D ROESY. Two-dimensional double-quantum filtered phase-sensitive ^1H–^1H correlated spectra (DQF ^1H–^1H COSY) and rotating-frame nuclear Overhauser enhancement spectroscopy (ROESY) were recorded at 303 K using a Bruker AMX 400 (mixing time of 400 ms). All samples submitted for elemental analyses were dried for 48 h under vacuum over P_2O_5 at room temperature. Elemental analyses were performed by Robertson Laboratory, Madison, NJ, USA. Dichloromethane (CH_2Cl_2) and 1,2-dichloroethane were kept over 4 Å molecular sieves. Pyridine was redistilled over potassium hydroxide.

Benzyl (6-*O*-trimethylacetyl-β-D-galactopyranosyl)-(1→3)-4,6-*O*-benzylidene-2-acetamido-2-deoxy-α-D-galactopyranoside (20).[16] ^1H NMR ($CDCl_3$, 400 MHz): δ = 7.54–7.48 (m, 2H, ArH), 7.40–7.26 (m, 8H, ArH), 5.83 (d, 1H, *J* = 9.2 Hz, NHAc), 5.12 (s, 1H, benzylidene proton), 5.08 (d, 1H, *J* 4.1 Hz, H-1), 4.71 (d, 1H, *J* = 11.4 Hz, OCH_APh, ABq), 4.64 (dd, 1H, H-2), 4.55 (d, 1H, *J* = 12.2 Hz, OCH_BPh, ABq), 4.22–4.10 (m, 4H), 3.97 (dd, 1H), 3.77 (dd, 1H, *J* = 3.3, 10.9 Hz), 3.65 (d, 1H, *J* = 2.2 Hz, H′-4), 3.58–3.56 (m, 2H), 3.50 (t, 1H, *J* = 6.2 Hz), 3.32 (dd, 1H), 1.91 (s, 3H, Ac), 1.20 (s, 9H, *t*-Bu); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 178.61 (C=O), 174.35 (C=O), 137.94, 137.12, 128.63, 128.63, 128.47, 126.96, 105.81, 101.43, 98.16, 76.17, 73.16, 72.87, 70.59, 69.24, 68.84, 63.55, 48.43, 38.89, 27.39 ($3CH_3$), 23.68 (NAc). Anal. calcd for $C_{33}H_{43}O_{12}N{\cdot}3H_2O$: C, 56.64, H, 7.06, N, 2.00. Found: C, 56.11, H, 6.41, N, 1.68.

Benzyl[methyl(*N*-acetyl-5-diacetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosyl)onate]-(2→3)-(6-*O*-trimethylacetyl-β-D-galactopyranosyl)-(1→3)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside (21). A solution of compound **10** (582 mg, 0.93 mmol), compound **20** (540 mg, 0.84 mmol), *N*-iodosuccinimide (NIS, 625 mg, 2.78 mmol) in dry dichloromethane–acetonitrile (20 mL, 1:1) containing 3 Å MS (2 g) was stirred at −70 to −50 °C for 2 h under N_2 atmosphere. Trifluoromethanesulfonic acid (TfOH) (100 μL) in 2 mL dry acetonitrile was then added dropwise and stirring continued for 6 h. Additional portions of donor **10** (220 mg) and trifluoromethanesulfonic acid (30 μL) were then added and stirring was continued for a total of 16 h. The mixture was neutralized with satd aq sodium bicarbonate. Solids were filtered off and the organic layer washed with satd aq $NaHCO_3$, 10% $Na_2S_2O_3$, water, dried (Na_2SO_4) and concentrated under reduced pressure to a crude product. The product was applied to a column of silica gel eluted with dichloromethane/methanol (30:1) to give a pure compound **21** (430 mg, 45%) as a glass white solid. ^1H NMR ($CDCl_3$, 400 MHz): δ = 7.81–7.35 (m, 10H, ArH), 6.40 (d, 1H, *J* = 8.4 Hz, NHAc), 5.60–5.40 (m, 3H, benzylidene proton, H″-4, H″-8), 5.21 (d, 1H, *J* = 3.6 Hz, H-1), 5.10 (dd, 1H, H″-7), 4.95 (dd, 1H, H″-6), 4.80–4.65 (m, 2H, H-2, OCH_APh), 4.55 (d, 1H, OCH_BPh, Abq), 4.40–4.20 (m, 7H, H″-1, H-4, H″-9b, H″-5, H′-6b, H-6b, H′-6a), 4.12 (dd, 1H, H′-3) 4.00 (dd, 1H, H-6a), 3.95–3.80 (m, 5H, H″-9a, H-3, $COOCH_3$), 3.70–3.60 (m, 3H, H′-2, H′-5, H-5), 3.55 (t, 1H, H′-4), 2.85 (dd, 1H, *J* = 4.6, 12.4 Hz, H″-3e), 2.38 (s, 3H, NAc), 2.30 (s, 3H, NAc), 2.12 (s, 3H, Ac), 2.10 (s, 3H, Ac), 2.00 (s, 3H, Ac), 1.98 (s, 3H, Ac), 1.89 (s, 3H, Ac), 1.20 (s, 9H, *t*-Bu); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 178.10 (C=O), 174.52 (C=O), 171.20 (C=O), 170.98 (C=O), 170.65 (C=O), 170.33 (C=O), 169.93 (C=O), 168.33 (C=O), 138.01, 137.65, 129.04, 128.71, 128.57, 128.42, 128.29, 128.26, 126.79, 106.47, 101.26, 98.56, 97.38, 77.76, 77.54, 76.91, 76.20, 72.18, 70.63, 70.09, 69.47, 69.32, 68.95, 68.26, 67.94, 67.49, 66.76, 63.60, 63.03, 62.65, 56.69, 53.30, 48.77, 38.70, 27.39 ($3CH_3$), 23.42 (NAc), 21.38 (Ac), 21.05 (Ac), 20.94 (Ac), 20.72 (Ac). Anal. calcd for $C_{56}H_{71}O_{25}N_2$: C, 57.38, H, 6.11, N, 2.39. Found: C, 56.62, H, 5.93, N, 2.23.

Benzyl[methyl(*N*-acetyl-5-diacetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosyl)onate]-(2→3)-(6-*O*-trimethylacetyl-2,4-*O*-di-acetyl-β-d-galactopyranosyl)-(1→3)-2-acetamido-2-deoxy-α-d-galactopyranoside (5). A solution of compound **21** (300 mg, 0.26 mmol) and DMAP (3 mg) in acetic anhydride (3 mL) and dry pyridine (3 mL) was stirred at room temperature overnight. The reaction mixture was concentrated to a crude residue which was passed through a short column of silica gel eluted with dichloromethane/methanol (40:1) to give an amorphous solid. This product was treated with 60% acetic acid at 60–65 °C for 1.5 h and concentrated then passed through a short column of silica gel eluted with dichloromethane/methanol (25:1) to give a pure compound **5** (200 mg, 72%) as an amorphous solid. ^1H NMR ($CDCl_3$, 400 MHz): δ = 7.37–7.31 (m, 5H, ArH), 6.42 (d, 1H, *J* = 8.4 Hz, NHAc), 5.65 (ddd, 1H, H″-8), 5.54 (ddd, 1H, H″-4), 5.11–5.07 (m, 2H, H″-7, H-1), 5.05–4.99 (m, 2H, H′-2, H′-4), 4.71–4.68 (m, 2H, $PhCH_AO$, H′-1), 4.63–4.56 (m, 3H, H″-6, H′-3, H-2), 4.46 (d, 1H, J_{gem} = 12.0 Hz, $PhCH_BO$, ABq), 4.36–4.30 (m, 2H, H′-5, H″-9b), 4.17 (d, 1H, *J* = 2.8 Hz, H-4), 4.11 (m, 1H, H-6b), 4.00–3.90 (m, 2H, H-6a, H′-6b), 3.90–3.76 (m, 7H, H′-5, H-5, $COOCH_3$, H′-6a, H-3), 2.66 (dd, 1H, *J* = 5.2, 12.6 Hz, H″-3e), 2.35 (s, 3H, NAc), 2.25 (s, 3H, NAc), 2.20 (s, 3H, Ac), 2.17 (s, 3H, Ac), 2.10 (s, 3H, Ac), 2.06 (s, 3H, Ac), 2.05 (s, 3H, Ac), 1.99 (s, 3H, Ac), 1.96 (s, 3H, Ac), 1.92 (s, 3H, Ac), 1.82 (s, 3H, Ac), 1.58 (t, 1H, J_{gem} = 12.4 Hz, H″-3a), 1.19 (s, 9H, *t*-Bu); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 177.76 (C=O), 174.11 (C=O), 173.74 (C=O), 171.55 (C=O), 171.24 (C=O), 170.05 (C=O), 169.99 (C=O), 169.86 (C=O), 169.80 (C=O), 167.97 (C=O), 137.39, 128.75, 128.64, 128.26, 102.76, 97.57, 96.66, 78.59, 71.41, 70.74, 70.22, 69.93, 69.90, 69.36, 69.28, 69.23, 67.57, 67.41, 67.16, 67.01, 63.23, 63.01, 61.06, 55.88, 53.10, 48.35, 38.37, 28.17 (NAc), 27.14 (3 CH_3), 26.82 (NAc), 23.15 (NAc), 21.52 (Ac), 21.12 (Ac), 21.02 (Ac), 20.79 (Ac). Anal. calcd for

$C_{53}H_{71}O_{27}N_2$: C, 54.49, H, 6.13, N, 2.40. Found: C, 54.98, H, 6.23, N, 2.42.

Phenyl(3-*O*-methyl-β-D-galactopyranosyl)-(1→4)-6-*O*-trimethylacetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (23). A solution of compound **22** (210 mg, 0.29 mmol) in dry methanol (10 mL) received dibutyltin oxide (72 mg, 0.29 mmol) and was refluxed for 4 h. This mixture was concentrated and the residue treated with tetrabutylammonium iodide (62 mg, 0.44 mmol) and methyl iodide (12.12 g, 54.86 mmol) in dry benzene/DMF (1:1) (10 mL) at 40–45 °C for 4 days. The mixture was concentrated under reduced pressure and the crude residue was applied to a column of silica gel and eluted first with hexane/ethyl acetate (4:1), and then with hexane/ethyl acetate (1:1) to give compound **23** (148 mg, 68%) as an amorphous solid. ^{1}H NMR (CD_3OD, 400 MHz): δ = 8.00–7.80 (m, 4H, ArH), 7.40–7.20 (m, 5H, ArH), 5.51 (d, 1H, *J* = 8.4 Hz, H-1), 4.65 (dd, 1H), 4.40–4.30 (m, 3H), 4.10 (t, 1H), 4.00 (s, 1H), 3.82 (m, 1H), 3.78–3.54 (m, 4H), 3.51 (m, 3H), 3.44 (s, 3H, OCH_3), 3.10 (dd, 1H), 1.10 (s, 9H, *t*-Bu); ^{13}C NMR (CD_3OD, 100.6 MHz): δ = 147.57, 147.36, 147.15, 135.85, 133.86, 133.49, 130.12, 129.22, 124.66, 124.39, 105.16 (C′-1), 84.97 (C-1), 84.34, 80.80, 78.69, 77.39, 72.40, 71.61, 66.23, 64.30, 64.28, 62.85, 57.56, 56.81, 27.86 ($3CH_3$). Anal. calcd for $C_{32}H_{39}O_{12}NS\cdot H_2O$: C, 56.54, H, 6.08, N, 2.07, S, 4.72. Found: C, 56.77, H, 5.49, N, 1.99, S, 4.83.

Phenyl(2,4,6-tri-*O*-acetyl-3-*O*-methyl-β-D-galactopyranosyl)-(1→4)-4-*O*-acetyl-6-*O*-trimethyl-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (4). A solution of compound **23** (140 mg, 0.19 mmol) and DMAP (5 mg) in acetic anhydride (6 mL) and dry pyridine (6 mL) was stirred at room temperature overnight. The reaction mixture was concentrated to a crude mixture, which was applied to a short column of silica gel eluted with hexane/ethyl acetate (1:1) to give a pure compound **4** (90%) as an amorphous solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.78–7.72 (m, 2H, ArH), 7.41–7.36 (m, 2H, ArH), 7.28–7.20 (m, 3H, ArH), 5.80–5.70 (m, 2H, H-3, H-1), 5.42 (d, 1H, *J* = 2.8 Hz, H′-4), 4.98 (dd, 1H, H′-2), 4.52 (dd, 1H), 4.42 (d, 1H, *J* = 7.8 Hz, H′-1), 4.30–4.15 (m, 2H), 4.10–4.00 (m, 2H), 3.90–3.70 (m, 3H), 3.35 (s, 3H, OCH_3), 3.25 (dd, 1H), 2.28 (s, 3H, Ac), 2.20 (s, 3H, Ac), 2.15 (s, 3H, Ac), 1.84 (s, 3H, Ac), 1.10 (s, 9H, *t*-Bu); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 178.09 (C=O), 174.28 (C=O), 170.61 (C=O), 170.36 (C=O), 169.97 (CO), 169.36 (C=O), 167.44 (C=O), 134.63, 134.38, 133.26, 131.91, 131.48, 131.44, 129.10, 128.44, 123.97, 123.75, 101.29 C′-1), 83.22 (C-1), 80.10, 76.91, 76.74, 71.99, 71.20, 70.80, 65.19, 62.76, 61.69, 58.27, 54.09, 39.05 [$C(CH_3)_3$], 27.45 ($3CH_3$), 20.91 (Ac), 20.85 (Ac), 20.68 (Ac). Anal. calcd for $C_{40}H_{47}O_{16}NS\cdot H_2O$: C, 56.66, H, 5.88, N, 1.65, S, 3.78. Found: C, 55.97, H, 5.03, N, 1.45, S, 3.69.

Glycosylation procedure for preparation of 24, 27, and 28 with NIS–TfOH promoter

A solution of acceptor (1 mmol), donor (1.05–1.1 mmol), *N*-iodosuccinimide (NIS, 3.0 mmol) in dry dichloromethane containing 4 Å MS (500–800 mg/mL) was stirred at −65 to −60 °C for 2 h under N_2 atmosphere. Trifluoromethanesulfonic acid (TfOH) (25–32 μL/mmol NIS) in dry dichloromethane (2 mL) was then added dropwise and stirring was continued at the same temperature for 1.5–2 h. Reaction monitored by TLC. The mixture was neutralized with satd aq sodium bicarbonate, solids were filtered off and the organic layer washed with satd aq $NaHCO_3$, 10% $Na_2S_2O_3$, water, dried (Na_2SO_4) and concentrated under reduced pressure. The resultant mixture was purified on a column of silica gel eluted with dichloromethane/methanol to give pure product.

Pentasaccharide (24). As an amorphous solid, yield 65%. ^{1}H NMR ($CDCl_3$, 600 MHz): δ = 7.80–7.10 (m, 9H, ArH), 5.70 (dd, 1H, *J* = 8.3, 7.9 Hz, H‴-3), 5.58–5.49 (m, 2H, H″-4, H″-8), 5.47 (d, 1H, *J* = 2.8 Hz, H⁗-4), 5.43 (d, 1H, *J* = 8.7 Hz, H‴-l), 5.14 (dd, 1H, *J* = 2.6, 9.6 Hz, H″-7), 5.02 (d, 1H, *J* = 3.2 Hz, H′-4), 4.92–4.85 (m, 2H, H⁗-2, H′-2), 4.68 (d, 1H, *J* = 7.3 Hz, H′-1), 4.64–4.56 (m, 3H, H″-6, H⁗-1, H′-3), 4.49 (d, 1H, *J* = 3.0 Hz, H-1), 4.38–4.32 (m, 2H, H-2, H″-5), 4.28–4.20 (m, 2H, H″-9b, H⁗-2), 4.20–3.84 (m, 15H, H″-9b, $PhCH_AO$, H‴-2, H′-6b, H′-6a, H-6b, H⁗-5, H-4, H‴-4, H′-5, H-5, H″-9a, $COOCH_3$), 3.76 (d, 1H, J_{gem} = 11.0 Hz, $PhCH_BO$, ABq), 3.73–3.68 (m, 2H, H-6a, H-3), 3.52 (dd, 1H, H⁗-3), 3.32 (s, 3H, OCH_3), 2.59 (dd, 1H, *J* = 5.1, 12.5 Hz, H″-3e), 2.35 (s, 3H, NAc), 2.34 (s, 3H, NAc), 2.33 (s, 3H, NAc), 2.20 (s, 3H, Ac), 2.18 (s, 3H, Ac), 2.12 (s, 3H, Ac), 2.08 (s, 3H, Ac), 2.06 (s, 3H, Ac), 2.04 (s, 3H, Ac), 1.97 (s, 3H, Ac), 1.96 (s, 3H, Ac), 1.90 (s, 3H, Ac), 1.83 (s, 3H, Ac), 1.48 (t, 1H, *J* = 11.7 Hz, H″-3a), 1.28 (s, 9H, *t*-Bu), 1.16 (s, 9H, *t*-Bu). Anal. calcd for $C_{83}H_{108}O_{41}N_3$: C, 55.27, H, 6.04, N, 2.33. Found: C, 55.15, H, 6.13, N, 2.15.

A general procedure B for deprotection of compound 24, 27, and 29

To a solution of compound **24** (32 mg, 17 μmol) in dry pyridine (3 mL), was added lithium iodide (Lil, 50 mg, 0.37 mmol). The mixture was refluxed at 120–125 °C for 6 h under N_2 atmosphere. The dark yellow solution was then concentrated to dryness and co-evaporated with toluene to a corresponding carboxylic acid as a dark yellow amorphous solid which was used directly for the next reaction. A solution of the above in methanol (5 mL), was treated with NH_2–$NH_2\cdot H_2O$ (1 mL) for 4 h at 80–85 °C, the mixture was concentrated under reduced pressure, co-evaporated with toluene then acetylated with acetic anhydride–pyridine (1:1) in the presence of a catalytic amount of DMAP at room temperature overnight. The acetylated mixture was concentrated and passed through a short column of silica gel eluted with dichloromethane/methanol (10:1) to give a bright yellow film. To a solution of this bright yellow film in methanol–water (1 mL, 1:1), was added a catalytic amount of 1 M sodium methoxide (150 μL). The mixture was stirred at room temperature for 48 h and concentrated under reduced pressure to give a crude mixture which was then applied to a short column of silica gel eluted with n-C_3H_7OH/HOAc/H_2O (3:1:1) to give a pure compound.

Deprotected pentasaccharide (1). See general procedure B, (11 mg, 57%) as an amorphous solid. ^{1}H NMR (D_2O, 600 MHz) δ = 7.40–7.20 (m, 5H, ArH), 4.88 (d, 1H, J = 3.6 Hz, H-1), 4.61 (d, 1H, J = 11.4 Hz, $PhCH_AO$, ABq), 4.45 (d, 1H, J = 8.4 Hz, H‴-l), 4.41–4.36 (m, 3H, H′-1, $PhCH_BO$, H⁗-1), 4.18 (dd, 1H, H-2), 4.14–4.10 (dd, 2H, H-4, H⁗-4), 4.06 (dd, 1H, H-5), 3.96–3.92 (m, 3H, H′-3, H-6b, H-3), 3.87 (dd, 1H, H‴-2), 3.81 (d, 1H, J = 2.3 Hz, H′-4), 3.75–3.40 (m, 20H, H-6a, H″-4, H′-5, H⁗-2, H′-2), 3.34 (s, 3H, OCH_3), 3.26 (dd, 1H, J = 3.1, 10.0 Hz, H⁗-3), 2.64 (dd, 1H, J = 4.6, 2.4 Hz, H″-3e), 1.92 (s, 3H, Ac), 1.85 (s, 3H, Ac), 1.81 (s, 3H, Ac), 1.67 (*t*, 1H, J = 11.8 Hz, H″-3a); ^{13}C NMR (D_2O, 100.6 MHz) δ = 182.40 (C=O), 176.21 (C=O), 175.60 (C=O), 175.40 (C=O), 138.03, 129.84, 129.69, 129.47, 105.45, 103.87, 102.58, 100.81 (C″-2), 97.32, 82.78, 79.50, 78.23, 76.75, 76.35, 75.86, 73.91, 73.59, 72.88, 71.65, 70.87, 70.68, 70.50, 70.19, 69.78, 69.43, 69.20, 68.52, 65.22, 63.62, 62.18, 62.08, 61.16, 57.34, 56.20, 52.81, 40.85 (C″-3), 23.45 (NAc), 23.28 (NAc), 23.12 (NAc); ESIMS (*m*/*z*) (negative ion mode) $C_{47}H_{73}O_{29}N_3$: 1143.3; found: 1142 $[M-H]^-$.

Benzyl(2,4-*O*-di-acetyl-3-*O*-methyl-β-D-galactopyranosyl)-(1→3)-2-acetamido-2-deoxy-α-D-galactopyranoside (7). To a solution of compound **25**[1b] (370 mg, 0.57 mmol) in dry methanol (15 mL) was added dibutyltin oxide (142 mg, 0.57 mmol), and refluxed for 2 h. The reaction mixture was concentrated to a crude residue which was treated with tetrabutylammonium iodide (142 mg) and methyl iodide (54 uL, 0.86 mmol) in dry benzene–DMF (1:1) (10 mL) at 40–43 °C for 4–5 days. The reaction mixture was concentrated to a crude residue which was passed through a short of column of silica gel and eluted with dichloromethane/methanol (10:1) to give a pure compound (240 mg) in 56% yield as an amorphous solid. A solution of the above compound **26** (240 mg) was treated with a mixture of dry pyridine (4 mL) and anhydrous acetic anhydride (4 mL) in the presence of DMAP (5 mg) at room temperature overnight. The reaction was concentrated under reduced pressure to a crude residue which was purified by a short column of silica gel eluted with dichloromethane/methanol (40:1) to give a pure acetylated compound **26** (250 mg) which was treated with 60% HOAc at 65 °C for 1 h. The reaction mixture was concentrated under reduced pressure to a crude mixture which was passed through a short column of silica gel eluted with dichloromethane/methanol (20:1) to give a pure compound **7** (147 mg) in 66% yield as an amorphous solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 7.41–7.29 (m, 5H, ArH), 5.48 (d, 1H, J = 3.1 Hz), 4.96 (dd, 1H, J = 8.1, 8.0 Hz), 4.83 (d, 1H, J = 4.6 Hz), 4.76 (dd, 2H, $PhCH_2O$), 4.71 (d, 1H, J = 7.9 Hz), 4.50–4.45 (m, 2H), 4.15–4.09 (m, 3H), 3.95–3.87 (m, 3H), 3.76–3.70 (m, 2H), 3.51 (dd, 1H), 3.32 (s, 3H, OCH_3), 2.09 (s, 3H, Ac), 2.06 (s, 3H, Ac), 2.01 (s, 3H, Ac), 1.95 (s, 3H, Ac). Anal. calcd for $C_{28}H_{39}O_{14}N$: C, 54.81, H, 6.40. Found: C, 54.77, H, 5.90.

Pentasaccharide (27). The compound **27** (95 mg) was obtained from acceptor **7** (67 mg) according to general glycosylation procedure **A** in 49% yield as amorphous solid. ^{1}H NMR ($CDCl_3$, 600 MHz): δ = 7.52–7.49 (m, 5H, ArH), 7.41–7.32 (m, 8H, ArH), 5.70 (dd, 1H, J = 8.3, 7.9 Hz, H‴-3), 5.59–5.48 (m, 2H, H″-4, H″-8), 5.48 (d, 1H, J = 2.9 Hz, H⁗-4), 5.45 (d, 1H, J = 8.7 Hz, H‴-l), 5.15 (dd, 1H, J = 2.8, 9.6 Hz, H″-7), 5.03 (d, 1H, J = 2.8 Hz, H′-4), 4.93–4.86 (m, 2H, H⁗-2, H′-2), 4.69 (d, 1H, J = 7.3 Hz, H′-1), 4.65–4.57 (m, 3H, H″-6, H⁗-l, H′-3), 4.48 (d, 1H, J = 3.1 Hz, H-1), 4.39–4.20 (m, 7H, H-2, H″-5, H″-9b, H⁗-2), 4.21–3.85 (m, 15H, H″-9b, $PhCH_AO$, H‴-2, H′-6b, H′-6a, H-6b, H⁗-5, H-4, H‴-4, H′-5, H-5, H″-9a, $COOCH_3$), 3.75 (d, 1H, J_{gem} = 12.6 Hz, $PhCH_BO$, ABq), 3.74–3.69 (m, 2H, H-6a, H-3), 3.53 (dd, 1H, H⁗-3), 3.34 (s, 3H, OCH_3), 2.61 (dd, 1H, J = 4.8, 12.6 Hz, H″-3e), 2.36 (s, 3H, NAc), 2.34 (s, 3H, NAc), 2.32 (s, 3H, NAc), 2.21 (s, 3H, Ac), 2.16 (s, 3H, Ac), 2.13 (s, 3H, Ac), 2.09 (s, 3H, Ac), 2.06 (s, 3H, Ac), 2.05 (s, 3H, Ac), 1.96 (s, 3H, Ac), 1.95 (s, 3H, Ac), 1.91 (s, 3H, Ac), 1.85 (s, 3H, Ac), 1.51 (t, 1H, J = 11.7 Hz, H″-3a), 1.27 (s, 9H, *t*-Bu). Anal. calcd for $C_{92}H_{113}O_{42}N_3$: C, 57.16, H, 5.89. Found: C, 57.70, H, 5.95.

Deprotection of pentasaccharide 27 to target oligosaccharide (2)

To a solution of compound **27** (63 mg, 0.037 mmol) in a mixture of dichloromethane–methanol (5 mL, 4:1) was added DDQ (12 mg). The reaction mixture was stirred at room temperature for 20 h. The reaction was concentrated under reduced pressure to a crude residue which was then redissolved in dichloromethane (50 mL) and washed with satd $NaHCO_3$ solution (3×50 mL). The organic phase was dried with Na_2SO_4 and concentrated to a crude residue which was purified by a short column of silica gel eluted with dichloromethane–methanol (20:1) to give a pure compound (41 mg) in 70% yield as amorphous solid. The compound thus obtained was subjected to general procedure **B** to give target oligosaccharide **2** (8.0 mg) as an amorphous solid. ^{1}H NMR (D_2O, 600 MHz) δ = 7.48–7.40 (m, 5H, ArH), 4.96 (d, 1H, J = 3.3 Hz, H-1), 4.70 (d, 1H, J = 11.3 Hz, $PhCH_AO$, ABq), 4.54 (d, 1H, J = 8.6 Hz, H″-1), 4.52 (d, 1H, J = 7.9 Hz, H‴-l), 4.50 (d, 1H, J = 11.3 Hz, OCH_BPh, Abq), 4.44 (d, 1H, J = 7.8 Hz, H′-1), 4.30 (dd, 1H, J = 3.4, 11.2 Hz, H-2), 4.20 (d, 1H, J = 2.8 Hz, H-4), 4.17 (d, 1H, J = 3.1 Hz, H′-4), 4.13 (dd, 1H), 4.10 (dd, 1H, J = 3.0, 9.9 Hz, H‴-3), 4.06–3.96 (m, 3H, H-6a, H-3,), 3.95 (d, 1H, J = 2.6 Hz, H‴-4), 3.92–3.48 (m, 22H, H-6b, H⁗-4, H‴-2, H′-2), 3.40 (s, 3H, OCH_3), 3.29 (dd, 1H, H′-3), 2.75 (dd, 1H, J = 4.6, 12.4 Hz, H‴-3e), 2.04 (s, 3H, Ac), 1.98 (s, 6H, 2Ac), 1.79 (t, 1H, J = 11.9 Hz, H″-3a); ^{13}C NMR (D_2O, 100.6 MHz) δ = 182.50 (C=O), 176.50 (C=O), 175.50 (C=O), 175.10 (C=O), 138.20, 130.00, 129.98, 129.80, 105.60, 103.80, 102.65, 101.00 (C″-2), 97.45, 82.80, 79.62, 78.20, 76.65, 76.40, 76.10, 75.98, 74.10, 73.62, 70.89, 70.80, 70.78, 70.60, 70.58, 70.10, 69.49, 69.25, 68.80, 68.72, 65.21, 63.80, 62.20, 61.25, 56.15, 56.05, 53.00, 49.55, 40.98, 23.44 (NAc), 23.22 (NAc), 23.15 (NAc); ESIMS (*m*/*z*) (negative ion mode) $C_{47}H_{73}O_{29}N_3$: 1143.3; found: 1141.9 $[M-H]^-$.

Sialylated Lewis^x tetrasaccharide (9).[9] TLC: R_f = 0.48 (hexane/ethyl acetate 1:1), ^{1}H NMR (DMF-d_7, 600 MHz) δ = 7.84–7.60 (m, 8H, ArH), 7.52–7.36 (m,

6H, ArH), 7.12–7.00 (m, 17H, ArH), 5.57–5.50 (m, 3H, H″-8, H″-4, H-1, $J_{1,2}$ = 10.9 Hz), 5.19 (dd, 1H, J = 2.3, 9.5 Hz, H″-7), 5.00 (d, 1H, J = 3.8 Hz, H′-4), 4.97 (d, 1H, $J_{1,2}$ = 9.1 Hz, H′-1), 4.91 (dd, 1H, H′-2), 4.87–4.85 (m, 2H, OCHAr, H‴-1), 4.82–4.65 (m, 5H, OCHAr, H-3, OCHAr, H′-3, H‴-5), 4.62 (dd, 2H, OCH_2Ar, ABq), 4.59–4.47 (m, 3H, H″-9b, OCHAr, H-2), 4.41 (d, 1H, J_{gem} = 12.1 Hz, OCHAr, ABq), 4.33–4.17 (m, 4H, H″-9a, OCHAr, H″-6, H-4), 4.09 (dd, 1H, H″-5), 4.04–3.95 (m, 4H, H-6b, H′-6b, H′-5, H-6a), 3.92–3.78 (m, 6H, H‴-3, H′-6a, $COOCH_3$, H‴-2), 3.80 (dd, 1H, H‴-5), 3.70 (dd, 1H, H-5), 3.62 (d, 1H, J = 2.8 Hz, H‴-4), 2.60 (dd, 1H, J = 4.9, 12.3 Hz, H″-3e), 2.35 (s, 3H, Ac), 2.28 (s, 3H, Ac), 2.22 (s, 3H, Ac), 2.10 (s, 3H, Ac), 1.98 (s, 3H, Ac), 1.97 (s, 3H, Ac), 1.96 (s, 3H, Ac), 1.79 (s, 3H, Ac), 1.58 (t, 1H, J = 11.0 Hz, H″-3a), 1.25 (d, 3H, J = 6.7 Hz, $CH‴_3$), 1.10 (s, 9H, *t*-Bu); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ = 177.31 (C=O), 174.24 (C=O), 173.81 (C=O), 170.74 (C=O), 170.38 (C=O), 170.13 (C=O), 169.86 (C=O), 169.27 (C=O), 167.99 (C=O), 139.17, 138.88, 138.56, 136.17, 134.31, 133.47, 133.03, 132.51, 128.94, 128.31, 128.25, 128.23, 128.10, 128.04, 127.83, 127.81, 127.51, 127.29, 127.18, 127.03, 126.12, 125.94, 125.81, 125.67, 123.82, 99.79, 97.54, 96.79 (C^c-2), 84.07 (C^a-1), 80.02, 79.89, 77.69, 74.88, 74.79, 74.41, 73.60, 73.05, 73.03, 72.50, 71.90, 70.74, 70.35, 69.49, 68.47, 67.37, 67.30 (2C), 67.00, 66.65, 62.14, 60.12, 56.05, 55.60, 53.05, 38.65 [$C(CH_3)_3$], 38.55 [$(CH_2)''$], 28.29 (Ac), 27.13 (CH_3), 26.94 (Ac), 21.52 (Ac), 21.22 (Ac), 21.07 (Ac), 20.88 (Ac), 20.75 (Ac), 16.92 ($CH‴_3$). Anal. calcd for $C_{95}H_{106}O_{31}N_2S$: C, 63.25, H, 5.92, N, 1.55, S, 1.78. Found: C, 63.17, H, 6.40, N, 1.40, S, 1.70.

Hexaccharide (28). As an amorphous solid yield 67%. ^{1}H NMR ($CDCl_3$, 600 MHz): δ = 7.95–7.88 (m, 4H, ArH), 7.71–7.68 (m, 2H, ArH), 7.55–7.51 (m, 4H, ArH), 7.29–7.10 (m, 16H, ArH), 6.09 (d, J = 9.6 Hz, 1H, NHAc), 5.66 (ddd, 1H, H⁗-8), 5.58 (ddd, 1H, H⁗-4), 5.15–5.11 (m, 3H; H‴-1, H⁗-7, H⁗-4), 5.06–4.99 (m, 3H; H′-2, H⁗-2, OCHAr, ABq), 4.86–4.74 (m, 4H; H-4, $J_{3,4}$ = 3.6 Hz, OCHAr, ABq, H″-3, H″-1, $J_{1,2}$ = 8.0 Hz), 4.73–4.71 (m, 2H, $J_{1,2}$ = 5.2 Hz; H-1, H′-1), 4.68–4.57 (m, 3H; OCHAr, J_{gem} = 12.0 Hz, H⁗-6, OCHAr, J_{gem} 11.6 Hz, ABq), 4.46 (d, J_{gem} = 12.8 Hz, 1H; OCHAr, ABq), 4.38–4.18 (m, 7H; H⁗′-1, H⁗′-4, H⁗′-5, H⁗′-9b, H-2, H‴-4), 4.12–3.90 (m, 14H; H″-6b, H′-6b, H-6b, H⁗′-3, H‴-6b, H^1-6a, H″-6a, $COOCH_3$, H‴-6a, H⁗-9a, H′-5, H-3), 3.73–3.56 (m, 2H; H-6a, H⁗′-2), 3.36 (t, J = 6.8, 7.2 Hz, 1H; H″-5), 2.84 (s, 3H; OCH_3), 2.67 (dd, J = 5.2, 12.8 Hz, 1H; H⁗′-3e), 2.38 (s, 3H; Ac), 2.35 (s, 3H; Ac), 2.26 (s, 3H; Ac), 2.19 (s, 3H; Ac), 2.10 (s, 3H; Ac), 2.07 (s, 3H; Ac), 2.04 (s, 3H; Ac), 1.97 (s, 3H; Ac), 1.95 (s, 3H; Ac), 1.94 (s, 3H; Ac), 1.93 (s, 3H; Ac), 1.91 (s, 3H; Ac), 1.59 (t, J = 12.4 Hz, 1H; H⁗-3a), 1.21 (s, 12H; *t*-Bu, $CH_3{}'''''$), 1.20 (s, 9H, *t*-Bu); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 178.70 (C=O), 174.20 (C=O), 173.70 (C=O), 173.17 (C=O), 171.38 (C=O), 171.16 (C=O), 170.38 (C=O), 170.17 (C=O), 170.08 (C=O), 170.03 (C=O), 169.88 (C=O), 169.78 (C=0), 169.70 (C=O), 169.61 (C=O), 168.82 (C=O), 167.96 (C=O), 139.11, 138.90, 138.51, 135.41, 134.15, 133.21, 128.70, 128.31, 128.27, 128.20, 128.09, 127.95, 127.51, 127.28, 127.21, 127.19, 126.83, 126.45, 126.13, 125.99, 102.62, 99.71, 99.23, 98.25, 97.47, 96.72, 79.91, 78.48, 75.39, 75.27, 74.59, 74.39, 73.91, 72.83, 72.41, 72.33, 71.51, 71.44, 71.18, 70.85, 70.68, 70.42, 69.45, 69.28, 69.25, 69.11, 68.72, 68.18, 67.49, 67.45, 67.43, 67.08, 68.83, 66.52, 63.13, 60.89, 60.19, 56.65, 55.92, 54.39, 53.08, 48.25, 38.37, 27.21 ($3CH_3$), 23.24 (Ac), 21.55 (Ac), 21.15 (Ac), 21.05 (Ac), 20.81 (Ac), 20.69 (Ac), 16.81 (CH_3). Anal. calcd (%) for $C_{115}H_{141}O_{46}N_3$: C, 59.94, H, 6.17, N, 1.82. Found: C, 59.64, H, 5.86, N, 1.52.

Deprotected hexasaccharide (3). A solution of compound **28** (292 mg, 0.13 mmol) and DMAP (4 mg) in acetic anhydride (3 mL) and dry pyridine (3 mL) was stirred at room temperature overnight. The reaction mixture was concentrated to a crude residue which was passed through a short column of silica gel eluted with dichloromethane/methanol (40:1) to give a pure compound (264 mg, 87%). To a solution of this compound (264 mg, 0.112 mmol) in a mixture of dichloromethane–methanol (8 mL, 4:1) was added DDQ (38 mg) and stirred at room temperature overnight. Another additional portion of DDQ (20 mg) was added and stirred for a total 22 h. The reaction was concentrated to a crude residue which was dissolved in dichloromethane and washed with satd $NaHCO_3$, water, and dried over Na_2SO_4. The organic layer was concentrated to a crude mixture (95 mg) which was treated with SO_3–pyridine complex (15 mg) in dry pyridine (2 mL) at 0 °C overnight. The reaction mixture was concentrated to a crude residue which was purified by a short column of silica gel eluted with dichloromethane/methanol (20:1) to give a pure compound (64 mg). A solution of the above obtained compound (82 mg) and Pd/C(10%) (80 mg) in a mixture of dichloromethane–methanol (10 mL) was stirred overnight under H_2 atmosphere. The filtrate was concentrated to a crude residue which was treated with acetic anhydride (3 mL) and pyridine (3 mL) in the presence of DMAP (3 mg) at room temperature overnight. The reaction mixture was concentrated to a crude product, which was passed through a short column of silica gel eluted with CH_2Cl_2/MeOH (20:1) to give pure compound **29** (60 mg) as an amorphous solid which was directly subjected to general deprotection procedure **B** to give compound **3** (14 mg). ^{1}H NMR (D_2O, 600 MHz): δ = 5.13 (d, 1H, J = 3.1 Hz, H⁗′-1), 4.81 (dd, 1H, H⁗′-5), 4.78 (d, 1H, J = 3.6 Hz, H-1), 4.66–4.60 (dd, 2H, H‴-1, H″-1), 4.48 (d, 1H, J = 7.5 Hz, H′-1), 4.40 (t, 2H, H″-6b, H″-6a), 4.33 (dd, 1H, H-20, 4.22 (d, 1H, H-4), 4.12 (dd, 2H, H-5), 4.08–3.06 (m, 26H, H-6b, H″-4, H-3, H″-2, H″-3, H-6a), 3.56–3.50 (m, 2H, H′-2, H‴-2), 3.38 (s, 3H, OCH_3), 2.78 (dd, 1H, H⁗-3e), 2.06 (s, 3H, Ac), 2.02 (s, 3H, Ac), 1.82 (t, 1H, H⁗-3a), 1.20 (d, 3H, J = 5.9 Hz, $CH⁗'''_3$); ^{13}C NMR (D_2O, 100.6 MHz): δ = 180.58 (C=O), 174.68 (C=O), 174.25 (C=O), 173.89 (C=O), 104.36, 101.35, 100.90, 99.36, (C⁗-2), 98.25, 97.85, 76.92, 75.15, 74.61, 74.50, 74.44, 72.62, 72.41, 72.55, 72.41, 72.25, 71.62, 71.06, 70.41, 70.35, 69.12, 69.05, 68.91, 68.64, 68.29, 68.10, 68.05, 67.82, 67.45, 66.97, 66.40, 65.65, 62.27, 61.16, 60.63, 55.34, 54.68, 51.48, 48.24, 39.58, 21.96 (NAc), 21.76 (NAc), 21.72 (NAc), 14.98 ($CH⁗'''_3$); ESIMS (*m*/*z*) (negative ion mode) calcd for $C_{46}H_{76}O_{36}N_3SNa$ [M]: 1301; found 1278.3 $[M-Na]^-$, 638.9 $[M-2H]^-$.

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References and Notes

1. (a) Nicolaou, K. C.; Bockovich, N. J. D.; Carcanague, R. *J. Am. Chem. Soc.* **1993**, *115*, 8843. (b) Yamada, H.; Harada, T.; Takahashi, T. *J. Am. Chem. Soc.* **1994**, *116*, 7919. (c) Kanie, O.; Barresi, A.; Ding, Y.; Otter, Y.; Forsberg, L. S.; Ernst, B.; Hindsgaul, O. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2720. (d) Boons, G. J. *Tetrahedron* **1996**, *52*, 1095. (e) Sanders, W. J.; Manning, D. D.; Koeller, K. M.; Kiessling, L. L. *Tetrahedron* **1997**, *51*, 5643. (f) Shimanel, E. E.; McGarvey, G. J.; Jablonowski, J. A.; Wong, C.-H. *Chem. Rev.* **1998**, *98*, 833. (g) Lengenmuller, M.; Ito, Y.; Ogawa, T. *Tetrahedron* **1998**, *54*, 1381. (h) Wong, C.-H. *Acc. Chem. Res.* **1999**, *32*, 376. (i) Zhang, Z.; Ollmann, I. R.; Ye, X.-S.; Wischnat, R.; Baasov, T.; Wong, C.-H. *J. Am. Chem. Soc.* **1999**, *121*, 734. (j) Demchenko, A.; Boons, G. J. *Chem. Eur. J.* **1999**, *5*, 1278. (k) Danishefsky, S. J.; Allen, J. R. *Angew. Chem., Int. Ed.* **2000**, *39*, 836. (l) Gege, C.; Vogel, J.; Bendas, G.; Rothe, U.; Schmidt, R. R. *Chem. Eur. J.* **2000**, *6*, 111. (m) Cook, B. N.; Bhakta, S.; Biegel, T.; Bowman, K. G.; Armstrong, J. I.; Hemmerich, S.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2000**, *122*, 8612.

2. (a) Koeller, K. M.; Wong, C.-H. *Chem. Eur. J.* **2000**, *6*, 1243. (b) Koeller, K. M.; Smith, M. E. B.; Huang, R. F.; Wong, C.-H. *J. Am. Chem. Soc.* **2000**, *122*, 4241. (c) Mehta, S.; Gilbert, M.; Wakarchuk, W. W.; Whitfield, D. M. *Org. Lett.* **2000**, *2*, 751. (d) Wittmann, V.; Datta, A. K.; Koeller, K. M.; Wong, C.-H. *Chem. Eur. J.* **2000**, *6*, 162. (e) Mukherjee, A.; Palcic, M. M.; Hindsgaul, O. *Carbohydr. Res.* **2000**, *326*, 1. (f) Sujino, K.; Uchiyama, T.; Hindsgaul, O. *J. Am. Chem. Soc.* **2000**, *122*, 1261. (g) Xian, X. P.; Sujino, K.; Otter, A.; Hindsgaul, O. *J. Am. Chem. Soc.* **1999**, *121*, 12606. (h) Sujino, K.; Ochiyama, T.; Hindsgaul, O.; Seto, N. O. L.; Wakarchuk, W. W.; Palcic, M. M. *J. Am. Chem. Soc.* **2000**, *122*, 1261. (i) Koeller, K. M.; Smith, M. E. B.; Wong, C.-H. *J. Am. Chem. Soc.* **2000**, *122*, 742.

3. (a) Varki, A. *Glycobiology* **1993**, *3*, 97. (b) Dwek, R. D. *Chem. Rev.* **1996**, *96*, 683. (c) Ganem, B. *Acc. Chem. Res.* **1996**, *29*, 340.

4. Hemmerich, S.; Leffier, H.; Rosen, S. D. *J. Biol. Chem.* **1995**, *270*, 12035.

5. Capon, C.; Wieruszeski, J.-M.; Lemoine, J.; Byrd, J. C.; Leffier, H.; Kim, Y. S. *J. Biol. Chem.* **1997**, *272*, 31957.

6. Capon, C.; Laboisse, C. L.; Wieruszeski, J.-M.; Maoret, J. J.; Auheron, C.; Fournet, B. *J. Biol. Chem.* **1992**, *267*, 19248.

7. (a) Chandrasekaran, E. V.; Jam, R. K.; Vig, R.; Matta, K. L. *Glycobiology* **1997**, *7*, 753. (b) Chandrasekaran, E. V.; Jam, R. K.; Rhodes, J. M.; Chawda, R.; Piskorz, C. F.; Matta, K. L. *Glycoconjugates J.* **1999**, *16*, 523.

8. (a) Xia, J.; Piskorz, C. F.; Alderfer, J. L.; Locke, R. D.; Matta, K. L. *Tetrahedron Lett.* **2000**, *41*, 2773. (b) Xia, J.; Alderfer, J. L.; Piskorz, C. F.; Matta, K. L. *Chem. Eur. J.* **2000**, *6*, 3442. (c) Xia, J.; Srikrishnan, T.; Alderfer, J. L.; Jain, R. K.; Piskorz, C. F.; Matta, K. L. *Carbohydr. Res.* **2000**, *329*, 561. (d) Xia, J.; Alderfer, J. L.; Matta, K. L. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2485. (e) Xia, J.; Alderfer, J. L.; Piskorz, C. F.; Matta, K. L. *Chem. Eur. J.* **2001**, *7*, 356.

9. Xia, J.; Abbas, S. A.; Piskorz, C. F.; Alderfer, J. L.; Matta, K. L. *Tetrahedron Lett.* **2000**, *41*, 169.

10. Johnson, C. K. *ORTEPII Report ORNL-5138*; Oak Ridge National Laboratory: TN, USA, 1976.

11. Okamoto, K.; Kondo, T.; Goto, T. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 637.

12. Hori, H.; Nakajima, T.; Nishida, Y.; Ohrui, H. *Tetrahedron Lett.* **1988**, *29*, 6317.

13. (a) David, S.; Hannessian, S. *Tetrahedron* **1985**, *41*, 643. (b) Guibe, F. *Tetrahedron* **1997**, *53*, 13509. (c) Hanessian, S.; Reddy, G.; Huynh, H. K.; Pan, J.; Pedatella, S. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2729.

14. Sheldrick, G. M. *SHELXL96. Program for the Solution of Crystal Structures*; University of Göttingen: Germany, 1996.

15. (a) Veeneman, G. H.; Van Leeuwen, S. H.; Van Boom, J. H. *Tetrahedron Lett.* **1990**, *31*, 1331. (b) Konradsson, P.; Udodong, U. E.; Fraser-Reid, B. *Tetrahedron Lett.* **1990**, *31*, 4313.

16. (a) Jain, R. K.; Vig, R.; Locke, R. D.; Mohammad, A.; Matta, K. L. *Chem. Commun.* **1996**, 65. (b) Jain, R. K.; Huang, B.-G.; Chandrasekaran, E. V.; Matta, K. L. *Chem. Commun.* **1997**, 23.