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Synthesis of a Forssman antigen derivative for use in a conjugate vaccine

Jianhao Feng, Rachel Hevey, Chang-Chun Ling *

Alberta Ingenuity Center for Carbohydrate Science, Department of Chemistry, University of Calgary, Calgary AB, Canada T2N 1N4

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ABSTRACT

The total chemical synthesis of a Forssman antigen analog is described. The pentasaccharide contains a functionalized tether which should facilitate future conjugation with immunogenic proteins. We found that the total synthesis can be efficiently achieved by following a convergent 2+3 strategy, and using *N*-Troc protected GalNAc thioglycoside as a donor.

1. Introduction

The differential expression of carbohydrates on cell surfaces is widely recognized to be an integral component of many biological pathways and recognition processes. One such example is the glycolipid Forssman antigen, αGalNAc(1→3)βGalNAc(1→3)αGal(1→4)βGal(1→4)βGal(1→4)βGlc1-ceramide (**1**), an example of a sphingolipid which is an important class of glycolipids universally expressed in higher organisms such as humans, but typically not in lower organisms such as bacteria and yeast (Fig. 1). The Forssman antigen is a pentasaccharide member of the *globo*-series of glycosphingolipids, and very closely resembles the Globo H antigen as they share an identical Gb_4 tetrasaccharide core.

Initial studies dating back to 1911 demonstrated that expression of the Forssman antigen is species specific: the antigen is expressed in some mammals (e.g., sheep, dogs, horses, chickens, etc.) but not in others (rabbits, pigs, humans, etc.), referred to as Forssman positive species and Forssman negative species, respectively.[1] Forssman negative species instead express the tetrasaccharide precursor (Gb_4), which is a substrate for the final enzyme in the biosynthetic pathway, a β(1→3)GalNAc transferase referred to as Forssman synthetase; the human genome contains the gene for this synthetase but it is not actively expressed.[2] Together with playing an important role on the surface of Forssman positive mammalian cells as well as viral coats, interestingly the Forssman antigen is also present in several forms of human cancers (e.g., gastric, colon, lung).[3] For individuals with Forssman positive tumors, chemical and immunological detection has confirmed the absence of this antigen on their healthy tissues.[2] The identity of this pentasaccharide as a tumor-specific antigen makes it an interesting candidate for cancer therapy. An analogous target pentasaccharide which incorporated a 6-aminohexyl linker at the reducing end (**2**) was designed as a synthetic target; such a linker would facilitate conjugation with an immunogenic protein or peptide that would be capable of generating the desired T-cell dependant immune response (Fig. 1).

Although a few syntheses of the antigen were already available in the literature,[4–6] none of the established methods had integrated a linker molecule onto the oligosaccharide. In addition, we sought to improve some of the limitations of the other methods. A chemical method was chosen over an enzymatic approach in order to be less restricted by the high substrate specificity,[7–9] increased cost of glycosyl donors, and the limited number of glycosyltransferases available from commercial sources, commonly associated with enzymatic syntheses. A key consideration in the chemical synthesis involves the stereocontrol of the α- and β-linkages at the GalNAc residues. The glycan fragment of the Forssman antigen was first published by Paulsen et al. in 1980, in which glycosyl bromides and a 2+3 synthetic approach were utilized.[4] This was followed by Ogawa's group in 1989 synthesizing the glycosphingolipid in its entirety, utilizing an azide/silyl ether combination as a ceramide synthon and a glycopentaosyl fluoride donor.[5] An alternative chemical synthesis was published by Magnusson et al. in 1994, which utilized AgOTf-mediated glycosylations to afford the pertinent α-linkage at the non-reducing end.[6]

2. Results and discussion

In an attempt to develop a more efficient chemical synthesis, we envisioned using an exceptionally stable glycosyl donor (as compared to Paulsen's and Magnusson's glycosyl halides) and to

* Corresponding author.
E-mail address: ccling@ucalgary.ca (C.-C. Ling).

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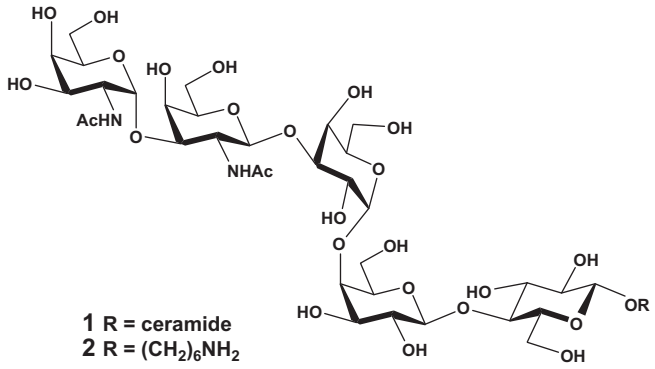

Figure 1. Structure of Forssman antigen (**1**) and the designed synthetic analog (**2**) for use in conjugate vaccines.

decrease the number of multistep functional group transformations that were present throughout Ogawa's synthesis. Two different strategies were explored, an iterative 2+1+1+1 approach and a convergent 2+3 approach (Fig. 2). The first strategy relied on a 2,3-oxazolidinone group on the sugar at the terminal non-reducing position (**4**) in order to direct the desired α-linkage, since it had been established in the literature to give the appropriate stereochemistry under particular activation conditions.[10,11] An *N*-2,2,2-trichloroethoxycarbonyl (Troc) protected 2-amino group was envisioned to direct the β-linkage via anchimeric assistance (**5**), and a general advantage of this strategy was that it used only monosaccharide donors (more easily obtained and synthesized at a lower cost), their improved availability over more complex donors allowing them to be used in excess to improve glycosylation yields. Alternatively, the second strategy utilized the same trisaccharide core (**6**) with an *N*-Troc group to direct the β-linkage, but coupled the terminal non-reducing disaccharide (**10**) together before combining it with the acceptor to offer a convergent approach. In principle, the α(1→3)GalNAc linkage found in **10** could be obtained using the protected glycosyl donor **4**; however, we finally decided to employ the more accessible 2-azido imidate donor **11**, due to potential problems that might occur during glycosylation between **4**

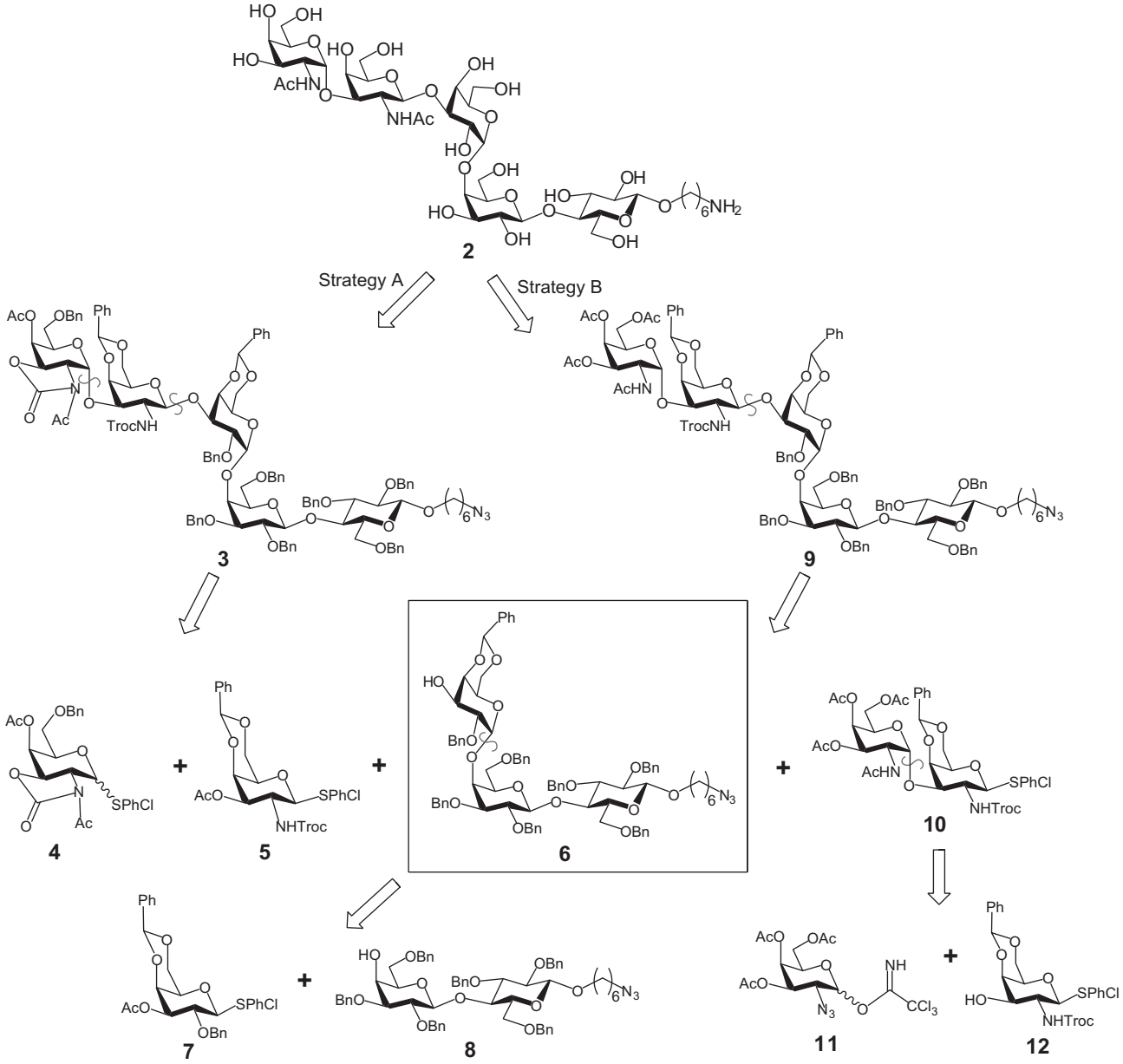

Figure 2. Retrosynthesis for target (**2**): iterative approach (strategy A) and convergent 2+3 approach (strategy B).

and **12**, as both parts contain a thioglycoside functionality, and compound **12** appeared to be more reactive than **4**—this would not provide us with the desired chemoselectivity. Our analysis concluded that retaining the *N*-Troc protecting group in disaccharide donor (**10**) is advantageous because this would render the thiodisaccharide **10** more reactive thus increasing the efficiency of the final glycosylation step. The only downside of using the 2-azido imidate **11** as a donor is that the glycosylation step would produce an anomeric mixture, which we hoped to separate by careful chromatography.

The desired oxazolidinone-protected GalNAc donor **4** was synthesized over five steps (Fig. 3) beginning from the known partially protected D-glucosamine thioglycoside **13**.[12] Removal of the phthalimido protection using 1,2-ethylenediamine-mediated conditions afforded the desired aminoalcohol **14** in 89% yield. A subsequent installation of the oxazolidinone moiety was realized using *p*-nitrophenyl chloroformate as a reagent; the amide bond formation followed by a ring closure afforded the oxazolidinone derivative **15** in 88% yield. The 2-amido position of **15** was subsequently protected via an acetylation using NaH and acetyl chloride (→**16**, 91% yield), based on a method recently published by Crich.[13] Using either Ac_2O/pyridine/4-dimethylaminopyridine or acetyl chloride/*N*,*N*-diisopropylethylamine, compound **16** consistently resulted in disappointingly low yields.[14,15] A reductive regioselective acetal opening was then performed using Et_3SiH and $BF_3{\cdot}Et_2O$ to afford the free alcohol **17**. Interestingly, the conditions used for the acetal opening concomitantly resulted in scrambling of the anomeric center; the α-anomer 17α was isolated in 54% yield while the related β-isomer was obtained in 8% yield after column chromatography. This is in agreement with similar observations made previously by several other groups while performing acid-catalyzed acetal openings on sugars protected with a 2,3-oxazolidinone moiety, which has been supported by an endo-cleavage mechanism.[9,14,16–18] The α-thioglycoside 17α was then used for triflation of the free 4-OH position, followed by an inversion using sodium acetate via a nucleophilic substitution to afford the target building block (4α).

To obtain the *N*-Troc protected thioglycoside donor **5**, we started with compound **18** (Fig. 4), conveniently prepared from *N*-acetyl-D-glucosamine using our recently published method.[19] The acyl groups in GalNAc derivative **18** were deprotected via an overnight reflux in aqueous NaOH solution; after neutralization with HCl, the mixture was concentrated and purified by column chromatography to give **19** in excellent yield (94%). Treatment of **19** with TrocCl in the presence of triethylamine provided the crude *N*-Troc protected triol intermediate **20**, which was directly converted to the 4,6-benzylidene acetal **12** in 51% yield over two steps. A final acetylation afforded the orthogonally protected building block **5** in almost quantitative yield.

The synthesis of thiogalactosyl donor **7** was achieved in five steps from the previously known *p*-chlorophenyl thioglycoside **21** (Fig. 5). To introduce a non-participating group (Bn) at O-2 position as well as an orthogonal protecting group (Ac) at O-3, the tetraol **21**[12] was first refluxed in neat 2,2-dimethoxypropane with a catalytic amount of 10-camphorsulfonic acid (CSA) to form the 3,4-acetal and a mixed acetal at the O-6 position, which was then used directly for the subsequent O-benzylation step to afford protected intermediate **22**. The isopropylidene acetals were then fully

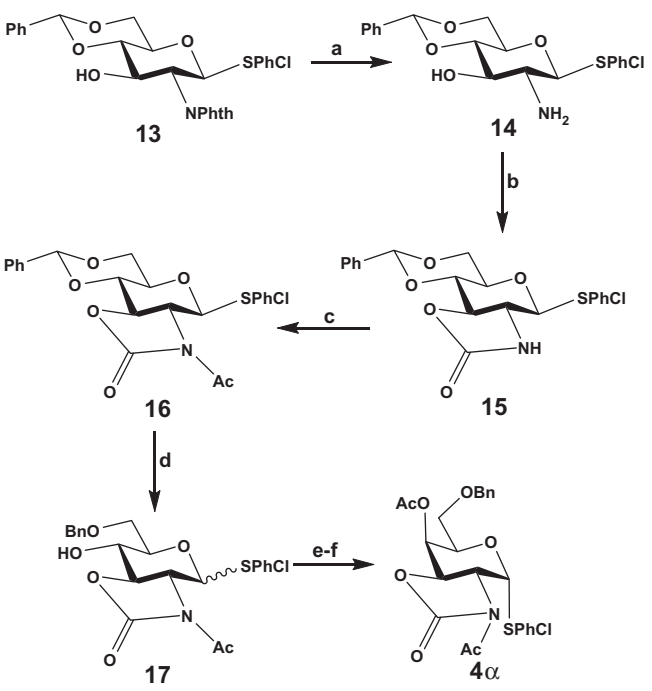

Figure 3. Synthesis of the galactosaminyl donor (**4α**) containing 2,3-oxazolidinone protection. (a) Ethylenediamine, EtOH, reflux; (b) *p*-nitrophenyl chloroformate, $NaHCO_3$, H_2O/CH_3CN; (c) AcCl, NaH, DMF, −10 °C; (d) Et_3SiH, $BF_3{\cdot}Et_2O$, DCM; (e) Tf_2O, Py/DCM; (f) NaOAc, DMF, 60 °C.

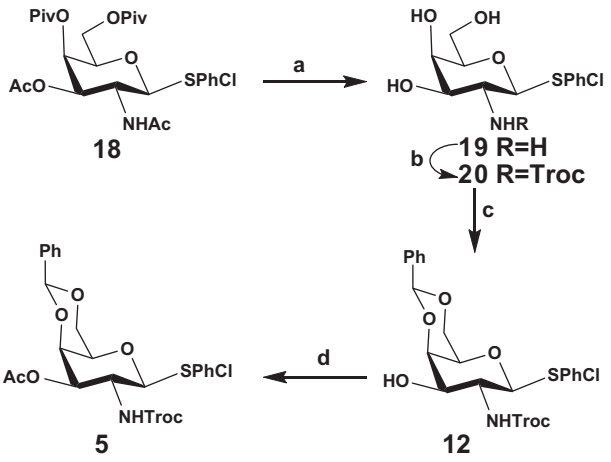

Figure 4. Synthesis of galactosaminyl donor **5**. (a) 1 M NaOH, reflux, o/n; (b) TrocCl, NEt_3, MeOH; (c) $PhCH(OMe)_2$,CSA, DMF; (d) Ac_2O, Py.

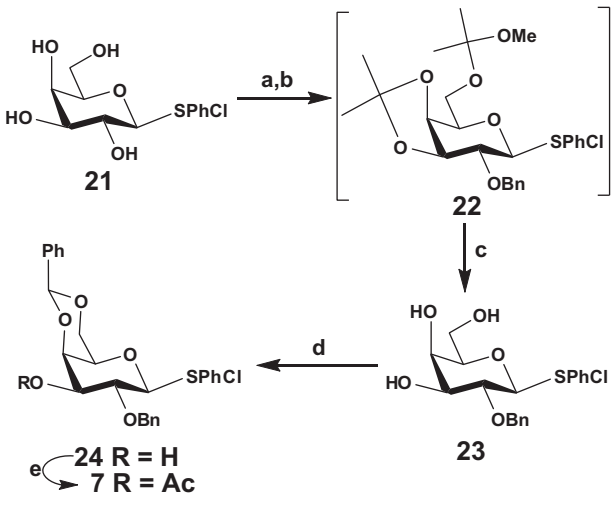

Figure 5. Synthesis of the galactosyl donor **7**. (a) 2,2-Dimethoxypropane,CSA; (b) BnBr, NaH, DMF; (c) CH_3CO_2H/H_2O; (d) $PhCH(OMe)_2$, CSA, CH_3CN; (e) Ac_2O, Py.

hydrolyzed to give the desired triol **23** in 52% yield over three steps, and the 4,6-benzylidene acetal subsequently introduced in 83% yield (→**24**). After a final acetylation step, the desired orthogonally protected building block (**7**) was obtained (95% yield).

Synthesis of the lactosyl acceptor **8** began via the glycosylation of perbenzoylated lactosyl bromide (**25**) with the 6-azido-hexan-1-ol using AgOTf promotion (Fig. 6);[20] the desired β-glycoside (**26**) was obtained in excellent yield (94%). Zemplén transesterification afforded the fully debenzoylated intermediate (**27**, 97% yield), which was then protected with a 4,6-benzylidene acetal (→**28**, 79% yield). Following a per-O-benzylation (**29**, 84% yield), a regioselective reductive opening of the acetal was achieved using Et_3SiH and $BF_3{\cdot}Et_2O$,[21] to afford the desired acceptor (**8**). When the reaction was carried out at room temperature, the majority of the start-

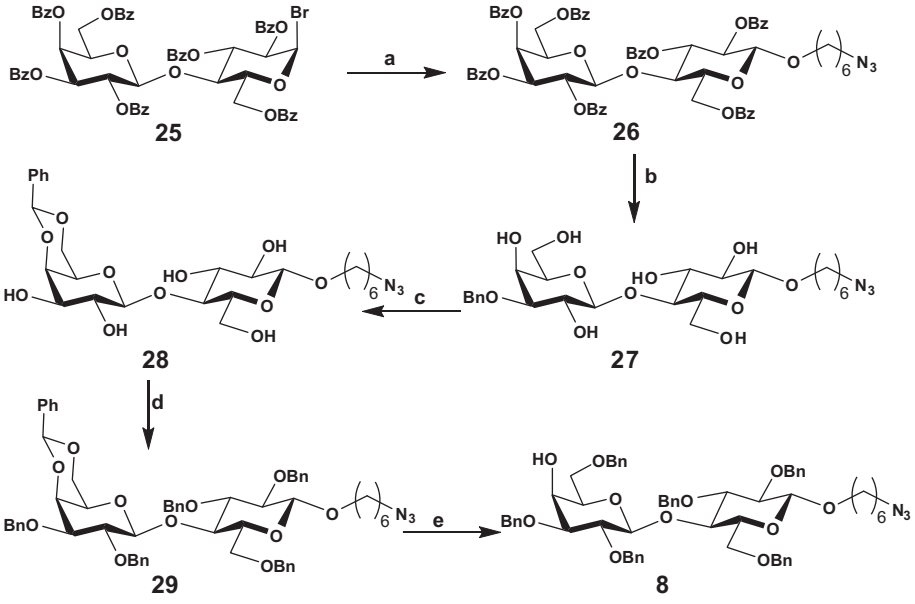

Figure 6. Synthesis of the lactosyl acceptor **8**. (a) 6-Azidohexan-1-ol, AgOTf, toluene; (b) NaOMe, MeOH; (c) $PhCH(OMe)_2$, CSA, DMF; (d) BnCl, NaH, DMF; (e) $BF_3{\cdot}Et_2O$, Et_3SiH, CH_2Cl_2.

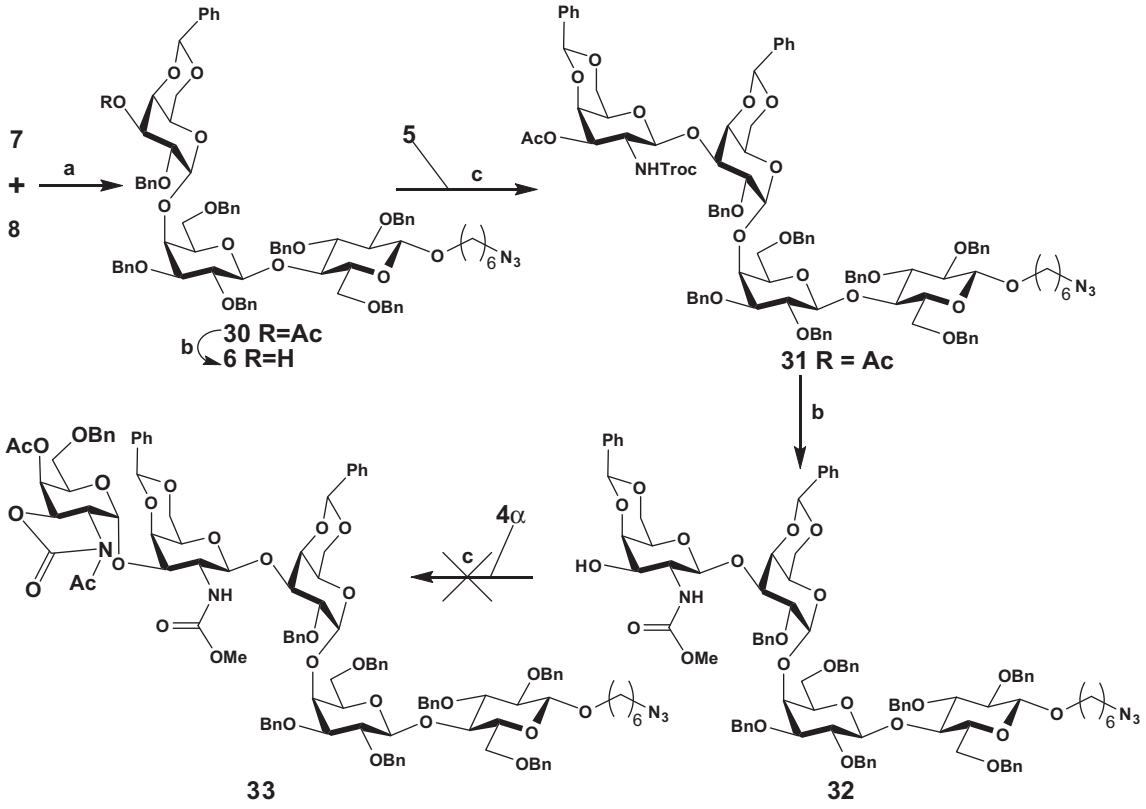

Figure 7. Attempt at assembling the pentasaccharide using the 2+1+1+1 iterative approach. (a) TfOH, NIS, CH_2Cl_2; (b) NaOMe, MeOH/CH_2Cl_2; (c) TfOH, NIS, CH_2Cl_2.

ing material was consistently found to be converted to a more polar by-product, presumably as a result of reduction of the azido group; when we kept the reaction at 0 °C overnight, the desired compound **8** was obtained as a majority and isolated in 56% yield by column chromatography on silica gel.

An initial attempt of assembling the building blocks in the iterative approach began by coupling lactosyl acceptor **8** to thiogalactosyl donor **7**; using *N*-iodosuccinimide/triflic acid (NIS/TfOH) promotion the desired trisaccharide **30** was obtained in high yield (87%) and excellent anomeric selectivity, as the α-isomer **30** was the only product isolated from the reaction (Fig. 7). Deacetylation to reveal the free OH afforded the required acceptor **6** in almost quantitative yield (97%). The coupling between the obtained alcohol **6** with building block **5** (2 equiv) proceeded with very high efficiency to produce the desired tetrasaccharide **31** in 96% yield. Unfortunately, attempts at selectively removing the OAc protecting group at the non-reducing end without affecting the Troc group proved to be more difficult than expected. For example, when using guanidine/guanidinium buffer in anhydrous methanol, the reaction was found to be sluggish;[22] however, when using more forcing conditions (catalytic NaOMe in $MeOH/CH_2Cl_2$) the reaction resulted in a complex product mixture. For the latter case, the major product was isolated in 47% yield and characterized to be compound **32**, which had the expected O-deacetylation at the non-reducing end galactosamine residue but unfortunately the *N*-Troc group had also been converted to the corresponding methyl carbamate via transesterification. Since the methyl carbamate could later be deprotected using saponification, we attempted to synthesize the pentasaccharide **33** by reacting **32** with 4α. Unfortunately multiple attempts at this final glycosylation step using NIS/TfOH activation were unsuccessful in delivering the desired product, as only decomposition products were obtained. As a result, our attention turned to the alternative convergent 2+3 approach.

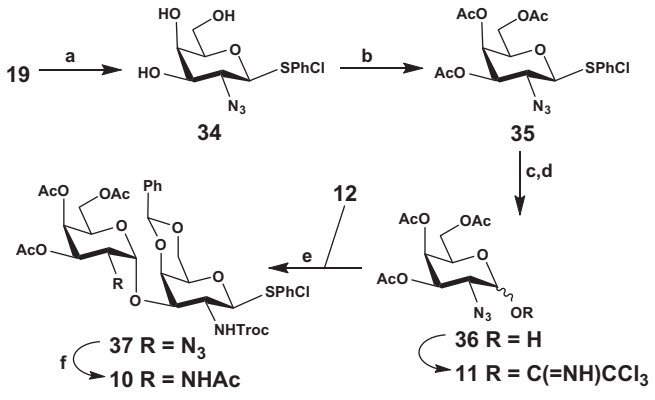

Figure 8. Synthesis of α(1→3)-linked thiodisaccharide donor **10**. (a) TfN_3, $CuSO_4$, NEt_3, Py; (b) Ac_2O, Py; (c) NIS, CH_3CN/H_2O; (d) K_2CO_3, CCl_3CN/CH_2Cl_2; (e) TMSOTf/CH_2Cl_2; (f) AcSH.

The key to succeed in the second approach was to prepare the α(1→3)-linked di-galactosamine donor **10**. The imidate building block **11** was first synthesized from thioglycoside intermediate **19** that had been previously generated during the synthesis of building block **5** (see Fig. 4). The free 2-amino group was converted to an azide using Tf_2O and NaN_3 in pyridine (**34**), based on a method introduced by C.-H. Wong's group[23] and later improved by Ye et al. (Fig. 8).[24] The crude material was then acetylated (**35**) and the anomeric thiophenyl group hydrolyzed using NIS and CH_3CN/H_2O to afford the desired glycosyl hemiacetal (**36**), which was directly converted into the imidate (**11**)[25] by reacting with trichloroacetonitrile in the presence of K_2CO_3 in anhydrous CH_2Cl_2. Coupling between this donor and acceptor **12** proceeded smoothly upon activation with trimethylsilyl triflate to afford the product as an anomeric mixture, of which the desired α-anomer (**37**) was obtained in 57% yield by column chromatography. A recent paper has suggested that the OAc protecting groups at C-3 and C-4 improve the α-selectivity of the glycosylation reaction, as computational studies suggest that transition state stabilities direct formation of the α-anomer.[26] Since the azide moiety needed to be differentiated from the azide at the linker terminus in the final product, we therefore converted it directly to the corresponding NHAc group prior to the final glycosylation step. The transformation was realized using neat thioacetic acid as a reagent based on a literature procedure,[27] and the desired disaccharide donor 10 was obtained in almost quantitative yield (98%).

With the desired disaccharide donor **10** and trisaccharide acceptor **6** in hand, the final glycosylation reaction was performed via NIS/TfOH activation (Fig. 9) using the acceptor **6** in slight excess (1.2 equiv). The glycosylation started at −78 °C and the temperature was raised to −30 °C; after 1 h, the desired pentasaccharide was isolated in excellent yield (89%). This demonstrates the high glycosylation efficiency of disaccharide donor **10**.

The identity of the final protected pentasaccharide (**9**) was confirmed by high-resolution mass spectrometry and one/two-dimensional NMR studies. For example, high resolution electrospray mass spectrometry showed a peak with *m/z* 2122.7337, which corresponds to the $[M+Na]^+$ adduct. In the HSQC spectra (Fig. 10), the C–H correlations indicated that two anomeric α-linkages were present (^1H: 4.9–5.2 ppm ^{13}C: 93–102 ppm) and three anomeric β-linkages were present (^1H: 4.3–4.6 ppm, ^{13}C: 100–105 ppm); the two benzylidene acetal C–H peaks were also observed. Two characteristic N-attached C–H bonds were present (4.55 pm/46 ppm and 4.10 ppm/53 ppm, $^1H/^{13}C$, respectively), as was the

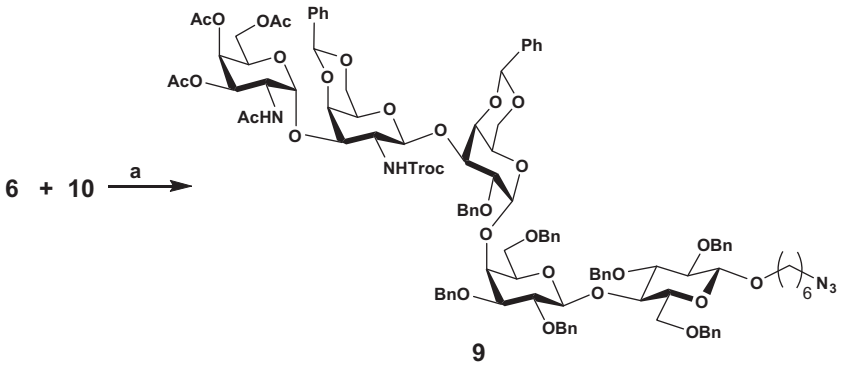

Figure 9. Synthesis of the fully protected pentasaccharide **9** via 2+3 assembly. (a) TfOH, NIS, CH_2Cl_2.

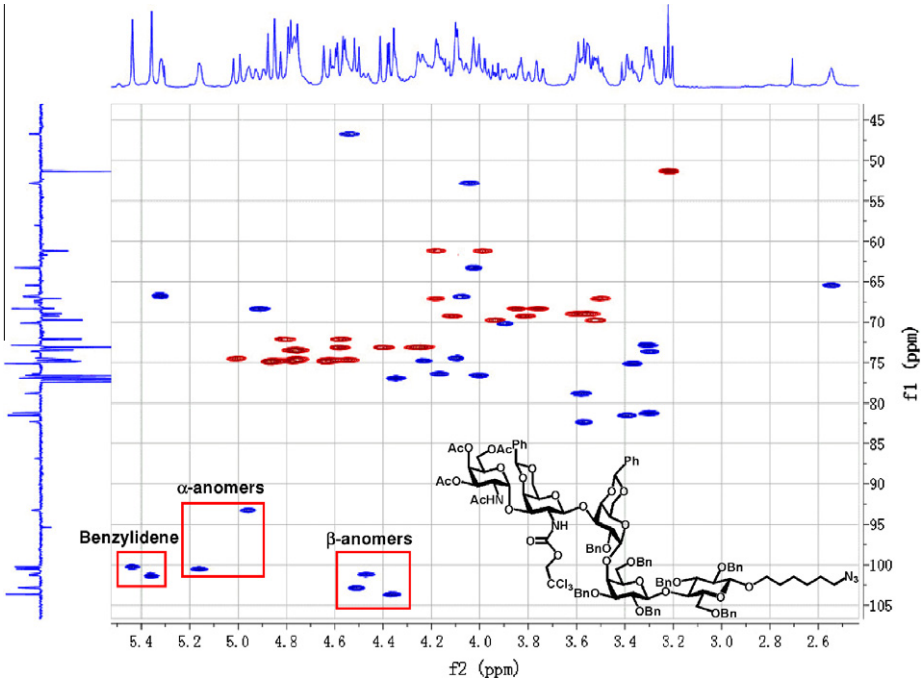

Figure 10. The ^{1}H–^{13}C HSQC spectra of fully protected pentasaccharide **9** in $CDCl_3$ (400 MHz).

distinct signal from the methylene unit attached to the azide of the linker (3.21 ppm/51 ppm, $^{1}H/^{13}C$, respectively).

Initial attempts to deprotect the fully-protected pentasaccharide began with Zemplén transesterification, which was expected to remove all the *O*-acetyl groups and concomitantly convert the Troc group to the methyl carbamate; after another base-catalyzed saponification step to remove the carbamate, the mixture was neutralized with acetic anhydride to afford the desired N-acetylated intermediate. However, the final hydrogenation disappointingly afforded a complex mixture. The multistep deprotections appeared to be complicated by the benzylidene groups which were removed by the hydrogenation at a much slower rate than the benzyl ethers. In an alternative approach (Fig. 11), an acid-catalyzed hydrolysis was first performed to remove the 4,6-benzylidene acetals, followed by a Zemplén transesterification step of the crude material and a subsequent

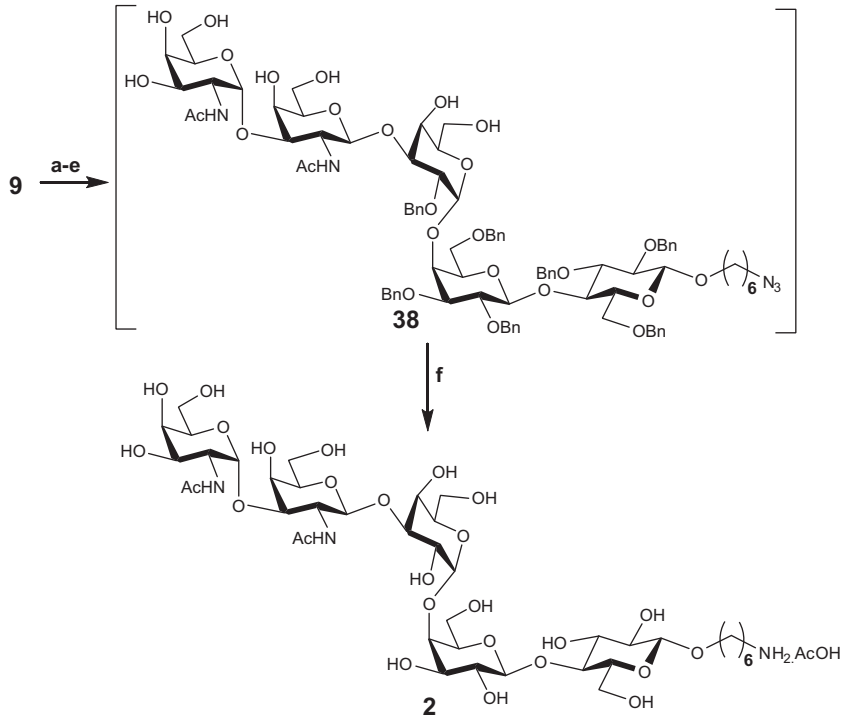

Figure 11. Deprotection of pentasaccharide **9** to obtain the desired Forssman antigen analog (**2**). (a) 80% $AcOH/H_2O$, 90 °C; (b) NaOMe, MeOH, 50 °C; (c) 2 N NaOH, reflux; (d) Ac_2O, Py; (e) NaOMe, MeOH; (f) H_2, 20% $Pd(OH)_2$-C, $MeOH/H_2O/AcOH$.

saponification of the carbamate as before; the crude mixture was then concentrated, fully acetylated, and then de-O-acetylated using NaOMe in MeOH to afford mainly the desired product (**38**). The final hydrogenation reaction was performed to afford 2 mg of the desired product **2** (11.3% yield from the fully protected pentasaccharide).

The ^{1}H–^{13}C HSQC NMR spectrum of the final compound (**2**) clearly confirmed the presence of five sets of anomeric C–H correlation peaks at (5.10 ppm, 93.4 ppm), (4.96 ppm, 102.6 ppm), (4.74 ppm, 100.3 ppm), (4.54 ppm, 103.2 ppm), and (4.51 ppm, 101.9 ppm), confirming that the obtained compound is a pentasaccharide. The anomeric protons at 5.10 and 4.96 ppm appear as doublets and have small coupling constants (<4 Hz), indicating that they both have an α-anomeric configuration; HSQC spectra suggests that they correspond to the anomeric protons of the αGalNAc and αGal, respectively. The three remaining anomeric protons also appear as doublets but with a large coupling constant (>7 Hz), confirming that they all have a β-anomeric configuration, with the anomeric proton resonating at 4.74 ppm corresponding to the βGalNAc residue, while those protons at 4.54 ppm, 4.51 ppm correspond to the βGal and βGlc residues, respectively. The structure of **2** was finally confirmed by high resolution electrospray mass spectrometry which showed a peak with *m/z* 1010.4390, which correlates with the expected $[M+H]^+$ adduct.

3. Conclusions

Since the Forssman antigen is a tumor-specific antigen closely integrated with cancer biology, it is desirable to have it readily accessible in order to incorporate it into carbohydrate conjugate-vaccines used to target different types of cancer. It could become immensely valuable if able to effectively raise an immune response against the Forssman pentasaccharide expressed on the surface of cancer cells. Although improvements need to be made to the deprotection sequence, we have successfully developed a synthetic methodology that allows for the incorporation of a linker at the reducing end of the pentasaccharide, in order to eventually conjugate it to an immunogenic protein or peptide fragment required for an effective vaccine.

4. Experimental data

4.1. General methods

Optical rotations were determined in a 5-cm cell at 25 ± 2 °C. $[\alpha]_D^{25}$ values are given in units of 10^{-1} deg cm^2 g^{-1}. Analytical TLC was performed on Silica Gel 60-F_{254} (E. Merck, Darmstadt) with detection by quenching of fluorescence and/or by charring with 5% sulfuric acid in water or with a ceric ammonium molybdate dip. All commercial reagents were used as supplied unless otherwise stated. Column chromatography was performed on Silica Gel 60 (Silicycle, Ontario). Organic solutions from extractions were dried with anhydrous Na_2SO_4 prior to concentration under vacuum at <60 °C (bath). NMR spectra were recorded at 400 MHz on Bruker spectrometers. The first-order proton chemical shifts δ_H and δ_C are reported in δ (ppm) for solutions in $CDCl_3$ and referenced to residual $CHCl_3$ (δ_H 7.27 and δ_C 77.0). ^{1}H and ^{13}C NMR spectra were assigned with the assistance of 2D GCOSY, 2D TOCSY, 2D GHSQC, 2D GHMBC, or 1D TOSCY experiments. High-resolution ESI-QTOF mass spectra were recorded on an Agilent 6520 Accurate Mass Quadrupole Time-of-Flight LC/MS spectrometer. Elemental analyses were obtained with a Perkin Elmer Series II 2400 CHNS/O Analyzer. All of the data were obtained by the analytical services of the Department of Chemistry, University of Calgary.

4.2. *p*-Chlorophenyl 2-amino-4,6-*O*-benzylidene-2-deoxy-1-thio-β-D-glucopyranoside (14)

Compound **13**[12] (8.1 g, 15.5 mmol) was dissolved in absolute ethanol (80 mL) and ethylene diamine (20.0 mL, 310 mmol) was added to the suspension. The reaction was refluxed at 95 °C overnight. HCl (2 N) was then used to neutralize the reaction to modify the pH to ~8 and the mixture was concentrated. The mixture was diluted by EtOAc, and washed by water and brine. The organic layer was dried over anhydrous Na_2SO_4 and evaporated to give the compound **14** (5.44 g, 89.3% yield). R_f: 0.13 (EtOAc/toluene 4:6). $[\alpha]_D$ +23.7 (*c* 1.5, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.56–7.28 (m, 9H, Ph), 5.55 (s, 1H, PhCH), 4.52 (d, 1H, *J* = 9.8 Hz, H-1), 4.36 (dd, 1H, *J* = 10.5, 4.4, H-6a), 3.79 (dd, 1H, *J* = 9.9, 1.3 Hz, H-6b), 3.64 (dd, 1H, *J* = 8.6, 8.6 Hz, H-3), 3.57–3.46 (m, 2H, H-4 + H-5), 2.79 (dd, 1H, *J* = 9.4, 9.4 Hz, H-2), 2.02 (s, 3H, OH + NH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 136.97 (Ph), 134.18 (Ph), 130.30 (Ph), 129.33 (Ph), 129.24 (Ph), 128.37 (Ph), 126.27 (Ph), 101.92 (PhCH), 90.00 (C-1), 80.88 (C-5), 74.79 (C-3), 70.53 (C-4), 68.58 (C-6), 56.47 (C-2). HRESIMS: Calcd for $C_{19}H_{20}NO_4Na$ ($M+Na^+$): *m/z* 416.0694. Found: 416.0679.

4.3. *p*-Chlorophenyl 4,6-*O*-benzylidene-2-deoxy-2,3-*N*,*O*-carbonyl-1-thio-β-D-glucopyranoside (15)

Compound **14** (4.28 g, 10.9 mmol) was added into a mixture of saturated $NaHCO_3$ (60 mL) and CH_3CN (60 mL) and stirred at 0 °C. *p*-Nitrophenyl chloroformate (4.6 g in 10 mL CH_3CN, 21.7 mmol) was added into the mixture. Then reaction was kept at 0 °C for 2 h and warmed to rt overnight. The mixture was then diluted by EtOAc and washed with H_2O. The organic layer was dried over anhydrous Na_2SO_4 and evaporated. The residue was purified by column chromatography on silica gel using 4% EtOAc–toluene as eluent to furnish the compound **15** (4.0 g, 87.7% yield). R_f: 0.35 (EtOAc/toluene 1:4). $[\alpha]_D$ +15.1 (*c* 1.8, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.51–7.45 (m, 4H, SPhCl), 7.42–7.33 (m, 5H, benzylidene_Ph), 5.60 (s, 1H, PhCH), 5.46–5.37 (m, 1H, NH), 4.82 (d, 1H, *J* = 9.8, H-1), 4.43–4.34 (m, 2H, H-6a + H-3), 4.06–3.98 (dd, 1H, *J* = 9.5, 8.7 Hz, H-4), 3.92 (dd, 1H, *J* = 10.4, 10.4 Hz, H-6b), 3.64 (ddd, 1H, *J* = 10.1, 8.6, 4.7, H-5), 3.46 (m, 1H, H-2). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 158.15 (oxazolidinone), 136.25 (Ph), 135.72 (Ph), 134.99 (Ph), 129.59 (Ph), 129.35 (Ph), 128.32 (Ph), 128.20 (Ph), 126.08 (Ph), 101.44 (PhCH), 85.24 (C-1), 80.53 (C-3), 78.58 (C-4), 73.26 (C-5), 68.24 (C-6), 59.55 (C-2). HREIMS: Calcd for $C_{20}H_{18}NO_5SCl$ (M^+): *m/z* 419.0594. Found: 419.0595.

4.4. *p*-Chlorophenyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-2,3-*N*,*O*-carbonyl-1-thio-β-D-glucopyranoside (16)

Compound **15** (3.0 g, 7.14 mmol) was dissolved in anhydrous DMF (20 mL) with 60% NaH (1.0 g, 41.7 mmol). Acetyl chloride (2.0 mL, 28.6 mmol) was then slowly added into the mixture at −10 °C and the reaction was stirred at rt overnight. $NaHCO_3$ was added and the mixture was extracted by CH_2Cl_2 and H_2O. The organic layer was dried over anhydrous Na_2SO_4 and evaporated. The residue was purified by column chromatography on silica gel using 1% EtOAc–CH_2Cl_2 as eluent to give **16** (3.0 g, 90.9% yield). R_f: 0.60 (EtOAc/toluene 1:4). $[\alpha]_D$ +36.9 (*c* 1.4, $CHCl_3$). ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 7.46–7.35 (m, 9H, Ph), 5.76 (s, 1H, PhCH), 5.55 (d, 1H, *J* = 8.8 Hz, H-1), 4.80 (dd, 1H, *J* = 10.5, 10.5 Hz, H-3), 4.31–4.22 (m, 2H, H-4 + H-2), 4.19 (dd, 1H, *J* = 10.0, 4.6 Hz, H-6a), 3.91 (dd, 1H, *J* = 10.2, 10.2 Hz, H-6b), 3.78 (ddd, 1H, *J* = 10.1, 8.4, 4.6 Hz, H-5), 2.44 (s, 3H, Ac). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C 173.01 (Ac), 154.20 (oxazolidinone), 137.42 (Ph), 134.37 (Ph), 131.93 (Ph), 131.32 (Ph), 129.61 (Ph), 129.42 (Ph), 128.67 (Ph), 126.67 (Ph), 100.86 (PhCH), 85.41 (C-1), 78.26 (C-4), 77.38 (C-3),

72.54 (C-5), 67.72 (C-6), 60.44 (C-2), 24.86 (Ac). HREIMS: Calcd for $C_{22}H_{20}NO_6SCl$ (M^+): m/z 461.0700. Found: 461.0719.

4.5. *p*-Chlorophenyl 2-acetamido-6-*O*-benzyl-2-deoxy-2,3-*N*,*O*-carbonyl-1-thio-α,β-D-glucopyranoside (17)

Triethylsilane (3.65 mL, 22.8 mmol) and $BF_3{\cdot}Et_2O$ (359 μL, 2.85 mmol) were added to a suspension of **16** (880 mg, 1.9 mmol) in anhydrous CH_2Cl_2 (30 mL) at 0 °C under argon. The reaction was stirred at 0 °C for 2 h and then diluted by CH_2Cl_2 and washed with H_2O. The organic layer was dried over anhydrous Na_2SO_4 and evaporated. The residue was purified by column chromatography on silica gel using 9% EtOAc–toluene as eluent to produce **17α** (481 mg, 54.4% yield) and **17β** (67 mg, 8.3% yield).

4.5.1. *p*-Chlorophenyl 2-acetamido-6-*O*-benzyl-2-deoxy-2,3-*N*,*O*-carbonyl-1-thio-α-D-glucopyranoside (17α)

R_f: 0.35 (EtOAc/toluene 1:4). $[\alpha]_D$ +204.1 (*c* 1.1, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ_H 7.43–7.39 (m, 2H, SPhCl), 7.38–7.29 (m, 5H, Bn), 7.28–7.22 (m, 2H, SPhCl), 6.12 (d, 1H, *J* = 4.4 Hz, H-1), 4.63 (d, 1H, *J* = 11.9 Hz, Bn_CH_2), 4.55 (d, 1H, *J* = 11.9 Hz, Bn_CH_2), 4.36 (dd, 1H, *J* = 12.1, 9.5 Hz, H-3), 4.16 (ddd, 1H, *J* = 8.4, 4.1, 4.1 Hz, H-5), 4.10 (dd, 1H, *J* = 9.3, 3.3 Hz, H-4), 4.04 (dd, 1H, *J* = 12.2, 4.5 Hz, H-2), 3.86 (dd, 1H, *J* = 10.5, 4.4 Hz, H-6a), 3.77 (dd, 1H, *J* = 10.5, 3.9 Hz, H-6b), 3.46 (d, 1H, *J* = 3.3 Hz, OH-4), 2.53 (s, 3H, Ac). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 171.28 (Ac), 153.02 (oxazolidinone), 137.38 (Ph), 134.57 (Ph), 133.96 (Ph), 130.92 (Ph), 129.36 (Ph), 128.57 (Ph), 128.07 (Ph), 127.78 (Ph), 86.34 (C-1), 78.30 (C-3), 73.80 (Bn_CH_2), 72.89 (C-5), 69.83 (C-4), 69.13 (C-6), 59.57 (C-2), 23.79 (Ac). HRESIMS: Calcd for $C_{22}H_{22}NO_6SClNa$ ($M+Na^+$): m/z 486.0748. Found: 486.0756. Anal. Calcd for $C_{22}H_{22}NO_6SCl$: C, 56.96; H, 4.78; N, 3.02. Found: C, 57.10; H, 4.94; N, 3.00.

4.5.2. *p*-Chlorophenyl 2-acetamido-6-*O*-benzyl-2-deoxy-2,3-*N*,*O*-carbonyl-1-thio-β-D-glucopyranoside (17β)

R_f: 0.27 (EtOAc/toluene 1:4). $[\alpha]_D$ −62.5 (*c* 2.1, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ_H 7.45–7.41 (m, 2H, SPhCl), 7.40–7.29 (m, 5H, Bn), 7.22–7.17 (m, 2H, SPhCl), 4.83 (d, 1H, *J* = 8.4 Hz, H-1), 4.60–4.52 (m, 2H, Bn_CH_2), 4.19–3.99 (m, 3H, H-3 + H-4 + H-2), 3.82–3.73 (m, 2H, H-6a + H-6b), 3.55 (ddd, 1H, *J* = 8.1, 5.0, 5.0 Hz, H-5), 3.20 (d, 1H, *J* = 2.0 Hz, OH-4), 2.57 (s, 3H, Ac). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 173.10 (Ac), 153.75 (oxazolidinone), 137.25 (Ph), 134.27 (Ph), 133.83 (Ph), 132.24 (Ph), 129.00 (Ph), 128.60 (Ph), 128.12 (Ph), 127.85 (Ph), 87.00 (C-1), 81.64 (C-3), 79.53 (C-5), 73.85 (Bn_CH_2), 69.79 (C-6), 69.77 (C-4), 59.57 (C-2), 24.69 (Ac). HREIMS: Calcd for $C_{22}H_{22}NO_6SCl$ (M^+): m/z 463.0856. Found: 463.0853. Anal. Calcd for $C_{22}H_{22}NO_6SCl$: C, 56.96; H, 4.78; N, 3.02. Found: C, 57.00; H, 4.89; N, 2.95.

4.6. *p*-Chlorophenyl 2-acetamido-4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2,3-*N*,*O*-carbonyl-1-thio-α-D-glucopyranoside (4α)

Compound **17α** (122 mg, 0.26 mmol) was dissolved in a mixture of anhydrous CH_2Cl_2 (0.6 mL) and anhydrous pyridine (0.6 mL). At −10 °C, trifluoromethanesulfonic anhydride (132.7 μL, 0.78 mmol) was added and the reaction was stirred at 0 °C for 3.5 h. The solution was diluted by CH_2Cl_2 and washed with H_2O. The organic layer was dried over anhydrous Na_2SO_4 and evaporated below 40 °C to afford the crude triflate. This residue was then dissolved in anhydrous DMF (1 mL) and NaOAc (215.7 mg, 2.6 mmol) was added. The reaction was stirred at 60 °C overnight. The mixture was diluted by CH_2Cl_2 and washed by H_2O. The organic layer was dried over anhydrous Na_2SO_4 and evaporated. The residue was purified by column chromatography on silica gel using 10% acetone–hexane as eluent to give **4α** (94 mg, 70.7% yield). R_f: 0.50 (EtOAc/toluene 1:4). $[\alpha]_D$ +168.5 (*c* 2.1, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ_H 7.45–7.40 (m, 2H, SPhCl), 7.35 (m, 5H, Bn), 7.24–7.19 (m, 2H, SPhCl), 6.17 (d, 1H, *J* = 3.8 Hz, H-1), 5.72 (dd, 1H, *J* = 1.2, <1 Hz, H-4), 4.61–4.41 (m, 5H, H-3 + H-2 + H-5 + Bn_CH_2), 3.68–3.49 (m, 2H, H-6a + H-6b), 2.54 (s, 3H, Ac), 2.08 (s, 3H, Ac). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 171.43 (Ac), 169.18 (Ac), 152.45 (oxazolidinone), 137.43 (Ph), 134.76 (Ph), 134.36 (Ph), 130.35 (Ph), 129.36 (Ph), 128.48 (Ph), 127.95 (Ph), 127.84 (Ph), 87.07 (C-1), 74.32 (C-5), 73.65 (Bn_CH_2), 70.12 (C-3), 67.99 (C-6), 65.85 (C-4), 56.03 (C-2), 23.87 (Ac), 20.55 (Ac). HRCIMS: Calcd for $C_{24}H_{28}N_2O_7SCl$ ($M+NH_4^+$): m/z 523.1306. Found: 523.1330. Anal. Calcd for $C_{24}H_{24}NO_7SCl$: C, 56.97; H, 4.78; N, 2.77. Found: C, 57.41; H, 4.78; N, 2.70.

4.7. *p*-Chlorophenyl 2-amino-2-deoxy-1-thio-β-D-galactopyranoside (19)

Compound **18** (10.4 g, 18.6 mmol) was stirred in aq NaOH (500 mL, 1 N) and the suspension was refluxed overnight. HCl (2 N) was added to modify the pH to 8. Then the solvent was evaporated and MeOH was added to wash the residue. The precipitate was filtered and purified by flash column chromatography on silica gel using CH_2Cl_2/MeOH/H_2O 80:20:2 as eluent to give the compound **19** (5.35 g, 94.1% yield). R_f: 0.30 (MeOH/CH_2Cl_2 2:8). $[\alpha]_D$ −43.6 (*c* 5.2, MeOH). ^1H NMR (400 MHz, CD_3OD) δ_H 7.60–7.55 (m, 2H, Ph), 7.34–7.29 (m, 2H, Ph), 4.58 (d, 1H, *J* = 10.0 Hz, H-1), 3.85 (dd, 1H, *J* = 3.1, <1 Hz, H-4), 3.79 (dd, 1H, *J* = 11.5, 6.9 Hz, H-6a), 3.72 (dd, 1H, *J* = 11.5, 5.1 Hz, H-6b), 3.59 (ddd, 1H, *J* = 6.7, 5.1, 1.0 Hz, H-5), 3.47 (dd, 1H, *J* = 9.8, 3.2 Hz, H-3), 3.04 (dd, 1H, *J* = 9.9, 9.9 Hz, H-2). ^{13}C NMR (100 MHz, CD_3OD) δ_C 133.25 (Ph), 132.72 (Ph), 132.23 (Ph), 128.65 (Ph), 88.25 (C-1), 79.45 (C-5), 73.98 (C-3), 68.11 (C-4), 61.36 (C-6), 51.75 (C-2). HRESIMS: Calcd for $C_{12}H_{17}NO_4SCl$ ($M+H^+$): m/z 306.0561. Found: 306.0556.

4.8. *p*-Chlorophenyl 2-deoxy-2-*N*-(2,2,2-trichloroethoxycarbonylamino)-1-thio-β-D-galactopyranoside (20)

Compound **19** (700 mg, 2.29 mmol) was dissolved in a mixture of anhydrous MeOH (6 mL) and Et_3N (274.4 μL, 2.98 mmol). 2,2,2-Trichloroethoxycarbonyl chloride (461.8 μL, 3.44 mmol) was added and stirred at 0 °C and the reaction was stirred at rt overnight. The solution was concentrated and H_2O was then added and precipitate formed. The solid was collected by the vacuum filtration and a following evaporation with toluene gave the compound **20**. The crude product was used for next step without further purification. R_f = 0.63 (MeOH/CH_2Cl_2 1.5:8.5).

4.9. *p*-Chlorophenyl 4,6-*O*-benzylidene-2-deoxy-2-*N*-(2,2,2-trichloroethoxycarbonylamino)-1-thio-β-D-galactopyranoside (12)

$PhCH(OMe)_2$ (436.6 μL, 2.91 mmol) and 10-camporsulfonic acid were added to the solution of **20** (850 mg, crude) in anhydrous DMF (3 mL). The reaction was stirred at rt overnight and Et_3N was then added to the mixture. The solvent was evaporated and the residue was purified by column chromatography on silica gel using 35% EtOAc–toluene as eluent to afford the compound **12** (662.1 mg, 50.8% yield over two steps). R_f: 0.67 (MeOH/CH_2Cl_2 5:95). $[\alpha]_D$ −48.5 (*c* 0.84, acetone). ^1H NMR (400 MHz, acetone-d_6) δ_H 7.70–7.57 (m, 2H, SPh), 7.56–7.44 (m, 2H, benzylidene), 7.44–7.30 (m, 3H, benzylidene), 7.30–7.19 (m, 2H, SPh), 6.94 (d, 1H, *J* = 8.8 Hz, NH), 5.66 (s, 1H, PhCH), 4.94 (d, 1H, *J* = 9.7 Hz, H-1), 4.86 (d, 1H, *J* = 12.3 Hz, Troc_CH_2), 4.72 (d, 1H, *J* = 12.3 Hz, Troc_CH_2), 4.34 (dd, 1H, *J* = 2.3, <1 Hz, H-4), 4.22 (dd, 1H, *J* = 12.3, 1.6 Hz, H-6a), 4.17–4.11 (m, 2H, OH-3 + H-6b), 3.99–3.82 (m, 2H, H-3 + H-2), 3.79–3.69 (m, 1H, H-5). ^{13}C NMR (100 MHz, acetone-d_6) δ_C 154.51 (CO), 138.79 (Ph), 133.81 (Ph), 132.74 (Ph), 132.33

(Ph), 128.63 (Ph), 128.59 (Ph), 127.83 (Ph), 126.53 (Ph), 100.56 (PhCH), 96.15 (CCl_3), 85.78 (C-1), 75.65 (C-4), 73.93 (CH_2_Troc), 71.42 (C-3), 70.06 (C-5), 69.01 (C-6), 53.16 (C-2). HREIMS: Calcd for $C_{16}H_{17}NO_6Cl_3$ (M−SC_6H_4Cl): *m*/*z* 426.0092. Found: 426.0072.

4.10. *p*-Chlorophenyl 3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-2-*N*-(2,2,2-trichloroethoxycarbonylamino)-1-thio-β-D-galactopyranoside (5)

Compound **12** (50 mg, 0.088 mmol) was dissolved in a mixture of pyridine (1.3 mL) and Ac_2O (1.0 mL). The reaction was stirred at rt overnight. The mixture was evaporated to dry to give the compound **5** as a white solid (53 mg, 99% yield). R_f: 0.84 (MeOH/CH_2Cl_2 3:97). $[\alpha]_D$ −34.5 (*c* 0.91, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.58–7.67 (m, 2H, SPhCl), 7.35–7.45 (m, 5H, benzylidene), 7.15–7.24 (m, 2H, SPhCl), 5.52 (s, 1H, benzylidene_CH), 5.29 (d, 1H, *J* = 3.4, 11.3 Hz, H-3), 4.95–5.07 (m, 2H, H-1 + NH), 4.76 (s, 1H, Troc_CH_2), 4.33–4.42 (m, 2H, H-6a + H-4), 4.05 (dd, 1H, *J* = 0.8, 12.1 Hz, H-6b), 3.88 (m, 1H, H-2), 3.64 (m, 1H, H-5), 2.05 (s, 3H, Ac). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 170.68 (Ac), 137.48 (Ph), 135.46 (Ph), 129.25 (Ph), 129.06 (Ph), 128.23 (Ph), 126.33 (Ph), 100.83 (PhCH), 96.52 (CCl_3), 84.56 (C-1), 74.37 (Troc_CH_2), 73.15 (C-4), 71.32 (C-3), 69.74 (C-5), 69.19 (C-6), 50.30 (C-2), 20.85 (Ac). HRCIMS: Calcd for $C_{24}H_{27}N_2O_7SCl_4$ (M+NH_4^+): *m*/*z* 629.0264. Found: 629.0247.

4.11. *p*-Chlorophenyl 2-*O*-benzyl-1-thio-β-D-galactopyranoside (23)

Compound **21**[12] (5.4 g, 17.6 mmol) was dissolved in 2,2-dimethoxypropane (30 mL). After adding 10-camphorsulfonic acid (300 mg), the reaction was refluxed overnight. Et_3N was then added and the solvent was evaporated. The crude was dissolved in anhydrous DMF (30 mL) containing NaH (60%, 2.1 g, 52.5 mmol). At 0 °C, benzyl bromide (2.7 mL, 22.7 mmol) was added and the reaction was then warmed up to rt. After 1 h, MeOH was added dropwise and the mixture was extracted with EtOAc–H_2O. The organic layer was dried over anhydrous Na_2SO_4 and to solvent was evaporated to obtain crude **22** (6.9 g, crude). R_f = 0.53 (EtOAc/toluene 1:4). The residue was used for next step without any further purification.

The obtained crude **22** was dissolved in 70% AcOH–H_2O (70 mL) and refluxed for 1 h. The solvent was evaporated and the residue was washed by CH_2Cl_2. After filtration, the filtrate was concentrated and the residue was purified by column chromatography on silica gel using 4% MeOH–CH_2Cl_2 as eluent to give the compound **23** (3.62 g, 51.9% yield over three steps). R_f = 0.32 (MeOH/CH_2Cl_2 5:95). $[\alpha]_D$ +18.4 (*c* 1.3, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.53–7.48 (m, 2H, SPh), 7.41–7.32 (m, 5H, Bn), 7.32–7.28 (m, 2H, SPh), 4.95 (d, 1H, *J* = 11.1 Hz, PhCH_2), 4.68 (d, 1H, *J* = 11.1 Hz, PhCH_2), 4.62 (d, 1H, *J* = 9.0 Hz, H-1), 4.05 (dd, 1H, *J* = 2.9, <1 Hz, H-4), 3.97 (dd, 1H, *J* = 11.9, 5.9 Hz, H-6a), 3.86 (dd, 1H, *J* = 11.9, 4.2 Hz, H-6b), 3.67 (dd, 1H, *J* = 8.8, 3.1 Hz, H-3), 3.63(dd, 1H, *J* = 8.9, 8.9 Hz, H-2), 3.54 (m, 1H, H-5). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 137.82 (Ph), 133.80 (Ph), 132.98 (Ph), 132.05 (Ph), 129.15 (Ph), 128.67 (Ph), 128.21 (Ph), 87.37 (C-1), 77.88 (C-5), 77.82 (C-2), 75.35 (PhCH_2), 74.81 (C-3), 69.78 (C-4), 62.99 (C-6). HRCIMS: Calcd for $C_{19}H_{25}NO_5SCl$ (M+NH_4^+): *m*/*z* 414.1142. Found: 414.1126. Anal. Calcd for $C_{19}H_{21}O_5SCl$: C, 57.50; H, 5.33; N, 0.00. Found: C, 57.54; H, 5.15; N, −0.04.

4.12. *p*-Chlorophenyl 2-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (24)

Compound **23** (3.42 g, 8.62 mmol) was stirred in anhydrous CH_3CN (50 mL). Benzaldehyde dimethyl acetal (2.6 mL, 17.3 mmol) and 10-camphorsulfonic acid were added. The reaction was stirred for 30 min and Et_3N was then added. The solvent was evaporated and the residue was purified by column chromatography on silica gel using 3% MeOH–CH_2Cl_2 as eluent to yield the compound **24** (3.50 g, 82.0% yield). R_f = 0.23 (EtOAc/Hexane 3:7). $[\alpha]_D$ −6.22 (*c* 0.91, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.67–7.63 (m, 2H, SPh), 7.52–7.28 (m, 10H, benzylidene + Bn), 7.23–7.18 (m, 2H, SPh), 5.57 (s, 1H, Ph*CH*), 4.76 (d, 1H, *J* = 10.7 Hz, PhCH_2), 4.72 (d, 1H, *J* = 10.7 Hz, PhCH_2), 4.59 (d, 1H, *J* = 9.4 Hz, H-1), 4.38 (dd, 1H, *J* = 12.3, 1.4 Hz, H-6a), 4.22 (dd, 1H, *J* = 3.5, <1 Hz, H-4), 4.04 (dd, 1H, *J* = 12.4, 1.4 Hz, H-6b), 3.81 (dd, 1H, *J* = 9.0, 3.6 Hz, H-3), 3.61 (dd, 1H, *J* = 9.2, 9.2 Hz, H-2), 3.53–3.50 (m, 1H, H-5). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 138.13 (Ph), 137.51 (Ph), 134.29 (Ph), 133.91 (Ph), 130.96 (Ph), 129.39 (Ph), 129.01 (Ph), 128.41 (Ph), 128.29 (Ph), 128.04 (Ph), 127.86 (Ph), 126.41 (Ph), 101.26 (PhCH), 85.83 (C-1), 77.00 (C-2), 75.64 (C-4), 75.28 (PhCH_2), 74.37 (C-3), 69.82 (C-5), 69.19 (C-6). HREIMS: Calcd for $C_{20}H_{21}O_5$ (M−$SC_6H_5Cl^+$): *m*/*z* 341.1389. Found: 341.1393.

4.13. *p*-Chlorophenyl 3-*O*-acetyl-2-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (7)

To a solution of **24** (3.50 g, 7.22 mmol) in pyridine (30 mL), acetic anhydride (20 mL) was added dropwise. The solution was stirred at rt for 3 h and then the solvent was evaporated. The residue was purified by column chromatography on silica gel using 25% EtOAc–hexane as eluent to give the compound **7** (3.62 g, 95.3% yield). R_f = 0.38 (EtOAc/Hexane 3:7). $[\alpha]_D$ −5.83 (*c* 0.96, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.68–7.63 (m, 2H, SPh), 7.52–7.28 (m, 10H, benzylidene + Bn), 7.18–7.12 (m, 2H, SPh), 5.52 (s, 1H, PhCH), 4.98 (dd, 1H, *J* = 9.7, 3.3 Hz, H-3), 4.76 (d, 1H, *J* = 10.9 Hz, PhCH_2), 4.68 (d, 1H, *J* = 9.5 Hz, H-1), 4.54 (d, 1H, *J* = 10.9 Hz, PhCH_2), 4.43–4.34 (m, 2H, H_4 + H-6a), 4.04 (dd, 1H, *J* = 12.4, 1.5 Hz, H-6b), 3.90 (dd, 1H, *J* = 9.6, 9.6 Hz, H-2), 3.61–3.57 (m, 1H, H-5), 2.02 (s, 3H, Ac). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 170.50 (Ac), 138.03 (Ph), 137.66 (Ph), 134.26 (Ph), 133.95 (Ph), 130.71 (Ph), 129.18 (Ph), 129.06 (Ph), 128.36 (Ph), 128.20 (Ph), 127.77 (Ph), 127.63 (Ph), 126.33 (Ph), 100.82 (PhCH), 86.10 (C-1), 75.48 (C-3), 75.30 (PhCH_2), 73.77 (C-2), 73.72 (C-4), 69.51 (C-5), 69.13 (C-6), 20.96 (CH_3). HRCIMS: Calcd for $C_{28}H_{31}NO_6SCl$ (M+NH_4^+): *m*/*z* 544.1561. Found: 544.1570. Anal. Calcd for $C_{28}H_{27}O_6SCl$: C, 63.81; H, 5.16; N, 0.00. Found: C, 63.42; H, 5.03; N, −0.06.

4.14. 6-Azidohexyl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (26)

Perbenzoylated lactosyl bromide **25**[28] (20.0 g, 17.6 mmol) and 6-azidohexan-1-ol (4 mL, 26.5 mmol) were dissolved in anhydrous toluene (35 mL). After adding molecular sieves (3.5 g), the mixture was stirred for 20 min. At −78 °C, AgOTf (7.0 g, 29.9 mL) was added into the mixture and the reaction was kept in darkness, and then warmed up to rt. After 4 h, Et_3N was added and the mixture was filtrated. The solution was then extracted with EtOAc/$NH_3{\cdot}H_2O$. The organic layer was dried over anhydrous Na_2SO_4 and the solvent was evaporated. The residue was purified by column chromatography on silica gel using 3% EtOAc–toluene as eluent to give **26** as white solid (19.7 g, 93.6% yield). R_f: 0.84 (EtOAc/toluene 1:4). $[\alpha]_D$ +25.7 (*c* 1.2, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.07–7.95 (m, 10H, Bz), 7.95–7.89 (m, 2H, Bz), 7.77–7.72 (m, 2H, Bz), 7.67–7.54 (m, 3H, Bz), 7.54–7.46 (m, 5H, Bz), 7.46–7.36 (m, 6H, Bz), 7.36–7.29 (m, 3H, Bz), 7.26–7.13 (m, 4H, Bz), 5.82 (dd, 1H, *J* = 9.7, 9.7 Hz, Glc_H-3), 5.78–5.71 (m, 2H, Gal_H-2 + Gal_H-4), 5.47 (dd, 1H, *J* = 9.9, 8.0 Hz, Glc_H-2), 5.39 (dd, 1H, *J* = 10.3, 3.4 Hz, Gal_H-3), 4.90 (d, 1H, *J* = 7.9 Hz, Gal_H-1), 4.69 (d, 1H, *J* = 7.9 Hz, Glc_H-1), 4.62 (dd, 1H, *J* = 12.1, 1.7 Hz, Glc_H-6a), 4.50 (dd, 1H, *J* = 12.1, 4.4 Hz,

Glc_H-6b), 4.28 (dd, 1H, J = 9.5 Hz, Glc_H-4), 3.91 (ddd, 1H, J = 6.8, 6.8, <1 Hz, Gal_H-5), 3.88–3.82 (m, 2H, Glc_H-5 + OCH_{2a}), 3.80–3.67 (m, 2H, Gal_H-6a + Gal_H-6b), 3.45 (ddd, 1H, J = 13.1, 7.1, 5.9 Hz, OCH_{2b}), 3.04 (t, 2H, J = 6.9 Hz, CH_2N_3), 1.57–1.39 (m, 2H, chain), 1.36–1.26 (m, 2H, chain), 1.24–1.10 (m, 4H, $CH_2 \times 2$_chain). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 165.86 (Bz), 165.59 (Bz), 165.42 (Bz × 2), 165.24 (Bz), 165.14 (Bz), 164.81 (Bz), 133.55 (Ph), 133.41 (Ph), 133.38 (Ph), 133.27 (Ph), 133.23 (Ph), 133.18 (Ph), 130.01 (Ph), 129.76 (Ph), 129.70 (Ph), 129.67 (Ph), 129.60 (Ph), 129.46 (Ph), 129.44 (Ph), 128.89 (Ph), 128.72 (Ph), 128.65 (Ph), 128.59 (Ph), 128.57 (Ph), 128.53 (Ph), 128.40 (Ph), 128.26 (Ph), 101.22 (Glc_ C-1), 101.00 (Gal_ C-1), 76.10 (Glc_ C-4), 73.03 (Glc_ C-5), 72.90 (Glc_ C-3), 71.82 (Glc_ C-2 + Gal_ C-3), 71.43 (Gal_ C-5), 70.01 (OCH_2), 69.93 (Gal_ C-4), 67.56 (Gal_ C-2), 62.44 (Glc_ C-6), 61.11 (Gal_ C-6), 51.16 (CH_2N_3), 29.18 (chain), 28.57 (chain), 26.22 (chain), 25.34 (chain). HRESIMS: Calcd for $C_{67}H_{61}N_3O_{18}K$ (M+K$^+$): m/z 1234.3587. Found: 1234.3593. Anal. Calcd for $C_{67}H_{61}N_3O_{18}$: C, 67.27; H, 5.14; N, 3.51. Found: C, 66.99; H, 5.33; N, 3.49.

4.15. 6-Azidohexyl β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (27)

A solution of **26** (19.7 g, 16.5 mmol) in anhydrous MeOH (200 mL) was refluxed at 60 °C in the presence of NaOMe (2.0 mL, 1.5 M in MeOH) for 3 h. Amberlite IR-120 resin (H$^+$) was then added into the solution to neutralize the base. The mixture was filtrated and the solvent was evaporated. The solid was washed with acetone and filtrated to produce **27** as white solid (7.43 g, 96.5% yield). R_f: 0.71 (MeOH/CH_2Cl_2 1:4). $[\alpha]_D$ −4.2 (c 1.0, MeOH). ^{1}H NMR (400 MHz, CD_3OD) δ_H 4.37 (d, 1H, J = 7.8 Hz, Gal_ H-1), 4.28 (d, 1H, J = 7.8 Hz, Glc_ H-1), 3.95–3.85 (m, 2H, OCH_2a + Glc_H-6a), 3.85–3.67 (m, 4H, Glc_H-6b + Gal_ H-4 + Gal_H-6a + Gal_H-6b), 3.63–3.47 (m, 6H, Gal_ H-5 + Gal_ H-2 + OCH_2b + Gal_ H-3 + Glc_ H-3 + Glc_ H-4), 3.40 (ddd, 1H, J = 9.2, 3.9, 2.6 Hz, Glc_ H-5), 3.29 (t, 2H, J = 6.9 Hz, CH_2N_3), 3.25 (dd, 1H, J = 8.0, 7.3 Hz, Glc_ H-2), 1.70–1.36 (m, 8H, chain). ^{13}C NMR (100 MHz, D_2O) δ_C 102.93 (Gal_C-1), 102.02 (Glc_C-1), 78.43 (Gal_C-2), 75.35 (Gal_C-5), 74.75 (Glc_C-3), 74.46 (Glc_C-5), 72.85 (Glc_C-4), 72.53 (Glc_C-2), 70.96 (Gal_C-3), 70.54 (OCH_2), 68.55 (Gal_C-4), 61.02 (Gal_C-6), 60.12 (Glc_C-6), 51.14 (CH_2N_3), 28.58 (chain), 27.87 (chain), 25.65 (chain), 24.60 (chain). Anal. Calcd for $C_{18}H_{33}N_3O_{11}$: C, 46.25; H, 7.12; N, 8.99. Found: C, 46.02; H, 7.11; N, 8.81.

4.16. 6-Azidohexyl 4,6-*O*-benzylidene-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (28)

To a solution of **27** (3.0 g, 6.42 mmol) in anhydrous DMF (20 mL), $PhCH(OMe)_2$ (3 mL, 19.3 mmol) and 10-camphorsulfonic acid (550 mg, 2.37 mmol) were added. After stirring for 1 h at rt, Et_3N was used to neutralize the acid. The solvent was evaporated and the residue was purified by column chromatography on silica gel using 5% MeOH–CH_2Cl_2 as eluent to yield the compound **28** (2.8 g, 78.6% yield). R_f: 0.36 (MeOH/CH_2Cl_2 1:9). $[\alpha]_D$ −27.1 (c 1.0, MeOH). ^{1}H NMR (400 MHz, CD_3OD) δ_H 7.58–7.30 (m, 5H, Ph), 5.63 (s, 1H, PhCH), 4.49 (d, 1H, J = 7.3 Hz, Gal_H-1), 4.30 (d, 1H, J = 7.9 Hz, Glc_H-1), 4.25–4.18 (m, 2H, Gal_H-4 + Gal_H-6a), 4.16 (dd, 1H, J = 1.5, 12.5 Hz, Gal_H-6b), 3.97–3.86 (m, 3H, Glc_H-6a + Glc_H-6b + OCH_2a), 3.73–3.52 (m, 6H, Gal_H-2 + Gal_H-3 + Gal_H-5 + Glc_H-3 + Glc_H-4 + OCH_2b), 3.41 (ddd, 1H, J = 2.7, 2.7, 9.3 Hz, Glc_H-5), 3.30–3.23 (m, 2H, CH_2N_3 + Glc_H-2), 1.70–1.55 (m, 4H, chain), 1.50–1.36 (m, 4H, chain). ^{13}C NMR (100 MHz, CD_3OD) δ_C 138.15 (Ph), 128.49 (Ph), 127.63 (Ph), 126.10 (Ph), 103.43 (Gal_C-1), 102.87 (Glc_C-1), 100.88 (PhCH), 78.66 (Glc_C-3), 75.95 (Gal_C-4), 75.04 (Glc_C-5), 74.94 (Glc_C-4), 73.43 (Glc_C-2), 72.10 (Gal_C-3), 70.36 (Gal_C-2), 69.36 (OCH_2), 68.79 (Gal_C-6), 66.92 (Gal_C-5), 60.35 (Glc_C-6), 51.00 (CH_2N_3), 29.22 (chain), 28.45 (chain), 26.21 (chain), 25.23 (chain). Anal. Calcd for $C_{25}H_{37}N_3O_{11}\cdot 1/4H_2O$: C, 53.61; H, 6.75; N, 7.50. Found: C, 54.11; H, 6.70; N, 7.01.

4.17. 6-Azidohexyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (29)

Compound **28** (1.0 g, 1.8 mmol) was dissolved in anhydrous DMF (6 mL), and NaH (480 mg, 10.8 mmol) was added into the solution. At 0 °C, BnCl (2.1 mL, 18.0 mmol) was added into the mixture. After stirring overnight at rt for 3.5 h, MeOH was added to quench the reaction. The mixture was diluted by EtOAc, and washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4 and evaporated. The residue was purified by column chromatography on silica gel using 5% EtOAc–toluene as eluent to furnish the compound **29** (1.5 g, 83.5% yield). R_f: 0.59 (EtOAc/toluene 0.5:9.5). $[\alpha]_D$ +14.6 (c 0.6, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.17–7.58 (m, 30H, PhCH × 5 + Bn × 25), 5.49 (s, 1H, PhC*H*), 5.21 (d, 1H, J = 10.7 Hz, PhCH_2), 4.93 (d, 1H, J = 11.0 Hz, PhCH_2), 4.87 (d, 1H, J = 11.2 Hz, PhCH_2), 4.73–4.84 (m, 5H, PhCH_2 × 5), 4.58 (d, 1H, J = 12.1 Hz, PhCH_2), 4.49 (d, 1H, J = 7.9 Hz, Gal_H-1), 4.41 (d, 1H, J = 7.9 Hz, Glc_H-1), 4.36 (d, 1H, J = 12.1 Hz, PhCH_2), 4.23 (dd, 1H, J = 1.0, 12.4 Hz, Gal_H-6a), 4.05 (dd, 1H, J = <1, 3.4 Hz, Gal_H-4), 3.83–4.03 (m, 4H, Glc_H-4 + OCH_2a + Glc_H-6a + Gal_H-6b), 3.72–3.82 (m, 2H, Gal_H-2 + Glc_H-6b), 3.66 (dd, 1H, J = 9.1, 9.1 Hz, Glc_H-3), 3.55 (ddd, 1H, J = 6.7, 9.5, 13.5 Hz, OCH_2b), 3.36–3.49 (m, 3H, Glc_H-2 + Gal_H-3 + Glc_H-5), 3.23 (t, 1H, J = 6.9 Hz, CH_2N_3), 2.96 (s, 1H, Gal_H-5), 1.35–1.74 (m, 8H, CH_2 × 8). ^{13}C NMR (100 MHz, CDCl3) δ_C 139.05 (Ph), 138.69 (Ph), 138.68 (Ph), 138.37 (Ph), 138.28 (Ph), 138.21 (Ph), 129.11 (Ph), 128.37 (Ph), 128.31 (Ph), 128.28 (Ph), 128.23 (Ph), 128.13 (Ph), 128.03 (Ph), 127.91 (Ph), 127.86 (Ph), 127.77 (Ph), 127.58 (Ph), 127.54 (Ph), 127.51 (Ph), 127.42 (Ph), 127.28 (Ph), 126.27 (Ph), 103.65 (Glc_C-1), 103.24 (PhCH), 101.79 (Gal_C-1), 82.96 (Glc_C-2), 81.87 (Gal_C-5), 80.27 (Gal_C-4), 77.95 (Gal_C-3), 76.71 (Gal_C-2), 75.33 (PhCH$_2$), 75.07 (Glc_C-5), 74.89 (PhCH$_2$), 73.80 (Glc_C-4), 73.41 (PhCH$_2$), 73.38 (PhCH$_2$), 73.23 (PhCH$_2$), 72.22 (Glc_C-3), 69.73 (OCH_2), 69.03(Gal_C-6), 68.28 (Glc_C-6), 51.36 (CH_2N_3), 29.61 (chain), 28.77 (chain), 26.52 (chain), 25.74 (chain). HRESIMS: Calcd for $C_{60}H_{67}N_3O_{11}Na$ (M+Na$^+$): m/z 1028.4668. Found: 1028.4677.

4.18. 6-Azidohexyl 2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (8)

Compound **29** (1.24 g, 1.23 mmol) was dissolved in anhydrous CH_2Cl_2 (50 mL). At 0 °C, Et_3SiH (2.0 mL, 12.3 mmol) was added and then $BF_3\cdot Et_2O$ (232.4 μL, 1.85 mmol) was added dropwise into the mixture. The reaction was stirred overnight keeping the temperature at 0 °C overnight. Et_3N was then added to quench the reaction. The solvent was evaporated and the residue was purified by column chromatography on silica gel using 15% EtOAc–hexane as eluent to give the compound **8** as a colorless gel (690 mg, 55.6% yield). R_f: 0.67 (EtOAc/toluene 1:3). $[\alpha]_D$ +13.3 (c 3.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.42–7.19 (m, 30H, Bn × 30), 4.99 (d, 1H, J = 10.9 Hz, PhCH_2), 4.89 (d, 1H, J = 11.0 Hz, PhCH_2), 4.80–4.70 (m, 5H, PhCH_2 × 5), 4.67 (d, 1H, J = 11.7 Hz, PhCH_2), 4.56 (d, 1H, J = 12.1 Hz, PhCH_2), 4.46 (d, 1H, J = 12.0 Hz, PhCH_2), 4.44 (d, 1H, J = 7.8 Hz, Gal_H-1), 4.43–4.39 (m, 2H, PhCH_2 × 2), 4.38 (d, 1H, J = 7.9 Hz, Glc_H-1), 4.03 (dd, 1H, J = 2.5, 2.5 Hz, Gal_H-4), 4.00–3.90 (m, 2H, Glc_H-3 + OCH_2a), 3.81 (dd, 1H, J = 4.5, 11.0 Hz, Gal_H-6a), 3.73 (dd, 1H, J = 1.7, 10.9 Hz, Gal_H-6b), 3.67 (dd, 1H, J = 7.2, 9.7 Hz, Glc_H-6a), 3.63–3.46 (m, 4H, Gal_H-2 + Glc_H-4 + OCH_2b + Glc_H-6b), 3.44–3.36 (m, 3H, Glc_H-2 + Gal_H-3 + Gal_H-5), 3.33 (m, 1H, Glc_H-5), 3.23 (t, 1H, J = 7.0 Hz,

CH_2N_3), 2.40 (d, 1H, J = 2.1 Hz, Gal_OH), 1.70–1.35 (m, 8H, $CH_2 \times 8$). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 139.17 (Ph), 138.75 (Ph), 138.67 (Ph), 138.38 (Ph), 138.23 (Ph), 137.96 (Ph), 128.47 (Ph), 128.37 (Ph), 128.27 (Ph), 128.07 (Ph), 128.06 (Ph), 127.90 (Ph), 127.86 (Ph), 127.80 (Ph), 127.77 (Ph), 127.66 (Ph), 127.61 (Ph), 127.53 (Ph), 127.47 (Ph), 127.23 (Ph), 103.63 (Glc_C-1), 102.56 (Gal_C-1), 82.93 (Glc_C-4), 81.84 (Glc_C-2), 81.16 (Gal_C-5), 79.43 (Gal_C-2), 76.67 (Glc_C-3), 75.34 ($PhCH_2$), 75.25 ($PhCH_2$), 75.15 (Gal_C-3), 74.89 ($PhCH_2$), 73.52 ($PhCH_2$), 73.14 ($PhCH_2$), 72.80 (Glc_C-5), 72.03 ($PhCH_2$), 69.73 (OCH_2), 68.46 (Glc_C-6), 68.36 (Gal_C-6), 66.17 (Gal_C-4), 51.37 (CH_2N_3), 29.62 (chain), 28.79 (chain), 26.54 (chain), 25.75 (chain). HRESIMS: Calcd for $C_{60}H_{69}N_3O_{11}Na$ (M+Na^+): m/z 1030.4824. Found: 1030.4866. Anal. Calcd for $C_{60}H_{69}N_3O_{11}$: C, 71.48; H, 6.90; N, 4.17. Found: C, 71.24; H, 6.79; N, 4.64.

4.19. 6-Azidohexyl 3-*O*-acetyl-2-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (30)

Compound **7** (235.2 mg, 0.45 mmol) and **8** (300 mg, 0.30 mmol) were dissolved in anhydrous CH_2Cl_2 (3 mL) containing molecular sieves (200 mg). The mixture was stirred at rt for 15 min and then cooled down to −78 °C. N-Iodosuccinimide (133.9 mg, 0.60 mmol) and trifluoromethanesulfonic acid was added and the reaction was slowly warmed up to rt. Et_3N was then added and the residue was purified by column chromatography on silica gel using 15% EtOAc–hexane as eluent to yield the compound **30** (362.5 mg, 86.9% yield). R_f: 0.34 (EtOAc/toluene 1:9, developed twice). $[\alpha]_D$ +60.0 (c 0.99, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ_H 7.45–7.14 (m, 40H, Ph), 5.33 (s, 1H, PhCH), 5.28 (dd, 1H, J = 10.7, 3.4 Hz, αGal_H-3), 5.18 (d, 1H, J = 3.3 Hz, αGal_H-1), 5.04 (d, 1H, J = 11.8 Hz, $PhCH_2$), 4.94–4.80 (m, 3H, $PhCH_2$), 4.80–4.67 (m, 3H, $PhCH_2$), 4.65–4.52 (m, 4H, $PhCH_2$), 4.48 (d, 1H, J = 7.6 Hz, βGal_H-1), 4.42–4.34 (m, 3H, $PhCH_2$ + αϖ Gal_H-4 + Glc_H-1), 4.34–4.22 (m, 2H, $PhCH_2$), 4.19–4.06 (m, 4H, αGal_H-2 + αGal_H-5 + βGal_H-6a + βGal_H-4), 3.99–3.88 (m, 2H, Glc_H-3 + OCH_2), 3.83 (dd, 1H, J = 10.8, 4.3 Hz, Glc_H-6a), 3.75 (dd, 1H, J = 9.3, 1.7 Hz, Glc_H-6b), 3.66 (dd, 1H, J = 9.9, 7.7 Hz, βGal_H-2), 3.60 (dd, 1H, J = 9.0, 9.0 Hz, Glc_H-4), 3.56–3.42 (m, 4H, OCH_2 + αGal_H-6a + βGal_H-6b + αGal_H-6b), 3.42–3.26 (m, 4H, Glc_H-2 + βGal_H-5 + Glc_H-5 + βGal_H-3), 3.21 (t, 2H, J = 6.9 Hz, CH_2N_3), 1.71–1.50 (m, 4H, chain_CH_2), 1.46–1.32 (m, 4H, chain_CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 170.55 (Ac), 139.46 (Ph), 138.76 (Ph), 138.46 (Ph), 138.20 (Ph), 137.92 (Ph), 128.77 (Ph), 128.41 (Ph), 128.38 (Ph), 128.26 (Ph), 128.21 (Ph), 128.18 (Ph), 128.14 (Ph), 128.00 (Ph), 127.98 (Ph), 127.94 (Ph), 127.86 (Ph), 127.62 (Ph), 127.59 (Ph), 127.55 (Ph), 127.53 (Ph), 127.50 (Ph), 127.40 (Ph), 127.05 (Ph), 126.89 (Ph), 126.16 (Ph), 103.55 (Glc_C-1), 103.01 (βGal_C-1), 100.46 (αGal_C-1), 100.39 (PhCH), 82.56 (Glc_C-4), 81.71 (βGal_C-5), 81.19 (βGal_C-3), 78.74 (βGal_C-2), 77.43 (Glc_C-3), 75.08 ($PhCH_2$), 75.03 (Glc_C-2), 74.98 ($PhCH_2$), 74.83 ($PhCH_2$), 74.21 (αGal_C-4), 74.05 (αGal_C-2), 73.73 ($PhCH_2$), 73.66 (αGal_C-5) , 73.09 ($PhCH_2$), 73.04 ($PhCH_2$), 72.80 (Glc_C-5), 72.16 ($PhCH_2$), 71.73 (αGal_C-3), 69.70 (OCH_2), 68.97 (αGal_C-6), 68.39 (Glc_C-6), 67.10 (βGal_C-6), 62.36 (βGal_C-4), 51.34 (CH_2N_3), 29.58 (chain), 28.75 (chain), 26.50 (chain), 25.72 (chain), 21.04 (Ac). HRESIMS: Calcd for $C_{82}H_{91}N_3O_{17}Na$ (M+Na^+): m/z 1412.6241. Found: 1412.6221.

4.20. 6-Azidohexyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (6)

NaOMe (100 μL, 1.5 M in MeOH) was added to a solution of **30** (340 mg, 0.24 mmol) in a mixture of CH_2Cl_2 (1 mL) and MeOH (3 mL). The reaction was stirred at rt for 2 h. The mixture was then neutralized by Amberlite IR-120 resin (H^+). After filtration, the solvent was evaporated. The residue was purified by column chromatography on silica gel using 20% EtOAc–hexane as eluent to afford the compound **6** as a colorless gel. (318.2 mg, 96.5% yield). R_f: 0.56 (EtOAc/toluene 1:4). $[\alpha]_D$ +48.2 (c 0.86, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 7.48–7.21 (m, 40H, Ph), 5.42 (s, 1H, PhCH), 5.23 (d, 1H, J = 3.2 Hz, αGal_H-1), 5.11 (d, 1H, J = 11.7 Hz, Bn), 4.95–4.88 (m, 2H, Bn), 4.87–4.70 (m, 6H, Bn), 4.67 (d, 1H, J = 11.5 Hz, Bn), 4.61 (d, 1H, J = 12.1 Hz, Bn), 4.54 (d, 1H, J = 7.6 Hz, βGal_H-1), 4.47–4.40 (m, 2H, Bn + Glc_H-1), 4.36 (d, 1H, J = 11.8 Hz, Bn), 4.30 (d, 1H, J = 11.8 Hz, Bn), 4.22 (dd, 1H, J = 9.5, <1 Hz, αGal_H-3), 4.18–4.09 (m, 4H, βGal_H-4 + αGal_H-4 + βGal_H-6a + βGal_H-5), 4.06–3.93 (m, 2H, Glc_H-3 + OCH_2), 3.92–3.85 (m, 2H, αGal_H-2 + Glc_H-6a), 3.80 (dd, 1H, J = 10.7, 1.5 Hz, Glc_H-6b), 3.70–3.52 (m, 5H, Glc_H-4 + βGal_H-2 + αGal_H-6a + αGal_H-6b + OCH_2), 3.51–3.42 (m, 3H, βGal_H-6b + Glc_H-5 + Glc_H-2), 3.40–3.32 (m, 2H, αGal_H-5 + βGal_H-3), 3.25 (t, 2H, J = 6.9 Hz, N_3CH_2), 2.18 (s, br, 1H, OH), 1.75–1.65 (m, 2H, chain), 1.65–1.55 (m, 2H, chain), 1.50–1.38 (m, 4H, chain). HRESIMS: Calcd for $C_{80}H_{89}N_3O_{16}Na$ (M+Na^+): m/z 1370.6135. Found: 1370.6115.

4.21. 6-Azidohexyl 3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-2-*N*-(2,2,2-trichloroethoxycarbonylamino)-β-D-galactopyranosyl-(1→3)-2-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (31)

Compound **5** (235.7 mg, 0.38 mmol) and **6** (260 mg, 0.19 mmol) were dissolved in anhydrous CH_2Cl_2 (3 mL) containing molecular sieves (120 mg). The mixture was stirred at rt for 30 min. At −78 °C, N-iodosuccinimide (108.4 mg, 0.38 mmol) and trifluoromethanesulfonic acid were added into the mixture. The reaction was slowly warmed up to −20 °C for 1 h. Et_3N was then added to quench the reaction. The mixture was purified by column chromatography on silica gel using 22% EtOAc–hexane as eluent to furnish the compound 31 (330 mg, 95.8% yield). R_f: 0.52 (EtOAc/toluene 1:4). $[\alpha]_D$ +47.8 (c 4.0, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ_H 7.56–7.14 (m, 45H, Ph), 5.44 (s, 1H, PhCH), 5.42 (s, 1H, PhCH), 5.21 (d, 1H, J <1 Hz, αGal_H-1), 5.06 (d, 1H, J = 11.1 Hz, Bn), 4.93–4.77 (m, 5H, Bn), 4.77–4.64 (m, 3H, Bn), 4.64–4.56 (m, 2H, Bn), 4.56–4.49 (m, 2H, βGal_H-1 + Bn), 4.49–4.40 (m, 2H, Bn + Troc), 4.40–4.34 (m, 3H, αGal_H-4 + Glc_H-1 + GalN_H-1), 4.34–4.11 (m, 8H, Bn + Troc + βGal_H-6a + αGal_H-3 + αGal_H-2 + GalN_H-6a), 4.11–4.01 (m, 3H, αGal_H-5 + Glc_H-3 + βGal_H-4), 4.01–3.91 (m, 2H, GalN_H-4 + OCH_2), 3.91–3.73 (m, 3H, Glc_H-6a + GalN_H-6b + Glc_H-6b), 3.68–3.49 (m, 6H, βGal_H-2 + αGal_H-6a + αGal_H-6b + Glc_H-4 + βGal_H-6b + OCH_2), 3.42–3.29 (m, 4H, Glc_H-2 + Glc_H-5 + βGal_H-3 + βGal_H-5), 3.23 (t, 2H, J = 6.9 Hz, N_3CH_2), 2.67–2.56 (m, 1H, GalN_H-5), 2.02 (s, 3H, Ac), 1.71–1.52 (m, 4H, chain), 1.50–1.34 (m, 4H, chain). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.69, 153.87, 139.28, 138.62, 138.43, 138.34, 138.30, 138.13, 138.05, 138.03, 137.61, 129.51, 128.93, 128.64, 128.60, 128.55, 128.40, 128.38, 128.27, 128.22, 128.11, 127.96, 127.90, 127.86, 127.80, 127.69, 127.65, 127.61, 127.57, 127.50, 127.47, 127.15, 126.36, 126.28, 126.21, 103.60, 102.63, 101.71, 100.74, 100.51, 100.24, 95.58, 81.89, 81.53, 81.16, 78.85, 76.85, 76.36, 75.06, 74.87, 74.80, 74.65, 74.47, 74.06, 73.46, 73.06, 72.77, 72.66, 72.01, 71.13, 69.69, 68.99, 68.27, 67.06, 65.58, 63.24, 51.91, 51.31, 50.71, 47.94, 29.88, 29.56, 28.72, 26.47, 25.69, 20.80. HRESIMS: Calcd for $C_{98}H_{107}N_4O_{23}Cl_3Na$ (M+Na^+): m/z 1837.6292. Found: 1837.6323.

4.22. 6-Azidohexyl 4,6-*O*-benzylidene-2-deoxy-2-*N*-methoxycarbonylamino-β-D-galactopyranosyl-(1→3)-2-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (32)

Compound **31** (240 mg, 0.13 mmol) was dissolved in a mixture of CH_2Cl_2 (0.7 mL) and MeOH (2.0 mL). NaOMe (100 μL, 1.5 M in MeOH) was added into the mixture. Then the reaction was stirred at rt for 2 h. The mixture was then neutralized by Amberlite IR-120 resin (H^+). After filtration, the solution was concentrated. The residue was purified by column chromatography on silica gel using 40% EtOAc–hexane as eluent to give compound **32** (102 mg, 46.5% yield). R_f: 0.17 (EtOAc/toluene 1:3). $[\alpha]_D$ +43.1 (*c* 1.8, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ_H 7.52–7.15 (m, 45H), 5.45 (s, 1H), 5.44 (s, 1H), 5.23 (d, 1H, *J* <1), 5.01 (d, 1H, *J* = 10.9 Hz), 4.89–4.72 (m, 6H), 4.72–4.63 (m, 2H), 4.63–4.55 (m, 2H), 4.51 (d, 1H, *J* = 7.6 Hz), 4.41 (d, 1H, *J* = 12.1 Hz), 4.38–4.19 (m, 6H), 4.17–4.08 (m, 4H), 4.06–3.98 (m, 2H), 3.94 (ddd, 1H, *J* = 9.5, 9.5, 6.3 Hz), 3.89–3.79 (m, 3H), 3.78–3.67 (m, 2H), 3.66–3.49 (m, 6H), 3.43–3.28 (m, 4H), 3.23 (t, 2H, *J* = 6.9 Hz,), 3.09 (s, 1H), 2.61–2.54 (m, 1H), 1.70–1.52 (m, 4H), 1.47–1.34 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 157.96, 139.15, 138.67, 138.44, 138.33, 138.13, 138.10, 138.07, 137.62, 129.39, 129.06, 128.52, 128.50, 128.44, 128.41, 128.26, 128.18, 127.93, 127.88, 127.80, 127.74, 127.69, 127.66, 127.61, 127.58, 127.53, 127.51, 127.48, 127.22, 126.36, 126.32, 103.64, 102.74, 102.05, 101.12, 100.42, 100.38, 82.03, 81.62, 81.21, 78.84, 76.42, 75.11, 74.93, 74.81, 74.71, 74.69, 74.62, 73.18, 73.11, 72.80, 72.07, 69.73, 69.11, 69.03, 68.31, 67.13, 66.01, 63.33, 55.00, 52.20, 51.35, 29.59, 28.76, 26.51, 25.73. HRESIMS: Calcd for $C_{95}H_{106}N_4O_{22}Na$ ($M+Na^+$): *m*/*z* 1677.7191. Found: 1677.7157.

4.23. *p*-Chlorophenyl 2-azido-2-deoxy-1-thio-β-D-galactopyranoside (34)

Sodium azide (306 mg, 4.71 mmol) was suspended in anhydrous pyridine (3 mL). At 0 °C, trifluoromethanesulfonic anhydride (659 μL, 3.92 mmol) was added dropwise. After 2 h, at 0 °C, the solution was carefully added into an anhydrous pyridine solution (2.5 mL) containing **19** (1.0 g, 3.27 mmol), Et_3N (1.14 mL. 8.18 mmol) and $CuSO_4$ (5.2 mg, 0.03 mmol). The reaction was then warmed up to rt and stirred for 5 h. The solution was concentrated to produce crude **34** and the residue used for the next step without further purification. R_f = 0.48 ($MeOH/CH_2Cl_2$ 8:92). ^1H NMR (400 MHz, CD_3OD) δ_H 7.60–7.54 (m, 2H, Ph), 7.36–7.30 (m, 2H, Ph), 4.52 (d, 1H, *J* = 9.8 Hz, H-1), 3.86 (dd, 1H, *J* = 2.7, <1 Hz, H-4), 3.78 (dd, 1H, *J* = 11.5, 6.9 Hz, H-6a), 3.71 (dd, 1H, *J* = 11.5, 5.1 Hz, H-6b), 3.57–3.47 (m, 3H, H-5 + H-3 + H-2). ^{13}C NMR (100 MHz, CD_3OD) δ_C 133.41 (Ph), 133.24 (Ph), 131.77 (Ph), 128.61 (Ph), 86.30 (H-1), 79.36 (H-5), 73.80 (H-3), 68.30 (H-4), 62.96 (H-2), 61.24 (H-6). HRESIMS: Calcd for $C_{12}H_{14}N_3O_4SClNa$ ($M+Na^+$): *m*/*z* 354.0286. Found: 354.0284.

4.24. *p*-Chlorophenyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-1-thio-β-D-galactopyranoside (35)

Acetic anhydride (5 mL) was added to a solution of **34** (~1.2 g, crude) in pyridine (7 mL) and the reaction was stirred at rt overnight. The solution was then evaporated and the residue was purified by column chromatography on silica gel using 15% EtOAc–hexane as eluent to give the compound **35** (1.23 g, 82.3% yield over two steps). R_f = 0.75 (EtOAc/Hexane 4:6). $[\alpha]_D$ +13.5 (*c* 1.2, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ_H 7.60–7.54 (m, 2H, Ph), 7.37–7.32 (m, 2H, Ph), 5.37 (dd, 1H, *J* = 3.2, <1 Hz, H-4), 4.88 (dd, 1H, *J* = 10.2, 3.2 Hz, H-3), 4.50 (d, 1H, *J* = 10.1 Hz, H-1), 4.18 (dd, 1H, *J* = 11.4, 6.9 Hz, H-6a), 4.11 (dd, 1H, *J* = 11.3, 6.2 Hz, H-6b), 3.94–3.86 (m, 1H, H-5), 3.63 (dd, 1H, *J* = 10.2, 10.2 Hz, H-2), 2.11 (s, 3H, Ac), 2.06 (s, 3H, Ac), 2.05 (s, 3H, Ac). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 170.32 (Ac), 169.84 (Ac), 169.66 (Ac), 135.10 (Ph), 135.04 (Ph), 129.34 (Ph), 129.16 (Ph), 86.25 (C-1), 74.52 (C-5), 72.89 (C-3), 66.49 (C-4), 61.53 (C-6), 59.29 (C-2), 20.64 (CH_3), 20.57 (CH_3), 20.53 (CH_3). HRESIMS: Calcd for $C_{18}H_{20}N_3O_7SClNa$ ($M+Na^+$): *m*/*z* 480.0603. Found: 480.0597.

4.25. 3,4,6-Tri-*O*-acetyl-2-azido-2-deoxy-α,β-D-galactopyranosyl 2,2,2-trichloroacetimidate (11)

Thioglycoside **35** (745 mg, 1.62 mmol) was dissolved in a mixture of CH_3CN (5 mL)–H_2O (0.6 mL) and treated with *N*-iodosuccinimide (1.46 g, 6.92 mmol) by following a previous procedure[12] to afford the hemiacetal **36** as a crude material. Part of the crude material (~200 mg) was converted to the previously known imidate **11** by reacting with CCl_3CN (0.3 mL) and K_2CO_3 (166 mg) in anhydrous CH_2Cl_2 (1.5 mL) as before.[25]

4.26. *p*-Chlorophenyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-*N*-(2,2,2-trichloroethoxycarbonylamino)-1-thio-β-D-galactopyranoside (37)

Compound **11** (83.6 mg, 0.18 mmol) and **12** (50 mg, 0.09 mmol) were dissolved in anhydrous CH_2Cl_2 (1 mL) containing molecular sieves. The mixture was stirred at rt for 15 min. At −78 °C, trimethylsilyl trifluoromethanesulfonate was added and the reaction was warmed up to 0 °C. Et_3N was then added and the mixture was purified by column chromatography on silica gel using 1% acetone–CH_2Cl_2 as eluent to give the α anomer **37** (45.6 mg, 57.4% yield for α). R_f: 0.71 ($EtOAc/CH_2Cl_2$ 1:4). $[\alpha]_D$ +36.7 (*c* 0.77, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ_H 7.65–7.57 (m, 2H, SPhCl), 7.49–7.36 (m, 5H, benzylidene), 7.21–7.13 (m, 2H, SPhCl), 5.66–5.56 (m, 2H, NH + PhCH), 5.39 (d, 1H, *J* = 2.2 Hz, H-4′), 5.31–5.23 (m, 2H, H-3′ + H-1), 5.17 (d, 1H, *J* = 3.4 Hz, H-1′), 4.86 (d, 1H, *J* = 12.0 Hz, Troc_CH_2), 4.64 (d, 1H, *J* = 12.0 Hz, Troc_CH_2), 4.52 (d, 1H, *J* = 9.9 Hz, H-3), 4.45–4.37 (m, 2H, H-6a + H-4), 4.21 (dd, 1H, *J* = 6.6 Hz, H-5′), 4.15–4.00 (m, 3H, H-6′a + H-6b + H-6′b), 3.65–3.53 (m, 3H, H-5 + H-2′ + H-2), 2.14 (s, 3H, Ac), 2.09 (s, 3H, Ac), 2.02 (s, 3H, Ac). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 169.91 (Ac × 2), 169.57 (Ac), 153.75 (Troc), 137.45 (Ph), 135.02 (Ph), 134.59 (Ph), 133.54 (Ph), 129.17 (Ph), 129.06 (Ph), 128.12 (Ph), 126.17 (Ph), 100.71 (PhCH), 95.86 (C-1′). 95.52 (CCl_3), 83.50 (C-1), 74.55 (Troc_CH_2), 74.45 (C-3), 71.89 (C-4), 69.74 (C-5), 69.32 (C-6), 67.53 (C-3′), 67.23 (C-4′), 66.97 (C-5′), 61.36 (C-6′), 56.97 (C-2), 51.44 (C-2′), 20.83 (Ac), 20.56 (Ac × 2). HRESIMS: Calcd for $C_{34}H_{36}N_4O_{13}SCl_4Na$ ($M+Na^+$): *m*/*z* 903.0646. Found: 903.0664.

4.27. *p*-Chlorophenyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-*N*-(2,2,2-trichloroethoxycarbonylamino)-1-thio-β-D-galactopyranoside (10)

Compound **37** (30 mg, 0.034 mmol) was dissolved in thioacetic acid (2 mL). The solution was stirred at rt for 15 h and then diluted by toluene and evaporated. The residue was purified by column chromatography on silica gel using 65% EtOAc–hexane as eluent to give the compound **10** (30.0 mg, 98.2% yield). R_f: 0.46 (EtOAc/hexane 9:1). $[\alpha]_D$ +55.7 (*c* 0.43, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ_H 7.69–7.63 (m, 2H, Ph), 7.42–7.37 (m, 3H, Ph), 7.33–7.28 (m, 4H, Ph), 5.55 (d, 1H, *J* = 9.5 Hz, NH), 5.45 (s, 1H, PhCH), 5.38 (d, 1H, *J* = 8.8 Hz, NH), 5.31 (dd, 1H, *J* = 2.2, <1 Hz, H-4′), 5.05 (d, 1H, *J* = 3.7 Hz, H-1′), 4.96–4.87 (m, 3H, H-3′ + Troc_CH_2 + H-1), 4.69 (d, *J* = 12.1 Hz, 1H, Troc_CH_2), 4.53 (ddd, 1H, *J* = 11.2, 9.8, 3.7 Hz,

H-2′), 4.38 (dd, 1H, J = 12.4, 1.3 Hz, H-6a), 4.30 (dd, 1H, J = 3.0, <1 Hz, H-4), 4.18 (dd, 1H, J = 6.7, 6.7 Hz, H-3), 4.11–4.02 (m, 4H, H-5′ + H-6′a + H-6′b + H-6b), 3.83 (ddd, 1H, J = 19.4, 9.9, 2.4 Hz, H-2), 3.61–3.56 (m, 1H, H-5), 2.15 (s, 3H, OAc), 2.10 (s, 3H, OAc), 1.91 (s, 3H, OAc), 1.26 (s, 3H, NAc). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 171.00 (Ac), 170.46 (Ac), 170.43 (Ac), 170.23 (Ac), 137.31 (Ph), 136.16 (Ph), 135.10 (Ph), 129.65 (Ph), 129.15 (Ph), 128.43 (Ph), 128.37 (Ph), 126.44 (Ph), 101.34 (PhCH), 95.34 (CCl_3), 93.01 (C-1′), 84.08 (C-1), 74.69 (Troc_CH_2), 73.19 (C-5′), 70.22 (C-4), 69.57 (C-5), 69.47 (C-6), 68.39 (C-3′), 66.93 (C-3), 66.89 (C-4′), 61.80 (C-6′), 50.67 (C-2), 46.82 (C-2′), 22.33 (NAc), 20.91 (OAc), 20.67 (OAc), 20.60 (OAc). HRESIMS: Calcd for $C_{36}H_{40}N_2O_{14}SCl_4Na$ (M+Na$^+$): m/z 919.0847. Found: 919.0862.

4.28. 6-Azidohexyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-*N*-(2,2,2-trichloroethoxycarbonylamino)-β-D-galactopyranosyl-(1→3)-2-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (9)

Compound **10** (22 mg, 0.024 mmol) and **6** (40 mg, 0.028 mmol) were dissolved in anhydrous CH_2Cl_2 (0.7 mL) containing molecular sieves (50 mg). At −78 °C, *N*-iodosuccinimide (8 mg, 0.06 mmol) and trifluoromethanesulfonic acid were added. The reaction was warmed up to −30 °C for 1 h. Et_3N was then added and the mixture was purified by column chromatography on silica gel using 43% EtOAc–hexane as eluent to give the compound **9** (45 mg, 88.6% yield). R_f: 0.35 (EtOAc/hexane 7:3). $[\alpha]_D$ +125.0 (c 0.2, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.52–7.14 (m, 45H), 5.80 (d, 1H, J = 8.8 Hz), 5.44 (s, 1H), 5.36 (s, 1H), 5.31 (d, 1H, J = 2.0 Hz), 5.16 (d, 1H, J <1 Hz), 5.01 (d, 1H, J = 11.1 Hz), 4.97–4.81 (m, 5H), 4.81–4.70 (m, 5H), 4.66–4.44 (m, 8H), 4.44–4.32 (m, 3H), 4.30–4.11 (m, 7H), 4.11–3.81 (m, 11H), 3.80–3.71 (m, 2H), 3.66–3.45 (m, 7H), 3.44–3.34 (m, 2H), 3.33–3.26 (m, 3H), 3.22 (t, 2H, J = 6.9 Hz), 2.58–2.51 (m, 1H), 2.16 (s, 3H), 2.03 (s, 3H), 1.92 (s, 3H), 1.72–1.51 (m, 4H), 1.47–1.35 (m, 4H), 1.27 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 170.54, 170.44, 170.24, 170.21, 154.54, 139.40, 138.62, 138.37, 138.30, 138.16, 138.07, 138.05, 138.04, 137.37, 133.79, 129.41, 129.31, 129.28, 128.63, 128.48, 128.44, 128.42, 128.26, 128.00, 127.93, 127.90, 127.88, 127.71, 127.67, 127.62, 127.60, 127.56, 127.53, 127.46, 127.17, 126.39, 126.18, 103.62, 102.83, 101.34, 101.17, 100.50, 100.24, 95.38, 93.28, 82.32, 81.52, 81.20, 78.80, 76.92, 76.56, 76.38, 75.09, 74.86, 74.80, 74.62, 74.48, 73.45, 73.12, 72.84, 72.10, 69.75, 69.25, 68.97, 68.36, 68.33, 67.10, 66.85, 66.75, 65.44, 63.27, 61.20, 51.34, 29.59, 28.75, 26.50, 25.72, 22.35, 20.77, 20.70, 20.62. HRESIMS: Calcd for $C_{110}H_{124}N_5O_{30}Cl_3Na$ (M+Na$^+$): m/z 2122.7289. Found: 2122.7337.

4.29. 6-Aminohexyl 2-acetamido-2-deoxy-α-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→3)-α-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside, acetic acid salt (2)

Compound **9** (35 mg, 0.017 mmol) was dissolved in AcOH (1.8 mL) and H_2O (200 μL) was added. The reaction was stirred at 90 °C for 2.5 h. The solution was then concentrated and the residue was dissolved in anhydrous MeOH (4 mL) and NaOMe (100 μL, 1.5 M in MeOH) was added. The reaction was stirred at 50 °C for 2 h and was concentrated. The residue was mixed in aq. NaOH (2 N, 4 mL) and the mixture was kept at 100 °C to reflux overnight. After evaporating the solvent, the residue was stirred in a mixture of Ac_2O (2 mL)/pyridine (3 mL) and followed by the treatment of MeOH (4 mL)/NaOMe (100 μL, 1.5 M in MeOH). The mixture was concentrated and the residue was purified by column chromatography on silica gel using 2% MeOH–CH_2Cl_2 as eluent to afford the intermediate **38**, which was then dissolved in a mixture of MeOH (5 mL), H_2O (50 μL) and several drops of AcOH, and was treated by $Pd(OH)_2$/C/H_2 at rt for 2 days. Then the $Pd(OH)_2$/C was filtered and the solution was concentrated to afford the compound **2** (2 mg, 10% yield). R_f: 0.25 (IPA/H_2O/NH_3·H_2O 6:2:1). Selected ^{1}H NMR (400 MHz, D_2O) δ_H 5.10 (d, 1H, J = 3.7 Hz, H-1_αGalNAc), 4.96 (d, 1H, J = 4.0 Hz, H-1_αGal), 4.74 (overlaped with HDO, 1H, H-1_βGalNAc), 4.54 (d, 1H, J = 7.8 Hz, H-1_βGal), 4.51 (d, 1H, J = 8.2 Hz, H-1_αGalNAc), 3.53 (t, 2H, J = 5.1 Hz, $CH_2NH_3^+$), 2.09 (s, 3H, NHAc), 2.07 (s, 3H, NHAc). RESIMS: Calcd for $C_{40}H_{72}N_3O_{26}$ (M+H$^+$): m/z 1010.4398. Found: 1010.4390. Selected ^{13}C NMR (400 MHz, D_2O, from HSQC) δ_H 103.2 (C-1_βGal), 102.6 (C-1_αGal), 101.9 (C-1_βGlc), 100.3 (C-1_βGalNAc), 93.4 (C-1_αGalNAc), 50.8 (C-2_βGalNAc), 50.0 ($CH_2NH_3^+$), 49.2 (C-1_αGalNAc).

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