Carbohydrate Research 343 (2008) 2863–2869

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Chemical preparation of sialyl Lewis x using an enzymatically synthesized sialoside building block

Hongzhi Cao, Shengshu Huang, Jiansong Cheng, Yanhong Li, Saddam Muthana, Bryan Son, Xi Chen *

Department of Chemistry, University of California, One Shields Avenue, Davis, CA 95616, United States

ARTICLE INFO

Article history:
Received 26 May 2008
Received in revised form 20 June 2008
Accepted 26 June 2008
Available online 4 July 2008

Dedicated to Professor Yongzheng Hui on the occasion of his 70th birthday

Keywords:
Sialyl Lewis x
Sialoside
Oligosaccharide
Chemoenzymatic synthesis

ABSTRACT

The sialyl Lewis x tetrasaccharide with a propylamine aglycon was assembled by chemoselective glycosylation from a *p*-tolyl thioglycosyl donor obtained from an enzymatically synthesized sialodisaccharide. Combining the advantages of highly efficient enzymatic synthesis of sialoside building blocks, and diverse chemical glycosylation, this chemoenzymatic approach is practical for obtaining complex sialosides and their analogues.

1. Introduction

P-, E-, and L-selectins are C-type lectins that are involved in lymphocyte homing, initial process in leukocyte recruitment to inflammation sites, and cancer metathesis.[1–6] Sialyl Lewis x (SLe^x) and its sulfated derivatives in the presence or the absence of a peptide carrier are the most common selectin ligands. The sialyl Lewis x tetrasaccharide (α-Neu*p*5Ac-(2→3)-β-Gal*p*-(1→4)[α-Fuc*p*-(1→3)]-Glc*p*NAc-R) has been a leading structure for the development of new anti-inflammatory agents.[7] SLe^x is also a tumor-associated antigen; it has been found on cancer cells and in the sera from patients with breast, colon, lung, stomach, ovarian, pancreatic, prostate, or urinary bladder cancer. Therefore, it has been a key component for developing synthetic carbohydrate-based cancer vaccines.[8–10]

The important biological activity of SLe^x has attracted much interest in developing chemical, enzymatic, or chemoenzymatic approaches for the synthesis of this oligosaccharide and its derivatives.[6,11–24] Despite recent advances, the synthesis of SLe^x is still challenging.[25–27] Enzymatic synthesis of SLe^x using a number of recombinant enzymes proceeds with regio- and stereoselectively, but its application to large-scale synthesis is limited by the accessibility of biosynthetic enzymes, the strict substrate specificity of glycosyltransferases, and the involvement of multiple enzymes to generate and regenerate sugar nucleotides. Chemical glycosylation offers more diversity but is complicated by inherent multiple protection and deprotection processes and difficulties in choosing suitable protecting groups. For example, chemical sialylation on the 3-OH of Gal usually results in low yield and a mixture of α- and β-linked sialoside products due to the sterically hindered anomeric position and the lack of a participating group on the C3 of sialic acid.[25–27] In addition, the low reactivity of the hydroxyl groups in *N*-acetylglucosamine acceptor during glycosylation[28,29] and the lability of the α-L-Fuc linkage under acidic condition[30] complicate the total chemical synthesis of SLe^x.

Recently, chemically synthesized sialylated oligosaccharide building blocks have been successfully used in the assembly of complex sialosides.[31,32] These sialylated oligosaccharide donor building blocks can be easily accessed by enzyme-catalyzed approaches, which greatly simplify the overall synthesis of complex sialosides such as SLe^x. Several groups have successfully employed this method for synthesizing sialosides.[33–35] We present here the work showing that the SLe^x structure can be efficiently obtained by chemical glycosylation using a *p*-tolyl thioglycosyl donor formed by a sialyltransferase-catalyzed reaction.

2. Results and discussion

Recently, our group has developed an efficient one-pot three-enzyme chemoenzymatic approach for preparing natural and non-natural sialosides from simple sialic acid precursors using

* Corresponding author. Tel.: +1 530 754 6037; fax: +1 530 752 8995.
E-mail address: chen@chem.ucdavis.edu (X. Chen).

0008-6215/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2008.06.020

highly active and highly flexible recombinant bacterial sialoside biosynthetic enzymes.[36a,b,37] Due to the high expression level and reactivity of these bacterial enzymes, the synthesis can be routinely carried out in large scale (>100 mg) and can give various sialosides in high yields. In this chemoenzymatic method, chemical synthesis of the substrate derivatives is applied prior to the enzyme-catalyzed sialylation reaction. An alternative chemoenzymatic approach is to carry out enzymatic sialylation reaction to obtain sialoside building blocks before chemical glycosylation reactions for the synthesis of more complex oligosaccharides. The latter approach will be extremely useful, as a way to avoid the strict substrate specificity of some glycosyltransferases, and to provide more diverse carbohydrate or glycoconjugate structures that may not be accessible by enzymatic synthesis or the former chemoenzymatic method. The latter chemoenzymatic approach also avoids the difficulties in chemical construction of some glycosidic bonds including sialyl linkages.

Several examples of applying the alternative chemoenzymatic method in the synthesis of complex carbohydrates have been reported. Hayashi et al. used a sialotrisaccharide building block obtained from enzymatically synthesized oligosaccharide followed by protection and selective deprotection as a glycosyl acceptor for chemical fucosylation to provide SLe^x tetrasaccharide donors, which can be used for further introduction of different aglycons.[33] Similarly, Whitfield's group used phenyl thioglycosides formed from enzymatically assembled galactodisaccharide or sialotrisaccharide as glycosyl donors for the synthesis of more complex oligosaccharides.[35] In these examples, the β-Gal*p*-(1→4)-Glc*p*NAc moiety was assembled by a β-(1→4)-galactosyltransferase-catalyzed reaction. We present here that a SLe^x tetrasaccharide with a propylamine aglycon **1** (Fig. 1) can be obtained efficiently, without using a galactosyltransferase, from a *p*-tolyl thioglycosyl donor formed from a sialodisaccharide provided by a sialyltransferase-catalyzed reaction. A similar aryl thioglycoside disaccharide donor has been used by Whitfield's group for synthesizing α-(2→3)-linked sialyl LacNAc derivatives but has not been shown for the synthesis of SLe^x.[34] The propylamine aglycon in the target compound **1** can be used as a chemical handle for further conjugation to facilitate biological studies.

Retrosynthetic analysis of the target molecule **1** indicates that it can be chemically assembled from sialodisaccharide donor **2**, thiofucosyl donor **4**, and glucosamine acceptor **3** (Fig. 2).

Thioglycosides were chosen as donors due to their superior stability and their easy transferability to other glycosyl donors.[38,39]

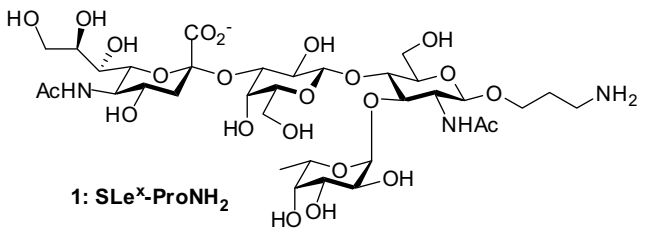


Figure 1. SLe^x tetrasaccharide with a propylamine aglycon.

The synthesis of sialodisaccharide donor **2** started from commercially available *N*-acetylmannosamine (ManNAc) and known thiogalactoside **5**[40] by the one-pot three-enzyme approach described previously.[36a,37] In this enzymatic reaction, ManNAc is converted by an *Escherichia coli* K-12 sialic acid aldolase to *N*-acetylneuraminic acid, which is activated by an *Neisseria meningitidis* CMP-sialic acid synthetase before being transferred to *p*-tolyl thiogalatoside **5** by a multifunctional *Pasteurella multocida* sialyltransferase (PmST1) (Scheme 1).[36a,b] Gram-scale synthesis of sialodisaccharide **6** was achieved in quantitative yield as shown by TLC analysis. The reaction was stopped by adding an equal volume of ethanol and the mixture was centrifuged to remove precipitates. The supernatant was concentrated by rotary evaporation and the sialoside **6** was converted to protected methyl ester **2** in a yield of 79% over three steps by following a reported method.[33]

2-*N*-Phthaloyl protected glycoside **3**, having a bulky silyl group at *O*-6 and two free hydroxy groups at C3 and C4, was chosen as the glycosylation acceptor for sialodisaccharide **2**. The bulky *N*-phthalimido protecting group in acceptor **3** provides steric hindrance to the neighboring C-3 hydroxyl group and enhances the reactivity of the C-4 hydroxyl group by masking the amide group, which disrupts amide-involved intermolecular hydrogen bonding.[29] Acceptor **3** was prepared in 81% overall yield by glycosylation of trichloroacetimidate donor **8**[41] with 3-chloropropanol followed by S_N2 substitution of the chlorine by an azido group, deacetylation, and selective silylation at *O*-6 with *tert*-butyldiphenylsilyl chloride (TBDPS-Cl) (Scheme 2). Thiofucosyl donor **4** was prepared by following a reported method.[42]

With building blocks **2–4** in hand, the next step was to explore the linear assembly of SLe^x tetrasaccharide **1** (Scheme 3). After screening different promoters and activation systems including BSP–Tf_2O[43] and *p*-TolSCl–AgOTf[44], only NIS–TfOH[45,46] gave the satisfactory yield in the chemoselective glycosylation of acceptor **3** using thiosialoside **2**. The low yields resulting from BSP–Tf_2O[43] or *p*-TolSCl–AgOTf[44] under pre-activation conditions may be due to the inherent low reactivity of the sialyl moiety in the glycosyl donor.[25] As shown in Scheme 3, using NIS–TfOH, glycosylation of **3** using **2** led to regioselective formation of a (1→4)-glycosidic bond between the anomeric carbon on the Gal*p* residue in sialodisaccharide donor **2** and the acceptor **3**. The regioselectivity was the result of the relatively low activity and the relatively large size of sialodisaccharide donor **2**, as well as the steric hindrance to C3–OH and the enhanced reactivity of C4–OH caused by the bulky *N*-phthalimido protecting group in acceptor **3**.[29] The stereoselective formation of the β-galactosyl linkage was the result of the neighboring group participation of the C-2 acetylated OH on the Gal in sialodisaccharide donor **2**. Trisaccharide **11** was achieved in 87% yield.

Subsequent α-fucosylation of the free hydroxyl group at C3 of glucosamine in compound **11** using NIS–TfOH gave tetrasaccharide **12** in a yield of 92%. In this reaction, thiofucoside **4** was used in an excess amount (2.5 equiv) to achieve a high yield. The efficient fucosylation in this step was due to the smaller size and the higher reactivity of thiofucosyl donor **4** compared to sialodisaccharide donor **2**. Removal of the *tert*-butyldiphenylsilyl and the 2-*N*-phthalyl groups by HF–pyridine and $N_2H_4{\cdot}H_2O$, respectively, followed by

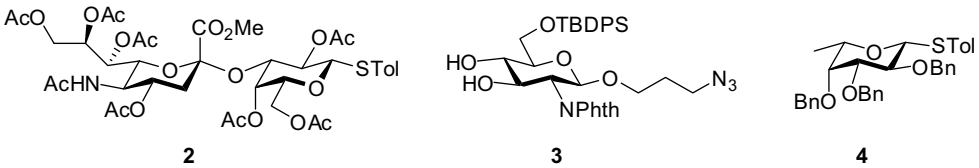
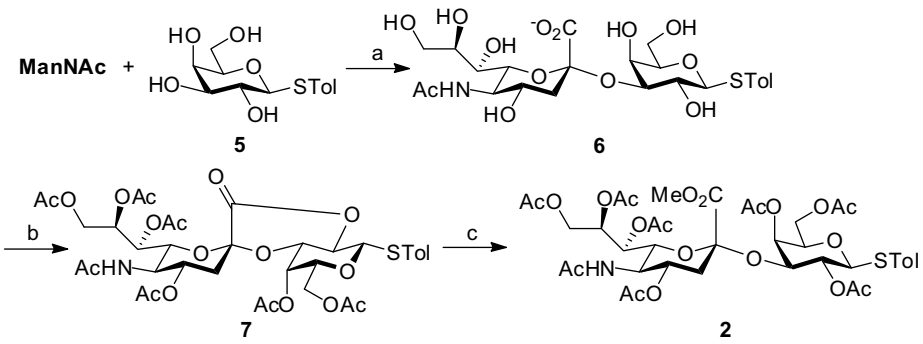
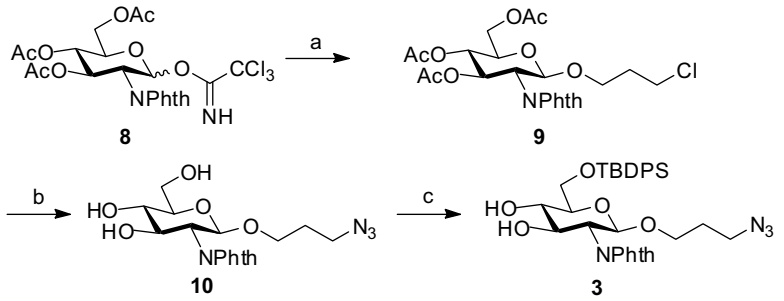


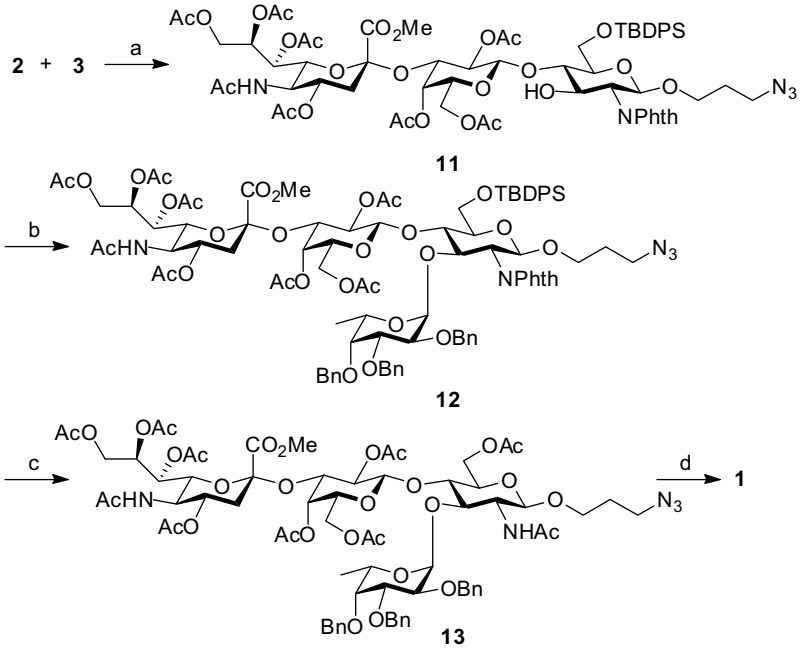
Figure 2. Building blocks for the synthesis of SLe^x tetrasaccharide **1**.



Scheme 1. Reagents and conditions: (a) sodium pyruvate, CTP, $MgCl_2$, Tris–HCl buffer (100 mM, pH 8.5), *E. coli* sialic acid aldolase, *N. meningitidis* CMP-sialic acid synthetase, *P. multocida* sialyltransferase, quantitative; (b) Ac_2O, pyridine, 91%; (c) (i) DMAP, MeOH, 90%; (ii) Ac_2O, pyridine, 97%.



Scheme 2. Reagents and conditions: (a) 3-chloropropanol, 4 Å molecular sieves, CH_2Cl_2, TMSOTf, rt, 95%; (b) (i) NaN_3, DMF, TBAI, 60 °C, 98%; (ii) NaOMe–MeOH, 100%; (c) TBDPSCl, pyridine, DMAP, 60 °C, 87%.



Scheme 3. Reagents and conditions: (a) NIS, TfOH, 4 Å molecular sieves, CH_2Cl_2, 0 °C to rt, 87%; (b) thiofucoside **4**, NIS, TfOH, 4 Å molecular sieve, CH_2Cl_2, 0 °C to rt, 92%; (c) (i) HF–pyridine, THF, pyridine, rt; (ii) $N_2H_4{\cdot}H_2O$, MeOH, 80 °C; (iii) Ac_2O, pyridine, 57% over three steps; (d) (i) 1,3-propanoldithiol, Et_3N, pyridine, H_2O, 50 °C; (ii) NaOMe–MeOH then add H_2O, rt; (iii) $Pd(OH)_2$, H_2, MeOH–H_2O–AcOH, rt, BioGel P-2, 61% over three steps.

acetylation, the fully protected tetrasaccharide **13** was produced in 57% over three steps.

Because hydrogenation of benzyl groups in the presence of azido group usually leads to low yield and complicated product mixtures, it was not used here. Instead, a two-step strategy was applied. In the first step, the azido group in **13** was reduced to an amino group using 1,3-propanoldithiol.[47] After deacetylation and saponification, benzyl groups were removed by hydrogenation using $Pd(OH)_2$ and H_2 to give the desired SLe^x tetrasaccharide **1**. After gel filtration purification, SLe^x tetrasaccharide **1** was

obtained in 61% yield over three steps of reduction and deprotection.

3. Conclusion

In conclusion, an alternative chemoenzymatic approach was used for efficient assembly of the biologically important SLe^x tetrasaccharide. Applying highly efficient and selective enzymatic synthesis to obtain oligosaccharide building blocks for diverse chemical synthesis was proven to be highly valuable in obtaining complex sialosides.

4. Experimental

4.1. General

Chemicals were purchased and used without further purification. ^{1}H NMR and ^{13}C NMR spectra were recorded on Mercury-300, Varian Inova-400, or Varian-600 spectrometer. Low and high resolution ESI mass spectra were obtained at the Mass Spectrometry Facility in the University of California at Davis. Silica Gel 60 Å (200–425 mesh, Sorbent technologies) was used for flash column chromatography. Thin-layer chromatography (TLC) was performed on silica gel plates 60 GF254 (Sorbent technologies) using anisaldehyde sugar stain or 5% sulfuric acid in ethanol stain for detection. Gel filtration chromatography was performed using a column (100 cm × 2.5 cm) packed with BioGel P-2 Fine resins (Bio-Rad, Hercules, CA).

4.2. *p*-Methylphenyl 1-thio-α-D-galactopyranoside (5)

$BF_3{\cdot}OEt_2$ (2.6 mL, 20.6 mmol) was added to a mixture of 1,2,3,4,6-penta-*O*-acetyl-D-galactopyranose (3.0 g, 7.7 mmol) and *p*-thiocresol (1.9 g, 15.5 mmol) in anhydrous CH_2Cl_2 (50 mL) at 0 °C. The mixture was stirred for 2 h at 0 °C before being allowed to warm to room temperature followed by continuous stirring overnight. The reaction mixture was diluted with CH_2Cl_2 (100 mL), washed with saturated $NaHCO_3$ and then with brine. It was then dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (1:2, EtOAc–hexane) to give *p*-methylphenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-galactopyranoside (3.2 g, 92%) as a syrup. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.36 (d, 2H, J = 8.4 Hz), 7.07 (d, 2H, J = 8.4 Hz), 5.35 (d, 1H, J = 3.0 Hz), 5.16 (t, 1H, J = 10.2 Hz), 4.99 (dd, 1H, J = 10.2, 3.6 Hz), 4.61 (d, 1H, J = 10.2 Hz), 4.13 (dd, 1H, J = 11.4, 6.6 Hz), 4.06 (dd, 1H, J = 11.4, 6.6 Hz), 3.87 (t, 1H, J = 7.2 Hz), 2.29, 2.06, 2.04, 1.99, 1.92(s, 3H for each); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.54, 170.38, 170.22, 169.60, 138.60, 133.30, 129.83, 128.82, 87.03, 74.52, 72.20, 67.46, 67.43, 61.80, 21.35, 21.05, 20.86, 20.82, 20.77. The NMR data were consistent with that reported.[40]

To a solution of *p*-methylphenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-galactopyranoside (3.2 g, 7.04 mmol) in dry MeOH (100 mL), was added NaOMe (200 mg). After stirring for 2 h, the reaction mixture was neutralized with Dowex 50W (H^+), filtered, and concentrated to give the *p*-methylphenyl 1-thio-α-D-galactopyranoside **5**[40] as white solid (2.0 g, 100%). The compound was used directly for the following enzyme reaction without any further purification. ^{1}H NMR (600 MHz, D_2O) δ 7.30 (d, 2H, J = 7.8 Hz), 7.07 (d, 2H, J = 7.8 Hz), 4.50 (d, 1H, J = 10.2 Hz), 3.79 (d, 1H, J = 3.0 Hz), 3.58 (dd, 1H, J = 12.6, 9.0 Hz), 3.54–3.51 (m, 2H), 3.49 (dd, 1H, J = 9.0, 3.0 Hz), 3.42 (t, 1H, J = 9.6 Hz), 2.15 (s, 3H); ^{13}C NMR (150 MHz, D_2O) δ 138.84, 131.99, 130.10, 128.84, 88.55, 79.08, 74.08, 69.31, 68.80, 61.05, 20.25. The NMR data were consistent with that reported.[40]

4.3. *p*-Methylphenyl (5-*N*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl)-(2→3)-1-thio-β-D-galactopyranoside (6)

The sialoside **6** was prepared using the one-pot three-enzyme approach as reported.[36a,37] ManNAc (464 mg, 2.1 mmol) was used as sialic acid precursor and thiogalactopyranoside **5** (500 mg, 1.8 mmol) was used as sialyltransferase acceptor. The reaction was carried out at room temperature (25 °C) in a total volume of 80 mL in the presence of CTP (1 equiv), sodium pyruvate (3 equiv), $MgCl_2$ (20 mM), Tris–HCl buffer (100 mM, pH 8.5), *E. coli* sialic acid aldolase (5 mg), *N. meningitidis* CMP-sialic acid synthetase (1 mg), and *P. multocida* sialyltransferase (PmST1, 1 mg). The reaction was quenched by adding the same volume of ethanol when TLC indicated the completion of the reaction. The reaction mixture was centrifuged at 7000*g* for 30 min. to remove precipitates. The supernatant was concentrated by rotary evaporation and lyophilized to white solid which was used directly for acetylation in the next step. Sialoside **6** was achieved in quantitative yield based on TLC and purifying partial sialoside **6** by passing through a BioGel P-2 gel filtration column. ^{1}H NMR (600 MHz, D_2O) δ 7.34 (d, 2H, J = 7.8 Hz), 7.11 (d, 2H, J = 7.2 Hz), 4.60 (d, 1H, J = 9.6 Hz), 3.98 (dd, 1H, J = 9.6, 3.6 Hz), 3.84 (d, 1H, J = 3.0 Hz), 3.73–3.68 (m, 3H), 3.57–3.44 (m, 8H), 2.61 (dd, 1H, J = 12.0, 4.8 Hz), 2.19 (s, 3H), 1.88 (s, 3H), 1.65 (t, 1H, J = 12.6 Hz); ^{13}C NMR (150 MHz, D_2O) δ 175.16, 174.02, 138.96, 132.38, 130.06, 128.30, 100.07, 87.69, 78.89, 77.16, 73.01, 71.91, 68.50, 68.20, 67.88, 67.54, 62.60, 59.51, 51.81, 39.75, 22.18, 20.30; ESIMS: *m/z* calcd for $[C_{24}H_{34}NO_{13}S]^+$: 576.1751, found: 576.1742.

4.4. *p*-Methylphenyl [(5-*N*-acetyl-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl-1′→2-lactone)]-(2→3)-4,6-di-*O*-acetyl-1-thio-β-D-galactopyranoside (7)

Crude thiosialoside **6** (1.2 g, 1.8 mmol, about 90% purity) was dissolved in pyridine (40 mL). To this solution at 0 °C was added Ac_2O (20 mL). The reaction mixture was allowed to warm to room temperature and stirred overnight. After concentration by rotary evaporation, the residue was purified by flash chromatography on silica gel (3:3:0.5 EtOAc–hexane–MeOH) to give lactone **7** as white solid (1.47 g, 91% yield base on the amount of pure thioglycoside **6**). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.39 (d, 2H, J = 7.8 Hz), 7.07 (d, 2H, J = 7.8 Hz), 6.04 (d, 1H, J = 10.2 Hz), 5.46 (d, 1H, J = 3.0 Hz), 5.39 (dt, 1H, J = 10.8, 4.8 Hz), 5.29 (d, 1H, J = 5.4 Hz), 5.18 (dt, 1H, J = 6.0, 2.4 Hz), 4.93 (t, 1H, J = 10.2 Hz), 4.58 (d, 1H, J = 9.6 Hz), 4.32 (dd, 1H, J = 12.6, 3.0 Hz), 4.19–4.13 (m, 2H), 4.09 (dd, 2H, J = 12.0, 6.6 Hz), 4.00 (dd, 1H, J = 10.2, 3.0 Hz), 3.86 (t, 1H, J = 6.0 Hz), 3.75 (d, 1H, J = 10.2 Hz), 2.47 (dd, 1H, J = 13.8, 6.0 Hz), 2.29, 2.22, 2.19, 2.07, 2.06, 2.05, 2.00, 1.98, 1.86 (s, 3H for each), 1.84 (t, 1H, J = 8.4 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.15, 171.11, 170.91, 170.79, 170.70, 170.62, 170.16, 169.95, 164.01, 138.85, 133.19, 130.04, 129.65, 129.23, 128.42, 97.35, 87.18, 75.71, 74.62, 73.39, 72.54, 70.51, 69.59, 67.82, 66.71, 62.80, 61.92, 49.17, 37.76, 23.32, 21.35, 21.23, 21.16, 21.09, 20.99, 20.85, 20.70; ESIMS: *m/z* calcd for $[C_{36}H_{45}NO_{18}S+Na]^+$: 834.2255, found: 834.2292.

4.5. *p*-Methylphenyl [methyl (5-*N*-acetyl-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-2,4,6-tri-*O*-acetyl-1-thio-β-D-galactopyranoside (2)

To a solution of lactone **7** (1.4 g, 1.7 mmol) in MeOH (41 mL) at 0 °C, was added 4-(dimethylamino)pyridine (4.6 mL of 10% solution; 0.46 mmol). The reaction mixture was stirred for 24 h at room temperature and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (3:3:0.5 hexane–EtOAc–MeOH) to give *p*-methylphenyl [methyl (5-*N*-acet-

yl-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl) onate]-(2→3)-4,6-di-*O*-acetyl-1-thio-β-D-galactopyranoside as a white solid (1.3 g, 90%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.47 (d, 2H, J = 8.4 Hz), 7.07 (d, 2H, J = 8.4 Hz), 5.42–5.39 (m, 1H), 5.34 (s, 1H), 5.31 (dd, 1H, J = 9.0, 2.4 Hz), 4.95–4.90 (m, 2H), 4.73 (d, 1H, J = 9.0 Hz), 4.39 (t, 1H, J = 4.2 Hz), 4.24 (dd, 1H, J = 12.0, 2.4 Hz), 4.04–3.97 (m, 4H), 3.83 (s, 3H), 3.82 (dd, 2H, J = 3.6, 2.4 Hz), 3.61 (t, 1H, J = 8.4 Hz), 2.63 (dd, 1H, J = 12.6, 4.2 Hz), 2.30, 2.11, 2.09, 2.02, 2.00, 1.99, 1.97, 1.84 (s, 3H for each), 1.85 (t, 1H, J = 12.6 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.00, 170.81, 170.64, 170.57, 170.45, 170.33, 170.19, 167.84, 137.88, 132.94, 129.65, 129.31, 97.17, 87.66, 75.35, 74.55, 72.61, 69.00, 68.13, 67.94, 66.92, 62.58, 62.46, 53.44, 49.49, 37.84, 23.36, 21.59, 21.33, 21.04, 21.00, 20.98, 20.94, 20.92, 20.80.

To a solution of the methyl ester (1.3 g, 1.5 mmol) containing a free C2–OH at Gal obtained from previous step in pyridine (30 mL) at 0 °C, was added Ac_2O (10 mL). The reaction mixture was stirred overnight at room temperature before it was concentrated and the residue was purified by flash chromatography (3:3:0.5 hexane–EtOAc–MeOH) to give compound **2** as white solid (1.3 g, 97%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.37 (d, 2H, J = 7.8 Hz), 7.05 (d, 2H, J = 8.4 Hz), 5.34 (d, 1H, J = 10.2 Hz), 5.48 (dt, 1H, J = 2.4, 8.4 Hz), 5.32 (dd, 1H, J = 8.4, 2.4 Hz), 4.98 (t, 1H, J = 9.6 Hz), 4.88 (d, 1H, J = 3.6 Hz), 4.80–4.84 (m, 1H), 4.76 (d, 1H, J = 10.2 Hz), 4.58 (dd, 1H, J = 9.6, 3.0 Hz), 4.33 (dd, 1H, J = 12.6, 3.0 Hz), 3.99–4.07 (m, 3H), 3.94 (dd, 1H, J = 12.6, 6.0 Hz), 3.85 (t, 1H, J = 7.2 Hz), 3.77 (s, 3H), 3.63 (dd, 1H, J = 10.2, 2.4 Hz), 2.27, 2.20, 2.10, 2.03, 2.02, 1.99, 1.95, 1.93, 1.80 (s, 3H for each), 1.63 (t, 1H, J = 12.6 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.95, 170.73, 170.67, 170.59, 170.45, 169.88, 169.86, 168.20, 138.03, 132.91, 129.66, 129.27, 96.96, 86.09, 74.37, 72.44, 72.28, 69.53, 68.17, 68.01, 67.37, 62.69, 62.47, 53.33, 49.12, 37.75, 24.20, 23.30, 21.65, 21.30, 21.19, 20.96, 20.89, 20.84; ESIMS: *m/z* calcd for $[C_{39}H_{51}NO_{20}S+Na]^+$: 908.2623, found: 908.2635.

4.6. 3-Chloropropyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (9)

A mixture of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-D-glucopyranosyl imidate (1.8 g, 3.1 mmol), 3-chloropropanol (0.52 mL, 6.2 mmol), and 4 Å molecular sieves (2.0 g) in dry CH_2Cl_2 (50 mL) was stirred at room temperature for 30 min. The reaction mixture was cooled down to −78 °C and TMSOTf (20 μL, 0.11 mmol) was added. After 1 h, the reaction mixture was allowed to warm to room temperature and stirred for another 2 h when the reaction was completed as indicated by TLC. Et_3N (0.1 mL) was added and the reaction mixture was filtered and concentrated. The residue was purified by flash chromatography (1:1 EtOAc–hexane) on silica gel to give the glycoside **9** as solid foam (1.51 g, 95%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.85 (dd, 2H, J = 5.7, 3.0 Hz), 7.74 (dd, 2H, J = 5.7, 2.7 Hz), 5.80 (dd, 1H, J = 10.5, 9.0 Hz), 5.37 (d, 1H, J = 8.7 Hz), 5.16 (dd, 1H, J = 9.9, 9.0 Hz), 4.36–4.27 (m, 2H), 4.17 (dd, 1H, J = 12.3, 2.4 Hz), 3.98–3.85 (m, 2H), 3.67–3.59 (m, 1H), 3.44–3.30 (m, 2H), 2.11, 2.03, 1.86 (s, 3H for each), 1.96–1.78 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.89, 170.31, 169.64, 134.50, 131.50, 123.77, 98.50, 72.03, 70.84, 69.10, 66.63, 62.16, 54.74, 41.35, 32.19, 20.93, 20.79, 20.62; ESIMS: *m/z* calcd for $[C_{23}H_{26}ClNO_{10}+Na]^+$: 534.1143, found: 534.1109.

4.7. 3-Azidopropyl 2-deoxy-2-phthalimido-β-D-glucopyranoside (10)

To a solution of **9** (2.5 g, 4.9 mmol) in dry DMF (50 mL) were added NaN_3 (3.2 g, 48.8 mmol) and TBAI (100 mg). After stirring overnight at 60 °C, the reaction mixture was diluted with EtOAc (150 mL), washed with brine, dried over $MgSO_4$, filtered and purified by flash chromatography (1:1 EtOAc–hexane) to give 3-azidopropyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (2.5 g, 98%) as white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.86 (dd, 2H, J = 6.0, 3.3 Hz), 7.74 (dd, 2H, J = 5.1, 2.7 Hz), 5.78 (dd, 1H, J = 10.8, 9.0 Hz), 5.37 (d, 1H, J = 8.4 Hz), 5.17 (dd, 1H, J = 10.2, 9.0 Hz), 4.35–4.28 (m, 2H), 4.18 (dd, 1H, J = 12.3, 2.1 Hz), 3.94–3.84 (m, 2H), 3.58–3.51 (m, 1H), 3.24–3.10 (m, 2H), 2.11, 2.03, 1.86 (s, 3H for each), 1.81–1.63 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.98, 170.42, 169.73, 134.64, 123.89, 98.41, 72.15, 70.96, 69.17, 66.83, 62.22, 54.80, 48.16, 29.01, 21.01, 20.88, 20.70; ESIMS: *m/z* calcd for $[C_{23}H_{26}N_4O_{10}+Na]^+$: 541.1547, found: 541.1529.

To a solution of 3-azidopropyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (2.5 g, 4.8 mmol) in MeOH (100 mL) was added NaOMe (200 mg). After stirring for 2 h at room temperature, the reaction mixture was neutralized with Dowex 50W (H^+), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (10:1 CH_2Cl_2–MeOH) to give glycoside **10** (1.9 g, 100%) as white solid. ^{1}H NMR (600 MHz, D_2O) δ 7.86 (dd, 2H, J = 4.8, 3.0 Hz), 7.81 (dd, 2H, J = 5.4, 3.0 Hz), 5.16 (d, 1H, J = 8.4 Hz), 4.26 (dd, 1H, J = 10.2, 7.8 Hz), 3.97 (dd, 1H, J = 10.2, 8.4 Hz), 3.94–3.88 (m, 2H), 3.74 (dd, 1H, J = 12.0, 5.4 Hz), 3.53–3.49 (m, 1H), 3.45–3.38 (m, 2H), 3.22 (t, 1H, J = 8.4 Hz), 3.14–3.09 (m, 2H), 1.69–1.62 (m, 2H), 1.00 (t, 2H, J = 7.2 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.72, 134.43, 131.81, 98.62, 75.79, 71.79, 71.54, 66.47, 61.93, 56.84, 48.17, 28.99; ESIMS: *m/z* calcd for $[C_{17}H_{20}N_4O_7+Na]^+$: 415.1230, found: 415.1228.

4.8. 3-Azidopropyl 6-*tert*-*O*-butyldiphenylsilyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (3)

To a solution of glycoside **10** (1.8 g, 4.6 mmol) in dry pyridine (34 mL), were added TBDPSCl (2.4 mL, mmol) and DMAP (100 mg). The reaction mixture was stirred overnight at 60 °C before it was concentrated and purified by flash chromatography (20:1 CH_2Cl_2–MeOH) to give compound **3** as syrup (2.5 g, 87%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.81 (dd, 2H, J = 6.0, 3.6 Hz), 7.70–7.68 (m, 6H), 7.45–7.38 (m, 6H), 5.18 (d, 1H, J = 8.4 Hz), 4.34 (dd, 1H, J = 10.8, 8.4 Hz), 4.09 (dd, 1H, J = 10.8, 9.0 Hz), 3.94 (dd, 2H, J = 4.8, 1.8 Hz), 3.81–3.78 (m, 1H), 3.66 (t, 1H, J = 9.0 Hz), 3.59–3.56 (m, 1H), 3.47–3.44 (m, 1H), 3.12 (dt, 2H, J = 6.0, 1.8 Hz), 1.73–1.60 (m, 2H), 1.06 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.67, 135.88, 135.82, 134.42, 131.84, 130.21, 128.12, 128.09, 123.70, 98.38, 74.64, 74.50, 71.93, 66.17, 65.24, 56.46, 48.27, 29.08, 27.03, 19.44; ESIMS: *m/z* calcd for $[C_{33}H_{38}N_4O_7Si+Na]^+$: 653.2408, found: 653.2398.

4.9. *p*-Methylphenyl 2,3,4-tri-*O*-benzyl-1-thio-α-L-fucopyranoside (4)

Compound **4** was prepared from L-fucose in 71% yield in four steps by following procedures as reported previously.[42] ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.49 (d, 2H, J = 7.8 Hz), 7.41–7.29 (m, 15H), 7.02 (d, 2H, J = 7.8 Hz), 5.01 (d, 1H, J = 12.0 Hz), 4.81 (d, 1H, J = 10.2 Hz), 4.76–4.72 (m, 3H), 4.67 (d, 1H, J = 12.0 Hz), 4.55 (d, 1H, J = 10.2 Hz), 3.90 (t, 1H, J = 9.6 Hz), 3.64 (d, 1H, J = 3.0 Hz), 3.59 (dd, 1H, J = 9.6, 3.0 Hz), 3.51 (dd, 1H, J = 12.6, 6.0 Hz), 2.30 (s, 3H), 1.27 (d, 3H, J = 6.6 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 139.00, 138.70, 138.62, 137.35, 132.42, 130.67, 129.76, 128.69, 128.61, 128.58, 128.39, 128.20, 127.95, 127.82, 127.69, 88.11, 84.81, 77.38, 76.80, 75.80, 74.80, 73.08, 21.38, 17.57. The ^{1}H and ^{13}C NMR spectra were consistent with those reported.[42]

4.10. 3-Azidopropyl [methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranonate)]-(2→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (11)

A mixture of sialoside donor **2** (360 mg, 0.41 mmol), glucosamine acceptor **3** (216 mg, 0.34 mmol), and 4 Å molecular sieve (300 mg) in dry CH_2Cl_2 was stirred for 30 min at room temperature. The mixture was cooled down to 0 °C and *N*-iodosuccinimide (NIS, 185 mg, 0.82 mmol) and triflic acid (11 μL, 0.12 mmol) were added. After stirring for 1 h at 0 °C, the reaction mixture was warmed to room temperature and stirred for another 2 h when TLC indicated the completion of the reaction. The reaction mixture was neutralized by Et_3N (0.2 mL), filtered, and concentrated under reduce pressure. The residue was purified by flash chromatography (3:3:0.5 EtOAc–hexane–MeOH) to give trisaccharide **11** as white solid (415 mg, 87%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.80–7.79 (m, 2H), 7.68–7.66 (m, 6H), 7.37–7.30 (m, 6H), 5.72 (d, 1H, *J* = 10.2 Hz), 5.35 (t, 1H, *J* = 8.4), 5.24 (d, 1H, *J* = 8.4 Hz), 5.16 (d, 1H, *J* = 8.4 Hz), 4.91 (t, 1H, *J* = 8.4 Hz), 4.78–4.74 (m, 2H), 4.61 (d, 1H, *J* = 9.6 Hz), 4.53–4.51 (m, 2H), 4.28 (dd, 1H, *J* = 10.2, 8.4 Hz), 4.20 (d, 1H, *J* = 12.6 Hz), 4.07–4.01 (m, 1H), 3.99–3.87 (m, 4H), 3.86–3.77 (m, 3H), 3.71 (s, 3H), 3.69–3.66 (m, 1H), 3.64 (t, 1H, *J* = 9.6 Hz), 3.56 (d, 1H, *J* = 10.8 Hz), 3.45 (t, 1H, *J* = 8.4 Hz), 3.36–3.32 (m, 1H), 3.08–3.01 (m, 2H), 2.46 (dd, 1H, *J* = 12.6, 4.2 Hz), 2.04, 1.99, 1.94, 1.91, 1.89, 1.76, 1.69, 1.65 (s, 3H for each), 1.63–1.56 (m, 2H), 1.53 (t, 1H, *J* = 12.0 Hz), 0.99 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.88, 170.66, 170.60, 170.58, 170.39, 169.78, 169.69, 168.14, 136.00, 135.86, 134.33, 134.12, 133.51, 131.83, 129.92, 129.77, 127.88, 127.81, 101.61, 98.02, 96.93, 83.64, 75.43, 72.34, 71.33, 71.07, 70.61, 69.83, 69.49, 68.26, 67.36, 67.29, 65.98, 63.48, 62.62, 62.49, 60.61, 56.12, 53.37, 48.96, 37.70, 28.98, 23.21, 21.59, 21.22, 20.98, 20.93, 20.74, 20.52, 20.24, 19.47; ESIMS: *m*/*z* calcd for $[C_{65}H_{81}N_5O_{27}Si+H]^+$: 1392.4966, found: 1392.4893.

4.11. 3-Azidopropyl [methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranonate)]-(2→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)[(1→3)-2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl]-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (12)

The mixture of thiofucosyl donor **4** (398 mg, 0.74 mmol), trisaccharide acceptor **11** (410 mg, 0.29 mmol) and 4 Å molecular sieve (400 mg) in dry CH_2Cl_2 was stirred for 30 min at room temperature. The mixture was cooled down to 0 °C, and *N*-iodosuccinimide (NIS, 333 mg, 1.5 mmol) and triflic acid (20 μL, 0.23 mmol) were added. After stirring for 1 h at 0 °C, the reaction mixture was warmed to room temperature and stirred for another 3 h when TLC indicated the completion of the reaction. The reaction mixture was neutralized by Et_3N (0.4 mL), filtered, and concentrated. The residue was purified by flash chromatography (3:3:0.4 EtOAc–hexane–MeOH) to give tetrasaccharide **12** as white solid (490 mg, 92%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.75–7.64 (m, 8H), 7.40–7.34 (m, 6H), 7.25–7.09 (m, 13H), 7.02 (d, 2H, *J* = 6.6 Hz), 5.43 (d, 1H, *J* = 10.2 Hz), 5.37–5.34 (m, 2H), 4.99–4.88 (m, 5H), 4.76 (d, 1H, *J* = 11.4 Hz), 4.71 (dd, 1H, *J* = 10.8, 9.0 Hz), 4.56–4.49 (m, 5H), 4.42 (d, 1H, *J* = 12.6 Hz), 4.29–4.25 (m, 2H), 4.16–4.08 (m, 4H), 4.00–3.94 (m, 3H), 3.91–3.83 (m, 3H), 3.78 (s, 3H), 3.77 (dd, 1H, *J* = 10.2, 2.4 Hz), 3.62 (d, 1H, *J* = 2.4 Hz), 3.60 (s, 1H), 3.57 (dd, 1H, *J* = 10.2, 3.6 Hz), 3.42 (dt, 1H, *J* = 6.0 Hz), 3.11–3.07 (m, 1H), 2.93 (ddd, 1H, *J* = 2.4, 1.2, 1.2 Hz), 2.48 (dd, 1H, *J* = 12.6, 4.8 Hz), 2.09, 2.05, 2.02, 2.01, 1.99, 1.95, 1.92, 1.84, 1.76 (s, 3H for each), 1.71 (t, 1H, *J* = 12.6 Hz), 1.53–1.41 (m, 2H), 1.21 (d, 3H, *J* = 7.2 Hz), 1.08 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 171.23, 170.86, 170.50, 170.24, 170.18, 169.83, 169.32, 168.12, 139.17, 138.99, 138.52, 136.28, 135.87, 134.38, 134.35, 133.27, 129.92, 129.87, 128.33, 128.27, 128.19, 128.10, 127.78, 127.76, 127.56, 127.37, 127.28, 127.17, 123.69, 99.69, 97.94, 97.60, 97.31, 79.84, 77.78, 76.64, 75.68, 75.15, 74.49, 73.24, 72.89, 72.71, 72.43, 71.79, 70.91, 70.73, 69.71, 68.80, 67.99, 67.37, 66.81, 65.63, 63.27, 62.08, 61.63, 60.66, 56.54, 53.70, 53.31, 49.32, 37.47, 28.94, 27.21, 23.33, 21.41, 21.27, 21.10, 21.05, 20.92, 20.91, 20.85, 20.72, 19.78, 16.95; ESIMS: *m*/*z* calcd for $[C_{92}H_{109}N_5O_{31}Si+Na]^+$: 1830.6774, found: 1830.6538.

4.12. 3-Azidopropyl [methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranonate)]-(2→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)[(1→3)-2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl]-6-*O*-acetyl-2-deoxy-2-*N*-acetyl-β-D-glucopyranoside (13)

To the solution of tetrasaccharide **12** (490 mg, 0.27 mmol) in pyridine (0.6 mL) and THF (2.4 mL) at 0 °C, was added HF–pyridine (65–70%, 0.26 mL). The reaction mixture was stirred overnight at room temperature when TLC indicated the completion of the reaction. The reaction mixture was cooled down to 0 °C and neutralized using saturated $NaHCO_3$ (10.0 mL). It was then extracted with EtOAc and the combined organic phase was dried with Na_2SO_4, filtered, and concentrated to give crude selectively deprotected tetrasaccharide as white solid (480 mg). The white solid residue was dissolved in MeOH (30.0 mL) and $N_2H_4{\cdot}H_2O$ (6.0 mL) was added. The reaction mixture was heated at reflux at 80 °C for 10 h when TLC indicated the completion of the reaction. The reaction mixture was concentrated and co-evaporated with toluene. The solid residue was dissolved in dry pyridine (20.0 mL) and Ac_2O (5.0 mL) was added at 0 °C. It was allowed to warm to room temperature and stirred for overnight. The reaction mixture was concentrated and purified by flash chromatography (3:3:0.5 EtOAc–hexane–MeOH) to give compound **13** as white solid (235 mg, 57% for three steps). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.37–7.34 (m, 4H), 7.30–7.21 (m, 11H), 5.95 (d, 1H, *J* = 7.8 Hz), 5.63 (dt, 1H, *J* = 3.0 Hz), 5.43 (dd, 1H, *J* = 9.6, 2.4 Hz), 5.12 (d, 1H, *J* = 10.2 Hz), 5.04 (d, 1H, *J* = 3.6 Hz), 4.92 (d, 2H, *J* = 12.0 Hz), 4.83 (d, 1H, *J* = 12.0 Hz), 4.79–4.61 (m, 7H), 4.57 (d, 1H, *J* = 11.4, 3.6 Hz), 4.53 (d, 1H, *J* = 10.2, 3.0 Hz), 4.26 (d, 1H, *J* = 12.6, 3.0 Hz), 4.20 (dd, 1H, *J* = 12.0, 5.4 Hz), 4.14 (d, 1H, *J* = 10.8 Hz), 4.12–4.06 (m, 3H), 3.95 (dd, 1H, *J* = 10.8, 2.4 Hz), 3.94 (d, 1H, *J* = 3.6 Hz), 3.87 (d, 2H, *J* = 7.8 Hz), 3.85 (dd, 1H, *J* = 11.4, 3.6 Hz), 3.82–3.79 (m, 2H), 3.69 (t, 2H, *J* = 7.2 Hz), 3.65 (s, 3H), 3.46–3.43 (m, 1H), 3.26–3.21 (m, 2H), 2.69 (dd, 1H, *J* = 13.2, 4.8 Hz), 2.23, 2.22, 2.06, 2.03, 2.02, 1.98, 1.92, 1.84, 1.81, 1.76 (s, 3H for each), 1.73–1.66 (m, 2H), 1.12 (d, 3H, *J* = 6.6 Hz); ^{13}C NMR (150 MHz, $CDCl_3$): δ 171.02, 171.00, 170.88, 170.66, 170.58, 170.38, 170.18, 170.08, 169.76, 169.63, 166.35, 161.67, 139.01, 138.99, 138.78, 128.69, 128.60, 128.54, 128.42, 128.08, 127.91, 127.80, 127.59, 127.44, 100.00, 99.79, 97.72, 95.24, 79.93, 77.25, 76.60, 74.61, 74.52, 73.83, 73.55, 72.85, 72.80, 70.86, 70.48, 69.54, 69.10, 67.39, 67.15, 66.90, 66.77, 66.25, 63.14, 62.24, 60.58, 49.07, 48.33, 38.41, 29.06, 23.39, 23.34, 22.21, 21.07, 21.05, 20.99, 20.97, 20.96, 20.80, 20.66, 16.94 ESIMS: *m*/*z* calcd for $[C_{72}H_{95}N_5O_{32}+Na]^+$: 1564.5858, found: 1564.6082.

4.13. 3-Aminopropyl (5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→3)-β-D-galactopyranosyl-(1→4)[(1→3)-α-L-fucopyranosyl]-2-acetamido-2-deoxy-β-D-glucopyranoside (1)

Tetrasaccharide **13** (230 mg, 0.15 mmol) was dissolved in the mixture of pyridine (15 mL) and H_2O (3 mL), then 1,3-propanol-

dithiol (0.55 mL) and Et_3N (0.56 mL) were added. The reaction mixture was stirred at 50 °C for 24 h when TLC indicated the completion of the reaction. The mixture was concentrated and co-evaporated with 5:1 toluene–ethanol, and purified by flash chromatography (8:1 CH_2Cl_2–MeOH) to give amine intermediate as white solid (224 mg). The amine intermediate (224 mg) was dissolved in dry MeOH (10 mL), and NaOMe (540 mg) was added. After stirring for 10 h, H_2O (10 mL) was added and the mixture was stirred for another 4 h at room temperature. The reaction mixture was neutralized with Dowex H^+ resin, filtered, and concentrated. The white solid residue (163 mg) was dissolved in 5:5:1 MeOH–H_2O–AcOH, and $Pd(OH)_2$ (120 mg) was added. The mixture was stirred at room temperature under atmospheric pressure H_2 for 24 h. The mixture was filtered through Celite and concentrated. The residue was purified by BioGel P-2 to give the targeted compound **1** as white solid (83 mg, 61% for three steps). ^1H NMR (600 MHz, D_2O): δ 4.92 (d, 1H, J = 4.0 Hz), 4.61 (m, 1H), 4.34 (d, 2H, J = 8.4 Hz), 3.90 (dd, 1H, J = 9.6, 2.4 Hz), 3.84–3.81 (m, 2H), 3.78–3.65 (m, 9H), 3.59 (d, 1H, J = 3.0 Hz), 3.53–3.39 (m, 10H), 3.35 (dd, 1H, J = 10.8, 9.6 Hz), 2.90 (t, 2H, J = 7.2 Hz), 2.58 (dd, 1H, J = 11.4, 4.2 Hz), 1.86 (s, 3H), 1.84 (s, 3H), 1.82–1.79 (m, 2H), 1.61 (t, 1H, J = 12.6 Hz), 0.98 (d, 3H, J = 6.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 175.21, 174.52, 101.52, 101.14, 99.68, 98.68, 75.48, 75.27, 74.78, 73.91, 73.38, 72.00, 71.37, 69.39, 69.29, 68.13, 67.95, 67.71, 67.29, 66.77, 63.06, 61.40, 59.72, 55.92, 51.65, 38.06, 37.71, 23.39, 22.31, 15.39; ESIMS: *m/z* calcd for $[C_{34}H_{58}N_3O_{23}]^+$: 876.3461, found: 876.3455.

Acknowledgment

This work was supported by NIH Grant U01CA128442.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2008.06.020.

References

1. Kannagi, R. *Curr. Opin. Struct. Biol.* **2002**, *12*, 599–608.
2. Kannagi, R.; Izawa, M.; Koike, T.; Miyazaki, K.; Kimura, N. *Cancer Sci.* **2004**, *95*, 377–384.
3. Varki, A. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 7390–7397.
4. Kansas, G. S. *Blood* **1996**, *88*, 3259–3287.
5. Dube, D. H.; Bertozzi, C. R. *Nat. Rev. Drug Disc.* **2005**, *4*, 477–488.
6. Barthel, S. R.; Gavino, J. D.; Descheny, L.; Dimitroff, C. J. *Expert Opin. Ther. Targets* **2007**, *11*, 1473–1491.
7. Simanek, E. E.; McGarvey, G. J.; Jablonowski, J. A.; Wong, C. H. *Chem. Rev.* **1998**, *98*, 833–862.
8. Danishefsky, S. J.; Allen, J. R. *Angew. Chem., Int. Ed.* **2000**, *39*, 836–863.
9. Ouerfelli, O.; Warren, J. D.; Wilson, R. M.; Danishefsky, S. J. *Expert Rev. Vaccines* **2005**, *4*, 677–685.
10. Seeberger, P. H.; Werz, D. B. *Nature* **2007**, *446*, 1046–1051.
11. Nicolaou, K. C.; Caulfield, T. J.; Kataoka, H.; Stylianides, N. A. *J. Am. Chem. Soc.* **1990**, *112*, 3693–3695.
12. Kameyama, A.; Ishida, H.; Kiso, M.; Hasegawa, A. *Carbohydr. Res.* **1991**, *209*, c1–c4.
13. Danishefsky, S. J.; Gervay, J.; Peterson, J. M.; McDonald, F. E.; Koseki, K.; Oriyama, T.; Griffith, D. A.; Wong, C. H.; Dumas, D. P. *J. Am. Chem. Soc.* **1992**, *114*, 8329–8331.
14. Danishefsky, S. J.; Koseki, K.; Griffith, D. A.; Gervay, J.; Peterson, J. M.; McDonald, F. E.; Oriyama, T. *J. Am. Chem. Soc.* **1992**, *114*, 8331–8333.
15. Ellervik, U.; Magnusson, G. *J. Org. Chem.* **1998**, *63*, 9314–9322.
16. Gege, C.; Geyer, A.; Schmidt, R. R. *Chemistry* **2002**, *8*, 2454–2463.
17. Zhang, Z. Y.; Niikura, K.; Huang, X. F.; Wong, C. H. *Can. J. Chem.* **2002**, *80*, 1051–1054.
18. Palcic, M. M.; Venot, A. P.; Ratcliffe, R. M.; Hindsgaul, O. *Carbohydr. Res.* **1989**, *190*, 1–11.
19. Ball, G. E.; Oneill, R. A.; Schultz, J. E.; Lowe, J. B.; Weston, B. W.; Nagy, J. O.; Brown, E. G.; Hobbs, C. J.; Bednarski, M. D. *J. Am. Chem. Soc.* **1992**, *114*, 5449–5451.
20. Ichikawa, Y.; Lin, Y. C.; Dumas, D. P.; Shen, G. J.; Garciajunceda, E.; Williams, M. A.; Bayer, R.; Ketcham, C.; Walker, L. E.; Paulson, J. C.; Wong, C. H. *J. Am. Chem. Soc.* **1992**, *114*, 9283–9298.
21. Sears, P.; Wong, C. H. *Angew. Chem., Int. Ed.* **1999**, *38*, 2300–2324.
22. Unger, F. M. *Adv. Carbohydr. Chem. Biochem.* **2001**, *57*, 207–435.
23. Kaila, N.; Thomas, B. E., 4th *Med. Res. Rev.* **2002**, *22*, 566–601.
24. Magnani, J. L. *Arch. Biochem. Biophys.* **2004**, *426*, 122–131.
25. Boons, G. J.; Demchenko, A. V. *Chem. Rev.* **2000**, *100*, 4539–4566.
26. Halcomb, R. L.; Chappell, M. D. *J. Carbohydr. Chem.* **2002**, *21*, 723–768.
27. Ando, H.; Imamura, A. *Trends Glycosci. Glycotechnol.* **2004**, *16*, 293–303.
28. Bongat, A. F. G.; Demchenko, A. V. *Carbohydr. Res.* **2007**, *342*, 374–406.
29. Crich, D.; Dudkin, V. *J. Am. Chem. Soc.* **2001**, *123*, 6819–6825.
30. Kunz, H.; Unverzagt, C. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 1697–1699.
31. Ishida, H.; Kiso, M. *Trends Glycosci. Glycotechnol.* **2001**, *13*, 57–64.
32. Hanashima, S.; Castagner, B.; Esposito, D.; Nokami, T.; Seeberger, P. H. *Org. Lett.* **2007**, *9*, 1777–1779.
33. Hayashi, M.; Tanaka, M.; Itoh, M.; Miyauchi, H. *J. Org. Chem.* **1996**, *61*, 2938–2945.
34. Mehta, S.; Gilbert, M.; Wakarchuk, W. W.; Whitfield, D. M. *Org. Lett.* **2000**, *2*, 751–753.
35. Yan, F. Y.; Mehta, S.; Eichler, E.; Wakarchuk, W. W.; Gilbert, M.; Schur, M. J.; Whitfield, D. M. *J. Org. Chem.* **2003**, *68*, 2426–2431.
36. (a) Yu, H.; Chokhawala, H.; Karpel, R.; Yu, H.; Wu, B.; Zhang, J.; Zhang, Y.; Jia, Q.; Chen, X. *J. Am. Chem. Soc.* **2005**, *127*, 17618–17619; (b) Yu, H.; Huang, S.; Chokhawala, H.; Sun, M.; Zheng, H.; Chen, X. *Angew. Chem., Int. Ed.* **2006**, *45*, 3938–3944;; (c) Yu, H.; Yu, H.; Karpel, R.; Chen, X. *Bioorg. Med. Chem.* **2004**, *12*, 6427–6435.
37. Yu, H.; Chokhawala, H. A.; Huang, S.; Chen, X. *Nat. Protoc.* **2006**, *1*, 2485–2492.
38. Toshima, K.; Tatsuta, K. *Chem. Rev.* **1993**, *93*, 1503–1531.
39. Garegg, P. J. *Adv. Carbohydr. Chem. Biochem.* **1997**, *52*, 179–205.
40. Vargasberenguel, A.; Meldal, M.; Paulsen, H.; Jensen, K. J.; Bock, K. *J. Chem. Soc., Perkin Trans. 1* **1994**, 3287–3294.
41. Kretzschmar, G.; Stahl, W. *Tetrahedron* **1998**, *54*, 6341–6358.
42. Wang, Z.; Zhou, L.; El-Boubbou, K.; Ye, X.; Huang, X. *J. Org. Chem.* **2007**, *72*, 6409–6420.
43. Crich, D.; Smith, M. *J. Am. Chem. Soc.* **2001**, *123*, 9015–9020.
44. Huang, X.; Huang, L.; Wang, H.; Ye, X. *Angew. Chem., Int. Ed.* **2004**, *43*, 5221–5224.
45. Veeneman, G. H.; Vanleeuwen, S. H.; Vanboom, J. H. *Tetrahedron Lett.* **1990**, *31*, 1331–1334.
46. Konradsson, P.; Udodong, U. E.; Fraserreid, B. *Tetrahedron Lett.* **1990**, *31*, 4313–4316.
47. Cao, H. Z.; Yu, B. *Tetrahedron Lett.* **2005**, *46*, 4337–4340.