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Total synthesis of a sialyl Lewis^x derivative for the diagnosis of cancer

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ABSTRACT

The total synthesis of aminoethyl glycoside of sialyl Lewis^x (sLe^x) is described. A galactose donor was condensed with a diol of glucosamine to afford regioselectively a β1,4 linked disaccharide, which was further stereoselectively fucosylated to provide a protected Lewis^x trisaccharide. After chemical modification, the trisaccharide was sialylated to give regio- and stereoselectively an azidoethyl glycoside of sLe^x. Finally, deprotection and azide reduction afforded the target compound. This compound will be coupled with protein and then be used to conduct further preclinical studies for the diagnosis of cancer.

1. Introduction

Metastatic spread is a major complication of cancer responsible for the majority of deaths. A key adhesion molecule associated with the capture of metastatic cells is E-selectin that is located on the surface of endothelial cells.[1,2] It is noteworthy that E-selectin expression is often enhanced at the site proximal to or directly at tumor metastases.[3]

Several E-selectin ligands have been identified on tumor cells, including PSG-1, ESL-1, Death receptor-3, and CD44, which contain sialyl Lewis^x (sLe^x) or sialyl Lewis^a (sLe^a) structure as the recognition motif.[4] SLe^x (NeuAcα2,3Galβ1,4(Fucα1,3)GlcNAc, Fig. 1) was discovered in 1990 as glycosphingolipids and glycoproteins that play a significant role in the adhesion between tumor cells and blood endothelial cells in metastases, as well as the adhesion of leucocytes to vascular wall in inflammation.[5–8] From then on, several synthetic methodologies (chemical and enzymatic) toward this tetrasaccharide have emerged in the literature despite synthetic challenges about regio- and stereoselectivity, the acid instability of the fucoside bond, and side reactions during sialylation.[9–13] Crystal structure analysis of E-selectin binding sLe^x displayed interactions between several amino acids coordinated to Ca^{2+} and fucose OHs at C-2 and C-3. Galactose OH at C-6 and neuraminic acid COOH are also involved in the binding events.[14]

The objective of our study was to search a kind of molecular probe that is able to target E-selectin through sLe^x moiety and recognize metastatic spot by imaging. The efficient synthesis of aminoethyl glycoside of sLe^x (**1**) (Fig. 2) as the targeting part is described here in detail. As an unreported sLe^x derivative, it was efficiently prepared from four building blocks through only seven steps with 18% total yield in our group. In addition, Le^x trisaccharide is the terminal moiety of numerous cell surface glycoproteins and glycolipids, correlated with selectin-mediated cell-cell recognition and adhesion processes,[15] thus aminoethyl glycoside of Le^x was also synthesized (**2**) as control (Fig. 2).

2. Results and discussion

For synthesis of the targets **1** and **2**, compounds **3**–**6** were chosen as building blocks (Fig. 3). They were prepared respectively from D-galactose,[10,16,17] D-glucosamine,[18] L-fucose[19–21] and *N*-acetyl neuraminic acid.[22,23] Then, they were condensed by glycosylation reaction to form desired trisaccharide and tetrasaccharide.

As illustrated in Scheme 1, the donor 2,3,4-tri-*O*-acetyl-6-*O*-benzyl-D-galactopyranosyl fluoride **3a** was firstly designed to couple with the acceptor **4**, unfortunately, the designed disaccharide **7a** was not obtained despite changing reaction conditions (time, temperature, donor equivalent, solvent, and promoter), probably due to the disarmed effects and the weak reactivity (fluorine) of the donor.[24–26] Compound **3a** was then replaced by the trichloroacetimidate **3b**, which was coupled with **4** in CH_2Cl_2 using trimethylsilyl triflate (TMSOTf) as promoter to give regio- and

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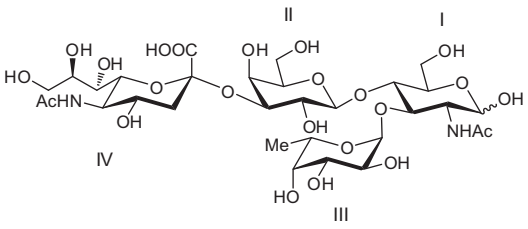


Figure 1. Structure of sLe^x.

stereoselectively β1,4 linked disaccharide **7a** in 77% yield. Such a selective behavior has been observed in our previous work.[27]

The ^{1}H NMR spectrum of **7a** displayed the presence of H-3^I of the glucosamine residue at δ 4.49 (dt, $J_{2,3}$, $J_{3,4}$ 10.4 Hz, $J_{3,OH}$ 1.8 Hz), indicating the position of the newly formed glycosidic linkage in the disaccharide **7a** to be at OH-4 of the acceptor **4**. This regioselectivity was further confirmed from the ^{1}H NMR spectrum of its acetylated derivative **7b**, which showed a deshielded signal for H-3^I at δ 5.71 (dd, $J_{2,3}$ 10.4 Hz, $J_{3,4}$ 8.9 Hz). Its stereochemistry was identified to be the desired β anomer on the basis of the H-1^{II}, H-2^{II} coupling constant ($J_{1,2}$ 7.9 Hz).

In order to construct the trisaccharide **8**, 2,3,4-tri-*O*-benzyl-L-fucopyranosyl fluoride (**5b**), synthesized from ethyl 2,3,4-tri-*O*-benzyl-1-thio-L-fucopyranoside **5a** (Scheme 2),[19] was used for the fucosylation of the disaccharide **7a** according to our previous work.[28] During preparation, it was observed that the reaction should be conducted at very low temperature (−90 °C), especially when the amount of **5a** was increased (more than 0.2 g). In addition, the reaction could be completed in only 10 min (Table 1).

Coupling of **5b** and **7a** in toluene/CH_2Cl_2 using silver triflate (AgOTf) and stannous chloride ($SnCl_2$) as promoters gave the trisaccharide **8** in 80% yield (Scheme 2).[28–30] The newly generated glycosidic linkage was confirmed to be α based on the low value of the Fuc H-1^{III}, H-2^{III} coupling constant ($J_{1,2}$ 3.5 Hz).

Subsequently, 2-azidoethanol, which was readily prepared from 2-bromoethanol,[31] was glycosylated with the trisaccharide **8** in the presence of *N*-iodosuccinimide (NIS)/trifluoromethanesulfonic acid (TfOH) at −15 °C, giving compound **9** in 90% yield, as shown in Scheme 3. Treatment of compound **9** with hydrazine and water in boiling ethanol for 4 h, followed by selective N-acetylation in a mild condition for another 4 h, provided compound **10** in 88% overall yield (Scheme 3).[32]

The last building block **6** was then coupled with compound **10** in CH_2Cl_2/CH_3CN using AgOTf, benzenesulfenyl chloride (PhSCl), and di-*tert*-butylpyridine (DTBP) as promoters to give α-product in 42% yield (Scheme 4).[23,33,34] Compound **10** could not completely react, which was quite close to the product on analytical thin-layer chromatography (TLC) in all tested eluants. It needed to be purified on silica gel column for two times with different eluants and then on Sephadex column (LH20) for one time to get the pure product **11a**. Fortunately, 44% of compound **10** could be recovered from the reaction.

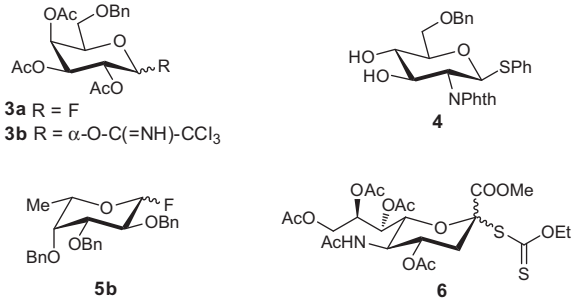


Figure 3. Key building blocks for synthesis of targets **1** and **2**.

According to our previous work, the sialylation was supposed to be regioselective at OH-3 of the acceptor **10**. This regioselectivity was confirmed from the ^{1}H NMR spectrum of its acetylated derivative **11b**. The ^{1}H NMR spectrum of **11a** displayed the presence of H-2^{II} and H-4^{II} of the galactose residue at δ 3.77–3.58, and the ^{1}H NMR spectrum of **11b** showed a deshielded signal at δ 4.95 for H-2^{II}, and another deshielded signal at δ 5.06 for H-4^{II}. Its stereochemistry was determined to be α on the basis of the chemical shift of H-3$^{IV}_e$ at δ 2.68, which was larger for α-glycosides (in the approximate range of 2.72 ± 0.05 ppm) than for β-anomers (in the approximate range of 2.32 ± 0.08 ppm).[35] In addition, the appearance of the NeuAc H-4 at ~4.8 ppm and the $J_{NeuAc7,8}$ >7.0 Hz also confirmed this stereochemistry.[36]

The acetyl groups of compound **11a** on hydroxyls were subsequently removed by reaction with NaOMe/MeOH. After adding several drops of water, compound **12** was obtained in 89% yield (Scheme 4).

We then performed reduction of azide and debenzylation of hydroxyl groups in order to get the targets **1** and **2** from compounds **12** and **10**, respectively. Surprisingly, the simultaneous debenzylation and azide reduction failed through conventional catalytic hydrogenation in MeOH. Instead, the intermediates **13a** and **13b** were generated, and they were quite unstable. No improvement was observed when Pd/C equivalent, hydrogen pressure, and reaction time were increased or acid (hydrochloric acid, acetic acid, formic acid) was added. On the basis of the structural feature of target compounds: solubility in water, toxicity of amine to Pd/C, and instability of fucoside bond to strong acid, the reaction condition was further improved. Finally, target compounds were obtained quantitatively when reaction was conducted with 1.5 equivalent of Pd/C in MeOH–H_2O–AcOH (3:1:0.5) or in THF–H_2O–AcOH (4:2:1) under an atmosphere of hydrogen (see Scheme 5).[37,38]

The compounds **1** and **2** were fully characterized by ^{1}H and ^{13}C NMR, as well as HRMS.

3. Conclusions

We have developed a concise total synthesis of aminoethyl glycoside of sLe^x with high overall yield. The disaccharide was successfully prepared through a highly regio- and stereoselective

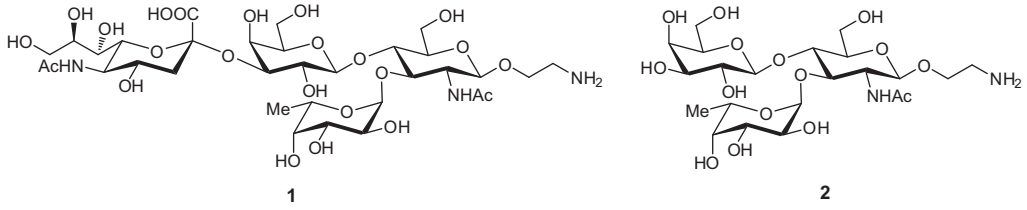
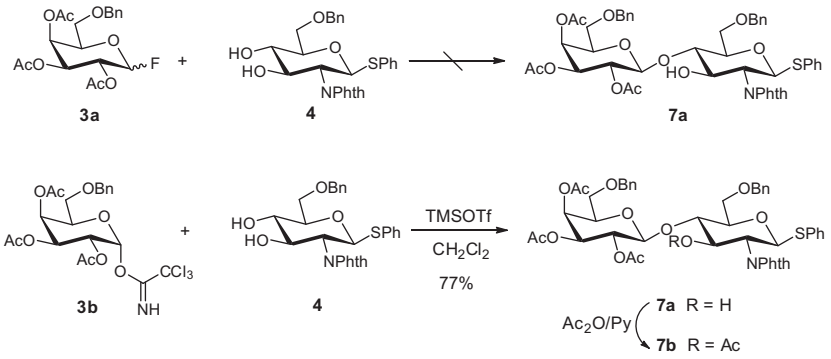
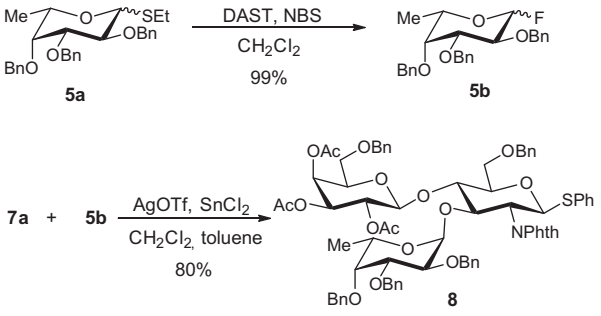


Figure 2. Aminoethyl glycoside of sLe^x **1** and aminoethyl glycoside of Le^x **2**.



Scheme 1. Synthesis of disaccharide **7a**.



Scheme 2. Synthesis of Le^x trisaccaride **8**.

Table 1
Preparation of fluoride **5b** from thioglycoside **5a**

5a[a] (g)	*T* (°C)	Time (min)	Yield of **5b** (%)
0.1	−15	30	92
0.2	−15	30	28
0.2	−20	30	39
0.1	−30	25	98
0.2	−30	15	73
0.2	−40	10	83
0.3	−80	10	84
0.4	−90	10	99

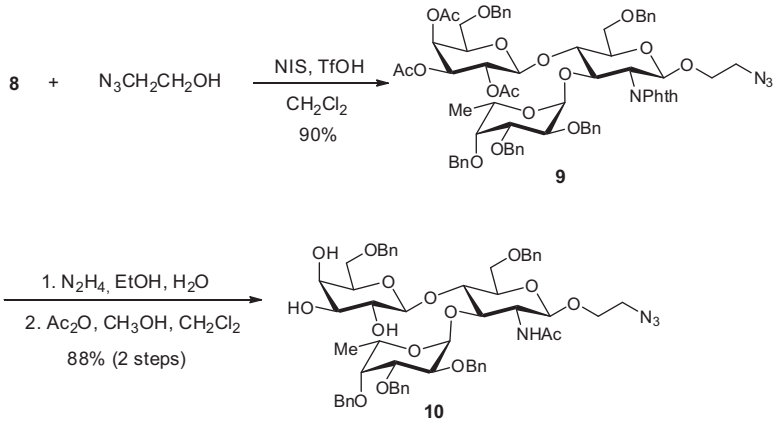
[a] Amount of reagents: DAST (1.5 equiv), NBS (1.3 equiv).

glycosylation to afford the β1,4 linked disaccharide in high yield owing to participating effect of the donor and stereo hindrance effect of the acceptor. The building block **5b** was prepared from compound **5a** in higher yield under the condition of lower temperature and shorter reaction time probably due to armed effects of benzyl groups. Fucosylation of disaccharide **7a** with the powerful donor **5b** provided the desired trisaccharide **8** in high yield. After coupling with 2-azidoethanol, deacetylation and acetylation on amino group, the last building block **6** was introduced by a highly regio- and stereoselective glycosylation to give tetrasaccharide **11a**, taking advantage of stereo hindrance effect of the OH-2^{II}, the difference of activity between OH-3^{II} and OH-4^{II}, and solvent effect of CH_3CN. Deacetylation and hydrolysis of the ester, followed by reduction of azide and debenzylation, finally provided the target compounds **1** and **2**. These biomolecules will be applied for in vivo imaging and biodistribution studies after coupling with protein and chelating with ^{99m}Tc for the investigation of early diagnosis of cancer (**2** will be used as a control).

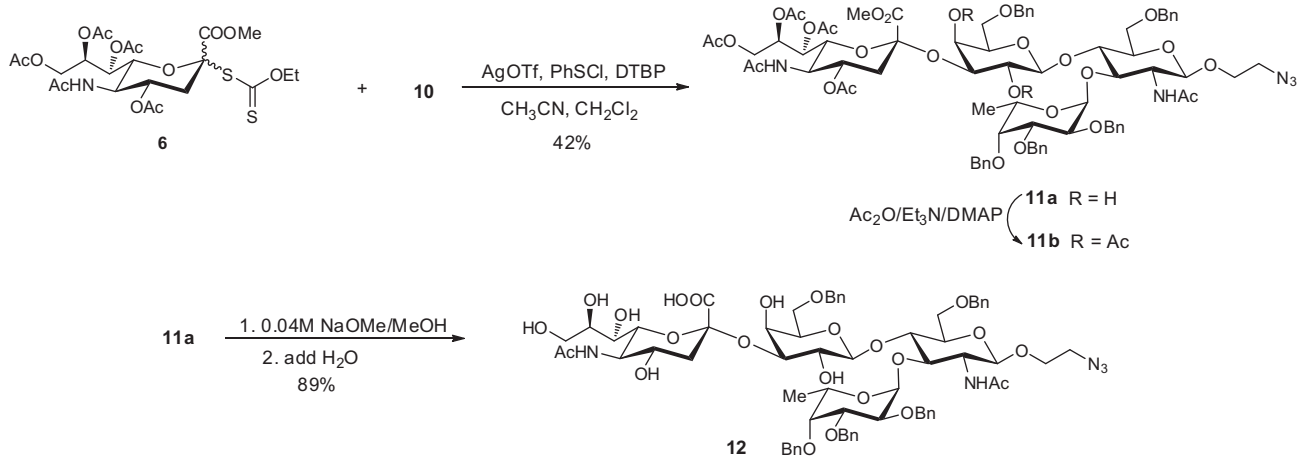
4. Experimental

4.1. General methods

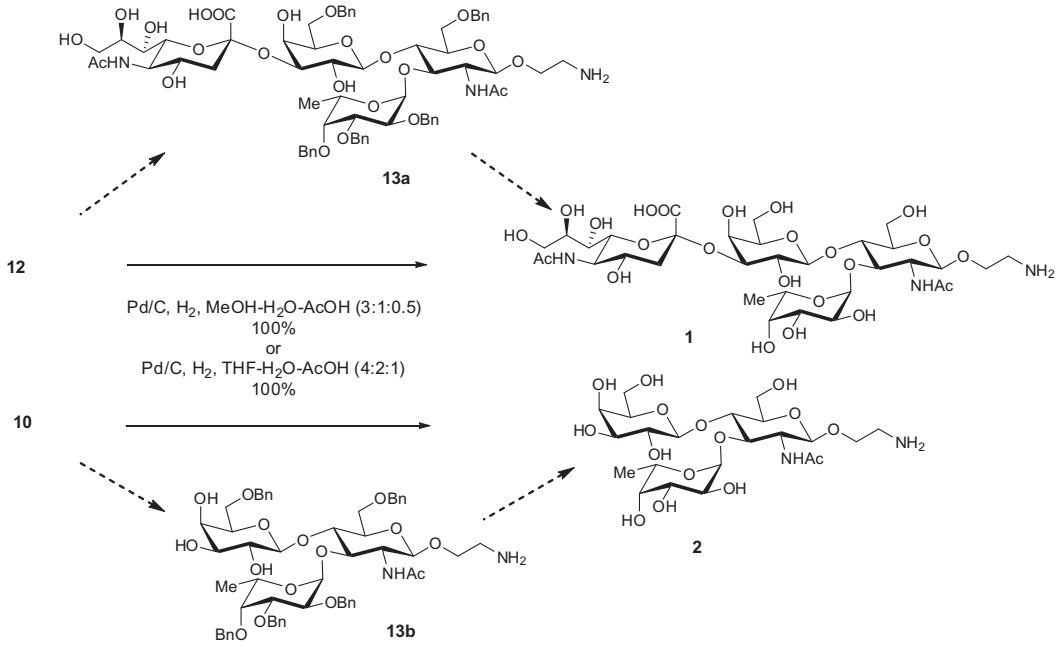
All reagents were purchased from commercial suppliers and used without further purification. CH_2Cl_2 was freshly distilled from phosphorus pentoxide (P_2O_5) and THF was distilled with sodium/benzophenone, and toluene was distilled from sodium. TLC was performed using silica gel 60 F_{254} (Merck, 0.2 mm). Flash chromatography was performed on silica gel 60 (230–400 mesh, Merck), Sephadex G25, and Sephadex LH20 (Sigma). ^{1}H NMR spectra and ^{13}C NMR spectra were recorded in $CDCl_3$, CD_3OD or D_2O with a Bruker DRX 400 spectrometer at ambient temperature. Assignments of



Scheme 3. Synthesis of azidoethyl glycoside of Le^x **10**.



Scheme 4. Synthesis of azidoethyl glycoside of sLe^x **12**.



Scheme 5. Synthesis of aminoethyl glycoside of sLe^x **1** and aminoethyl glycoside of Le^x **2**.

proton and carbon resonances were aided by 2D ^{1}H–^{1}H COSY and ^{13}C–^{1}H HSQC correlation experiments as well as 1D TOCSY experiments. Optical rotations were recorded at 20 °C with a Perkin–Elmer Model 343 digital polarimeter at 589 nm (Na line), using a 10 cm, 1 mL cell. High-resolution mass spectra (HRMS) were measured with a Bruker microTOF spectrometer in electrospray ionization (ESI) mode.

The building block **3b** was prepared from D-galactose according to the approaches in Refs. 10,16,17. The building block **4** was obtained from D-glucosamine on the basis of Ref. 18. The building block **5b** was prepared from L-fucose via **5a** based on Refs. 19–21. The synthesis of the building block **6** from *N*-acetyl neuraminic acid was described in Refs. 22,23.

4.2. Phenyl (2,3,4-tri-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-6-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (7a)

A mixture of **3b** (520 mg, 0.96 mmol), **4** (520 mg, 1.06 mmol), and 4 Å powdered molecular sieves was stirred in dry CH_2Cl_2 (20 mL) for 40 min at room temperature under N_2. TMSOTf (0.19 mL, 1.06 mmol) was added at 0 °C, and stir was continued for 1 h. The mixture was filtered through Celite. The filtrate was washed with saturated aqueous $NaHCO_3$ and then with brine, dried over $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (Cy-EtOAc 2:1), and then by a Sephadex column (LH20) using CH_2Cl_2–MeOH (1:1) as eluant to give **7a** (640 mg, 77%) as a white amorphous solid. R_f = 0.60 (Cy-EtOAc 1:1); $[\alpha]_D^{20}$ +13.6 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.91–7.16 (m, 19H, arom), 5.58 (d, 1H, $J_{1,2}$ 10.4 Hz, H-1^I), 5.36 (dd, 1H, $J_{3,4}$ 3.4 Hz, $J_{4,5}$ 1.1 Hz, H-4^{II}), 5.17 (dd, 1H, $J_{1,2}$ 8.0 Hz, $J_{2,3}$ 10.4 Hz, H-2^{II}), 4.94 (dd, 1H, $J_{2,3}$ 10.4 Hz, $J_{3,4}$ 3.4 Hz, H-3^{II}), 4.68, 4.52 (2d, 2H, J_{gem} 12.0 Hz, PhCH_2), 4.49 (dt, 1H, $J_{2,3}$, $J_{3,4}$ 10.4 Hz, $J_{3,OH}$ 1.8 Hz, H-3^I), 4.51 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1^{II}), 4.41, 4.30 (2d, 2H, J_{gem} 11.8 Hz, PhCH_2), 4.28 (t, 1H, $J_{1,2}$, $J_{2,3}$ 10.4 Hz, H-2^I), 4.18 (d, 1H, $J_{3,OH}$ 1.8 Hz, OH-3^I), 3.84 (td, 1H, $J_{4,5}$ 1.1 Hz, $J_{5,6}$ 6.0 Hz, H-5^{II}), 3.77–3.68 (m, 4H, H-5^I, H-4^I, H-6a^I, H-6b^I), 3.50–3.36 (m, 2H, H-6a^{II}, H-6b^{II}), 2.02, 2.00, 1.96 (3s, 9H, 3 × OAc); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 170.17, 170.02, 169.31 (3 × C=O, Ac), 168.25, 167.55 (2 × C=O, NPhth), 138.24, 137.09, 132.16, 131.94,

131.84 (arom C), 134.14, 132.79, 128.93, 128.58, 128.50, 127.98, 127.96, 127.90, 127.88, 123.82,123.32 (arom CH), 101.41 (C-1^{II}), 83.55 (C-1^{I}), 81.50 (C-4^{I}), 78.39 (C-5^{I}), 73,76 (PhCH$_2$), 73.66 (PhCH$_2$), 72.42 (C-5^{II}), 71.02 (C-3^{II}), 70.98 (C-3^{I}), 69.10 (C-2^{II}), 68.33 (C-6^{I}), 67.51 (C-6^{II}), 67.33 (C-4^{II}), 55.25 (C-2^{I}), 20.82, 20.67, 20.64 (3 × *C*H$_3$CO); ESI-HRMS (*m*/*z*) calcd for $C_{46}H_{47}NO_{14}SNa$ (M+Na$^+$) 892.2609, found 892.2648.

4.3. Phenyl (2,3,4-tri-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (7b)

A solution of **7a** (50 mg, 0.069 mmol) in 2 mL of pyridine and 1 mL of acetic anhydride was stirred at room temperature for 14 h. After concentration, the residue was purified by a column of silica gel (Cy-EtOAc 2:1), and then by a Sephadex column (LH20) using CH_2Cl_2–MeOH (1:1) as eluant. Compound **7b** was obtained (49 mg, 94%) as a white amorphous solid. R_f = 0.58 (Cy-EtOAc 1:1); $[\alpha]_D^{20}$ +3.4 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.87–7.19 (m, 19H, arom), 5.71 (d, 1H, $J_{1,2}$ 10.4 Hz, H-1^{I}), 5.71 (dd, 1H, $J_{2,3}$ 10.4 Hz, $J_{3,4}$ 8.9 Hz, H-3^{I}), 5.38 (dd, 1H, $J_{3,4}$ 3.5 Hz, $J_{4,5}$ 1.2 Hz, H-4^{II}), 5.00 (dd, 1H, $J_{1,2}$ 7.9 Hz, $J_{2,3}$ 10.4 Hz, H-2^{II}), 4.85 (dd, 1H, $J_{2,3}$ 10.4 Hz, $J_{3,4}$ 3.4 Hz, H-3^{II}), 4.74, 4.52 (2d, 2H, J_{gem} 12.0 Hz, PhC*H*$_2$), 4.51 (d, 1H, $J_{1,2}$ 7.9 Hz, H-1^{II}), 4.48, 4.36 (2d, 2H, J_{gem} 11.9 Hz, PhC*H*$_2$), 4.28 (t, 1H, $J_{1,2}$, $J_{2,3}$ 10.4 Hz, H-2^{I}), 4.00 (t, 1H, $J_{3,4}$, $J_{4,5}$ 8.9 Hz, H-4^{I}), 3.78 (m, 2H, H-$6a^{I}$, H-$6b^{I}$), 3.68 (m, 1H, H-5^{I}), 3.62 (td, 1H, $J_{4,5}$ 1.2 Hz, $J_{5,6}$ 6.4 Hz, H-5^{II}), 3.48–3.35 (m, 2H, H-$6a^{II}$, H-$6b^{II}$), 1.99, 1.95, 1.95, 1.79 (4s, 12H, 4 × OAc); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 170.21, 170.14, 170.00, 169.06 (4 × C═O, Ac), 167.84, 167.35 (2 × C═O, NPhth), 138.01, 137.46, 131.86, 131.50, 131.40 (arom C), 134.47, 134.22, 133.22, 128.99, 128.66, 128.61, 128.29, 128.07, 128.04, 128.01, 123.84, 123.62 (arom CH), 100.63 (C-1^{II}), 83.12 (C-1^{I}), 79.07 (C-5^{I}), 75.38 (C-4^{I}), 73.81 (PhCH$_2$), 73.56 (PhCH$_2$), 72.33 (C-3^{I}), 71.80 (C-5^{II}), 71.34 (C-3^{II}), 69.55 (C-2^{II}), 67.75 (C-6^{I}), 67.35 (C-4^{II}), 67.04 (C-6^{II}), 54.06 (C-2^{I}), 20.85, 20.74, 20.69, 20.61 (4 × *C*H$_3$CO); ESI-HRMS (*m*/*z*) calcd for $C_{48}H_{49}NO_{15}SNa$ (M+Na$^+$) 934.2715, found 934.2725.

4.4. Phenyl (2,3,4-tri-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→3)]-6-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (8)

A mixture of **7a** (300 mg, 0.34 mmol), **5b** (260 mg, 0.58 mmol), and 4 Å powdered molecular sieves was stirred in 10 mL of dry CH_2Cl_2 and 2 mL of dry toluene for 30 min at room temperature under N_2. A mixture of $SnCl_2$ (110 mg, 0.60 mmol) and AgOTf (150 mg, 0.60 mmol) was added at −15 °C, and stir was continued for 1 h at 0 °C. The mixture was filtered through Celite and washed with CH_2Cl_2. The filtrate was washed with a saturated $NaHCO_3$ solution, then with brine, dried over $MgSO_4$, and concentrated. After purification by a column of silica gel (Cy-EtOAc 4:1), and then by a Sephadex column (LH20) using CH_2Cl_2–MeOH (1:1) as eluant, compound **8** was obtained (360 mg, 80%) as a white amorphous solid. R_f = 0.41 (Cy-EtOAc 2:1); $[\alpha]_D^{20}$ −0.6 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.65–6.90 (m, 34H, arom), 5.37 (d, 1H, $J_{1,2}$ 10.4 Hz, H-1^{I}), 5.27 (d, 1H, $J_{3,4}$ 3.6 Hz, H-4^{II}), 4.89 (dd, 1H, $J_{1,2}$ 8.1 Hz, $J_{2,3}$ 10.4 Hz, H-2^{II}), 4.72 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1^{III}), 4.73–4.60 (m, 4H, H-3^{II}, H-3^{I}, PhC*H*$_2$), 4.60 (d, 1H, $J_{1,2}$ 8.1 Hz, H-1^{II}), 4.48 (q, 1H, $J_{5,6}$ 6.4 Hz, H-5^{III}), 4.46–4.07 (m, 9H, H-2^{I}, 4 × PhC*H*$_2$), 4.04 (dd, 1H, $J_{3,4}$ 9.7 Hz, $J_{4,5}$ 9.0 Hz, H-4^{I}), 3.79–3.65 (m, 4H, H-3^{III}, H-2^{III}, H-$6a^{I}$, H-$6b^{I}$), 3.46–3.42 (m, 3H, H-5^{I}, H-4^{III}, H-$6a^{II}$), 3.40 (td, 1H, $J_{4,5}$ 1.3 Hz, $J_{5,6}$ 5.2 Hz, H-5^{II}), 3.22 (m, 1H, H-$6b^{II}$), 1.88, 1.83, 1.66 (3s, 9H, 3×OAc), 1.06 (d, 3H, $J_{5,6}$ 6.4 Hz, H-6^{III}); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 170.01, 169.72, 168.83 (3 × C═O, Ac), 138.76, 138.70, 138.13, 137.88, 137.50, 132.38, 131.75 (arom C), 134.27, 132.58, 128.88, 128.64, 128.53, 128.32, 128.25, 128.17, 128.07, 128.05, 127.99, 127.92, 127.87, 127.83, 127.60, 127.52, 127.43, 127.28, 127.07, 123.73 (arom CH), 99.71 (C-1^{II}), 97.39 (C-1^{III}), 84.28 (C-1^{I}), 79.85 (C-3^{III}), 79.66 (C-5^{I}), 77.70 (C-4^{III}), 75.01 (C-2^{III}), 74.93 (C-4^{I}), 74.30 (PhCH$_2$), 73.62 (PhCH$_2$), 73.57 (C-3^{I}), 73.32 (PhCH$_2$), 72.95 (PhCH$_2$), 72.84 (PhCH$_2$), 71.58 (C-5^{II}), 71.32 (C-3^{II}), 69.32 (C-2^{II}), 67.84 (C-6^{I}), 67.40 (C-4^{II}), 66.60 (C-5^{III}), 66.58 (C-6^{II}), 55.59 (C-2^{I}), 20.81, 20.65, 20.63 (3×*C*H$_3$-CO), 16.73 (C-6^{III}); ESI-HRMS (*m*/*z*) calcd for $C_{73}H_{75}NO_{18}SNa$ (M+Na$^+$) 1308.4597, found 1308.4636.

4.5. 2-Azidoethyl (2,3,4-tri-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→3)]-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (9)

A mixture of **8** (120 mg, 0.09 mmol), 2-azidoethanol (200 mg, 0.20 mmol), and 4Å powdered molecular sieves (260 mg) was stirred in 9 mL of dry CH_2Cl_2 for 30 min at room temperature under N_2, then cooled to −15 °C. NIS (46 mg, 0.21 mmol) and TfOH (2.71 μL, 0.031 mmol) were added. The mixture was stirred at −15 °C for 1 h, then neutralized with Et_3N, filtered through Celite, and concentrated. The residue was washed with aqueous $Na_2S_2O_3$, water, and brine and then dried over $MgSO_4$ and concentrated. After purification by a column of silica gel (Cy-EtOAc 3:1), and then by a Sephadex column (LH20) using CH_2Cl_2–MeOH (1:1) as eluant, compound **9** was obtained (106 mg, 90%) as a white amorphous solid. R_f = 0.26 (Cy-EtOAc 3:2); $[\alpha]_D^{20}$ −12.4 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.71–7.00 (m, 29H, arom), 5.37 (d, 1H, $J_{3,4}$ 3.6 Hz, H-4^{II}), 5.15 (d, 1H, $J_{1,2}$ 8.5 Hz, H-1^{I}), 5.00 (dd, 1H, $J_{1,2}$ 8.0 Hz, $J_{2,3}$ 10.4 Hz, H-2^{II}), 4.84–4.77 (m, 4H, H-1^{III}, H-3^{II}, PhC*H*$_2$), 4.71 (dd, 1H, $J_{2,3}$ 8.0 Hz, $J_{3,4}$ 9.2 Hz, H-3^{I}), 4.67 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1^{II}), 4.59 (q, 1H, $J_{5,6}$ 6.4 Hz, H-5^{III}), 4.58–4.54 (m, 2H, PhC*H*$_2$), 4.50–4.35 (m, 5H, H-2^{I}, 2 × PhC*H*$_2$), 4.25–4.18 (m, 2H, PhC*H*$_2$), 4.15 (t, 1H, $J_{3,4}$, $J_{4,5}$ 9.2 Hz, H-4^{I}), 3.97–3.76 (m, 5H, OC*H*$_2$CH$_2$N$_3$, H-3^{III}, H-2^{III}, H-$6a^{II}$), 3.57–3.48 (m, 5H, H-5^{II}, H-4^{III}, H-5^{I}, H-$6b^{II}$, H-$6a^{I}$), 3.35–3.29 (m, 2H, H-$6b^{I}$, OCH$_2$C*H*N$_3$), 3.19–3.14 (m, 1H, OCH$_2$C*H*N$_3$), 2.00, 1.95, 1.78 (3s, 9H, 3 × OAc), 1.17 (d, 3H, $J_{5,6}$ 6.4 Hz, H-6^{III}); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 170.08, 169.81, 168.89 (3 × C═O, Ac), 138.86, 138.79, 138.22, 137.81, 137.56, 131.93 (arom C), 134.16, 128.76, 128.59, 128.38, 128.32, 128.26, 128.23, 128.10, 128.04, 128.01, 127.89, 127.66, 127.57, 127.47, 127.32, 127.13, 123.62 (arom CH), 99.73 (C-1^{II}), 98.53 (C-1^{I}), 97.37 (C-1^{III}), 79.89 (C-3^{III}), 77.78 (C-5^{II}), 75.51 (C-4^{III}), 75.22 (C-2^{III}), 75.18 (C-4^{I}), 74.35 (PhCH$_2$), 73.73 (PhCH$_2$), 73.39 (PhCH$_2$), 72.95 (2×PhCH$_2$), 72.47 (C-3^{I}), 71.60 (C-5^{I}), 71.36 (C-3^{II}), 69.42 (C-2^{II}), 68.10 (OCH$_2$CH$_2$N$_3$), 67.75 (C-6^{II}), 67.44 (C-4^{II}), 66.66 (C-6^{I}), 66.59 (C-5^{III}), 56.16 (C-2^{I}), 50.60 (OCH$_2$CH$_2$N$_3$), 20.86, 20.71, 20.69 (3 × *C*H$_3$CO), 16.80 (C-6^{III}); ESI-HRMS (*m*/*z*) calcd for $C_{69}H_{74}N_4O_{19}Na$ (M+Na$^+$) 1285.4839, found 1285.4807.

4.6. 2-Azidoethyl (6-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→3)]-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (10)

To a solution of compound **9** (300 mg, 0.238 mmol) in 37.6 mL of ethanol, 2.5 mL of hydrazine monohydrate and 2.5 mL of water were added. The mixture was refluxed at 80 °C for 4 h. After concentration, the residue was co-evaporated with toluene and dried, then dissolved in 25 mL of CH_2Cl_2–MeOH (1:1), to which 1.25 mL of acetic anhydride was introduced. The mixture was stirred at room temperature for 4 h. After concentration, the residue was purified by a column of silica gel (CH_2Cl_2–MeOH 40:1), and then by a Sephadex column (LH20) using CH_2Cl_2–MeOH (1:1) as eluant. Compound **10** was obtained (218 mg, 88%, 2 steps) as a white amorphous solid. R_f = 0.47 (CH_2Cl_2–MeOH 10:1); $[\alpha]_D^{20}$ −49.1 (*c*

1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.34–7.23 (m, 25H, arom), 5.93 (d, 1H, $J_{2,NH}$ 6.6 Hz, NH-2^I), 5.07 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1^{III}), 4.93–4.89 (m, 3H, H-1^I, PhCH_2), 4.73–4.43 (m, 9H, 4 × PhCH_2, H-1^{II}), 4.32 (q, 1H, $J_{5,6}$ 6.4 Hz, H-5^{III}), 4.27 (t, 1H, $J_{2,3}$, $J_{3,4}$ 8.8 Hz, H-3^I), 4.04 (dd, 1H, $J_{1,2}$ 3.6 Hz, $J_{2,3}$ 10.0 Hz, H-2^{III}), 4.04 (br s, 1H, OH-3^{II}), 3.96 (t, 1H, $J_{3,4}$, $J_{4,5}$ 8.8 Hz, H-4^I), 3.96–3.90 (m, 4H, H-3^{III}, H-4^{II}, OCHCH_2N_3, H-6a^I), 3.81–3.70 (m, 2H, H-6b^I, H-6a^{II}), 3.67–3.58 (m, 4H, H-3^{II}, H-5^I, H-6b^{II}, OCHCH_2N_3), 3.52–3.42 (m, 3H, H-2^{II}, H-5^{II}, H-4^{III}), 3.40–3.34 (m, 1H, OCH$_2$CHN$_3$), 3.24 (m, 1H, H-2^I), 3.22–3.16 (m, 1H, OCH$_2$CHN$_3$), 2.92 (br s, 1H, OH-2^{II}), 2.66 (br s, 1H, OH-4^{II}), 1.63 (s, 3H, OAc), 1.11 (d, 3H, $J_{5,6}$ 6.5 Hz, H-6^{III}); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 170.91 (C═O, Ac), 138.78, 138.65, 138.52, 138.20, 137.94 (arom C), 128.78, 128.59, 128.55, 128.48, 128.33, 128.21, 128.12, 127.98, 127.90, 127.77, 127.74, 127.69, 127.66, 127.40 (arom CH), 100.72 (C-1^{II}), 99.79 (C-1^I), 97.97 (C-1^{III}), 79.79 (C-3^{III}), 77.77 (C-3^{II}), 76.84 (C-2^{III}), 76.28 (C-3^I), 75.13 (PhCH$_2$), 74.84 (C-4^I), 74.68 (C-5^I), 74.40 (PhCH$_2$), 73.64 (C-4^{III}), 73.55 (PhCH$_2$), 73.51 (PhCH$_2$), 73.04 (C-5^{II}), 72.47 (PhCH$_2$), 72.12 (C-2^{II}), 69.00 (C-6^I, C-6^{II}), 68.38 (C-4^{II}), 68.28 (OCH$_2$CH$_2$N$_3$), 67.22 (C-5^{III}), 57.99 (C-2^I), 50.72 (OCH$_2$CH$_2$N$_3$), 23.38 (CH$_3$CO), 16.99 (C-6^{III}); ESI-HRMS (m/z) calcd for $C_{57}H_{68}N_4O_{15}Na$ (M+Na$^+$) 1071.4573, found: 1071.4604.

4.7. 2-Azidoethyl (5-*N*-acetyl-4,7,8,9-tetra-*O*-acetyl-1-methyl-α-neuraminyl)-(2→3)-(6-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→3)]-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (11a)

A mixture of **10** (108 mg, 0.103 mmol), **6** (152 mg, 81%, 0.206 mmol) and 4 Å powdered molecular sieves (520 mg) was stirred in 4.8 mL of dry CH_3CN and 2.4 mL of dry CH_2Cl_2 for 1 h at room temperature under N_2. AgOTf (79 mg, 0.309 mmol) and DTBP (81 μL, 0.360 mmol) were added, and the mixture was cooled to −65 °C and kept protected from light. PhSCl (42 μL, 0.360 mmol) in dry CH_2Cl_2 (0.2 mL) was added by running the solution down the cold wall of the reaction flask, and stir was continued for 2 h at −65 °C. The mixture was diluted with EtOAc, filtered through Celite, and washed with saturated aqueous $NaHCO_3$ and brine and then dried over $MgSO_4$ and concentrated. After purification by a column of silica gel (CH_2Cl_2–MeOH 40:1) and then by a column of silica gel (Cy–EtOAc–MeOH 10:10:1), and then by a Sephadex column (LH20) using CH_2Cl_2–MeOH (1:1) as eluant, compound **11a** was obtained (66 mg, 42%) as a white amorphous solid. R_f = 0.22 (CH_2Cl_2–MeOH 20:1); $[\alpha]_D^{20}$ −29.8 (c 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.36–7.20 (m, 25H, arom), 5.93 (d, 1H, $J_{2,NH}$ 7.3 Hz, NH-2^I), 5.42 (m, 1H, H-8^{IV}), 5.32 (dd, 1H, $J_{7,8}$ 8.7 Hz, $J_{6,7}$ 1.9 Hz, H-7^{IV}), 5.26 (d, 1H, $J_{5,NH}$ 9.0 Hz, NH-5^{IV}), 5.14 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1^{III}), 5.00–4.84 (m, 4H, H-4^{IV}, 2PhCH_2, H-1^I), 4.73–4.56 (m, 7H, 2 × PhCH_2, 2 × PhCH_2, H-1^{II}), 4.45 (s, 2H, PhCH_2), 4.42 (q, 1H, $J_{5,6}$ 6.5 Hz, H-5^{III}), 4.27 (dd, 1H, $J_{9a,9b}$ 12.5 Hz, $J_{8,9a}$ 2.7 Hz, H-9a^{IV}), 4.22 (t, 1H, $J_{2,3}$, $J_{3,4}$ 8.8 Hz, H-3^I), 4.13–3.91 (m, 9H, H-6^{IV}, H-2^{III}, H-4^I, H-3^{III}, H-9b^{IV}, H-5^{IV}, H-3^{II}, H-6a^I, H-6a^{II}), 3.86–3.82 (m, 1H, H-6b^I), 3.77 (s, 3H, COOCH_3), 3.77–3.58 (m, 7H, H-6b^{II}, H-4^{II}, H-5^I, H-4^{III}, H-2^{II}, OCH_2CH$_2$N$_3$), 3.51 (t, 1H, $J_{5,6}$ 6.2 Hz, H-5^{II}), 3.42 (m, 1H, H-2^I), 3.40–3.34 (m, 1H, OCH$_2$CHN$_3$), 3.23–3.18 (m, 1H, OCH$_2$CHN$_3$), 3.12 (s, 1H, OH-2^{II}),), 2.68 (dd, 1H, $J_{3e,3a}$ 13.0 Hz, $J_{3e,4}$ 4.6 Hz, H-3$_e^{IV}$), 2.51 (d, 1H, $J_{4,OH}$ 3.6 Hz, OH-4^{II}), 2.12–2.06 (m, 1H, H-3$_a^{IV}$), 2.10, 2.08, 2.03, 1.98 (4s, 12H, 4×OAc), 1.89, 1.66 (2s, 6H, 2×NAc), 1.13 (d, 3H, $J_{5,6}$ 6.5 Hz, H-6^{III}); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 170.96, 170.61, 170.48, 170.40, 170.07, 169.89 (6 × C═O, Ac), 168.24 (C-1^{IV}), 138.94, 138.80, 138.69, 138.66, 138.12 (arom C), 128.69, 128.49, 128.31, 128.26, 128.19, 128.07, 128.02, 127.78, 127.55, 127.49, 127.44, 127.29 (arom CH), 101.19 (C-1^{II}), 100.14 (C-1^I), 98.14 (C-2^{IV}), 97.99 (C-1^{III}), 80.01 (C-3^{III}), 78.00 (C-4^{III}), 77.36 (C-3^{II}), 77.06 (C-2^{III}), 75.47 (C-3^I), 75.12 (PhCH_2), 74.95 (C-5^I), 74.26 (PhCH_2), 74.04 (C-4^I), 73.39 (PhCH_2), 72.96 (PhCH_2), 72.79 (C-6^{IV}), 72.45 (C-5^{II}), 72.36 (PhCH_2), 70.01 (C-2^{II}), 68.91 (C-6^I), 68.73 (C-4^{IV}), 68.43 (C-6^{II}), 68.32 (C-8^{IV}), 67.99 (OCH$_2$CH$_2$N$_3$), 67.75 (C-4^{II}), 67.08 (C-7^{IV}), 66.98 (C-5^{III}), 62.33 (C-9^{IV}), 57.15 (C-2^I), 53.22 (COOCH_3), 50.74 (OCH$_2$CH$_2$N$_3$), 49.71 (C-5^{IV}), 37.47 (C-3^{IV}), 23.41, 23.30, 21.24, 20.95, 20.85, 20.80 (6×CH_3CO), 16.91 (C-6^{III}); ESI-HRMS (m/z) calcd for $C_{69}H_{74}N_4O_{19}Na$ (M+Na$^+$) 1544.6107, found: 1544.6110.

4.8. 2-Azidoethyl (5-*N*-acetyl-4,7,8,9-tetra-*O*-acetyl-1-methyl-α-neuraminyl)-(2→3)-(2,4-bis-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→3)]-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (11b)

A solution of **11a** (12 mg, 0.008 mmol) and a catalytic amount of DMAP in anhydrous CH_2Cl_2 (5 mL) was slowly added 0.03 mL of acetic anhydride and 0.03 mL of Et_3N. The reaction was stirred for 24 h at room temperature under N_2. The mixture was diluted with EtOAc, washed with brine for three times, and then dried over $MgSO_4$ and concentrated. After purification by a column of silica gel (Cy–EtOAc–MeOH 10:10:1), and then by a Sephadex column (LH20) using CH_2Cl_2–MeOH (1:1) as eluant, compound **11b** was obtained (8.4 mg, 66%) as a white amorphous solid. R_f = 0.3 (Cy–EtOAc–MeOH 10:10:2); $[\alpha]_D^{20}$ −4.5 (c 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.39–7.20 (m, 25H, arom), 6.13 (d, 1H, $J_{2,NH}$ 7.3 Hz, NH-2^I), 5.60 (m, 1H, H-8^{IV}), 5.36 (dd, 1H, $J_{7,8}$ 9.0 Hz, $J_{6,7}$ 2.7 Hz, H-7^{IV}), 5.12 (d, 1H, $J_{1,2}$ 3.7 Hz, H-1^{III}), 5.08 (d, 1H, $J_{5,NH}$ 8.4 Hz, NH-5^{IV}), 5.06 (d, 1H, $J_{3,4}$ 3.4 Hz, H-4^{II}), 4.95 (dd, 1H, $J_{1,2}$ 9.2 Hz, $J_{2,3}$ 8.8 Hz, H-2^{II}), 4.95–4.88 (m, 2H, H-4^{IV}, PhCH_2), 4.80–4.71 (m, 5H, PhCH_2, PhCH_2, H-1^{II}, H-1^I), 4.66–4.55 (m, 4H, PhCH_2, PhCH_2, H-3^{II}), 4.42 (2d, 2H, J_{gem} 9.4 Hz, PhCH_2), 4.36–4.31 (m, 2H, H-9a^{IV}, PhCH_2), 4.11–4.04 (m, 4H, H-5^{III}, H-3^I, H-5^{IV}, H-2^{III}), 4.01–3.95 (m, 2H, H-9b^{IV}, H-4^{III}), 3.92–3.77 (m, 7H, H-2^I, H-6a^I, H-4^I, OCHCH$_2$N$_3$, H-6b^I, H-3^{III}, H-5^{II}), 3.85 (s, 3H, COOCH_3), 3.63 (dd, 1H, $J_{5,6}$ 10.7 Hz, $J_{6,7}$ 2.7 Hz, H-6^{IV}), 3.59–3.54 (m, 1H, OCHCH$_2$N$_3$), 3.49–3.45 (m, 2H, H-5^I, H-6a^{II}), 3.35 (dd, 1H, $J_{5,6b}$ 9.6 Hz, $J_{6a,6b}$ 7.6 Hz, H-6b^{II}), 3.24–3.10 (m, 2H, OCH$_2$CH_2N$_3$), 2.57 (dd, 1H, $J_{3e,3a}$ 12.6 Hz, $J_{3e,4}$ 4.6 Hz, H-3$_e^{IV}$), 2.21, 2.11, 2.07, 2.01, 1.96, 1.95 (6s, 18H, 6 × OAc), 1.87, 1.85 (2s, 6H, 2 × NAc), 1.72 (t, 1H, $J_{3e,3a}$ 12.6 Hz, $J_{3a,4}$ 12.5 Hz, H-3$_a^{IV}$, 1.05 (d, 3H, $J_{5,6}$ 6.5 Hz, H-6^{III}); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 171.01, 170.66, 170.52, 170.42, 170.38, 170.29, 170.05, 169.82 (8 × C═O, Ac), 167.96 (C-1^{IV}), 139.04, 138.92, 138.80, 138.59, 137.86 (arom C), 128.51, 128.48, 128.46, 128.35, 128.33, 127.97, 127.82, 127.72, 127.70, 127.60, 127.52, 127.39, 127.23 (arom CH), 99.97 (C-1^{II}), 99.21 (C-1^I), 97.12 (C-1^{III}), 97.05 (C-2^{IV}), 79.62 (C-3^{III}), 77.66 (C-5^I), 76.67 (C-2^{III}), 74.65 (PhCH_2), 74.39 (C-4^I), 73.70 (C-3^I), 73.42 (PhCH_2), 73.28 (PhCH_2), 73.18 (C-4^{III}), 73.04 (PhCH_2), 72.93 (PhCH_2), 72.16 (C-6^{IV}), 71.84 (C-5^{II}), 71.43 (C-3^{II}), 70.40 (C-2^{II}), 69.62 (C-6^I), 69.50 (C-4^{IV}), 68.04 (C-4^{II}), 67.83 (C-8^{IV}), 67.59 (C-6^{II}, OCH$_2$CH$_2$N$_3$), 67.33 (C-7^{IV}), 66.92 (C-5^{III}), 62.55 (C-9^{IV}), 53.33 (C-2^I, COOCH_3), 50.64 (OCH$_2$CH_2N$_3$), 49.26 (C-5^{IV}), 37.70 (C-3^{IV}), 23.32, 21.52, 21.05, 20.93, 20.89, 20.74 (8 × CH_3CO), 16.77 (C-6^{III}); ESI-HRMS (m/z) calcd for $C_{81}H_{99}N_5O_{29}Na$ (M+Na$^+$) 1628.6318, found 1628.6323.

4.9. 2-Azidoethyl (5-*N*-acetyl-α-neuraminyl)-(2→3)-(6-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→3)]-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (12)

A solution of compound **11a** (48 mg, 0.032 mmol) in 8 mL of NaOMe/MeOH (0.04 M) was stirred at room temperature for 2 h. Then cooled to 0 °C, 0.5 mL NaOMe/MeOH (0.4 M) and 10 drops H_2O were added. The solution was stirred at room temperature for another 1 h. The mixture was neutralized by Amberlite IR 120/H$^+$ ion exchange resin. After filtration and concentration, the

residue was purified by a column of silica gel (EtOAc–*i*-PrOH 10:4), and then by a Sephadex column (LH20) using CH_2Cl_2–MeOH (1:1) as eluant. Compound **12** was obtained (38 mg, 89%) as a white amorphous solid. R_f = 0.25 (EtOAc–*i*-PrOH 10:6); $[\alpha]_D^{20}$ −4.1 (*c* 1.0, MeOH); ^{1}H NMR (400 MHz, CD_3OD): δ 7.42–7.23 (m, 25H, arom), 5.31 (d, 1H, $J_{1,2}$ 3.7 Hz, H-1^{III}), 4.79 (q, 1H, $J_{5,6}$ 6.4 Hz, H-5^{III}), 4.54–4.50 (m, 2H, H-1^{I}, H-1^{II}), 4.87–4.35 (m, 10H, 5 × PhCH_2), 4.15–4.08 (m, 3H, H-4^{I}, H-3^{III}, H-6a^{I}), 4.04–3.90 (m, 8H, H-3^{II}, H-3^{I}, H-2^{I}, H-7^{IV}, H-2^{III}, H-6a^{II}, H-8^{IV}, H-4^{III}), 3.89–3.84 (m, 2H, H-6b^{I}, H-9a^{IV}), 3.80–3.61 (m, 9H, H-5^{IV}, H-4^{IV}, H-9b^{IV}, OCH_2CH_2N_3, H-4^{II}, H-6b^{II}, H-5^{I}, H-6^{IV}), 5.57 (t, 1H, $J_{1,2}$ $J_{2,3}$ 8.5 Hz, H-2^{II}), 3.46–3.32 (m, 3H, OCH$_2$CH_2N_3, H-5^{II}), 2.93 (d, 1H, $J_{3e,3a}$ 12.4 Hz, H-3$_e^{IV}$), 2.04, 1.97 (2s, 6H, 2×NAc), 1.80 (m, 1H, H-3$_a^{IV}$), 1.16 (d, 3H, $J_{5,6}$ 6.4 Hz, H-6^{III}); ^{13}C NMR (100.6 MHz, CD_3OD): δ 175.47, 173.21 (2 × C═O, Ac, C-1^{IV}), 140.53, 140.26, 139.86, 139.71 (arom C), 129.44, 129.33, 129.21, 129.20, 129.18, 129.12, 128.75, 128.59, 128.47, 128.40, 128.27 (arom CH), 103.28 (C-1^{II}), 102.62 (C-1^{I}), 97.64 (C-1^{III}), 79.94 (C-3^{III}), 79.84 (C-4^{III}), 78.21 (C-3^{I}), 76.97 (C-2^{III}), 76.53 (C-5^{I}), 76.35 (PhCH$_2$), 75.43 (C-3^{II}), 74.98 (C-4^{II}), 74.70 (C-4^{I}, C-5^{II}), 74.11 (2PhCH$_2$), 73.48 (PhCH$_2$), 73.09 (PhCH$_2$), 72.90 (C-7^{IV}), 71.08 (C-2^{II}), 70.16 (OCH$_2$CH$_2N_3$), 69.70 (C-6^{IV}), 69.69 (C-6^{I}), 69.19 (C-4^{IV}), 68.92 (C-6^{II}), 68.44 (C-8^{IV}), 67.62 (C-5^{III}), 64.12 (C-9^{IV}), 57.26 (C-2^{I}), 54.07 (C-5^{IV}), 51.82 (OCH$_2$CH$_2N_3$), 42.21 (C-3^{IV}), 23.42, 22.73 (2×CH_3CO), 16.93 (C-6^{III}); ESI-HRMS (*m*/*z*) calcd for $C_{68}H_{84}N_5O_{23}$ (M−H$^+$) 1338.5563, found 1338.5605.

4.10. 2-Aminoethyl (5-*N*-acetyl-α-neuraminyl)-(2→3)-(β-D-galactopyranosyl)-(1→4)-[α-L-fucopyranosyl-(1→3)]-2-acetamido-2-deoxy-β-D-glucopyranoside (1)

A solution of compound **12** (40 mg, 0.030 mmol) in a mixture of MeOH–H_2O–AcOH (6:2:1, 13.5 mL) or THF–H_2O–AcOH (4:2:1, 14 mL) was added 10% Pd/C (48 mg, 0.045 mmol), and the reaction mixture was stirred for 20 h at room temperature under an atmosphere of hydrogen gas. After completion of the reaction, the mixture was filtered, and concentrated. The residue was purified by a Sephadex column (G25) using water as eluant. After lyophilisation compound **1** was obtained (28 mg, 100%) as its acetate in form of white amorphous solid. R_f = 0.17 (EtOAc–*i*-PrOH–H_2O–AcOH 5:10:6:0.1); $[\alpha]_D^{20}$ −37.4 (*c* 0.4, H_2O); ^{1}H NMR (400 MHz, D_2O): δ 5.12 (d, 1H, $J_{1,2}$ 3.9 Hz, H-1^{III}), 4.84 (q, 1H, $J_{5,6}$ 6.6 Hz, H-5^{III}), 4.60 (d, 1H, $J_{1,2}$ 8.3 Hz, H-1^{I}), 4.54 (d, 1H, $J_{1,2}$ 7.9 Hz, H-1^{II}), 4.12–3.84 (m, 13H, H-3^{II}, H-2^{I}, H-6a^{I}, H-6b^{I}, OCH_2CH$_2$NH$_2$, H-4^{I}, H-7^{IV}, H-4^{II}, H-3^{III}, H-3^{I}, H-9a^{IV}, H-5^{IV}), 3.80 (d, 1H, $J_{3,4}$ 3.0 Hz, H-4^{III}), 3.74–3.59 (m, 9H, H-6a^{II}, H-6b^{II}, H-2^{III}, H-4^{IV}, H-9b^{IV}, H-8^{IV}, H-5^{II}, H-5^{I}, H-6^{IV}), 3.55 (dd, 1H, $J_{1,2}$ 7.9 Hz, $J_{2,3}$ 9.7 Hz, H-2^{II}), 3.25 (m, 2H, OCH$_2$CH_2NH$_2$), 2.78 (dd, 1H, $J_{3e,3a}$ 12.3 Hz, $J_{3e,4}$ 4.6 Hz, H-3$_e^{IV}$), 2.05 (s, 6H, 2 × NAc), 1.93 (s, 1.6H, *Ac*OH), 1.81 (t, 1H, $J_{3e,3a}$, $J_{3a,4}$ 12.3 Hz, H-3$_a^{IV}$), 1.19 (d, 3H, $J_{5,6}$ 6.6 Hz, H-6^{III}); ^{13}C NMR (100.6 MHz, D_2O): δ 175.03, 174.59, 173.82 (2 × C═O, Ac, C-1^{IV}), 101.61 (C-1^{II}), 100.74 (C-1^{I}), 99.65 (C-2^{IV}), 98.62 (C-1^{III}), 75.67 (C-3^{II}), 75.19 (C-5^{I}), 74.95 (C-5^{II}), 74.74 (C-3^{I}), 73.19 (C-4^{I}), 72.91 (C-8^{IV}), 71.87 (C-4^{III}, C-3^{III}), 69.22 (C-2^{II}), 69.18 (C-4^{II}), 68.25 (C-4^{IV}), 68.10 (C-6^{IV}), 67.63 (C-2^{III}), 67.31 (C-7^{IV}), 66.70 (C-5^{III}), 65.68 (OCH$_2$CH$_2$NH$_2$), 62.61 (C-9^{IV}), 61.49 (C-6^{II}), 59.53 (C-6^{I}), 55.56 (C-2^{I}), 51.69 (C-5^{IV}), 39.80 (C-3^{IV}), 39.41 (OCH$_2$CH$_2$NH$_2$), 23.26 (*C*H$_3$COOH), 22.23, 22.03 (2 × *C*H$_3$CO), 15.27 (C-6^{III}); ESI-HRMS (*m*/*z*) calcd for $C_{33}H_{56}N_3O_{23}$ (M−H$^+$) 862.3310, found 862.3294.

4.11. 2-Aminoethyl (β-D-galactopyranosyl)-(1→4)-[α-L-fucopyranosyl-(1→3)]-2-acetamido-2-deoxy-β-D-glucopyranoside (2)

A solution of compound **10** (66 mg, 0.063 mmol) in a mixture of MeOH–H_2O–AcOH (6:2:1, 18 mL) or THF–H_2O–AcOH (4:2:1, 21 mL) was added 10% Pd/C (100 mg, 0.094 mmol), and the reaction mixture was stirred for 20 h at room temperature under an atmosphere of hydrogen gas. After completion of the reaction, the mixture was filtered, and concentrated. The residue was purified by a Sephadex column (G25) using water as eluant. After lyophilisation compound **2** was obtained (38 mg, 100%) as its acetate in form of white amorphous solid. R_f = 0.18 (EtOAc–*i*-PrOH–H_2O–AcOH 5:10:6:0.1); $[\alpha]_D^{20}$ −65.8 (*c* 0.4, H_2O); ^{1}H NMR (400 MHz, D_2O): δ 5.12 (d, 1H, $J_{1,2}$ 3.8 Hz, H-1^{III}), 4.84 (q, 1H, $J_{5,6}$ 6.6 Hz, H-5^{III}), 4.60 (d, 1H, $J_{1,2}$ 8.4 Hz, H-1^{I}), 4.46 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1^{II}), 4.03–3.86 (m, 9H, H-2^{I}, H-6a^{I}, H-6b^{I}, OCH_2CH$_2$NH$_2$, H-4^{I}, H-4^{II}, H-3^{III}, H-3^{I}), 3.80 (d, 1H, $J_{3,4}$ 2.8 Hz, H-4^{III}), 3.75–3.59 (m, 6H, H-6a^{II}, H-6b^{II}, H-2^{III}, H-3^{II}, H-5^{II}, H-5^{I}), 3.50 (dd, 1H, $J_{1,2}$ 8.0 Hz, $J_{2,3}$ 8.4 Hz, H-2^{II}), 3.22 (m, 2H, OCH$_2$CH_2NH$_2$), 2.05 (s, 3H, NAc), 1.92 (s, 3H, *Ac*OH), 1.18 (d, 3H, $J_{5,6}$ 6.6 Hz, H-6^{III}); ^{13}C NMR (100.6 MHz, D_2O): δ 177.14 (C═O, Ac), 104.33 (C-1^{II}), 103.25 (C-1^{I}), 101.17 (C-1^{III}), 77.80 (C-5^{I}), 77.46 (C-5^{II}), 77.38 (C-3^{I}), 75.73 (C-4^{I}), 75.00 (C-3^{II}), 74.42 (C-4^{III}), 73.54 (C-2^{II}), 71.72 (C-3^{III}), 70.87 (C-4^{II}), 70.15 (C-2^{III}), 69.26 (C-5^{III}), 68.21 (OCH$_2$CH$_2$NH$_2$), 64.04 (C-6^{II}), 62.18 (C-6^{I}), 58.09 (C-2^{I}), 41.93 (OCH$_2$CH$_2$NH$_2$), 25.80 (*C*H$_3$COOH), 24.77 (*C*H$_3$CONH), 17.84 (C-6^{III}); ESI-HRMS (*m*/*z*) calcd for $C_{22}H_{41}N_2O_{15}$ (M+H$^+$) 573.2501, found 573.2509.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carres.2013.11.012.

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