Synthesis of Azido-Globo H Analogs for Immunogenicity Evaluation

Supporting information-1

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General Methods
All chemicals and reagents were purchased from Merck, Acros, and TCI without further purification. The reactions contained moisture-sensitive reagents were achieved under argon. Reactions were monitored by analytical thin layer chromatography (TLC) Silica gel 60 F\textsubscript{254} (Merck), virtualized by UV (254 nm) and stained with acidic ceric ammonium molybdate. No unexpected or unusually high safety hazards were encountered. Compounds were purified by flash column chromatography silica gel LiChroprep RP-18 (40-63 µm, Merck) or Sephadex LH-20 (GE Healthcare). \textsuperscript{1}H NMR spectra were recorded on a Bruker Topspin-600 spectrometer at 24 °C. The chemical shift was reported in ppm (\(\delta\) scale) and calibrated by the internal standard of CDCl\textsubscript{3} (\(\delta = 7.24\) ppm), D\textsubscript{2}O (\(\delta = 4.80\) ppm). \textsuperscript{13}C NMR spectra were recorded on a Bruker Topspin-600 (150 MHz) spectrometer at 24 °C. The chemical shift was reported in ppm (\(\delta\) scale) and calibrated by the internal standard of CDCl\textsubscript{3} (\(\delta = 77.00\) ppm). Coupling constants (Hz) were calculated from the chemical shift from \textsuperscript{1}H NMR spectra. Splitting patterns were recorded by following abbreviations: s = singlet, d = doublet, t = triplet, dd = double doublet, q = quartet, m = multiplet. \textsuperscript{1}H NMR was reported in the following order: chemical shift; multiplicity; proton number; coupling constant(s). High resolution MS were analyzed by Bruker ultraflex II TOF/TOF. Carrier protein conjugation numbers were analyzed by Bruker MALDI-TOF with sinapinic acid as the matrix. Protein concentration was determined by the NanoDrop Lite (Thermo).
1. Scheme S1. Enzymatic synthesis of GH series glycans and the sugar nucleotides regeneration

All the materials and methods were followed by previous study. In brief, to synthesize Gb3, the starting material Lac was dissolved in the working solution in 50mM as final concentration. The working solution contained 30 mM Tris-HCl (pH 7), 15 mM MgCl₂, 144 mM PEP, 12 mM UTP disodium salt, 6 mM ATP disodium salt and 60 mM Gal as a fixed final concentration after enzymes were added. Then, the working solution were added the regeneration enzymes including β1,3-N-acetylgalactosaminyltransferase (MalE-LgtD, acceptor:enzyme = 2000:1), galactose kinase (GalK, acceptor:enzyme = 8000:1), UDP-Sugar pyrophosphorylase (AtUSP, acceptor:enzyme = 8000:1), pyruvate kinase (PK, acceptor:enzyme = 16000:1), pyrophosphatase (PPA, acceptor:enzyme = 8000:1). The reaction was incubated at 28 °C for overnight with shaking (300 rpm) and monitored by TLC using butanol/acetone/H₂O = 5:3:2 as the developing solvent.

The starting material Gb3 was dissolved in the working solution in 50 mM as final concentration. The working solution contained 15 mM Tris-HCl (pH 7), 15 mM MgCl₂, 144 mM PEP, 12 mM UTP disodium salt, 6 mM ATP disodium salt, and 60 mM N-acetylgalactosamine as a fixed final concentration after enzymes were added. Then, the working solution were added the regeneration enzymes including β1,3-N-acetylgalactosaminyltransferase (MalE-LgtD, acceptor:enzyme = 1500:1), N-acetylhexosamine 1-kinase (NahK, acceptor:enzyme = 2000:1), N-acetylglucosamine 1-phosphate uridylyltransferase (GlmU, acceptor:enzyme = 1500:1), pyruvate kinase
(PK, acceptor:enzyme = 16000:1), and pyrophosphatase (PPA, acceptor:enzyme = 8000:1). The reaction was incubated at 28 °C for overnight with shaking (300 rpm) and monitored by TLC using butanol/AcOH/H₂O = 5:3:2 as the developing solvent.

The starting material **Gb4** was dissolved in the working solution in 50mM as final concentration. The working solution contained 30 mM Tris-HCl (pH 7), 15 mM MgCl₂, 144 mM PEP, 12 mM UTP disodium salt, 6 mM ATP disodium salt and 60 mM galactose as a fixed final concentration after enzymes were added. Then, the working solution were added the regeneration enzymes including β1,3-N-acetylgalactosaminytransferase (MalE-LgtD, acceptor:enzyme = 2000:1), galactose kinase (GalK, acceptor:enzyme = 8000:1), UDP-Sugar pyrophosphorylase (AtUSP, acceptor:enzyme = 8000:1), pyruvate kinase (PK, acceptor:enzyme = 16000:1), pyrophosphatase (PPA, acceptor:enzyme = 8000:1). The reaction was incubated at 28 °C for overnight with shaking (300 rpm) and monitored by TLC using butanol/AcOH/H₂O = 5:3:2 as the developing solvent.

The starting material **SSEA3** was dissolved in the working solution in 50mM as final concentration. The working solution contained 25 mM Tris-HCl (pH 7), 5 mM MgCl₂, 120 mM PEP, 5 mM GTP disodium salt, 5 mM ATP disodium salt and 55 mM fucose as a fixed final concentration after enzymes were added. Then, the working solution were added the regeneration enzymes including α1,2-fucosyltransferase (FutC, acceptor:enzyme = 4000:1), L-fucose-1-P-guanylyltransferase (FKP, acceptor:enzyme = 8000:1), pyruvate kinase (PK, acceptor:enzyme = 16000:1), pyrophosphatase (PPA, acceptor:enzyme = 8000:1). The reaction was incubated at 28 °C for overnight with shaking (300 rpm) and monitored by TLC using butanol/AcOH/H₂O = 5:3:2 as the developing solvent.

2. Scheme S2. Attempted synthesis of azido-GH precursors

Reagents and conditions: (i) LgtC, GalK, AtUSP, PK, PPA, and buffer containing Tris-HCl, MgCl₂, PEP, UTP, and ATP; (ii) MalE-LgtD, NahK, GlmU, PK, PPA, and buffer
containing Tris-HCl, MgCl₂, PEP, UTP, and ATP. (iii) LgtC, GalK, AtUSP, PK, PPA, and buffer containing Tris-HCl, MgCl₂, PEP, UTP, and ATP.

3. General method of attempted synthesis of azido-GH precursors

To prepare the acceptors, we started from Lac and used Gal as donor to elongate Lac into Gb₃ by LgtC and Sugar nucleotides regeneration (Scheme S1). We then modified Gal and GalNAc to N₃-Gal² and N₃-GalNAc³ (in overall 48% and 8% yield, respectively) and used them as donors for enzymatic synthesis of azido-GH derivatives.

To synthesize N₃-Gb₃ (7), The starting material Lac was dissolved in the working solution in 50mM as final concentration. The working solution contained 30 mM Tris-HCl (pH 7), 15 mM MgCl₂, 144 mM PEP, 12 mM UTP disodium salt, 6 mM ATP disodium salt and 60 mM N₃-Gal as a fixed final concentration after enzymes were added. Then, the working solution were added the regeneration enzymes including β1,3-N-acetylgalactosaminyltransferase (MalE-LgtD, acceptor:enzyme = 200:1), galactose kinase (GalK, acceptor:enzyme = 8000:1), UDP-Sugar pyrophosphorylase (AtUSP, acceptor:enzyme = 8000:1), pyruvate kinase (PK, acceptor:enzyme = 16000:1), pyrophosphatase (PPA, acceptor:enzyme = 8000:1) (Scheme S1). The reaction was incubated at 28 °C for two days with shaking (300 rpm) and monitored by TLC using butanol/AcOH/H₂O = 5:3:2 as the developing solvent. However, no product was found.

To synthesize N₃-Gb₄ (8), The starting material Gb₃ was dissolved in the working solution in 50 mM as final concentration. The working solution contained 15 mM Tris-HCl (pH 7), 15 mM MgCl₂, 144 mM PEP, 12 mM UTP disodium salt, 6 mM ATP disodium salt, and 60 mM N₃-GalNAc as a fixed final concentration after enzymes were added. Then, the working solution were added the regeneration enzymes including β1,3-N-acetylgalactosaminyltransferase (MalE-LgtD, acceptor:enzyme = 1500:1), N-acetylhexosamine 1-kinase (NahK, acceptor:enzyme = 2000:1), N-acetylglucosamine 1-phosphate uridylyltransferase (GlmU, acceptor:enzyme = 1500:1), pyruvate kinase (PK, acceptor:enzyme = 16000:1), and pyrophosphatase (PPA, acceptor:enzyme = 8000:1) (Scheme S1). The reaction was incubated at 28 °C for 2 days with shaking (300 rpm). However, no product was found.
4. **Scheme S3. Synthesis of C6 hydrate on terminal Gal and GalNAc of Globo H precursors**

![Chemical structures and reactions]

Reagents and conditions: (i) Galactose oxidase, Peroxidase, Catalase, and H₂O.

5. **General method of synthesizing aldehyde-modified Globo H precursors.**

The starting materials (10 mg, lactose: 22.5 μmol, GB3: 16.5 μmol, GB4: 12.3 μmol, SSEA3: 10.3 μmol) were dissolved in ddH₂O (0.75 mL), and GO (1.04 U), catalase (2240 U), and HRP (9 U) were added and stirred for 14 h. The mixture was heated under 100 °C for 30 min, filtered by filter paper, and concentrated. The residue can be put in the next reaction without purification. The yield of the oxidation was directly evaluated by crude ¹H NMR.

Formula: The integral of C’6 H on product / the integral of C’1 H on starting material + product (the terminal galactose or N-acetylgalactosamine).
6. Scheme S4. Synthesis of benzylamine-modified Globo H precursors

Reagents and conditions: (i) BnNH₂, NaBH₃CN, AcOH, and MeOH.

7. General procedure of synthesizing of benzylamine-modified Globo H precursors

To a solution of starting material in dry MeOH, benzylamine (4 equiv.), sodium cyanoborohydride (4 equiv.), and AcOH (1.3 equiv.) were added and stirred at room temperature for 16 h. The mixture was concentrated and purified by reverse phase column chromatography (RP-18) and eluted by a gradient from 100% H₂O to 60% MeOH in ddH₂O to afford NHBn-Globo H precursors. The fractions with product were collected, lyophilized, and characterized by NMR spectroscopy and HRMS (80-96%).
8. Scheme S5. Synthesis of amine-modified Globo H precursors

Reagents and conditions: (i) NIS, MeOH, and H₂O.

9. General procedure of synthesizing of amine-modified Globo H precursors

To a solution of starting material in MeOH and ddH₂O was added NIS (10 equiv.) and stirred at room temperature for 16 h. The reaction was monitored by TLC using butanol/AcOH/H₂O = 5:3:2 as a developing solvent. After the optimal yield was achieved, the mixture was concentrated, purified by reverse phase column chromatography (RP-18), and eluted by a gradient from 100% H₂O with 1% AcOH to 30% MeOH in H₂O with 1% AcOH to afford NH₂-Globo H precursors. The fractions with product were collected, lyophilized, and characterized by NMR spectroscopy and HRMS (57-72%).
10. Scheme S6. Synthesis of azide-modified Globo H precursors

Reagents and conditions: (i) TfN₃, K₂CO₃, CuSO₄, CH₂Cl₂, MeOH, and H₂O.

11. General procedure of synthesizing azide-modified Globo H precursors

To a stirred solution of NaN₃ (595 mg, 9.15 mmol) in 1.5 mL ddH₂O and 2.5 mL CH₂Cl₂ under an ice bath, Tf₂O (400 μL, 845 mmol) was slowly added and stirred under ice bath for 2 h. The organic phase was separated and the water phase was extracted with CH₂Cl₂ for two times. The collected organic layer was extracted with saturated NaHCO₃ solution and can be used without further purification. To a solution of starting material (0.22 mmol) in ddH₂O (1 mL) was added K₂CO₃ (100 mg, 0.72 mmol) and CuSO₄ hydrate (10 mg, 62.9 μmol). The mixture was added MeOH and previously prepared TfN₃ solution. More MeOH was added until the mixture is homogeneous. After stirring for 16 h, the mixture was monitored by TLC using butanol/AcOH/H₂O = 5:3:2 as a developing solvent. The mixture was concentrated, purified by reverse phase column chromatography (RP-18), and eluted by a gradient from 100% H₂O to 50% MeOH in H₂O to afford N₃ Globo H precursors (47-62%).
12. Scheme S7. Chemical synthesis of azido-Gb3 analog

Reagents and conditions: (i) potassium phthalimide and DMF; (ii) 80% AcOH, reflux; (iii) TsCl and pyridine; (iv) NaN₃ and DMF, 110 °C; (v) NIS, TfOH, and CH₂Cl₂; (vi) hydrazine hydrate; (vii) Boc anhydride and pyridine; (viii) Pd(OH)₂, H₂, MeOH, and H₂O; (ix) TfN₃, CuSO₄, K₂CO₃, MeOH, and H₂O

Compound S10 was synthesized by previously reported method.⁴ We replaced the Cl group at linker on S10 by N-phthalimide to avoid nucleophilic substitution of azide in the subsequent step to get compound S11. Benzylidene was deprotected under acetic acid to get diol compound S12. Azide was selectively installed at C6 of Gal through p-toluenesulfonate leaving group to get compound S12, which was used as acceptor for glycosylation with compound S14⁵ to afford fully protected Gb3 (S15). To ensure the amino protecting group in the linker is retained after hydrogenation, N-phthalimide in the linker was replaced by NHBoc to get compound S16, and compound S17 was afforded after global deprotection. Finally, the amine at nonreducing end C6 on Lac was converted into the azide group to obtain compound 15 with NHBoc protection at linker. This final product was well dissolved in water and used as the acceptor for enzymatic synthesis.
13. Scheme S8. Enzymatic synthesis of Gb4 analogs.

Reagents and conditions: (i) N-acetylgalactosamine, MalE-LgtD, NahK, GlmU, PK, PPA, and buffer containing Tris-HCl, MgCl₂, PEP, UTP, and ATP.

14. General procedure of synthesizing Gb4 analogs

The starting material Gb3 analog was dissolved in the working solution in 50 mM as final concentration. The working solution contained 15 mM Tris-HCl (pH 7), 15 mM MgCl₂, 144 mM PEP, 12 mM UTP disodium salt, 6 mM ATP disodium salt, and 60 mM N-acetylgalactosamine as a fixed final concentration after enzymes were added. Then, the working solution were added the regeneration enzymes including β1,3-N-acetylgalactosaminyltransferase (MalE-LgtD, acceptor:enzyme = 1500:1), N-acetylhexosamine 1-kinase (NahK, acceptor:enzyme = 2000:1), N-acetylglucosamine 1-phosphate uridylyltransferase (GlmU, acceptor:enzyme = 1500:1), pyruvate kinase (PK, acceptor:enzyme = 16000:1), and pyrophosphatase (PPA, acceptor:enzyme = 8000:1). The reaction was incubated at 28 °C for overnight with shaking (300 rpm) and monitored by TLC using butanol/AcOH/H₂O = 5:3:2 as the developing solvent. If the starting material (Gb3 analog) was not totally consumed, more MalE-LgtD and PEP were added until Gb3 analog was totally used up. After the optimized yield was achieved, the reaction was heated at 90 °C for 10 min, centrifuged under 10000 rpm, 4 °C for 30 min. The supernatant was concentrated, and purified by reverse phase column chromatography (RP-18) and eluted by a gradient from 100% H₂O to 40% MeOH in H₂O to afford GB4 analogs (72-74%). The fractions with product were collected, lyophilized, and characterized by NMR spectroscopy and HRMS.
15. Scheme S9. Enzymatic synthesis of SSEA3 analogs

Reagents and conditions: (i) Gal, MalE-LgtD, GalK, AtUSP, PK, PPA, and buffer containing Tris-HCl, MgCl₂, PEP, UTP, and ATP.

16. General procedure of synthesizing SSEA3 analogs

The starting material Gb4 analog was dissolved in the working solution in 50mM as final concentration. The working solution contained 30 mM Tris-HCl (pH 7), 15 mM MgCl₂, 144 mM PEP, 12 mM UTP disodium salt, 6 mM ATP disodium salt and 60 mM galactose as a fixed final concentration after enzymes were added. Then, the working solution were added the regeneration enzymes including β1,3-N-acetylgalactosaminyltransferase (MalE-LgtD, acceptor:enzyme = 375-500:1), galactose kinase (GalK, acceptor:enzyme = 8000:1), UDP-Sugar pyrophosphorylase (AtUSP, acceptor:enzyme = 8000:1), pyruvate kinase (PK, acceptor:enzyme = 16000:1), pyrophosphatase (PPA, acceptor:enzyme = 8000:1). The reaction was incubated at 28 °C for overnight with shaking (300 rpm) and monitored by TLC using butanol/AcOH/H₂O = 5:3:2 as the developing solvent. If the starting material (Gb4 analogs) was not totally consumed, more MalE-LgtD and PEP were added until Gb4 analog was totally used up. After the optimized yield was achieved, the reaction was heated at 90 °C for 10 min, centrifuged under 10000 rpm, 4 °C for 30 min. The supernatant was concentrated, purified by reverse phase column chromatography (RP-18), and eluted by a gradient from 100% H₂O to 40% MeOH in H₂O to afford SSEA3 analogs (58-80%). The fractions with product were collected, lyophilized, and characterized by NMR spectroscopy and HRMS.
17. Scheme S10. Enzymatic synthesis of Globo H analogs

Reagents and conditions: (i) Fucose, FutC, FKP, PK, PPA and buffer containing Tris-HCl, MgCl₂, PEP, UTP, and ATP

18. General procedure of synthesizing Globo H analogs

The starting material SSEA3 analog was dissolved in the working solution in 50mM as final concentration. The working solution contained 25 mM Tris-HCl (pH 7), 5 mM MgCl₂, 120 mM PEP, 5 mM GTP disodium salt, 5 mM ATP disodium salt and 55 mM fucose as a fixed final concentration after enzymes were added. Then, the working solution were added the regeneration enzymes including α1,2-fucosyltransferase (FutC, acceptor:enzyme = 4000:1), L-fucose-1-P-guanlyltransferase (FKP, acceptor:enzyme = 8000:1), pyruvate kinase (PK, acceptor:enzyme = 16000:1), pyrophosphatase (PPA, acceptor:enzyme = 8000:1). The reaction was incubated at 28 °C for overnight with shaking (300 rpm) and monitored by TLC using butanol/AcOH/H₂O = 5:3:2 as the developing solvent. If the starting material (SSEA3 analogs) was not totally consumed, more FutC and PEP were added until SSEA3 analog was totally used up. After the
optimized yield was achieved, the reaction was heated at 90 °C for 10 min, centrifuged under 10000 rpm, 4 °C for 30 min. The supernatant was concentrated, purified by reverse phase column chromatography (RP-18), and eluted by a gradient from 100% H₂O to 40% MeOH in H₂O to afford Globo H analogs (58-80%). The fractions with product were collected, lyophilized, and characterized by NMR spectroscopy and HRMS.

19. Scheme S11. NHBoc deprotection at Globo H linker

Reagents and conditions: (i) TFA:H₂O = 9:1, 0 °C

20. General procedure of NHBoc deprotection at Globo H linker
The starting material was dissolved in TFA:H₂O = 9:1 and stirred under an ice bath for 2 h. The reaction was monitored by TLC using butanol/AcOH/H₂O = 3:2:2 as the developing solvent. The mixture was concentrated and the residue was purified by Sephadex LH-20 chromatography. The fractions with product were collected, lyophilized, and characterized by NMR spectroscopy and HRMS (76-95%).
21. Scheme S12. Conversion of Cl to NH$_2$ at linker on Globo H analogs

Reagents and conditions: (i) NH$_4$OH, reflux.

22. General procedure of converting Cl to NH$_2$ at linker on Globo H analogs

A solution of starting material in NH$_4$OH was stirred under reflux for overnight. The reaction was monitored by TLC using butanol/ACOH/H$_2$O = 3:2:2 as the developing solvent. The mixture was concentrated and the residue was purified by Sephadex LH-20 chromatography. The fractions with product were collected, lyophilized, and characterized by NMR spectroscopy and HRMS (76%-quant).
23. Scheme S13. Synthesis of GH-analogs monoester

Reagents and conditions: (i) p-nitrophenyl adipate linker, TEA, and DMF

24. General procedure of synthesizing GH-analogs monoester

To a solution of GH analogs (4-8 mg, 1 equiv.) in anhydrous DMF (5 mL) was added p-nitrophenyl adipate linker (7-15 mg, 5 equiv.) and triethylamine (5-6 µL, 10 equiv.) and stirred at room temperature for 16 h. The reaction was monitored by TLC by using butanol/AcOH/H₂O = 3:2:2 as the developing solvent. After the optimal yield was achieved, the mixture was concentrated without heating. The residue was purified by C18 chromatography and gradually eluted with H₂O containing 1% acetic acid to MeOH:H₂O = 6:4. The fractions containing product were lyophilized and characterized by NMR spectroscopy and HRMS (2-4 mg, 50-68%).
Synthesis and characterization of new compounds

5-chloropentyl 6-deoxy-6-aldehyde-α-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (S1)

By following with the general procedure of synthesizing aldehyde-modified Globo H precursors (Supporting information 5), starting material Gb3 (10 mg, 16.5 μmol) was converted into compound S1 in 74% (determination by crude NMR) yield as a light yellow foam. 

$^1$H NMR (600 MHz, D$_2$O) δ 5.10 ($J = 7.1$ Hz, 1H), 4.98 ($J = 3.5$ Hz, 1H), 4.51 ($J = 7.8$ Hz, 1H), 4.48 ($J = 8.0$ Hz, 1H), 4.16 (m, 1H), 4.09 – 4.07 (m, 1H), 4.03 – 3.98 (m, 2H), 3.95 – 3.78 (m, 7H), 3.75 – 3.57 (m, 8H), 3.29 (t, $J = 8.4$ Hz, 1H), 1.83 – 1.78 (m, 2H), 1.68 – 1.64 (m, 2H), 1.53 – 1.48 (m, 2H). $^{13}$C NMR (150 MHz, D$_2$O) δ 103.20, 101.95, 100.26, 88.45, 78.67, 77.67, 75.38, 75.32, 74.74, 74.45, 72.85, 72.53, 72.38, 71.00, 70.32, 69.05, 68.51, 68.45, 68.36, 60.42, 60.00, 45.45, 31.51, 30.18, 27.97, 22.51. HRMS (ESI–TOF, M+Na$^+$) calcd for C$_{23}$H$_{41}$ClO$_{17}$Na$^+$ 646.1924, found 647.1939.

5-chloropentyl 6-deoxy-6-aldehyde-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→3)-α-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (S2)

By following with the general procedure of synthesizing aldehyde-modified Globo H precursors (Supporting information 5), starting material Gb4 (10 mg, 12.3 μmol) was converted into compound S2 in 89% (determination by crude NMR) yield as a light yellow foam. 

$^1$H NMR (600 MHz, D$_2$O) δ 5.14 (d, $J = 7.3$ Hz, 1H), 4.91 (d, $J = 4.02$ Hz, 1H), 4.48 (d, $J = 8.5$ Hz, 1H), 4.51 (d, $J = 7.8$ Hz, 1H), 4.48 (d, $J = 8.1$ Hz, 1H), 4.39 (t, $J = 6.5$ Hz, 1H), 4.27 (d, $J = 2.6$ Hz, 1H), 4.09 (d, $J = 3.1$ Hz, 1H), 4.03 (d, $J = 3.1$ Hz, 1H), 4.00 – 3.88 (m, 6H), 3.85 – 3.73 (m, 5H), 3.70 – 3.57 (m, 9H), 3.38 (d, $J = 7.7$ Hz, 1H), 3.30 (t, $J = 8.4$ Hz, 1H), 2.04 (s, 3H), 1.83 – 1.78 (m, 2H), 1.68 – 1.64
(m, 2H), 1.53 – 1.49 (m, 2H). $^{13}$C NMR (150 MHz, D$_2$O) $\delta$ 175.16, 103.26, 101.94, 100.38, 88.09, 78.81, 78.68, 77.13, 76.58, 75.42, 74.76, 74.48, 72.92, 72.05, 70.82, 70.69, 70.32, 70.16, 68.84, 67.53, 67.28, 60.30, 60.25, 60.02, 52.36, 45.46, 31.51, 27.98, 22.52, 22.20. HRMS (ESI–TOF, M+Na$^+$) calcd for C$_{31}$H$_{54}$ClNO$_{22}$Na$^+$ 850.2718, found 850.2749.

5-chloropentyl 6-deoxy-6-aldehyde-$\beta$-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-$\beta$-D-galactopyranosyl-(1→3)-$\alpha$-D-galactopyranosyl-(1→4)-$\beta$-D-galactopyranosyl-(1→4)-$\beta$-D-glucopyranoside (S3)

By following with the general procedure of synthesizing aldehyde-modified Globo H precursors (Supporting information 5), starting material SSEA3 (10 mg, 10.3 μmol) was converted into compound S3 in 88% (determination by crude NMR) yield as a light yellow foam. $^1$H NMR (600 MHz, D$_2$O) $\delta$ 5.12 (d, $J = 7.3$ Hz, 1H), 4.91 (d, $J = 3.9$ Hz, 1H), 4.69 (d, $J = 8.5$ Hz, 1H), 4.51 (d, $J = 7.7$ Hz, 1H), 4.48 (d, $J = 8.0$ Hz, 1H), 4.45 (d, $J = 7.7$ Hz, 1H), 4.39 (t, $J = 6.5$ Hz, 1H), 4.25 (d, $J = 2.8$ Hz, 1H), 4.20 (d, $J = 3.0$ Hz, 1H), 4.08 – 4.04 (m, 3H), 4.00 – 3.89 (m, 6H), 3.86 – 3.77 (m, 4H), 3.76 – 3.73 (m, 2H), 3.71 – 3.68 (m, 4H), 3.66 – 3.57 (m, 7H), 3.54 (dd, $J = 9.8$, 2.0 Hz, 1H), 3.37 (d, $J = 7.3$ Hz, 1H), 3.30 (t, $J = 8.5$ Hz, 1H), 2.03 (s, 3H), 1.83 – 1.78 (m, 2H), 1.68 – 1.64 (m, 2H), 1.53 – 1.48 (m, 2H). $^{13}$C NMR (150 MHz, D$_2$O) $\delta$ 175.11, 104.83, 103.25, 102.92, 101.94, 100.36, 88.11, 79.79, 78.70, 78.66, 77.14, 76.57, 75.40, 74.76, 74.52, 74.48, 72.91, 72.37, 72.06, 70.83, 70.37, 70.32, 70.22, 68.89, 68.08, 67.84, 67.57, 60.90, 60.31, 60.26, 60.01, 51.37, 45.46, 31.51, 27.97, 22.51, 22.22. HRMS (ESI–TOF, M+Na$^+$) calcd for C$_{37}$H$_{64}$ClNO$_{27}$Na$^+$ 1012.3246, found 1012.3281.

5-chloropentyl 6-deoxy-6-benzylamino-$\alpha$-D-galactopyranosyl-(1→4)-$\beta$-D-galactopyranosyl-(1→4)-$\beta$-D-glucopyranoside (S4)

5-chloropentyl 6-deoxy-6-aldehyde-$\beta$-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-$\beta$-D-galactopyranosyl-(1→3)-$\alpha$-D-galactopyranosyl-(1→4)-$\beta$-D-galactopyranosyl-(1→4)-$\beta$-D-glucopyranoside (S3)

By following with the general procedure of synthesizing aldehyde-modified Globo H precursors (Supporting information 5), starting material SSEA3 (10 mg, 10.3 μmol) was converted into compound S3 in 88% (determination by crude NMR) yield as a light yellow foam. $^1$H NMR (600 MHz, D$_2$O) $\delta$ 5.12 (d, $J = 7.3$ Hz, 1H), 4.91 (d, $J = 3.9$ Hz, 1H), 4.69 (d, $J = 8.5$ Hz, 1H), 4.51 (d, $J = 7.7$ Hz, 1H), 4.48 (d, $J = 8.0$ Hz, 1H), 4.45 (d, $J = 7.7$ Hz, 1H), 4.39 (t, $J = 6.5$ Hz, 1H), 4.25 (d, $J = 2.8$ Hz, 1H), 4.20 (d, $J = 3.0$ Hz, 1H), 4.08 – 4.04 (m, 3H), 4.00 – 3.89 (m, 6H), 3.86 – 3.77 (m, 4H), 3.76 – 3.73 (m, 2H), 3.71 – 3.68 (m, 4H), 3.66 – 3.57 (m, 7H), 3.54 (dd, $J = 9.8$, 2.0 Hz, 1H), 3.37 (d, $J = 7.3$ Hz, 1H), 3.30 (t, $J = 8.5$ Hz, 1H), 2.03 (s, 3H), 1.83 – 1.78 (m, 2H), 1.68 – 1.64 (m, 2H), 1.53 – 1.48 (m, 2H). $^{13}$C NMR (150 MHz, D$_2$O) $\delta$ 175.11, 104.83, 103.25, 102.92, 101.94, 100.36, 88.11, 79.79, 78.70, 78.66, 77.14, 76.57, 75.40, 74.76, 74.52, 74.48, 72.91, 72.37, 72.06, 70.83, 70.37, 70.32, 70.22, 68.89, 68.08, 67.84, 67.57, 60.90, 60.31, 60.26, 60.01, 51.37, 45.46, 31.51, 27.97, 22.51, 22.22. HRMS (ESI–TOF, M+Na$^+$) calcd for C$_{37}$H$_{64}$ClNO$_{27}$Na$^+$ 1012.3246, found 1012.3281.
By following with the general method of synthesizing benzylamine-modified Globo H precursors (Supporting information 7), starting material S1 (125 mg, 179.2 μmol) was converted into compound S4 (122 mg, 85%) as a light yellow foam. ¹H NMR (600 MHz, D₂O) δ 7.31 – 7.18 (m, 5H), 4.90 (d, J = 2.9 Hz, 1H), 4.37 (d, J = 7.1 Hz, 1H), 4.24 (d, J = 7.8 Hz, 1H), 3.96 (d, J = 1.9 Hz, 1H), 3.86 – 3.60 (m, 11H), 3.56 – 3.46 (m, 7H), 3.36 – 3.25 (m, 2H), 3.20 (t, J = 8.2 Hz, 1H), 2.83 – 2.78 (m, 2H), 1.74 – 1.71 (m, 2H), 1.61 – 1.57 (m, 2H), 1.49 – 1.46 (m, 2H). ¹³C NMR (150 MHz, D₂O) δ 129.70, 129.67, 129.61, 128.43, 105.39, 104.28, 102.27, 80.98, 78.50, 76.87, 76.56, 76.49, 74.94, 74.52, 72.72, 72.62, 71.30, 70.73, 70.63, 70.55, 62.02, 61.44, 54.62, 50.71, 45.82, 33.66, 30.09, 24.58.

HRMS (ESI–TOF, M+H⁺) calcd for C₃₀H₄₈ClNO₁₅H⁺ 698.2785, found 698.2789.

5-chloropentyl 6-deoxy-6-benzylamino-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→3)-α-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (S5)

By following with the general method of synthesizing benzylamine-modified Globo H precursors (Supporting information 7), starting material S2 (85.4 mg, 94.9 μmol) was converted into compound S5 (80.0 mg, 85%) as a light yellow foam. ¹H NMR (600 MHz, D₂O) δ 7.49 – 7.42 (m, 5H), 4.94 (d, J = 3.4 Hz, 1H), 4.65 (d, J = 8.6 Hz, 1H), 4.54 (d, J = 7.7 Hz, 1H), 4.42 (d, J = 8.0 Hz, 1H), 4.35 (t, J = 6.3 Hz, 1H), 4.15 (s, 1H), 4.06 (d, J = 2.8, 1H), 3.99 – 3.91 (m, 6H), 3.89 – 3.83 (m, 4H), 3.81 – 3.79 (m, 2H), 3.78 – 3.75 (m, 3H), 3.73 – 3.70 (m, 1H), 3.68 – 3.63 (m, 4H), 3.59 – 3.54 (m, 3H), 3.47 – 3.45 (m, 1H), 3.30 (t, J = 8.3 Hz, 3H), 2.83 (d, J = 13.0, 1H), 2.07 (s, 3H), 1.86 – 1.81 (m, 2H), 1.71 – 1.66 (m, 2H), 1.56 – 1.51 (m, 2H). ¹³C NMR (150 MHz, D₂O) δ 175.18, 128.87, 128.65, 127.69, 103.37, 103.15, 101.95, 100.50, 79.04, 78.60, 77.36, 75.43, 74.68, 74.56, 72.87, 72.62, 72.16, 71.01, 70.83, 70.36, 70.25, 69.00, 68.64, 67.62, 60.35, 60.26, 60.07, 52.50, 51.84, 47.53, 45.52, 31.54, 28.00, 22.54, 22.55, 22.24.

HRMS (ESI–TOF, M+H⁺) calcd for C₃₈H₆₁ClN₂O₂₀H⁺ 901.3579, found 901.3597.
5-chloropentyl 6-deoxy-6-benzylamino-β-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 (S6)

By following with the general method of synthesizing benzylamine-modified Globo H precursors (Supporting information 7), starting material S3 (133 mg, 136.8 μmol) was converted into compound S6 (140.0 mg, 96%) as a light yellow foam. 1H NMR (600 MHz, D2O) δ 7.49-7.42 (m, 5H), 4.94 (d, J = 3.72 Hz, 1H), 4.70 (d, J = 8.6 Hz, 1H), 4.54 (d, J = 7.7 Hz, 1H), 4.50 (d, J = 7.9 Hz, 1H), 4.46 (d, J = 7.8 Hz, 1H), 4.41 (t, J = 6.4 Hz, 1H), 4.27 (d, J = 2.5 Hz, 1H), 4.08 – 4.04 (m, 4H), 3.98 – 3.92 (m, 4H), 3.88 – 3.85 (m, 5H), 3.82 – 3.76 (m, 4H), 3.73 – 3.70 (m, 4H), 3.68 – 6.60 (m, 8H), 3.56 – 3.50 (m, 2H), 3.03 (dd, J = 12.84, 9.36 Hz, 1H), 2.85 (d, J = 2.85 Hz, 1H), 2.05 (s, 3H), 1.85 – 1.81 (m, 2H), 1.71 – 1.66 (m, 2H), 1.56 – 1.51 (m, 2H). 13C NMR (150 MHz, D2O) δ 175.14, 129.78, 129.65, 129.35, 104.88, 103.26, 102.79, 101.99, 100.42, 80.03, 78.76, 78.67, 77.24, 75.41, 74.80, 74.52, 74.40, 72.92, 72.10, 72.03, 70.90, 70.34, 70.26, 70.03, 69.96, 69.11, 68.92, 67.82, 67.57, 60.77, 60.32, 60.08, 51.49, 51.31, 47.02, 45.52, 31.53, 28.00, 22.55, 22.28. HRMS (ESI–TOF, M+H+) calcd for C44H71ClN2O25H+ 1063.4107, found 1063.4082.

5-chloropentyl 6-deoxy-6-amino-α-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (S7)

By following with the general method of synthesizing amine-modified Globo H precursors (Supporting information 9), starting material S4 (73 mg, 104.6 μmol) was converted into compound S7 (42.6 mg, 67%) as a light yellow foam. 1H NMR (600 MHz, D2O) δ 5.01, (d, J = 3.7 Hz, 1H), 4.54 (d, J = 7.8 Hz, 1H), 4.51 (d, J = 8.0 Hz, 1H), 4.12 (d, J = 2.6 Hz, 1H), 4.05 (d, J = 2.6 Hz, 1H), 4.02 – 3.94 (m, 4H), 3.89 – 3.83 (m, 3H), 3.82 – 3.80 (m, 2H), 3.73 – 7.59 (m, 8H), 3.34 – 3.26 (m, 3H), 1.86 – 1.81 (m,
2H), 1.71 – 1.67 (m, 2H), 1.56 – 1.51 (m, 2H). $^{13}$C NMR (150 MHz, D$_2$O) δ 103.16, 101.98, 100.45, 78.66, 76.54, 75.57, 74.78, 74.50, 72.96, 72.12, 72.11, 71.00, 70.35, 69.90, 68.84, 68.28, 66.54, 60.05, 45.53, 40.45, 31.54, 28.01, 22.55. HRMS (ESI–TOF, M+H$^+$) calcd for C$_{23}$H$_{42}$ClNO$_15$H$^+$ 608.2316, found 608.2314.

5-chloropentyl
6-deoxy-6-amino-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→3)-α-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (S8)
By following with the general method of synthesizing amine-modified Globo H precursors (Supporting information 9), starting material S5 (150 mg, 166.4 μmol) was converted into compound S8 (97 mg, 72%) as a light yellow foam. $^1$H NMR (600 MHz, D$_2$O) δ 4.94 (d, $J = 3.6$ Hz, 1H), 4.73 (d, $J = 8.4$ Hz, 1H), 4.55 (d, $J = 7.7$ Hz, 1H), 4.51 (d, $J = 8.0$ Hz, 1H), 4.42 (t, $J = 6.4$ Hz, 1H), 4.25 (s, 1H), 4.07 (d, $J = 2.6$ Hz, 1H), 4.03 – 3.93 (m, 8H), 3.88 – 3.78 (m, 5H), 3.74 – 3.58 (m, 9H), 3.40 – 3.32 (m, 3H), 2.08 (s, 3H), 1.86 – 1.81 (m, 2H), 1.74 – 1.67 (m, 2H), 1.56 – 1.51 (m, 2H). $^{13}$C NMR (150 MHz, D$_2$O) δ 175.21, 103.21, 103.14, 102.00, 100.40, 78.57, 78.51, 77.22, 75.41, 74.82, 74.51, 73.00, 72.04, 70.91, 70.59, 70.36, 70.28, 70.14, 68.83, 68.47, 67.65, 60.31, 60.11, 60.06, 52.21, 45.53, 40.11, 31.53, 28.01, 22.55, 22.25. HRMS (ESI–TOF, M+H$^+$) calcd for C$_{31}$H$_{55}$ClN$_2$O$_2$H$^+$ 811.3109, found 811.3133.

5-chloropentyl
6-deoxy-6-amino-β-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 (S9)
By following with the general method of synthesizing amine-modified Globo H precursors (Supporting information 9), starting material S6 (130 mg, 122.2 μmol) was converted into compound S9 (67 mg, 57%) as a light yellow foam. $^1$H NMR (600 MHz,
D₂O) δ 4.91 (d, J = 3.7 Hz, 1H), 4.71 (d, J = 8.4 Hz, 1H), 4.51 – 4.47 (m, 3H), 4.36 (t, J = 6.0 Hz, 1H), 4.24 (d, J = 2.2 Hz, 1H), 4.16 (d, J = 2.9 Hz, 1H), 4.06 – 4.03 (m, 2H), 3.99 – 3.88 (m, 9H), 3.85 – 3.71 (m, 7H), 3.70 – 3.62 (m, 10H), 3.59 – 3.53 (m, 3H), 3.33 – 3.27 (m, 3H), 2.02 (s, 3H), 1.82 – 1.78 (m, 2H), 1.68 - 1.63 (m, 2H), 1.52 – 1.47 (m, 2H). ¹³C NMR (150 MHz, D₂O) δ 175.11, 104.80, 103.26, 102.80, 101.94, 100.36, 79.74, 78.70, 77.17, 75.38, 74.74, 74.48, 74.41, 72.91, 72.06, 72.00, 70.85, 70.51, 70.30, 70.22, 70.12, 69.20, 68.89, 67.80, 67.55, 60.33, 60.26, 60.02, 51.48, 45.51, 40.04, 31.52, 27.98, 22.53, 22.28. HRMS (ESI–TOF, M+H⁺) calcd for C₃₇H₆₅ClN₂O₂₅H⁺ 973.3638, found 973.3646.

![Phthalimidyl-aminopentyl-2,3-di-O-benzyl-4,6-O-benzylidene-β-D-galactopyranosyl-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (S11)](image)

**Phthalimidyl-aminopentyl-2,3-di-O-benzyl-4,6-O-benzylidene-β-D-galactopyranosyl-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (S11)**

To a solution of S10 (1.05 g, 1.07 mmol) in DMF (15 mL) was added potassium phthalimide (986.6 mg, 5.33 mmol) and stirred at 100 °C for overnight. The mixture was concentrated and purified by silica gel chromatography (EtOAc:Hexane = 1:2) to afford compound 17 (940 mg, 80%) as a white foam. ¹H NMR (600 MHz, CDCl₃) δ 7.82 – 7.80 (m, 2H), 7.67 – 7.66 (m, 2H), 7.56 – 7.49 (m, 4H), 7.41 – 7.27 (m, 19H), 7.25 – 7.19 (m, 7H), 5.49 (s, 1H), 5.20 (d, J = 10.6 Hz, 1H), 4.92 (d, J = 10.9 Hz, 1H), 4.87 (d, J = 11.2 Hz, 1H), 4.82 – 4.72 (m, 5H), 4.57 (d, J = 12.1 Hz, 1H), 4.49 (d, J = 7.9 Hz, 1H), 4.40 (d, J = 7.9 Hz, 1H), 4.35 (d, J = 12.1 Hz, 1H), 4.23 (d, J = 11.7 Hz, 1H), 4.05 (d, J = 3.6 Hz, 1H), 4.01 – 3.94 (m, 2H), 3.91 (dd, J = 11.0, 4.1 Hz, 1H), 3.86 (dd, J = 12.2, 1.4 Hz, 1H), 3.79 (dd, J = 9.5, 7.9 Hz, 1H), 3.76 – 3.74 (m, 1H), 3.68 – 3.63 (m, 3H), 3.57 (m, 1H), 3.47 – 3.38 (m, 3H), 3.45 – 3.38 (m, 3H), 3.05 (s, 1H), 1.75 – 1.70 (m, 4H), 1.50 – 1.45 (m, 2H). ¹³C NMR (150 MHz, CDCl₃) δ 168.23, 138.85, 138.71, 138.55, 138.41, 138.28, 137.98, 134.23, 133.70, 131.95, 128.70, 128.46, 128.22, 128.15, 128.09, 128.04, 127.97, 127.94, 127.90, 127.64, 127.56, 127.46, 127.38, 127.30, 127.21, 127.1, 126.43, 123.57, 122.99, 103.46, 102.71, 101.19, 82.90, 81.67, 79.49, 78.68, 77.51, 75.62, 75.51, 74.94, 74.82, 73.50, 72.82, 71.45, 69.52, 68.80, 68.15, 66.18, 37.66, 29.12, 28.23, 23.30. HRMS (ESI–TOF, M+Na⁺) calcd for C₆₇H₉₉NO₁₃Na⁺ 1118.4661, found 1118.4657.
**Phthalimidyl-aminopentyl-2,3-di-O-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (S12)**

To a solution of S11 (250 mg, 228 μmol) in 80% AcOH with H2O was stirred at 50 °C for overnight. The mixture was neutralized with saturated NaHCO3(aq) and the resulting solution was extracted with EtOAc. The organic layer was separated, concentrated and purified by silica gel chromatography (EtOAc:hexane = 1:1) to afford compound S12 (185 mg, 80%) as a colorless oil.  

$^1$H NMR (600 MHz, CDCl3) δ 7.80 – 7.79 (m, 2H), 7.67 – 7.65 (m, 2H), 7.41 – 7.40 (m, 2H), 7.32 – 7.27 (m, 16H), 7.26 – 7.23 (m, 6H), 7.20 – 7.18 (m, 1H), 4.96 (d, $J = 10.74$ Hz, 1H), 4.87 (d, $J = 11.4$ Hz, 1H), 4.77 – 4.75 (m, 3H), 4.72 – 4.63 (m, 3H), 4.55 (d, $J = 12.18$ Hz, 1H), 4.39 – 4.36 (m, 3H), 3.94 – 3.88 (m, 3H), 3.79 (dd, $J = 9.3$, 3.3 Hz, 1H), 3.14 (dd, $J = 6.3$, 4.5 Hz, 1H), 1.72 – 1.66 (m, 4H), 1.48 – 1.40 (m, 2H).  

$^{13}$C NMR (150 MHz, CDCl3) δ 168.30, 138.72, 138.50, 138.45, 138.15, 137.79, 133.75, 131.99, 128.38, 128.21, 128.20, 128.17, 128.12, 128.01, 127.93, 127.79, 127.73, 127.65, 127.62, 127.47, 127.45, 127.38, 123.05, 103.49, 102.41, 82.68, 81.41, 80.87, 79.14, 76.52, 75.52, 75.14, 74.93, 74.74, 74.00, 73.05, 71.98, 69.51, 68.04, 66.99, 62.11, 37.71, 29.14, 28.25, 23.34.  

HRMS (ESI–TOF, M+Na+) calced for C60H65NO13Na+1030.4348, found 1030.4345.

**Phthalimidyl-aminopentyl-2,3-di-O-benzyl-6-deoxy-6-azido-β-D-galactopyranosyl-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (S13)**

To a solution of S12 (120 mg, 119 μmol) in pyridine (5 mL) and CH2Cl2 (5 mL) was added 4-toluenesulfonyl chloride (45.3 mg, 238 μmol) at 0 °C. The mixture was stirred under argon at room temperature for overnight, concentrated and purified by silica gel chromatography (EtOAc:hexane = 1:2). To a solution of purified compound in DMF (5 mL) was added sodium azide (78 mg, 1.2 mmol) and stirred at 100 °C for overnight. The mixture was concentrated and purified by silica gel chromatography (EtOAc:hexane = 1:2) to afford compound S13 (121 mg, 91% over 2 steps as a colorless oil.  

$^1$H NMR (600 MHz, CDCl3) δ 7.82 – 7.81 (m, 2H), 7.68 – 7.67 (m, 2H), 7.44 – 7.43 (m, 2H), 7.35 – 7.22 (m, 23H), 4.95 (d, $J = 10.9$ Hz, 1H), 4.87 (d, $J = 11.0$ Hz, 1H), 4.80 – 4.67 (m, 6H), 4.62 (d, $J = 12.1$ Hz, 1H), 4.42 – 4.37 (m, 3H). 4.01, (t, $J =
9.4 Hz, 1H), 3.94 (dt, \( J = 9.5, 6.5 \) Hz, 1H), 3.73 – 3.72 (m, 1H), 3.68 – 3.66 (m, 2H),
3.57 – 3.52 (m, 3H), 3.44 – 3.26 (m, 5H), 3.16 (t, \( J = 6.8 \) Hz, 1H), 1.74 – 1.69 (m, 4H),
1.49 – 1.43 (m, 2H). \(^{13}\)C NMR (150 MHz, CDCl\(_3\)) \( \delta \) 168.28, 139.12, 138.54, 138.40,
138.23, 137.57, 133.73, 131.98, 128.42, 128.18, 127.95, 127.94, 127.89, 127.86,
127.68, 127.66, 127.57, 127.48, 127.40, 127.39, 127.15, 123.03, 103.49, 102.06, 82.66,
81.52, 80.48, 78.97, 76.17, 75.10, 75.09, 74.91, 74.80, 73.03, 72.47, 72.18, 69.51, 68.02,
66.06, 49.93, 37.70, 29.15, 28.25, 23.33. HRMS (ESI–TOF, M+Na\(^+\)) calcd for
C\(_{60}\)H\(_{64}\)N\(_4\)O\(_{12}\)Na\(^+\) 1055.4413, found 1055.4445.

Phthalimidyl-aminopentyl-2,3,4,6-tetra-O-benzyl-\( \alpha \)-\( \beta \)-galactopyranosyl-(1→4)-
2,3-di-O-benzyl-6-deoxy-6-azido-\( \beta \)-\( \beta \)-galactopyranosyl-(1→4)-2,3,6-tri-O-benzyl-
\( \beta \)-d-glucopyranoside (S15)

To a solution of donor perbenzylated thioglycoside S14 (130 mg, 200.98 \( \mu \)mol) and
acceptor S13 (115 mg, 111.65 \( \mu \)mol) in CH\(_2\)Cl\(_2\) (5 mL) was added flame-dried
powdered molecular sieves (4Å, 500 mg) and stirred under argon at room temperature
for 1 h. The reaction mixture was cooled to -70 °C and was added NIS (62 mg, 279.13
\( \mu \)mol) and TfOH (70 \( \mu \)L, 33.5 \( \mu \)mol). The reaction was stirred under at -70 °C for 1h.
After complete consumption of the acceptor, the reaction mixture was filtered. The
filtrate was quenched with Na\(_2\)S\(_2\)O\(_4\) and the whole mixture was extracted with NaHCO\(_3\).
The organic layer was concentrated and purified by silica gel chromatography
(EtOAc:hexane = 1:4) to afford compound S15 (157 mg, 90%) as a colorless oil. \(^{1}\)H NMR
(600 MHz, CDCl\(_3\)) 7.84 – 7.82 (m, 2H), 7.71 – 7.69 (m, 2H), 7.40 – 7.12 (m,
45H), 5.02 (d, \( J = 10.9 \) Hz, 1H), 4.97 (d, \( J = 3.4 \) Hz, 1H), 4.85 – 4.67 (m, 8H), 4.60 –
4.42 (d, 6H), 4.36 – 4.33 (m, 2H), 4.29 – 4.27 (m, 1H), 4.17 – 4.12 (m, 2H), 4.09 – 4.04
(m, 2H), 3.99 – 3.90 (m, 3H), 3.86 – 3.83 (m, 2H), 3.80 – 3.76 (m, 1H), 3.72 – 3.66 (m,
3H), 3.61 – 3.49 (m, 4H), 3.41 – 3.34 (m, 3H), 3.25 (dd, \( J = 10.0, 2.8 \) Hz, 1 H), 3.19
(dd, \( J = 8.3, 4.6 \) Hz, 1H), 3.12 – 3.10 (m, 1H), 1.74 – 1.68 (m, 4H), 1.49 – 1.42 (m, 2H).
\(^{13}\)C NMR (150 MHz, CDCl\(_3\)) \( \delta \) 168.36, 139.03, 138.79, 138.62, 138.58, 138.37, 138.34,
138.31, 137.92, 137.79, 132.07, 128.31, 128.24, 128.21, 128.19, 128.16, 128.10,
128.03, 127.75, 127.66, 127.59, 127.56, 127.51, 127.43, 127.39, 127.37, 127.32,
127.17, 127.10, 123.11, 103.50, 102.48, 100.98, 82.64, 81.51, 81.10, 79.29, 79.11,
75.51, 75.20, 74.95, 74.91, 74.83, 74.56, 74.11, 73.20, 73.15, 72.99, 72.34, 72.06, 69.58,
68.18, 67.74, 49.58, 37.77, 29.66, 29.21, 28.33, 23.39, HRMS (ESI–TOF, M+Na\(^+\))
calcd for C_{94}H_{88}O_{17}Na^+1578.6852, found 1578.6918.

5-tert-Butylcarbamateaminopentyl-2,3,4,6-tetra-O-benzyl-α-D-galactopyranosyl-(1→4)-2,3-di-O-benzyl-6-deoxy-6-azido-β-D-galactopyranosyl-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (S16)

To a solution of S15 (250 mg, 160.69 μmol) in butanol (5 mL) was added hydrazine monohydrate (234 μL, 4.82 mmol) and stirred at 90 °C for overnight. The mixture was concentrated and directly put in next reaction without purification. To a solution of the resulting mixture (total from last step) in CH2Cl2 were added Di-tert-butyl dicarbonate (105 mg, 482.05 mmol) and pyridine (39 μL, 482.05 μmol) and stirred at room temperature for overnight. The reaction mixture was concentrated and purified by silica gel chromatography (EtOAc:hexane = 1:4) to afford compound S16 (201 mg, 82%) as a colorless oil. 1H NMR (600 MHz, CDCl3) δ 7.40 – 7.39 (m, 2H), 7.37 – 7.23 (m, 33H), 7.21 – 7.12 (m, 10H), 5.02 (d, J = 10.9 Hz, 1H), 4.96 (d, J = 3.5 Hz, 1H), 4.90 (d, J = 11.2 Hz, 1H), 4.86 – 4.80 (m, 3H), 4.77 – 4.69 (m, 5H), 4.59 (d, J = 12.1 Hz, 1H), 4.54 – 4.42 (m, 6H), 4.36 – 4.34 (m, 2H), 4.29 – 4.26 (m, 1H), 4.16 – 4.12 (m, 2H), 4.08 (dd, J = 10.1, 3.4 Hz, 1H), 3.99 – 3.90 (m, 3H), 3.86 – 3.83 (m, 2H), 3.78 (dd, J = 12.1, 8.1 Hz, 1H), 3.73 – 3.71 (m, 1H), 3.61 – 3.49 (m, 4H), 3.42 – 3.34 (m, 3H), 3.25 (dd, J = 10.0, 2.6 Hz, 1H), 3.19 (dd, J = 8.4, 4.7 Hz, 1H), 3.12 – 3.10 (m, 2H), 1.69 – 1.63 (m, 2H), 1.50 – 1.48 (m, 2H), 1.46 (s, 9H), 1.42 – 1.38 (m, 2H). 13C NMR (150 MHz, CDCl3) δ 139.02, 138.80, 138.65, 138.63, 138.37, 138.35, 138.33, 137.93, 128.32, 128.25, 128.23, 128.20, 128.11, 127.90, 127.77, 127.67, 127.60, 127.57, 127.53, 127.47, 127.45, 127.40, 127.38, 127.35, 127.18, 127.10, 103.53, 102.50, 101.02, 82.66, 81.55, 81.10, 79.32, 79.12, 75.54, 75.23, 75.20, 74.96, 74.89, 74.84, 74.56, 74.13, 73.24, 73.16, 73.00, 72.35, 72.07, 69.68, 69.60, 68.22, 67.76, 49.62, 29.74, 29.32, 28.40, 23.33. HRMS (ESI–TOF, M+H+) calcd for C_{91}H_{104}N_{4}O_{17}H^+1526.7502, found 1526.7539.

5-tert-Butylcarbamateaminopentyl-α-D-galactopyranosyl-(1→4)-6-deoxy-6-
amino-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (S17)

To a solution of S16 (200 mg, 131.07 μmol) in EtOAc, MeOH and AcOH (9 mL, 4:4:1) was added Pd(OH)$_2$ (200 mg) and stirred under hydrogen balloon at room temperature for overnight. The solid Pd(OH)$_2$ was filtered and the resulting solution was concentrated. To a solution of resulting mixture in MeOH, H$_2$O and AcOH (9 mL, 6:2:1) was added Pd(OH)$_2$ (200 mg) and stirred under hydrogen balloon at room temperature for another overnight. The solid Pd(OH)$_2$ was filtered and the resulting solution was concentrated. To a solution of resulting mixture in MeOH, H$_2$O and AcOH (9 mL, 4:4:1) was added Pd(OH)$_2$ (200 mg) and stirred under hydrogen balloon at room temperature for another overnight. After filtration, the resulting solution was concentrated and purified by reverse phase column chromatography (RP-18, 40% MeOH in H$_2$O with 1% AcOH) to afford compound S17 (41 mg, 45%) as a white foam.

$^1$H NMR (600 MHz, D$_2$O) δ 4.99 (d, $J = 3.6$ Hz, 1H), 4.53 (d, $J = 7.7$ Hz, 1H), 4.45 (d, $J = 8.0$ Hz, 1H), 4.06 (d, $J = 2.8$ Hz, 1H), 4.01 (d, $J = 2.4$ Hz, 1H), 3.98 – 3.96 (m, 2H), 3.91 – 3.87 (m, 3H), 3.73 – 3.65 (m, 5H), 3.63 – 3.52 (m, 3H), 3.48 (dd, $J = 13.6$, 7.2 Hz, 1H), 3.36 (dd, $J = 13.6$, 3.4 Hz, 1H), 3.27 (dd, $J = 9.2$, 8.2 Hz, 1H), 3.04 (t, $J = 6.7$ Hz, 2H), 1.64 – 1.59 (m, 2H), 1.50 – 1.45 (m, 2H), 1.40 (s, 9H), 1.37 – 1.33 (m, 2H).

$^{13}$C NMR (150 MHz, D$_2$O) δ 158.31, 102.75, 102.05, 79.97, 76.91, 74.85, 74.21, 73.03, 71.83, 71.35, 70.65, 70.44, 69.06, 68.77, 68.21, 60.49, 59.63, 40.04, 28.56, 28.32, 27.65, 22.25, 21.55. HRMS (ESI–TOF, M+H$^+$) calcd for C$_{28}$H$_{52}$N$_2$O$_{17}$H$^+$ 689.3339, found 689.3379.

6-Aza-7,12-dioxo-12-(4-nitrophenoxy) α-L-fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→3)-α-D-Galactopyranosyl-(1→4)-6-azido-6-deoxy-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (S18)

By following with the general procedure of synthesizing GH-analogs monoester (Supporting information 24), starting material 1 (5.2 mg, 4.6 μmol) was converted into compound S18 (3.9 mg, 61%) as a yellow foam.$^1$H NMR (600 MHz, D$_2$O) δ 8.36 (d, $J = 9.1$ Hz, 2H), 7.39 (d, $J = 9.1$ Hz, 2H), 5.23 (d, $J = 5.2$ Hz, 1H), 4.90 (d, $J = 3.9$ Hz, 1H), 4.61 (d, $J = 7.7$ Hz, 1H), 4.55 (d, $J = 7.8$ Hz, 1H), 4.51 (d, $J = 7.8$ Hz, 1H), 4.41 – 4.38 (m, 2H), 4.26 – 4.23 (m, 2H), 4.10 (d, $J = 2.2$ Hz, 1H), 4.01 – 4.93 (m, 5H), 3.89 – 3.85 (m, 4H), 3.84 – 4.54 (m, 21H), 3.48 – 3.45 (m, 1H), 3.28 (t, $J = 8.4$ Hz, 1H), 3.20 (t, $J
= 6.7 Hz, 2H), 2.73 (t, J = 7.0 Hz, 2H), 2.31 (t, J = 6.5 Hz, 2H), 2.04 (s, 3H), 1.75 – 1.73 (m, 4H), 1.64 – 1.59 (m, 2H), 1.55 – 1.51 (m, 2H), 1.40 – 1.35 (m, 2H), 1.22 (d, J = 6.54 Hz, 3H). 13C NMR (150 MHz, D2O) δ 176.31, 174.33, 174.26, 155.18, 145.44, 125.58, 122.84, 103.98, 103.28, 102.00, 101.96, 100.63, 99.24, 79.17, 78.27, 77.29, 76.31, 76.07, 75.02, 74.59, 74.50, 73.53, 73.40, 72.81, 71.91, 71.81, 70.66, 70.36, 70.07, 69.47, 69.17, 69.07, 68.43, 67.99, 67.86, 66.75, 60.93, 60.88, 60.25, 60.01, 51.61, 50.22, 39.06, 35.36, 33.33, 28.30, 27.95, 24.70, 23.32, 22.36, 22.21, 15.28. HRMS (ESI–TOF, M+H+) calcd for C55H86N6O34H+ 1375.5258, found 1375.5265.

6-Aza-7,12-dioxo-12-(4-nitrophenoxy)

α-L-fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→3)-6-azido-6-deoxy-α-D-Galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (S19)

By following with the general procedure of synthesizing GH-analogs monoester (Supporting information 24), starting material 2 (8.0 mg, 7.1 μmol) was converted into compound S19 (6.9 mg, 71%) as yellow foam. 1H NMR (600 MHz, D2O) δ 8.35 (d, J = 9.0 Hz, 2H), 7.39 (d, J = 8.9 Hz, 2H), 5.23 (d, J = 3.8 Hz, 1H), 4.89 (d, J = 3.5 Hz, 1H), 4.61 (d, J = 7.7 Hz, 1H), 4.56 – 4.53 (m, 2H), 4.49 (d, J = 7.7 Hz, 1H), 4.40 (d, J = 8.0 Hz, 1H), 4.26 – 4.20 (m, 2H), 4.09 (m, 1H), 4.04 (d, J = 2.3 Hz, 1H), 3.99 – 3.94 (m, 4H), 3.90 – 3.72 (m, 14H), 3.72 – 3.69 (m, 2H), 3.65 – 3.50 (m, 9H), 3.42 – 3.39 (m, 1H), 3.27 (t, J = 8.3 Hz, 1H), 3.20 (t, J = 6.7 Hz, 2H), 2.73 (t, J = 6.7 Hz, 2H), 2.30 (t, J = 6.1 Hz, 2H), 2.04 (s, 3H), 1.73 – 1.73 (m, 4H), 1.62 – 1.60 (m, 2H), 1.54 – 1.52 (m, 2H), 1.38 – 1.37 (m, 2H), 1.21 (d, J = 6.4 Hz, 3H). 13C NMR (150 MHz, D2O) δ 176.31, 174.34, 174.26, 155.17, 145.43, 125.57, 122.82, 103.96, 103.32, 102.00, 101.94, 100.15, 99.25, 78.78, 77.93, 76.56, 76.30, 76.08, 75.53, 75.02, 74.72, 74.55, 74.51, 73.53, 72.88, 71.97, 71.81, 70.77, 70.34, 69.69, 69.47, 69.06, 68.92, 68.43, 67.98, 67.62, 66.75, 60.93, 60.86, 60.18, 60.00, 51.58, 50.64, 39.07, 35.35, 33.33, 28.30, 27.95, 24.70, 23.33, 22.36, 22.20, 15.27. HRMS (ESI–TOF, M+H+) calcd for C55H86N6O34H+ 1375.5258, found 1375.5241.
6-Aza-7,12-dioxo-12-(4-nitrophenoxy)

α-L-fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→3)-6-azido-6-deoxy-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→3)-α-D-Galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (S20)

By following with the general procedure of synthesizing GH-analogs monoester (Supporting information 24), starting material 3 (8 mg, 7.1 μmol) was converted into compound S20 (6.6 mg, 68%) as yellow foam. 

1H NMR (600 MHz, D2O) δ 8.36 (d, J = 8.9 Hz, 2H), 7.39 (d, J = 8.9 Hz, 2H), 5.22 (d, J = 4.0 Hz, 1H), 4.89 (d, J = 3.8 Hz, 1H), 4.67 (d, J = 7.7 Hz, 1H), 4.55 (d, J = 8.2 Hz, 1H), 4.49 (d, J = 7.7 Hz, 1H), 4.42 – 4.37 (m, 2H), 4.24 – 4.22 (m, 2H), 4.05 – 4.00 (m, 3H), 4.98 – 3.90 (m, 4H), 6.88 – 3.81 (m, 6H), 3.79 – 3.67 (m, 10H), 3.65 – 3.58 (m, 6H), 3.54 (t, J = 8.9 Hz, 1H), 3.49 (m, 1H), 3.26 (t, J = 8.7 Hz, 1H), 3.21 – 3.19 (m, 3H), 2.73 (t, J = 6.8 Hz, 2H), 2.31 (t, J = 6.1 Hz, 2H), 2.05 (s, 3H), 1.73 (m, 4H), 1.62 – 1.60 (m, 2H), 1.54 – 1.51 (m, 2H), 1.39 – 1.37 (m, 2H), 1.22 (d, J = 6.5 Hz, 3H). 13C NMR (150 MHz, D2O) δ 176.30, 174.31, 155.20, 145.43, 125.60, 122.86, 103.81, 103.42, 102.03, 101.94, 100.55, 99.23, 79.08, 77.70, 77.26, 76.10, 76.03, 75.45, 75.04, 74.66, 74.52, 73.70, 73.52, 72.83, 72.12, 71.81, 70.80, 70.38, 70.16, 69.47, 69.37, 69.08, 69.00, 67.98, 67.85, 66.75, 66.75, 60.95, 60.25, 60.22, 60.00, 51.45, 50.85, 39.05, 35.36, 33.34, 30.18, 28.30, 27.96, 24.70, 23.32, 22.36, 15.28. HRMS (ESI–TOF, M+H+) calcd for C55H86N6O34H+ 1375.5258, found 1375.5283.

6-Aza-7,12-dioxo-12-(4-nitrophenoxy)

α-L-fucopyranosyl-(1→2)-6-azido-6-deoxy-β-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 (S21)

By following with the general procedure of synthesizing GH-analogs monoester (Supporting information 24), starting material 4 (7.7 mg, 6.8 μmol) was converted into compound S21 (6.0 mg, 64%) as yellow foam. 1H NMR (600 MHz, D2O) δ 8.36 (d, J =
9.1 Hz, 2H), 7.39 (d, J = 9.1 Hz, 2H), 5.22 (d, J = 4.0 Hz, 1H), 4.89 (d, J = 3.8 Hz, 1H), 4.66 (d, J = 7.7 Hz, 1H), 4.55 (d, J = 8.2 Hz, 1H), 4.49 (d, J = 7.8 Hz, 1H), 4.41 (t, J = 6.5 Hz, 1H), 4.38 (d, J = 8.0 Hz, 1H), 4.25 – 4.22 (m, 2H), 4.05 – 3.90 (m, 8H), 3.88 – 3.58 (m, 23H), 3.54 (t, J = 9 Hz, 1H), 3.26 (t, J = 8.8 Hz, 1H), 3.20 (t, J = 6.1 Hz, 2H), 2.73 (t, J = 6.9 Hz, 2H), 2.31 (t, J = 6.2 Hz, 2H), 2.05 (s, 3H), 1.73 (m, 4H), 1.64 – 1.59 (m, 2H), 1.56 – 1.51 (m, 2H), 1.40 – 1.36 (m, 2H), 1.22 (d, J = 6.5 Hz, 3H).

$^{13}$C NMR (150 MHz, D$_2$O) δ 176.31, 174.32, 174.28, 155.20, 145.43, 125.60, 122.85, 103.93, 103.34, 101.95, 100.46, 99.29, 78.91, 78.22, 77.18, 76.27, 75.90, 75.42, 74.68, 74.61, 74.52, 74.37, 73.30, 72.83, 72.10, 71.81, 70.82, 70.36, 70.13, 69.56, 69.47, 69.19, 68.40, 67.96, 67.84, 66.77, 60.83, 60.31, 59.99, 51.57, 50.98, 39.06, 33.34, 28.29, 27.95, 24.71, 23.32, 22.36, 22.21, 15.27. HRMS (ESI–TOF, M+H$^+$) calcd for C$_{55}$H$_{86}$N$_6$O$_{34}$H$^+$ 1375.5258, found 1375.5293.

6-Aza-7,12-dioxo-12-(4-nitrophenoxy) 6-deoxy-6-azido-α-L-galactopyranosyl-(1→2)-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→3)-α-D-Galactopyranosyl-(1→4)-6-deoxy-6-azido-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (S23)

By following with the general procedure of synthesizing GH-analogs monoester (Supporting information 24), starting material 5 (5.0 mg, 4.3 μmol) was converted into compound S23 (3.9 mg, 64%) as yellow foam.$^1$H NMR (600 MHz, D$_2$O) δ 8.36 (J = 9.1 Hz, 2H), 7.39 (d, J = 9.0 Hz, 2H), 5.42 (d, J = 3.8 Hz, 1H), 4.90 (d, J = 3.7 Hz, 1H), 4.62 (d, J = 7.5 Hz, 1H), 4.57 (d, J = 8.3 Hz, 1H), 4.51 (d, J = 7.8 Hz, 1H), 4.41 – 4.38 (m, 2H), 4.27 – 4.26 (m, 2H), 4.14 (d, J = 2.4 Hz, 1H), 4.07 – 4.05 (m, 1H), 4.01 – 3.93 (m, 4H), 3.90 – 3.85 (m, 6H), 3.82 – 3.54 (m, 20H), 3.47 – 3.45 (m, 1H), 3.35 – 3.27 (m, 1H), 3.20 (t, J = 6.6 Hz, 2H), 2.73 (t, J = 6.9 Hz, 2H), 2.31 (t, J = 6.1 Hz, 2H), 2.05 (s, 3H), 1.73 (m, 4H), 1.64 – 1.59 (m, 2H), 1.56 – 1.51 (m, 2H), 1.40 – 1.36 (m, 2H).

$^{13}$C NMR (150 MHz, D$_2$O) δ 176.32, 174.36, 174.33, 155.19, 145.45, 125.59, 122.85, 103.91, 103.29, 102.26, 101.96, 100.63, 98.41, 79.19, 78.40, 77.30, 76.94, 75.09, 74.68, 74.60, 74.51, 74.41, 73.82, 73.40, 72.81, 71.92, 70.67, 70.37, 70.09, 69.66, 69.60, 69.10, 69.03, 68.52, 68.01, 67.84, 60.92, 60.85, 60.25, 60.01, 51.38, 51.23, 50.23, 39.06, 33.34, 30.18, 28.30, 27.95, 24.71, 23.32, 23.20, 22.36, 22.31. HRMS (ESI–TOF, M+H$^+$) calcd for C$_{55}$H$_{85}$N$_9$O$_{34}$H$^+$ 1416.5272, found 1416.5289.
6-Aza-7,12-dioxo-12-(4-nitrophenoxy) 6-deoxy-6-azido-α-L-galactopyranosyl-(1→2)-β-D-galactopyranosyl-(1→3)-6-deoxy-6-azido-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→3)-α-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (S24)

By following with the general procedure of synthesizing GH-analogs monoester (Supporting information 24), starting material 6 (6.8 mg, 5.8 μmol) was converted into compound S24 (4.7 mg, 57%) as yellow foam. ¹H NMR (600 MHz, D₂O) δ 8.36 (d, J = 9.1 Hz, 2H), 7.39 (d, J = 9.1 Hz, 2H), 5.43 (d, J = 3.9 Hz, 1H), 4.89 (d, J = 3.8 Hz, 1H), 4.62 (d, J = 7.4 Hz, 1H), 4.61 (d, J = 8.6 Hz, 1H), 4.49 (d, J = 7.7 Hz, 1H), 4.41 (t, J = 6.4 Hz, 1H), 4.37 (d, J = 8.0 Hz, 1H), 4.27 – 4.24 (m, 2H), 4.11 – 4.06 (m, 2H), 4.01 – 3.81 (m, 13H), 3.80 – 3.72 (m, 6H), 3.70 – 3.58 (m, 9H), 3.54 (t, J = 8.9 Hz, 1H), 3.44 – 3.42 (m, 1H), 3.34 (dd, J = 13, 3.6 Hz, 1H), 3.28 – 3.25 (m, 2H), 3.20 (t, J = 6.5 Hz, 2H), 2.73 (t, J = 6.8 Hz, 2H), 2.31 (t, J = 6.5 Hz, 2H), 2.06 (s, 3H), 1.74 – 1.70 (m, 4H), 1.64 – 1.59 (m, 2H), 1.56 – 1.51 (m, 2H), 1.40 – 1.36 (m, 2H). ¹³C NMR (150 MHz, D₂O) δ 176.31, 174.41, 174.31, 155.21, 145.44, 125.60, 122.86, 103.77, 103.42, 102.30, 101.95, 100.55, 98.41, 79.09, 77.81, 77.27, 76.77, 75.45, 75.09, 74.68, 74.66, 74.52, 73.82, 73.53, 72.83, 72.13, 70.81, 70.38, 70.19, 69.67, 69.61, 69.30, 69.11, 69.03, 68.00, 67.85, 60.93, 60.26, 60.01, 51.21, 50.84, 39.05, 35.36, 33.34, 30.18, 28.30, 27.96, 24.70, 23.32, 22.36, 22.28. HRMS (ESI-TOF, M+H⁺) calcd for C₅₅H₈₅N₉O₃₄H⁺1416.5272, found 1416.5285.

5-chloropentyl 6-deoxy-6-azido-α-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (7)

By following with the general method of synthesizing azide-modified Globo H precursors (supporting information 11), starting material S7 (73 mg, 120.1 μmol) was converted into compound 7 (40 mg, 53%) as a white foam. ¹H NMR (600 MHz, D₂O) δ 4.94 (d, J = 4.0 Hz, 1H), 4.51 (t, J = 7.8 Hz, 1H), 4.51 (d, J = 7.8 Hz, 1H), 4.48 (d, J = 8.0 Hz, 1H), 4.05 (d, J = 3.2 Hz, 1H), 4.00 – 3.98 (m, 2H), 3.95 – 3.90 (m, 3H), 3.85
– 3.81 (m, 3H), 3.79 – 3.75 (m, 2H), 3.71 – 3.67 (m, 1H), 3.66 – 3.62 (m, 4H), 3.61 –
3.52 (m, 3H), 3.43 (dd, J = 12.9, 5.3 Hz, 1H), 3.29 (t, J = 8.5 Hz, 1H), 1.83 – 1.78 (m,
2H), 1.68 – 1.64 (m, 2H), 1.53 – 1.48 (m, 2H). $^{13}$C NMR (150 MHz, D$_2$O) δ 103.24,
101.95, 99.99, 78.56, 76.65, 75.49, 74.77, 74.46, 72.90, 72.00, 70.82, 70.32, 69.51,
69.41, 68.85, 68.31, 60.22, 60.01, 50.80, 45.46, 31.51, 27.97, 22.51. HRMS (ESI–TOF,
M+Na$^+$) calcd for C$_{23}$H$_{40}$ClN$_3$O$_{15}$Na$^+$ 656.2040, found 656.2049.

5-chloropentyl

6-deoxy-6-azido-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→3)-α-D-
galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (8)

By following with the general method of synthesizing azide-modified Globo H
precursors (supporting information 11), starting material S8 (97 mg, 119.6 µmol) was
converted into compound 8 (62.8 mg, 62%) as a white foam. $^1$H NMR (600 MHz, D$_2$O)
δ 4.94 (d, J = 3.7 Hz, 1H), 4.72 (d, J = 8.5 Hz, 1H), 4.55 (d, J = 7.8 Hz, 1H), 4.52 (d, J
= 8.0 Hz, 1H), 4.42 (t, J = 6.3 Hz, 1H), 4.27 (d, J = 1.9 Hz, 1H), 4.06 – 3.94 (m, 8H),
3.90 – 3.76 (m, 6H), 3.73 – 3.66 (m, 8H), 3.64 – 3.61 (m, 2H), 3.36 – 3.32 (m, 2H),
2.08 (s, 3H), 1.87 – 1.82 (m, 2H), 1.72 – 1.67 (m, 2H), 1.57 – 1.52 (m, 2H). $^{13}$C NMR
(150 MHz, D$_2$O) δ 175.21, 103.34, 103.12, 101.98, 100.50, 78.90, 78.09, 77.24, 75.46,
74.77, 74.53, 73.90, 72.94, 72.12, 70.87, 70.58, 70.34, 70.32, 69.21, 68.30, 67.68, 60.35,
60.26, 60.10, 52.46, 50.92, 45.54, 31.54, 28.01, 22.56, 22.25. HRMS (ESI–TOF,
M+Na$^+$) calcd for C$_{31}$H$_{53}$ClN$_4$O$_{20}$Na$^+$ 859.2834, found 859.2838.

5-chloropentyl

6-deoxy-6-azido-β-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 (9)
By following with the general method of synthesizing azide-modified Globo H precursors (supporting information 11), starting material S9 (67 mg, 68.8 μmol) was converted into compound 9 (36 mg, 52%) as a white foam. $^1$H NMR (600 MHz, D$_2$O) $\delta$ 4.92 (d, $J$ = 3.8 Hz, 1H), 4.70 (d, $J$ = 8.5 Hz, 1H), 4.51 (d, $J$ = 7.8 Hz, 1H), 4.49 (d, $J$ = 7.7 Hz, 1H), 4.49 (d, $J$ = 8.0 Hz, 1H), 4.39 (t, $J$ = 6.5 Hz, 1H), 4.25 (d, $J$ = 2.5 Hz, 1H), 4.18 (d, $J$ = 2.9 Hz, 1H), 4.09 – 4.04 (m, 2H), 4.00 – 3.90 (m, 6H), 3.88 – 3.78 (m, 6H), 3.76 – 3.68 (m, 6H), 3.66 – 3.62 (m, 6H), 3.60 – 3.57 (m, 6H), 3.54 (dd, $J$ = 9.7, 7.8 Hz, 1H), 3.32 – 3.29 (m, 2H), 2.04 (s, 3H), 1.84 – 1.79 (m, 2H), 1.69 – 1.64 (m, 2H), 1.54 – 1.49 (m, 2H). $^{13}$C NMR (150 MHz, D$_2$O) $\delta$ 175.12, 104.79, 103.25, 102.87, 101.95, 100.37, 79.37, 78.70, 77.16, 75.39, 74.76, 74.62, 74.49, 73.83, 72.91, 72.18, 72.07, 70.84, 70.39, 70.31, 70.23, 69.01, 68.89, 68.09, 67.58, 60.94, 60.37, 60.23, 60.27, 60.23, 51.44, 51.01, 45.51, 31.53, 27.99, 22.54, 22.25. HRMS (ESI–TOF, M+Na$^+$) calcd for C$_{37}$H$_{63}$ClN$_4$O$_{25}$Na$^+$ 1021.3362, found 1021.3374.

5-chloropentyl 6-deoxy-6-azido-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (10)

By following with the general method of synthesizing azide-modified Globo H precursors (supporting information 11), starting material 13 (67 mg, 68.8 μmol) was converted into compound 10 (49 mg, 47%) as a white foam. $^1$H NMR (600 MHz, D$_2$O) $\delta$ 4.51 (d, $J$ = 8.0 Hz, 1H), 4.51 (d, $J$ = 7.9 Hz, 1H), 4.01 (dd, 12.3, 2.1 Hz, 1H), 3.97 – 3.65 (m, 2H), 3.88 – 3.82 (m, 2H), 3.74 – 7.66 (m, 6H), 3.65 – 3.56 (m, 4H), 3.37 – 3.34 (m, 1H), 1.87 – 1.82 (m, 2H), 1.72 – 1.67 (m, 2H), 1.57 – 1.52 (m, 2H). $^{13}$C NMR (150 MHz, D$_2$O) $\delta$ 102.92, 102.04, 78.77, 74.64, 74.40, 73.25, 72.81, 72.37, 70.73, 70.37, 68.82, 60.13, 50.84, 45.57, 31.56, 28.03, 22.57. HRMS (ESI–TOF, M+Na$^+$) calcd for C$_{17}$H$_{30}$ClN$_3$O$_{16}$Na$^+$ 494.1512, found 494.1466.

5-chloropentyl-6-deoxy-6-aldehyde-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (11)

By following with the general procedure of synthesizing aldehyde-modified Globo H precursors (supporting information 5), starting material Lac (10 mg, 22.5 μmol) was
converted into compound 11 in 80% (determination by crude NMR) yield as a light yellow foam. $^1$H NMR (600 MHz, D$_2$O) δ 5.14 (d, $J = 7.4$ Hz, 1H), 4.33 ($J = 7.2$ Hz, 1H), 4.46 ($J = 7.9$ Hz, 1H), 4.09 ($J = 3.1$ Hz, 1H), 3.98 – 3.91 (m, 2H), 3.81 – 3.78 (m, 1H), 3.71 – 3.54 (m, 8H), 3.46 (d, $J = 7.3$ Hz, 1H), 3.31 (t, $J = 8.5$ Hz, 1H), 1.83 – 1.78 (m, 2H), 1.68 – 1.64 (m, 2H), 1.53 – 1.48 (m, 2H). $^{13}$C NMR (150 MHz, D$_2$O) δ 103.18, 101.91, 87.93, 79.38, 76.88, 74.56, 72.72, 72.39, 70.63, 70.32, 67.94, 60.15, 45.46, 31.51, 27.97, 22.51. HRMS (ESI–TOF, M+Na$^+$) calcd for C$_{17}$H$_{31}$ClO$_{12}$Na$^+$ 485.1396, found 485.1399.

5-chloropentyl 6-deoxy-6-benzylamino-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (12)

By following with the general method of synthesizing benzylamine-modified Globo H precursors (supporting information 7), starting material 11 (220 mg, 494.5 μmol) was converted into compound 12 (211 mg, 80%) as a light yellow foam. $^1$H NMR (600 MHz, D$_2$O) δ 7.47 – 7.40 (m, 5H), 4.49 (d, $J = 8.0$ Hz, 1H), 4.44 (d, $J = 7.9$ Hz, 1H), 4.00 (d, $J = 12.4$, 2.0 Hz, 1H), 3.97 – 3.93 (m, 2H), 3.86 (d, $J = 3.2$ Hz, 1H), 3.84 – 3.80 (m, 2H), 3.79 – 3.76 (m, 2H), 3.73 – 3.70 (m, 1H), 3.65 – 3.61 (m, 4H), 3.61 – 3.57 (m, 1H), 3.55 (dd, $J = 9.8$, 7.8 Hz, 1H), 3.30 (t, $J = 8.5$ Hz, 1H), 2.98 (dd, $J = 13.0$, 9.1 Hz, 1H), 2.82 (dd, $J = 13.0$, 3.4 Hz, 1H), 1.86 – 1.81 (m, 2H), 1.70 – 1.66 (m, 2H), 1.56 – 1.52 (m, 2H). $^{13}$C NMR (150 MHz, D$_2$O) δ 128.75, 128.61, 127.53, 102.50, 102.11, 77.30, 74.93, 74.20, 72.99, 72.94, 72.62, 70.98, 70.35, 69.55, 59.97, 52.15, 48.08, 45.52, 31.54, 28.00, 22.55. HRMS (ESI–TOF, M+H$^+$) calcd for C$_{24}$H$_{38}$ClNO$_{10}$H$^+$ 536.2257, found 536.2257.

5-chloropentyl 6-deoxy-6-amino-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (13)

By following with the general method of synthesizing amine-modified Globo H precursors (supporting information 9), starting material 12 (211 mg, 393.7 μmol) was converted into compound 13 (100 mg, 57%) as a light yellow foam. $^1$H NMR (600 MHz, D$_2$O) δ 4.53 (d, $J = 7.9$ Hz, 1H), 4.51 (d, $J = 8.0$ Hz, 1H), 4.01 (dd, $J = 12.2$, 1.6 Hz,
1H), 3.97 – 3.92 (m, 3H), 3.85 (dd, J = 12.4, 4.6 Hz, 1H), 3.76 – 3.70 (m, 3H), 3.68 – 3.65 (m, 3H), 3.60 – 3.57 (m, 2H), 3.36 – 3.28 (m, 3H), 1.86 – 1.81 (m, 2H), 1.71 – 1.66 (m, 2H), 1.56 – 1.51 (m, 2H).\(^{13}\)C NMR (150 MHz, D\(_2\)O) δ 102.29, 102.16, 76.17, 74.89, 74.24, 72.97, 72.17, 70.91, 70.75, 70.40, 69.13, 59.71, 59.71, 45.53, 40.31, 31.53, 28.01, 22.55. HRMS (ESI–TOF, M+H\(^+\)) calcd for C\(_{17}\)H\(_{32}\)ClNO\(_{10}\)H\(^+\) 446.1788, found 446.1789.

5-\textit{tert}-Butylcarbamateaminopentyl-\(\alpha\)-\(\delta\)-galactopyranosyl-(1→4)-6-deoxy-6-azido-\(\beta\)-\(\delta\)-galactopyranosyl-(1→4)-\(\beta\)-\(\delta\)-glucopyranoside (15)

To a solution of NaN\(_3\) (595 mg, 9.15 mmol) in ddH\(_2\)O (1.5 mL) and CH\(_2\)Cl\(_2\) was added Tf\(_2\)O (400 μL, 845 mmol) and stirred at 0 °C for 2 h. The organic phase was separated and the water phase was extracted with CH\(_2\)Cl\(_2\) for two times. The collected organic layer was extracted with saturated NaHCO\(_3\) solution and can be used without further purification. To a solution of starting material S17 (40 mg, 58 μmol) in ddH\(_2\)O (1 mL) was added K\(_2\)CO\(_3\) (100 mg, 0.72 mmol) and CuSO\(_4\) hydrate (10 mg, 62.9 μmol). The mixture was added MeOH and previously prepared TfN\(_3\) solution. More MeOH was added until the mixture was homogeneous. The mixture was stirred for another 16 h, concentrated, purified by reverse phase column chromatography (RP-18) to afford compound 15. Yield: 73 %, white foam. \(^1\)H NMR (600 MHz, D\(_2\)O) δ 4.96 (d, J = 3.9 Hz, 1H), 4.53 (d, J = 7.9 Hz, 1H), 4.47 (d, J = 8.0 Hz, 1H), 4.35 (t, J = 6.4 Hz, 1H), 4.04 – 3.97 (m, 3H), 3.94 – 3.88 (m, 3H), 3.86 – 3.80 (m, 2H), 3.79 – 3.73 (m, 2H), 3.71 – 3.62 (m, 6H), 3.59 – 3.56 (m, 2H), 3.30 (t, J = 8.5 Hz, 1H), 3.07 (t, J = 6.7 Hz, 2H), 1.66 – 1.61 (m, 2H), 1.52 – 1.47 (m, 2H), 1.42 (s, 9H), 1.40 – 1.35 (m, 2H). \(^{13}\)C NMR (150 MHz, D\(_2\)O) δ 158.36, 103.18, 101.96, 100.45, 78.91, 77.37, 74.67, 74.47, 73.39, 72.85, 71.95, 70.76, 70.72, 70.41, 69.06, 68.87, 68.57, 60.40, 60.03, 50.23, 39.81, 28.55, 28.33, 27.65, 27.57, 22.26. HRMS (ESI–TOF, M+H\(^+\)) calcd for C\(_{28}\)H\(_{50}\)N\(_4\)O\(_{17}\)H\(^+\) 715.3244, found 715.3258.
5-tert-Butylcarbamateaminopentyl 2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→3)-α-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (16)

To a solution of 15 (22 mg, 30.8 μmol) in the working solution described in the general procedure of synthesizing Gb4 analogs (supporting information 14) were added MalE-LgtD (1.08 mg), NahK (560 μg), GlmU (970 μg), PK (90 μg) and PPA (80 μg). The reaction was followed with the general procedure described above to afford compound 16 (20.3 mg, 72%) as a white foam.

$^1$H NMR (600 MHz, D$_2$O) δ 4.92 (d, $J = 3.9$ Hz, 1H), 4.63 (d, $J = 8.5$ Hz, 1H), 4.54 (d, $J = 7.8$ Hz, 1H), 4.47 (d, $J = 8.0$ Hz, 1H), 4.38 (t, $J = 6.5$ Hz, 1H), 4.26 (d, 2.7 Hz, 1H), 4.00 – 3.88 (m, 8H), 3.83 – 3.73 (m, 6H), 3.71 – 3.62 (m, 7H), 3.60 – 3.56 (m, 2H), 3.31 (t, $J = 8.5$ Hz, 1H), 3.07 (t, $J = 6.7$ Hz, 1H), 2.04 (s, 3H), 1.66 – 1.61 (m, 2H), 1.52 – 1.47 (m, 2H), 1.42 (s, 9H), 1.35 – 1.35 (m, 2H).

$^{13}$C NMR (150 MHz, D$_2$O) δ 175.14, 158.35, 103.24, 103.21, 101.94, 100.55, 80.75, 79.01, 78.70, 77.22, 74.89, 74.67, 74.50, 73.40, 72.87, 71.87, 70.73, 70.68, 70.41, 70.15, 68.89, 67.71, 67.62, 60.96, 60.25, 60.04, 52.55, 50.19, 39.81, 28.55, 28.33, 27.65, 22.25, 22.23. HRMS (ESI–TOF, M+H$^+$) calcd for C$_{36}$H$_{63}$N$_5$O$_{22}$H$^+$ 918.4037, found 918.4057.

5-chloropentyl 2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→3)-6-deoxy-6-azido-α-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (17)

To a solution of 7 (29 mg, 47.4 μmol) in the working solution described in the general procedure of synthesizing Gb4 analogs (supporting information 14) were added MalE-LgtD (1.83 mg), NahK (950 μg), GlmU (1.64 mg), PK (160 μg) and PPA (130 μg). The reaction was followed with the general procedure described above to afford compound 17 (29.0 mg, 74%) as a white foam.

$^1$H NMR (600 MHz, D$_2$O) δ 4.90 (d, $J = 3.8$ Hz, 1H), 4.62 (d, $J = 8.5$ Hz, 1H), 4.54 (t, $J = 6.4$ Hz, 1H), 4.51 (d, $J = 7.8$ Hz, 1H), 4.48 (d, $J = 8.0$ Hz, 1H), 4.21 (d, $J = 2.6$ Hz, 1H), 4.05 (d, $J = 3.0$ Hz, 1H), 4.00 – 3.86 (m, 7H),
3.85 – 3.73 (m, 7H), 3.71 – 3.62 (m, 6H), 3.60 – 3.57 (m, 2H), 3.51 (dd, J = 12.8, 7.7 Hz, 1H), 3.41 (dd, J = 12.8, 5.4 Hz, 1H), 3.30 (t, J = 8.3 Hz, 1H), 2.04 (s, 3H), 1.83 – 1.78 (m, 2H), 1.68 – 1.63 (m, 2H), 1.53 – 1.48 (m, 2H). 13C NMR (150 MHz, D2O) δ 175.13, 103.27, 103.22, 101.94, 100.09, 78.67, 78.38, 76.55, 45.51, 74.86, 74.77, 74.50, 72.93, 71.95, 70.80, 70.74, 70.31, 69.41, 69.02, 67.71, 67.38, 60.94, 60.17, 60.03, 52.53, 50.64, 45.48, 31.51, 27.98, 22.52, 22.22. HRMS (ESI–TOF, M+H+) calcd for C31H53ClN4O20 H+ 837.3014, found 837.3039.

5-tert-Butylcarbamateaminopentyl β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→3)-α-D-galactopyranosyl-(1→4)-6-deoxy-6-azido-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (18)

To a solution of 16 (65 mg, 71.2 μmol) in the working solution described in the general procedure of synthesizing SSEA3 analogs (supporting information 16) were added MalE-LgtD (8.25 mg), GalK (370 μg), AtUSP (610 μg), PK (240 μg), PPA (200 μg). The reaction was followed with the general procedure described above to afford compound 18 (60.0 mg, 79%) as a white foam. 1H NMR (600 MHz, D2O) δ 4.92 (d, J = 3.9 Hz, 1H), 4.69 (d, J = 8.5 Hz, 1H), 4.54 (d, J = 7.9 Hz, 1H), 4.47 (d, J = 8.0 Hz, 1H), 4.45 (d, J = 7.8 Hz, 1H), 4.38 (t, J = 6.5 Hz, 1H), 4.26 (d, J = 2.6 Hz, 1H), 4.18 (d, J = 3.0 Hz, 1H), 4.06 (dd, J = 8.6, 2.2 Hz, 1H), 4.00 – 3.96 (m, 3H), 3.94 – 3.89 (m, 5H), 3.83 – 3.73 (m, 7H), 3.71 – 3.64 (m, 8H), 3.64 – 3.56 (m, 3H), 3.52 (dd, J = 7.9, 2.0 Hz, 1H), 3.31 (t, J = 8.3 Hz, 1H), 3.06 (t, J = 6.7 Hz, 2H), 2.02 (s, 3H), 1.66 – 1.61 (m, 2H), 1.52 – 1.35 (m, 13H). 13C NMR (150 MHz, D2O) δ 175.10, 158.34, 104.78, 103.21, 102.94, 101.95, 100.53, 80.73, 79.54, 79.01, 78.70, 77.20, 74.95, 74.87, 74.56, 74.50, 73.41, 72.87, 72.41, 71.88, 70.69, 70.55, 70.41, 70.15, 68.88, 68.53, 67.64, 67.62, 60.96, 60.92, 60.26, 60.05, 51.44, 50.18, 39.81, 28.56, 28.34, 27.67, 22.25. HRMS (ESI–TOF, M+H+) calcd for C42H73N5O27 H+ 1080.4566, found 1080.4590.

5-chloropentyl β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-
galactopyranosyl-(1→3)-6-deoxy-6-azido-α-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (19)

To a solution of 17 (29 mg, 34.6 μmol) in the working solution described in the general procedure of synthesizing SSEA3 analogs (supporting information 16) were added MalE-LgtD (5.36 mg), GalK (180 μg), AtUSP (290 μg), PK (110 μg), PPA (100 μg). The reaction was followed with the general procedure described above to afford compound 19 (27.0 mg, 80%) as a white foam. ¹H NMR (600 MHz, D₂O) δ 4.90 (d, J = 3.9 Hz, 1H), 4.68 (d, J = 8.5 Hz, 1H), 4.54 (t, J = 6.5 Hz, 1H), 4.51 (d, J = 7.8 Hz, 1H), 4.48 (d, J = 8.0 Hz, 1H), 4.46 (d, J = 7.7 Hz, 1H), 4.20 (d, J = 2.8 Hz, 1H), 4.18 (d, J = 3.1 Hz, 1H), 4.07 – 4.04 (m, 2H), 4.00 – 3.98 (m, 2H), 3.94 – 3.87 (m, 5H), 3.85 – 3.81 (m, 2H), 3.80 – 3.73 (m, 6H), 3.72 – 3.68 (m, 2H), 3.67 – 3.62 (m, 5H), 3.61 – 3.57 (m, 3H), 3.54 – 3.50 (m, 2H), 3.40 (dd, J = 13.0, 5.5 Hz, 1H), 3.30 (t, J = 8.5 Hz, 1H), 2.02 (s, 3H), 1.83 – 1.78 (m, 2H), 1.68 – 1.63 (m, 2H), 1.53 – 1.48 (m, 2H). ¹³C NMR (150 MHz, D₂O) δ 175.10, 104.78, 103.27, 102.92, 101.94, 100.08, 79.53, 78.67, 78.36, 76.53, 75.51, 74.95, 74.77, 74.52, 74.50, 72.93, 72.40, 71.94, 70.79, 70.54, 70.31, 69.40, 69.02, 68.52, 67.94, 67.37, 60.95, 60.89, 60.16, 60.03, 51.41, 50.63, 45.47, 31.51, 27.98, 22.52, 22.23. HRMS (ESI–TOF, M+H⁺) calcd for C₃₇H₆₃ClN₄O₂₅H⁺ 999.3543, found 999.3563.

5-chloropentyl β-D-galactopyranosyl-(1→3)-6-deoxy-6-azido-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→3)-α-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (20)

To a solution of 8 (37 mg, 44.2 μmol) in the working solution described in the general procedure of synthesizing SSEA3 analogs (supporting information 16) were added MalE-LgtD (5.13 mg), GalK (230 μg), AtUSP (380 μg), PK (150 μg), PPA (120 μg). The reaction was followed with the general procedure described above to afford compound 20 (26.0 mg, 58%) as a white foam. ¹H NMR (600 MHz, D₂O) δ 4.94 (d, J = 3.7 Hz, 1H), 4.77 (d, J = 8.8 Hz, 1H), 4.54 (d, J = 7.7 Hz, 1H), 4.52 (d, 8.0 Hz, 1H), 4.48 (d, J = 7.8 Hz, 1H), 4.41 (t, J = 6.4 Hz, 1H), 4.26 (s, 1H), 4.18 (d, J = 2.6 Hz, 1H), 4.11 – 4.01 (m, 4H), 3.97 – 3.90 (m, 6H), 3.89 – 3.81 (m, 3H), 3.79 – 3.75 (m, 3H), 3.73 – 3.61 (m, 12H), 3.56 (t, J = 8.1 Hz, 1H), 3.34 – 3.32 (m, 1H), 2.06 (s, 3H), 1.86 – 1.81 (m, 1H), 1.72 – 1.67 (m, 2H), 1.56 – 1.51 (m, 2H). ¹³C NMR (150 MHz, D₂O) δ 175.18, 104.83, 103.35, 102.81, 101.97, 100.50, 79.36, 78.90, 78.04, 77.23, 75.46, 74.98, 74.77, 74.53, 73.63, 72.94, 72.45, 72.11, 70.87, 70.58, 70.34, 70.32, 69.20, 68.57, 68.50, 67.68,
5-tert-Butylcarbamateaminopentyl α-L-fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→3)-α-D-galactopyranosyl-(1→4)-2-deoxy-6-azido-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (21)

To a solution of 18 (60 mg, 55.6 μmol) in the working solution described in the general procedure of synthesizing Globo H analogs (Supporting information 18) were added FutC (1.19 mg), FKP (830 μg), PK (180 μg), PPA (150 μg). The reaction was followed with the general procedure described above to afford compound 21 (55.0 mg, 80%) as a white foam. 1H NMR (600 MHz, D2O) δ 5.22 (d, J = 3.7 Hz, 1H), 4.89 (d, J = 3.5 Hz, 1H), 4.61 (d, J = 7.7 Hz, 1H), 4.54 (d, J = 7.1 Hz, 1H), 4.53 (d, J = 7.6 Hz, 1H), 4.46 (d, J = 8.0 Hz, 1H), 4.38 (t, J = 6.3 Hz, 1H), 4.24 – 2.22 (m, 2H), 4.09 (s, 1H), 4.00 – 3.82 (m, 9H), 3.70 – 3.60 (m, 1H), 3.70 – 3.55 (m, 2H), 3.30 (t, J = 8.4 Hz, 1H), 3.06 (t, J = 6.4 Hz, 2H), 2.04 (s, 3H), 1.65 – 1.61 (m, 2H), 1.51 – 1.46 (m, 2H), 1.42 (s, 9H), 1.37 – 1.34 (m, 2H), 1.21 (d, J = 6.42 Hz, 3H). 13C NMR (150 MHz, D2O) δ 174.25, 158.34, 103.97, 103.22, 102.01, 101.94, 100.58, 99.24, 80.73, 79.03, 78.28, 77.18, 76.30, 76.07, 75.02, 74.66, 74.59, 74.50, 73.53, 73.41, 72.88, 71.87, 71.81, 70.67, 70.40, 70.03, 69.47, 69.14, 69.07, 68.44, 67.99, 67.83, 66.75, 60.94, 60.25, 60.04, 51.60, 50.19, 48.83, 39.81, 28.56, 28.33, 27.67, 22.26, 22.22, 15.29. HRMS (ESI–TOF, M+H+) calcd for C48H83N5O31H+ 1226.5145, found 1226.5160.

5-chloropentyl α-L-fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→3)-6-deoxy-6-azido-α-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (22)

To a solution of 19 (26 mg, 26.0 μmol) in the working solution described in the general procedure of synthesizing Globo H analogs (Supporting information 18) were added
FutC (560 μg), FKP (390 μg), PK (90 μg), PPA (70 μg). The reaction was followed with the general procedure described above to afford compound 22 (24.1 mg, 81%) as a white foam.\(^1\)H NMR (600 MHz, D\(_2\)O) δ 5.22 (d, \(J = 4.1\) Hz, 1H), 4.88 (d, \(J = 3.9\) Hz, 1H), 4.61 (d, \(J = 7.7\) Hz, 1H), 4.55 (t, \(J = 6.7\) Hz, 1H), 4.53 (d, \(J = 7.7\) Hz, 1H), 4.51 (d, \(J = 7.9\) Hz, 1H), 4.48 (d, \(J = 8.0\) Hz, 1H), 4.23 (dd, \(J = 13.2, 6.5\) Hz, 1H), 4.20 (d, \(J = 2.7\) Hz, 1H), 4.10 (d, \(J = 2.3\) Hz, 1H), 4.04 (d, \(J = 3.2\) Hz, 1H), 4.00 – 3.88 (m, 7H), 3.85 – 3.74 (m, 11H), 3.73 – 3.68 (m, 3H), 3.67 – 3.57 (m, 9H), 3.51 (dd, \(J = 12.9, 7.7\) Hz, 1H), 3.40 (dd, \(J = 12.9, 5.3\) Hz, 1H), 3.30 (t, \(J = 8.6\) Hz, 1H), 2.04 (s, 3H), 1.83 – 1.78 (m, 2H), 1.68 – 1.63 (m, 2H), 1.53 – 1.48 (m, 2H), 1.21 (d, \(J = 6.6\) Hz, 3H). \(^{13}\)C NMR (150 MHz, D\(_2\)O) δ 174.26, 103.96, 103.28, 102.01, 101.93, 100.12, 99.25, 78.68, 77.94, 76.50, 76.30, 76.08, 75.55, 75.03, 74.77, 74.55, 74.50, 73.53, 72.93, 71.94, 71.82, 70.77, 70.31, 69.69, 69.47, 69.07, 68.91, 68.44, 67.99, 67.60, 66.75, 60.94, 60.90, 60.19, 60.02, 51.58, 50.64, 45.46, 31.51, 27.97, 22.52, 22.20, 15.28. HRMS (ESI–TOF, M+H\(^+\)) calcd for C\(_{43}\)H\(_{73}\)ClN\(_4\)O\(_29\)H\(^+\) 1145.4122, found 1145.4150.

5-chloropentyl α-L-fucopyranosyl-β-D-galactopyranosyl-(1→3)-6-deoxy-6-azido-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→3)-α-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (23)

To a solution of 20 (26 mg, 26.0 μmol) in the working solution described in the general procedure of synthesizing Globo H analogs (Supporting information 18) were added FutC (560 μg), FKP (390 μg), PK (90 μg), PPA (70 μg). The reaction was followed with the general procedure described above to afford compound 23 (28.0 mg, 94%) as a white foam.\(^1\)H NMR (600 MHz, D\(_2\)O) δ 5.26 (d, \(J = 4.1\) Hz, 1H), 4.92 (d, \(J = 3.8\) Hz, 1H), 4.64 (d, \(J = 7.7\) Hz, 1H), 4.62 (d, \(J = 7.6\) Hz, 1H), 4.54 (d, \(J = 7.8\) Hz, 1H), 4.51 (d, \(J = 7.0\) Hz, 1H), 4.42 (t, \(J = 6.5\) Hz, 1H), 4.48 – 4.24 (m, 2H), 4.11 (d, \(J = 1.6\) Hz, 1H), 4.05 – 3.97 (m, 5H), 3.97 – 3.89 (m, 5H), 3.88 – 3.84 (m, 3H), 3.83 – 3.76 (m, 5H), 3.75 – 3.66 (m, 12H), 3.64 – 3.60 (m, 2H), 3.35 – 3.31 (m, 2H), 2.08 (s, 3H), 1.86 – 1.81 (m, 2H), 1.72 – 1.67 (m, 2H), 1.56 – 1.51 (m, 2H), 1.25 (d, \(J = 6.5\) Hz, 3H). \(^{13}\)C NMR (150 MHz, D\(_2\)O) δ 174.33, 103.81, 103.37, 102.07, 101.97, 100.53, 99.26, 78.96, 77.74, 77.17, 76.16, 76.07, 75.51, 75.08, 74.77, 74.54, 73.69, 73.57, 72.95, 72.11, 71.85, 70.85, 70.34, 70.18, 69.52, 69.38, 69.12, 69.05, 68.03, 67.84, 66.78, 61.00, 60.31, 60.27, 60.10, 51.48, 50.88, 45.53, 31.54, 28.01, 22.55, 22.23, 15.32. HRMS (ESI–TOF, M+H\(^+\)) calcd for C\(_{43}\)H\(_{75}\)ClN\(_4\)O\(_{29}\)H\(^+\) 1145.4122, found 1145.4133.
To a solution of 9 (77 mg, 77.0 μmol) in the working solution described in the general procedure of synthesizing Globo H analogs (Supporting information 18) were added FutC (1.66 mg), FKP (1.16 mg), PK (260 μg), PPA (210 μg). The reaction was followed with the general procedure described above to afford compound 24 (57.0 mg, 65%) as a white foam.

\[ \text{1H NMR (600 MHz, D}_2\text{O) } \delta 5.22 (d, J = 4.1 Hz, 1H), 4.89 (d, J = 4.0 Hz, 1H), 4.66 (d, J = 7.8 Hz, 1H), 4.55 (d, J = 8.1 Hz, 1H), 4.51 (d, J = 7.7 Hz, 1H), 4.48 (d, J = 8.0 Hz, 1H), 4.39 (t, J = 6.5 Hz, 1H), 4.23 – 4.21 (m, 2H), 4.07 (d, J = 2.8 Hz, 1H), 4.03 (d, J = 3.2 Hz, 1H), 4.01 – 3.89 (m, 6H), 3.87 – 3.81 (m, 6H), 3.79 – 3.73 (m, 5H), 3.71 – 3.61 (m, 12H), 3.60 – 3.57 (m, 2H), 3.30 (t, J = 8.5 Hz, 1H), 3.23 (dd, J = 13.3, 2.9 Hz, 1H), 2.05 (s, 3H), 1.83 – 1.78 (m, 2H), 1.68 – 1.64 (m, 2H), 1.53 – 1.48 (m, 2H), 1.22 (d, J = 6.5 Hz, 3H). \]

\[ \text{13C NMR (150 MHz, D}_2\text{O) } \delta 174.26, 103.91, 103.26, 101.98, 101.94, 100.41, 99.28, 78.72, 78.24, 77.11, 76.29, 75.89, 75.44, 74.75, 74.60, 74.49, 74.33, 74.31, 72.92, 72.05, 71.81, 70.82, 70.31, 70.10, 69.57, 69.47, 69.14, 68.40, 67.96, 67.80, 66.76, 60.87, 60.31, 60.02, 51.56, 50.98, 45.48, 31.51, 27.98, 22.52, 22.22, 15.28. \]

HRMS (ESI–TOF, M+H\(^+\)) calcd for C\(_{43}\)H\(_{73}\)ClN\(_4\)O\(_{29}\)H\(^+\) 1145.4122, found 1145.4148.

By following with the general procedure of NHBoc deprotection at Globo H linker (Supporting information 20), starting material 18 (23.2 mg, 21.5 μmol) was converted into compound 25 (20 mg, 95%) as a white foam.

\[ \text{1H NMR (600 MHz, D}_2\text{O) } \delta 4.92 (d, J = 3.7 Hz, 1H), 4.69 (d, J = 8.5 Hz, 1H), 4.54 (J = 7.7 Hz, 1H), 4.48 (d, J = 8.0 Hz,} \]
1H), 4.45 (d, J = 7.7 Hz, 1H), 4.38 (t, J = 6.4 Hz, 1H), 4.26 (m, 1H), 4.18 (d, J = 2.3 Hz, 1H), 4.08 – 4.04 (m, 1H), 4.00 – 3.88 (m, 8H), 3.83 – 3.56 (m, 18H), 3.54 – 3.51 (m, 1H), 3.31 (t, J = 8.4 Hz, 1H), 3.00 – 2.99 (m, 2H), 2.03 (s, 3H), 1.69 – 1.65 (m, 4H), 1.46 – 1.45 (m, 2H). $^{13}$C NMR (150 MHz, D$_2$O) δ 175.12, 104.80, 103.25, 102.94, 101.97, 100.55, 79.56, 79.05, 78.71, 74.99, 74.71, 74.53, 73.45, 72.89, 72.44, 71.90, 70.71, 70.58, 70.18, 70.08, 68.90, 68.55, 67.97, 67.63, 60.95, 60.28, 60.05, 51.47, 50.23, 39.33, 28.15, 26.40, 22.28, 22.08. HRMS (ESI–TOF, M+H$^+$) calcd for C$_{37}$H$_{65}$N$_5$O$_{25}$H$^+$ 980.4041, found 980.4036.

5-chloropentyl 6-deoxy-6-azido-α-1-galactopyranosyl-(1→2)-β-D-galactopyranosyl-(1→3)-6-deoxy-6-azido-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→3)-α-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (26)

To a solution of 20 (23 mg, 23.0 μmol) in the working solution described in the general procedure of synthesizing Globo H analogs (supporting information 18) were added FutC (490 μg), FKP (3500 μg), PK (80 μg), PPA (60 μg). The reaction was followed with the general procedure described above to afford compound 26 (21 mg, 77%) as a white foam. $^1$H NMR (600 MHz, D$_2$O) δ 5.42 (J = 4.0 Hz, 1H), 4.88 (d, J = 3.9 Hz, 1H), 4.62 – 4.60 (m, 2H), 4.51 (d, J = 7.8 Hz, 1H), 4.48 (d, J = 8.0 Hz, 1H), 4.39 (t, J = 6.5 Hz, 1H), 4.27 – 4.23 (m, 2H), 4.11 (d, J = 2.9 Hz, 1H), 4.07 (q, J = 8.6, 2.3 Hz, 1H), 4.01 – 3.86 (m, 9H), 3.85 – 3.72 (m, 10H), 3.71 – 3.62 (m, 11H), 3.60 – 3.57 (m, 2H), 3.34 – 3.28 (m, 3H), 2.05 (s, 3H), 1.83 – 1.78 (m, 2H), 1.68 – 1.63 (m, 2H), 1.53 – 1.48 (m, 2H). $^{13}$C NMR (150 MHz, D$_2$O) δ 174.40, 103.74, 103.34, 102.30, 101.92, 100.48, 98.40, 78.89, 77.80, 77.11, 76.76, 75.48, 75.09, 74.73, 74.66, 74.50, 73.82, 73.49, 72.91, 72.06, 70.80, 70.31, 70.14, 69.67, 69.60, 69.27, 69.11, 69.02, 27.99, 67.79, 60.94, 60.24, 60.04, 51.20, 50.83, 45.47, 31.51, 27.97, 22.52, 22.27. HRMS (ESI–TOF, M+H$^+$) calcd for C$_{43}$H$_{72}$ClN$_7$O$_{29}$H$^+$ 1186.5136, found 1186.4151.
5-aminopentyl α-L-fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→3)-α-D-galactopyranosyl-(1→4)-6-deoxy-6-azido-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (1)

By following with the general procedure of NHBoc deprotection at Globo H linker (Supporting information 20), starting material 21 (30 mg, 24.5 μmol) was converted into compound 1 (21 mg, 76%) as a white foam. 1H NMR (600 MHz, D2O) δ 5.22 (d, J = 4.1 Hz, 1H), 4.89 (d, J = 4.0 Hz, 1H), 4.61 (d, J = 7.7 Hz, 1H), 4.54 (d, J = 7.6 Hz, 1H), 4.53 (d, J = 7.8 Hz, 1H), 4.48 (d, J = 8.0 Hz, 1H), 4.38 (t, J = 6.5 Hz, 1H), 4.25 – 4.22 (m, 2H), 4.10 (d, J = 2.3 Hz, 1H), 4.01 – 3.97 (m, 3H), 3.95 – 3.91 (m, 2H), 3.90 – 3.86 (m, 3H), 3.83 – 3.78 (m, 4H), 3.78 – 3.72 (m, 6H), 3.70 – 3.67 (m, 5H), 3.66 – 3.63 (m, 6H), 3.61 – 3.56 (m, 2H), 3.32 (t, J = 8.5 Hz, 1H), 3.00 – 2.95 (m, 2H), 2.04 (s, 3H), 1.71 – 1.64 (m, 2H), 1.48 – 1.44 (m, 2H), 1.21 (d, J = 6.6 Hz, 3H). 13C NMR (150 MHz, D2O) δ 174.24, 103.94, 103.22, 102.00, 101.92, 100.57, 99.23, 79.03, 78.23, 77.19, 76.29, 76.05, 75.02, 74.67, 74.58, 74.49, 73.53, 73.42, 72.86, 71.86, 71.80, 70.66, 70.05, 69.46, 69.12, 69.06, 68.43, 67.97, 67.82, 66.74, 66.93, 60.93, 60.91, 60.23, 60.01, 51.60, 50.21, 39.30, 28.12, 26.40, 23.21, 22.20, 22.04, 15.27. HRMS (ESI–TOF, M+H+) calcd for C43H75N5O29H+ 1126.4620, found 1126.4641.

5-aminopentyl α-L-fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→3)-6-deoxy-6-azido-α-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (2)

By following with the general procedure of converting Cl to NH2 at linker on Globo H analogs (Supporting information 22), starting material 22 (21 mg, 18.3 μmol) was converted into compound 2 (20.6 mg, quant) as a white foam. 1H NMR (600 MHz, D2O) δ 5.23 (d, J = 4.0 Hz, 1H), 4.89 (d, J = 2.8 Hz, 1H), 4.61 (d, J = 7.7 Hz, 1H), 4.56 – 4.53 (m, 2H), 4.51 (d, J = 7.8 Hz, 1H), 4.48 (d, J = 8.0 Hz, 1H), 4.26 (dd, J = 13.1, 6.5 Hz, 1H), 4.20 (d, J = 2.6 Hz, 1H), 4.10 (d, J = 1.9 Hz, 1H), 4.04 (d, J = 3.1 Hz, 1H), 4.00 – 3.88 (m, 7H), 3.85 – 3.74 (m, 11H), 3.73 – 3.68 (m, 3H), 3.66 – 3.57 (m, 7H), 3.51 (dd, J = 13.0, 7.8 Hz, 1H), 3.40 (dd, J = 13.0, 5.4 Hz, 1H), 3.30 (t, J = 8.4 Hz, 1H), 2.98 (t, J = 7.5 Hz, 2H), 2.04 (s, 3H), 1.71 – 1.65 (m, 4H), 1.48 – 1.45 (m, 2H), 1.21 (d, J = 6.5 Hz, 3H). 13C NMR (150 MHz, D2O) δ 174.25, 103.93, 103.29, 102.00, 101.92, 100.12, 99.24, 78.70, 77.90, 76.51, 76.30, 76.07, 75.57, 75.03, 7294.78, 74.55, 74.21, 73.53, 72.93, 71.95, 71.81, 70.77, 70.07, 69.68, 69.47, 69.07, 68.91, 68.44, 67.98, 67.59,
HRMS (ESI–TOF, M+H+) calcd for C_{43}H_{75}N_{5}O_{29}H^{+} 1126.4620, found 1126.4633.

5-aminopentyl α-L-fucopyranosyl-β-D-galactopyranosyl-(1→3)-6-deoxy-6-azido-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→3)-α-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (3)

By following with the general procedure of converting Cl to NH2 at linker on Globo H analogs (Supporting information 22), starting material 23 (28 mg, 24.4 μmol) was converted into compound 3 (27.5 mg, quant) as a white foam. ¹H NMR (600 MHz, D₂O) δ 5.23 (d, J = 4.1 Hz, 1H), 4.88 (d, J = 3.9 Hz, 1H), 4.61 (d, J = 7.7 Hz, 1H), 4.59 (d, J = 7.7 Hz, 1H), 4.51 (d, J = 7.8 Hz, 1H), 4.49 (d, J = 8.0 Hz, 1H), 4.39 (t, J = 6.7 Hz, 1H), 4.23 – 4.22 (m, 2H), 4.07 (d, J = 1.1 Hz, 1H), 4.02 – 3.86 (m, 10H), 3.85 – 3.81 (m, 3H), 3.71 – 3.57 (m, 12H), 3.31 – 3.27 (m, 2H), 2.99 (t, J = 7.6 Hz, 2H), 2.05 (s, 3H), 1.71 – 1.65 (m, 4H), 1.48 – 1.44 (m, 2H), 1.22 (d, J = 6.6 Hz, 3H). ¹³C NMR (150 MHz, D₂O) δ 174.31, 103.76, 103.35, 102.03, 101.91, 100.49, 99.23, 78.91, 77.64, 77.14, 76.09, 76.02, 75.50, 75.04, 74.75, 74.51, 73.67, 73.52, 72.91, 72.06, 71.82, 70.80, 70.12, 70.07, 69.47, 69.33, 69.08, 69.01, 67.98, 67.81, 66.74, 60.97, 60.24, 60.02, 51.46, 50.84, 39.35, 28.15, 26.59, 22.18, 22.06, 15.29. HRMS (ESI–TOF, M+H+) calcd for C_{43}H_{75}N_{5}O_{29}H^{+} 1126.4620, found 1126.4618.

5-aminopentyl α-L-fucopyranosyl-6-deoxy-6-azido-β-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 (4)

By following with the general procedure of converting Cl to NH2 at linker on Globo H analogs (Supporting information 22), starting material 24 (57 mg, 49.8 μmol) was converted into compound 4 (56.0 mg, quant) as a white foam. ¹H NMR (600 MHz, D₂O) δ 5.21 (d, J = 4.0 Hz, 1H), 4.88 (d, J = 3.8 Hz, 1H), 4.67 (d, J = 7.7 Hz, 1H), 4.55 (d, J = 7.9 Hz, 1H), 4.50 (d, J = 7.8 Hz, 1H), 4.48 (d, J = 8.1 Hz, 1H), 4.38 (t, J = 6.5 Hz,
1H), 4.23 – 4.21 (m, 2H), 4.06 – 4.02 (m, 2H), 4.00 – 3.97 (m, 2H), 3.94 – 3.89 (m, 3H), 3.86 – 3.82 (m, 6H), 3.79 – 3.72 (m, 5H), 3.70 – 3.56 (m, 13H), 3.29 (t, $J = 8.3$ Hz, 1H), 3.23 – 2.21 (m, 1H), 2.99 (m, 2H), 2.04 (s, 3H), 1.67 (m, 4H), 1.45 (m, 2H), 1.21 (d, $J = 6.5$ Hz, 3H). $^{13}$C NMR (150 MHz, D$_2$O) δ 174.27, 103.86, 103.26, 101.96, 101.91, 100.40, 99.28, 78.72, 78.22, 77.12, 76.27, 75.89, 75.44, 74.76, 74.49, 74.32, 73.30, 72.92, 72.05, 71.82, 71.00, 70.06, 69.57, 69.47, 69.14, 68.40, 67.96, 67.80, 66.76, 60.87, 60.32, 60.00, 51.57, 50.98, 39.34, 28.13, 26.49, 22.23, 22.05, 15.28. HRMS (ESI–TOF, M+H$^+$) calcld for C$_{43}$H$_{74}$N$_5$O$_{29}$H$^+$ 1126.4620, found 1126.4668.

5-aminopentyl

6-deoxy-6-azido-α-L-galactopyranosyl-(1→2)-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→4)-6-deoxy-6-azido-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (5)

To a solution of S22 (22 mg, 22.4 μmol) in the working solution described in the general procedure of synthesizing Globo H analogs (Supporting information 18) were added FutC (480 μg), FKP (340 μg), PK (70 μg), PPA (60 μg). The reaction was followed with the general procedure described above to afford compound 5 (15 mg, 57%) as a white foam. $^1$H NMR (600 MHz, D$_2$O) δ 5.42 ($J = 3.8$ Hz, 1H), 4.90 ($J = 3.7$ Hz, 1H), 4.62 ($J = 7.2$ Hz, 1H), 4.57 ($J = 8.2$ Hz, 1H), 4.54 ($J = 7.6$ Hz, 1H), 4.48 ($J = 7.8$ Hz, 1H), 4.38 (t, $J = 5.2$ Hz, 1H), 4.26 (m, 2H), 4.15 (m, 1H), 4.08 – 4.07 (m, 1H), 4.00 – 3.94 (m, 5H), 3.89 – 3.65 (m, 24H), 3.60 – 3.56 (m, 2H), 3.34 – 3.30 (m, 2H), 3.00 (t, $J = 7.3$ Hz), 2.05 (s, 3H), 1.70 – 1.66 (m, 4H), 1.47 – 1.46 (m, 2H). $^{13}$C NMR (150 MHz, D$_2$O) δ 174.35, 103.87, 103.24, 102.27, 101.94, 100.58, 98.40, 79.06, 78.37, 77.21, 76.92, 75.10, 74.66, 74.51, 74.41, 73.83, 73.44, 72.87, 71.88, 70.67, 70.06, 69.66, 69.60, 69.11, 69.03, 68.54, 68.00, 67.80, 60.89, 60.24, 60.03, 51.38, 51.23, 50.23, 39.31, 28.13, 26.39, 23.22, 22.32, 22.05. HRMS (ESI–TOF, M+H$^+$) calcld for C$_{43}$H$_{74}$N$_5$O$_{29}$H$^+$ 1167.4634, found 1167.4634.

5-aminopentyl

6-deoxy-6-azido-α-L-galactopyranosyl-(1→2)-β-D-galactopyranosyl-(1→3)-6-deoxy-6-azido-2-acetamido-2-deoxy-β-D-
**galactopyranosyl-(1→3)-α-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (6)**

By following with the general procedure of converting Cl to NH2 at linker on Globo H analogs (Supporting information 22), starting material S23 (20 mg, 17.1 μmol) was converted into compound 6 (15.0 mg, 76%) as a white foam. 

1H NMR (600 MHz, D2O) δ 5.43 (J = 3.9 Hz, 1H), 4.89 (d, J = 3.8 Hz, 1H), 4.63 – 4.60 (m, 2H), 4.51 (d, J = 7.7 Hz, 1H), 4.49 (d, J = 8.1 Hz, 1H), 4.39 (t, J = 6.5 Hz, 1H), 4.27 – 4.23 (m, 2H), 4.12 (d, J = 2.9 Hz, 1H), 4.07 (q, J = 8.7, 2.2 Hz, 1H), 4.02 – 3.98 (m, 3H), 3.96 – 3.87 (m, 6H), 3.86 – 3.73 (m, 10H), 3.71 – 3.63 (m, 9H), 3.60 – 3.57 (m, 2H), 3.35 – 3.29 (m, 3H), 3.01 (t, J = 7.6 Hz, 2H), 2.05 (s, 3H), 1.72 – 1.65 (m, 4H), 1.49 – 1.43 (m, 2H). 

13C NMR (150 MHz, D2O) δ 174.40, 103.72, 103.35, 102.29, 101.91, 100.48, 98.41, 78.91, 77.76, 77.14, 76.75, 75.49, 75.09, 74.75, 74.66, 74.51, 73.82, 73.49, 72.91, 72.06, 70.80, 70.15, 70.05, 69.67, 69.60, 69.26, 69.11, 69.02, 67.99, 67.79, 60.94, 60.25, 60.02, 51.23, 51.20, 50.83, 39.31, 28.13, 26.39, 22.28, 22.04. HRMS (ESI–TOF, M+H+) calcd for C43H74N8O29H+ 1167.4634, found 1167.4636.

**General strategy for synthesis of Globo H analog glycoconjugates**

To a solution of CRM197 (purchased from Reagent Proteins, 1 mg) in ddH2O (1 mL), different Globo H analog monoesters (60 eq) were added and the pH was adjusted to 7.9 by 100 mM disodium phosphate buffer. The reaction was stirred at room temperature for 24 h. The mixture was diluted with ddH2O and washed through 10K Amicon by ddH2O for five times. The obtained GH analog-CRM197 was monitored by MALDI-TOF (positive mode with sinapinic acid matrix) to characterize the glycan conjugation number. Then, the mixture was further washed through 10K Amicon by PBS for three times and lyophilized to white powder.

**Vaccine immunization dose and schedule**

Each glycoconjugate was intramuscularly immunized in BALB/c mice (8 weeks old female mice, BioLASCO, Taiwan) biweekly for three shots. Each shot contained 2 μg Globo H analog and 2 μg C34 as adjuvant in 200 μL PBS. The control mice were only immunized with the 20 μg CRM197 in 200 μL PBS for three shots. For the array analysis, mice blood was collected three days before the first shot and ten days after each shot. The sera were separated by centrifugation of the blood at 1500*g for 10 min at 4ºC.

**Glycan array slides preparation**

To prepare the glycan array slides, 6 different Globo H analogs and 27 different Globo
series glycans with aminopentyl linker (Table S1.) were dissolved in the printing buffer (100 μM glycan in 300 mM phosphate buffer with 0.005% triton X-100, pH 8.5). The prepared glycans were loaded into 96 well plate and 0.7 μL was printed on the NHS ester-coated glass slides (Nexterion H slide, SCHOTT, Germany). The slide was separated into 16 grids and each contained 16 column*10 rows of glycan spots. The prepared slides were reacted in an atmosphere of 80% humidity for 2 h, dried overnight, and stored at room temperature in drier before to use.

**Serologic analysis on glycan array**
The glycan array slides were blocked by Pierce™ blocking buffer (ThermoFisher, Waltham, Massachusetts, MA, USA) at room temperature for 1 hr. The sera were diluted with 3% BSA in PBST (0.05% Tween-20 in PBS). The blocking buffer was washed away from glycan slides which then incubated with sera diluents at room temperature for 2 hr. The sera diluents were washed away and the slides were further washed by PBST for three times. For the total IgG analysis, the slides were then incubated with Alexa Flour 647 conjugated goat anti-mouse IgG antibody (2.5 μg/mL PBST) at room temperature for 30 minutes. For the antibodies subtypes analysis, the slides were individually incubated with Alexa Flour 647 conjugated goat anti-mouse IgG1 antibody (2.5 μg/mL PBST), Alexa Flour 647 conjugated goat anti-mouse IgG2a antibody (2.5 μg/mL PBST), Alexa Flour 647 conjugated goat anti-mouse IgG2b antibody (2.5 μg/mL PBST), Alexa Flour 647 conjugated goat anti-mouse IgG2c antibody (2.5 μg/mL PBST) and Alexa Flour 647 conjugated goat anti-mouse IgG3 antibody (2.5 μg/mL PBST) at room temperature for 30 minutes (All the secondary antibodies used in glycan array are purchased from Lackson ImmunoResearch). The slides were rinsed in PBST and ddH₂O, centrifuged and scanned at 635 nm wavelength by microarray fluorescence chip reader (GenePix 4300A; Molecular Devices Corporation, San Jose, CA, USA). The result was quantified and analyzed by GenePix Pro-6.0 analysis software (Axon Instruments, Union City, CA, USA).

**Cell culture and Flow cytometry**
MCF-7 (human breast cancer cell line) were cultured in Dulbecco's Modified Eagle Medium (DMEM, ThermoFisher) with 10% FBS (ThermoFisher) and 1X Antibiotic-Antimycotic (ThermoFisher). For the Flow cytometry, cells were harvested, spun under at 200 g for 5 minutes, and resuspended by FACS buffer (1% FBS and 0.02% NaN₃ in PBS). All the sera were picked up for flow cytometry analysis. 2 * 10⁵ cells were incubated with the serum (5 μL serum + 45 μL FACS buffer) and VK9 (1 μg, ThermoFisher) at 4 ℃ for 1 h. Cells were then washed with 200 μL FACS buffer for 3 times and incubated...
with 0.2 μg Alexa Flour 647 labeled anti-mouse IgG antibody (Jackson ImmunoResearch) at 4 °C for 0.5 h. The cells were washed with 200 μL FACS buffer for 3 times again and analyzed by the FACSCanto (Becton Dickinson, Franklin Lakes, NJ, USA). The results were analyzed by FlowJo (Tree Star, Ashland, OR).

**Competitive Flow cytometry analyses**
As described above, 2 * 10^5 cells were incubated with Globo H or Globob H analogs in 10-fold serially diluted concentration in 45 μL FACS buffer in a 96 well plate. CRM197 (for negative control), different glycoconjugates immunized mice mixed sera (5 μL), or VK9 (for positive control, 1 μg) were added in the well and incubated at 4 °C for 1 h. Cells were then washed with 200 μL FACS buffer for 3 times and incubated with 0.2 μg Alexa Flour 647 labeled anti-mouse IgG antibody (Jackson ImmunoResearch) at 4 °C for 0.5 h. The cells were washed with 200 μL FACS buffer for 3 times again and analyzed by the FACSCanto (Becton Dickinson, Franklin Lakes, NJ, USA). The results were analyzed by FlowJo (Tree Star, Ashland, OR). The inhibition percentage was calculated by following formula:

For tested sera and VK9:

\[
\% \text{ inhibition} = \left[ 1 - \frac{(S_1 - D_0)}{(S_0 - D_0)} \right] \times 100
\]

For CRM197:

\[
\% \text{ inhibition} = \left[ \frac{(D_0 - D_1)}{D_0} \right] \times 100
\]

No competition CRM197 signal: \(D_0\); competition CRM197 signal: \(D_1\)

No competition sample signal: \(S_0\); competition sample signal: \(S_1\)

**Table S1. Characterization of glycan conjugated number on CRM197 by MALDI-TOF.**

| Glycoconjugates | After glycosylation | Average incorporation (n) | Carbohydrate percentage |
|-----------------|---------------------|---------------------------|-------------------------|
| GH-CRM197       | 66,109              | 6.3                       | 9.8%                    |
| 1-CRM197        | 67,130              | 7.0                       | 11.6%                   |
| 2-CRM197        | 67,488              | 7.3                       | 11.4%                   |
| 3-CRM197        | 67,318              | 7.2                       | 11.6%                   |
| 4-CRM197        | 67,201              | 7.1                       | 10.7%                   |
| N-GH-CRM197     | 65,035              | 5.6                       | 9.1%                    |
| 5-CRM197        | 64,871              | 5.3                       | 8.8%                    |
| 6-CRM197        | 64,750              | 5.2                       | 8.7%                    |
Figure S1. SDS PAGE of different GH analogs glycoconjugates.
A: CRM197; B: GH-CRM197; C: 1-CRM197; D: 2-CRM197; E:3-CRM197; F: 4-CRM197; G: N$_3$GH-CRM197; H: 5-CRM197; I: 6-CRM197
| Name | Glycan structure | Name | Glycan structure |
|------|------------------|------|------------------|
| TF   | ![TF structure](image) | SASSEA4 | ![SASSEA4 structure](image) |
| 70   | ![70 structure](image) | DSGb5 | ![DSGb5 structure](image) |
| Gb2  | ![Gb2 structure](image) | DSGG | ![DSGG structure](image) |
| Gb3  | ![Gb3 structure](image) | iSSEA4 | ![iSSEA4 structure](image) |
| Gb4  | ![Gb4 structure](image) | iGlobo H | ![iGlobo H structure](image) |
| SSEA3| ![SSEA3 structure](image) | Globo H-N3 | ![Globo H-N3 structure](image) |
| iGb4 | ![iGb4 structure](image) | 1 | ![1 structure](image) |
| iGb5 | ![iGb5 structure](image) | 2 | ![2 structure](image) |
| SAGb4| ![SAGb4 structure](image) | 3 | ![3 structure](image) |
| SSEA4| ![SSEA4 structure](image) | 4 | ![4 structure](image) |
| 2,6  | ![2,6 structure](image) | Globo H-N1 | ![Globo H-N1 structure](image) |
| SAGb5| ![SAGb5 structure](image) | 5 | ![5 structure](image) |
| SAGb5| ![SAGb5 structure](image) | 6 | ![6 structure](image) |
Table S3. Glycoconjugates induced IgG, IgM, and IgG/IgM against corresponding antigen on glycan array.

|                | GH-CRM197 | 1- CRM197 | 2- CRM197 | 3- CRM197 | 4- CRM197 | N,GH-CRM197 | 5- CRM197 | 6- CRM197 |
|----------------|-----------|-----------|-----------|-----------|-----------|-------------|-----------|-----------|
| IgG (1:100)    | 5,638,861 | 38,579,695| 45,815,171| 51,653,847| 32,692,221| 49,427,106  | 51,023,493| 52,007,939|
| IgM (1:100)    | 198,081   | 1,149,980 | 1,194,143 | 757,645   | 454,518   | 800,528     | 527,374   | 1,056,781 |
| IgG/IgM        | 28.5      | 33.5      | 38.4      | 68.2      | 71.9      | 61.7        | 96.8      | 49.2      |

The individual glycoconjugates induced mouse sera were mixed together and characterized by glycan array in 100X dilution. The glycan array procedure was follow the **Serologic analysis on glycan array** as described in the supporting information. Each glycan was spotted on the array slide for five repeats. The numbers are the average of five spots mean relative fluorescence unit.

Table S4. Positive ratio of different GH analog glycoconjugates immunized mice sera against GH, SSEA3 and SSEA4 antigens on glycan array.

|                | CRM197 | GH-CRM197 | 1- CRM197 | 2- CRM197 | 3- CRM197 | 4- CRM197 | N,GH-CRM197 | 5- CRM197 | 6- CRM197 |
|----------------|--------|-----------|-----------|-----------|-----------|-----------|-------------|-----------|-----------|
| Against GH     | 0/5    | 5/10      | 8/10      | 1/5       | 8/10      | 4/5       | 5/5         | 5/5       | 3/5       |
| Against SSEA3  | 0/5    | 4/10      | 8/10      | 1/5       | 8/10      | 4/5       | 5/5         | 5/5       | 3/5       |
| Against SSEA4  | 0/5    | 3/10      | 7/10      | 1/5       | 2/10      | 2/5       | 5/5         | 5/5       | 0/5       |
Array Results (Figure S2 – S11)

Figure S2. Characterization of CRM197 immunized mice sera binding profile to Globo series glycans. Each serum was analyzed by glycan array in 5 different dilution fold (200X: black bar; 600X: brown bar; 1800X: red bar; 5400X: green bar; 16200X: gray bar). Data are means ± SEM (standard error of the mean). RFU: relative fluorescence unit.
Figure S3. Characterization of GH-CRM197 immunized mice sera binding profile to Globo series glycans. Each serum was analyzed by glycan array in 5 different dilution fold (200X: black bar; 600X: brown bar; 1800X: red bar; 5400X: green bar; 16200X: gray bar). Data are means ± SEM (standard error of the mean). RFU: relative fluorescence unit.

Figure S4. Characterization of 1-CRM197 immunized mice sera binding profile to Globo series glycans. Each serum was analyzed by glycan array in 5 different dilution fold (200X: black bar; 600X: brown bar; 1800X: red bar; 5400X: green bar; 16200X: gray bar). Data are means ± SEM (standard error of the mean). RFU: relative fluorescence unit.
Figure S5. Characterization of 2-CRM197 immunized mice sera binding profile to Globo series glycans. Each serum was analyzed by glycan array in 5 different dilution fold (200X: black bar; 600X: brown bar; 1800X: red bar; 5400X: green bar; 16200X: gray bar). Data are means ± SEM (standard error of the mean). RFU: relative fluorescence unit.

Figure S6. Characterization of 3-CRM197 immunized mice sera binding profile to Globo series glycans. Each serum was analyzed by glycan array in 5 different dilution fold (200X: black bar; 600X: brown bar; 1800X: red bar; 5400X: green bar; 16200X: gray bar). Data are means ± SEM (standard error of the mean). RFU: relative
fluorescence unit.

Figure S7. Characterization of 4-CRM197 immunized mice sera binding profile to Globo series glycans. Each serum was analyzed by glycan array in 5 different dilution fold (200X: black bar; 600X: brown bar; 1800X: red bar; 5400X: green bar; 16200X: gray bar). Data are means ± SEM (standard error of the mean). RFU: relative fluorescence unit.

Figure S8. Characterization of N3GH-CRM197 immunized mice sera binding profile to Globo series glycans. Each serum was analyzed by glycan array in 5
different dilution fold (200X: black bar; 600X: brown bar; 1800X: red bar; 5400X: green bar; 16200X: gray bar). Data are means ± SEM (standard error of the mean). RFU: relative fluorescence unit.

Figure S9. Characterization of 5-CRM197 immunized mice sera binding profile to Globo series glycans. Each serum was analyzed by glycan array in 5 different dilution fold (200X: black bar; 600X: brown bar; 1800X: red bar; 5400X: green bar; 16200X: gray bar). Data are means ± SEM (standard error of the mean). RFU: relative fluorescence unit.
Figure S10. Characterization of 6-CRM197 immunized mice sera binding profile to Globo series glycans. Each serum was analyzed by glycan array in 5 different dilution fold (200X: black bar; 600X: brown bar; 1800X: red bar; 5400X: green bar; 16200X: gray bar). Data are means ± SEM (standard error of the mean). RFU: relative fluorescence unit.

Figure S11. Glycan array results
(A) Monoazido-GH analogs vaccines induced IgG level against SSEA3 on glycan array. Each serum was analyzed by glycan array in 5 different dilution fold (200X: black bar; 600X: brown bar; 1800X: red bar; 5400X: green bar; 16200X: gray bar). (B) Monoazido- and diazido-GH analogs vaccines induced IgG level against SSEA3 on glycan array. Data are means ± SEM (standard error of the mean). Each vaccine was compared with GH-CRM197 and the comparisons of titers were performed by Mann–Whitney U test (unpaired). (C) Monoazido- and diazido-GH analogs vaccines induced IgG subtypes against GH on glycan array. RFU: relative fluorescence unit. *P < 0.05; **P < 0.01.
Figure S12. Representative flow cytometry analysis of MCF-7 and U-87 cells interacting with antisera from mice immunized with various azido-GH glycoconjugates.
(A) Antisera against Globo H positive MCF-7 cells. (B) Antisera against Globo H negative U-87 cells. Red line: azido-GH glycoconjugates immunized mouse serum or VK9. Black line: CRM197 immunized mouse serum. (in 1:10 dilution)

Figure S13. Inhibition of the antisera binding to MCF-7.
Competitive flow with VK9, CRM197 and different GH analog immunized mice mixed sera. (A) GH- CRM197 immunized serum competition by GH; (B) 1-CRM197 immunized serum competition by GH or corresponding GH analog; (C) N3GH-CRM197 immunized serum competition by GH or corresponding GH analog; (D) 6-CRM197 immunized serum competition by GH or corresponding GH analog; (E) 2, 3, 4, and 5-CRM197 immunized serum competition by GH. Red line: competition by corresponding GH analog.
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