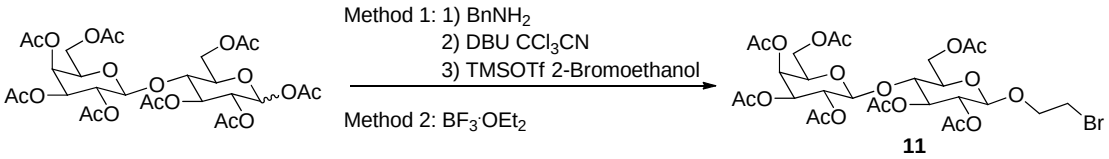
**Support information**

## Chemicals:

chemicals were purchased from Alfa Chemicals (Beijing, PR. China). Commercially available reagents were used without further purification. Nanopure water was used for all experiments. All other chemicals were reagent grade or better.

## Synthesis and characterization:

**Procedure to prepare N'-fluorenylmethyloxycarbonyl-(2-aminoethyl) 4-O-β-D-galactopyranosyl-β-D-glucopyranose (LacFmoc, compound 2)**




**11**

Method 1：

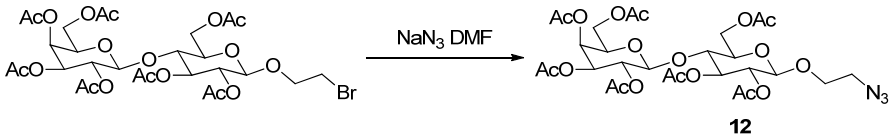
To a solution of 1-hydroxy sugar derivative (10 mmol) in 40 mL $CH_2Cl_2$ were added sequentially $CCl_3CN$ (5.68 g, 40 mmol, 4eq) and DBU (0.42 g, 3mmol, 0.3 eq) at -5ºC. The reaction mixture was stirred under argon for 10 min. The solvent was removed under reduced pressure, and the therefore obtained mixture was directly purified by flash chromatography to provide the trichloroacetimidate glycosylation donors. The trichloroacetimidate glycosylation donor (5 mmol) and 2-bormoethanol (6.5 mmol, 1.3eq) were dissolved in 40 mL dry $CH_2Cl_2$, to which a solution of TMSOTf (0.1M, 0.13 mL, 0.02eq) in $CH_2Cl_2$ was added. After being stirred at ambient temperature for 30 min, the mixture was treated with triethylamine (0.013 mL), and concentrated in vacuo. The residue was then purified by flash chromatography to afford the 2-bormoethyl glycoside product **11**. Flash chromatography (eluent，EtOAc/ $CH_2Cl_2$=1:15); 60% isolation yield from 2 steps.

Method 2：

Dissolve the lactose peracetate (3.45g, 5.1 mmol) and 2-bromoethanol (0.45 ml, 6.3 mmol) in DCM (9 ml) under nitrogen and cool to 0 $^{o}$C using the ice bath. Add the freshly distilled $BF_3 \cdot OEt_2$ (3.3 ml, 26.0 mmol) dropwise using the syringe and needle over the course of 15 min. After 1.5 h allow the solution to warm to room temperature. After 20 h pour the reaction solution into ice water (15 ml) and extract with DCM three times (15 ml,3). Combine these organic extracts, wash with water (15 ml), sat. $NaHCO_3$ solution (aq., 15 ml), water again (15 ml) and then dry over magnesium sulfate. Filter the dried solution and remove the solvent on the rotary evaporator. Purify the resulting residue by flash chromatography (EtOAc/hexane, 1:3) to give the compound **11** (2.2g,60%)

$^1$H NMR (400 MHz, $CDCl_3$) δ 5.35 (d, J = 3.8 Hz, 1H), 5.21 (dd, J1 = J2 = 9.4 Hz, 1H), 5.11 (dd, J = 10.4, 7.8 Hz, 1H), 4.96 (dd, J = 10.6, 3.4 Hz, 1H), 4.56-4.52 (m, 1H), 4.51-4.47 (m, 1H), 4.20 – 4.07 (m, 4H), 3.90 (dd, J = 11.8, 7.0 Hz, 1H), 3.84 (dd, J = 7.6, 4.5 Hz, 1H), 3.80 (dd, J = 10.2, 5.8 Hz, 1H), 3.49 – 3.42 (m, 1H), 2.16 (s, 3H), 2.16 (s, 3H), 2.13 (s, 3H), 2.07 (s, 3H), 2.06 (s, 3H),

2.05 (s, 3H), 1.97 (s, 3H).

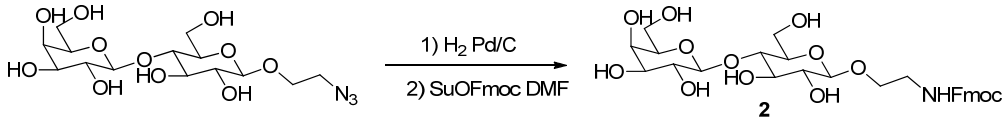



**12**

2-Bormoethyl glycoside **11** (2 mmol) was treated by $NaN_3$ (20mmol, 10eq) in 10ml DMF and stirred for 24h to get 2-azidoethyl-2,3,4,6-tetra-O-acetyl-glycoside **12** (quantitative yield). After deacetylation by MeONa, 2-azidoethyl glycoside was obtained as a colorless syrup.

Quantitative isolation yield from 2 steps.

$^1$H NMR (400 MHz, $D_2O$) δ 4.43 (d, J = 8.0 Hz, 1H), 4.35 (d, J = 7.8 Hz, 1H), 3.99 – 3.92 (m, 1H), 3.91- 3.86 (m, 1H), 3.82 (d, J = 3.3 Hz, 2H), 3.78 – 3.71 (m, 2H), 3.71 - 3.65 (m, 2H), 3.64-3.60 (m, 2H), 3.59 – 3.54 (m, 4H), 3.48 – 3.43 (m, 2H).




**2**

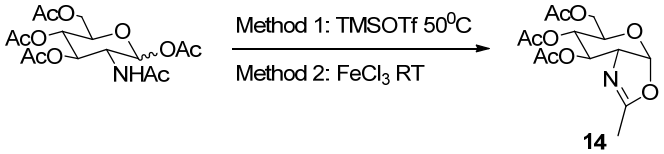
2-Azidoethyl glycoside (0.5 mmol) was dissolved in 7 mL MeOH and 50 mg 10% Pd/C was added. After stirring under 4 atm hydrogen for 4h, Pd/C was filtered out and the residue was concentrated in vacuo. The resulting syrup was dissolved in 10ml DMF. SuOFmoc (168 mg, 0.5 mmol, 1 eq) was added. After stirring for 8h, the solution was purified by flash chromatography to afford compound **2**

75% yield from 2 steps

$^1$H NMR (400 MHz, DMSO) δ 7.89 (d, J = 7.4 Hz, 2H), 7.84 (d, J = 7.4 Hz, 2H), 7.42 (dd, J = 7.4 Hz, 2H), 7.35 (dd, J = 7.3 Hz, 2H), 6.28 (s, 2H), 4.26 – 4.15 (m, 3H), 4.03 (dd, J = 14.1, 7.0 Hz, 1H), 3.84 – 3.70 (m, 2H), 3.69 – 3.41 (m, 9H), 3.39 – 3.24 (m, 7H), 3.23 – 2.98 (m, 4H).

$^{13}$C NMR (101 MHz, DMSO) δ 156.17, 143.86, 140.70, 127.58, 127.05, 125.14, 120.07, 103.82, 102.74, 80.62, 75.50, 74.85, 74.74, 73.25, 73.16, 70.56, 68.08, 67.91, 65.42, 60.45, 60.35, 59.70, 46.69. $C_{29}H_{37}NO_{13}$ M+H $^+$ 608.2337 calc. 608.2265

**Procedure to prepare N'-fluorenylmethyloxycarbonyl-(2-aminoethyl) 4-O-β-D-galactopyranosyl-β-D-glucopyranose**



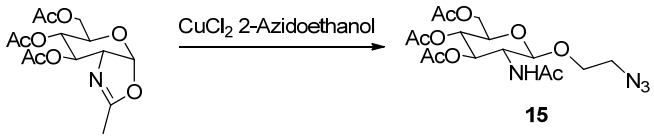

**14**

Method 1:

To a solution of acetate glucosamine (2.33 g, 5.99 mmol) in 50 ml of anhydrous 1, 2 –dichloroethane was added TMSOTf (1.2 ml). The mixture was stirred for 16 hours at 52 ℃, then

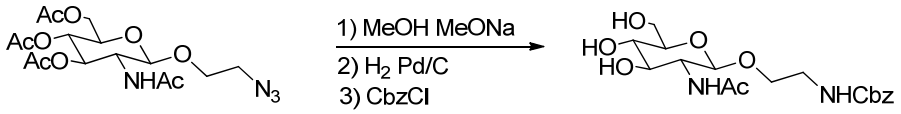
was diluted with $CH_2Cl_2$, washed with saturated aqueous $NaHCO_3$ and brine. The collected organic phase was dried over Na2SO4 and concentrated. The resultant residue was purified by silica gel chromatography eluting with AcOEt/$CH_2Cl_2$ = 2/1 to afford oxazoline **14** (1.69 g, 5.13 mmol, 85%).

Method 2:
Detailed procedure see Carbohydrate Research, 26(1), 215-18; 1973




**15**

oxazoline **14** (1.69 g, 5.13 mmol) and 2-azidoethanol (3.08 g, 30.46 mmol, 5.9 equiv.) were co-evaporated with toluene (15 ml × 2). The mixture was dissolved in 12 ml of anhydrous chloroform, and $CuCl_2$ (773.2 mg) was added. The resulting mixture was stirred for 5 hours at 50 ℃. At this stage the solid was filtered off, the filtrate was washed with saturated aqueous $NaHCO_3$ and brine. The collected organic phase was dried over Na2SO4 and concentrated. The resultant residue was purified by silica gel chromatography eluting with AcOEt/CH2Cl2 = 1/2 – 2/1 to AcOEt/MeOH = 19/1) to give rise to azide **15** (2.01 g, 4.67 mmol, 91%).




**8**

To a solution of azide **15** (7.25 g, 16.85 mmol) in 125 ml of MeOH was added a solution of 25 wt% of sodium methoxide in methanol (0.5 ml, 2.19 mmol, 0.1 equiv.). The mixture was stirred for 4 hours at room temperature, then the solution was neutralized with Amberlist-$H^+$ resin. After filtering off the solid, the filtrate was concentrated and dried in vacuum to furnish glycoside (4.76 g, 15.65 mmol, 93%).
$^1$H NMR (400MHz, $CDCl_3$) δ 4.50 (d, J= 8.8, 1 H), 4.06 (ddd, , J= 3.2, 5.6, 12, 1 H), 3.91(dd, J = 2.0, 12, 1 H), 3.72- 3.66 (m, 3 H), 3.49- 3.43 (m, 2 H), 3.36- 3.28 (m,3 H), 2.00 (s,3 H)
$^{13}$C NMR (100MHz, $CDCl_3$) δ 173.9, 102.5, 78.1, 76.1, 72.1, 69.3, 62.8, 57.3, 51.8, 23.1 .

2-Azidoethyl glycoside (1.4g, 4.8 mmol) was dissolved in 40 mL MeOH, 500mg 10% Pd/C was added. After stirring under 4 atm hydrogen for 4h, Pd/C was filtered out and the residue was concentrated in vacuo. The resulting syrup and $Na_2CO_3$ (750mg) was dissolved in 10ml $H_2O$ . CbzCl (1.6g, 9.6 mmol, 1.4 ml, 2 eq) was added. After stirring for 2h, the solution was purified by flash chromatography to afford compound **8**.

$^1$H NMR (400 MHz, MeOD) δ 7.54 – 7.09 (m, 5H), 5.09 (s, 2H), 4.40 (d, J = 8.4 Hz, 1H), 3.92 – 3.83 (m, 2H), 3.75 – 3.65 (m, 2H), 3.65 – 3.57 (m, 1H), 3.50 – 3.40 (m, 1H), 3.38 – 3.24 (m, 4H), 1.96 (s, 3H).
$^{13}$C NMR (101 MHz, MeOD) δ 174.06, 129.50, 129.03, 128.91, 102.92, 78.00, 76.00, 71.99, 69.53, 67.49, 62.70, 57.30, 41.94, 23.01.

**General Procedure to prepare trisaccharides**

Preparative scale (50–150 mg) synthesis: Reactions were typically carried out in a 50 mL centrifuge tube of Tris-Cl buffer solution (100mM, pH 7.5) containing acceptor **2**, donor (1.5equiv) and $MnCl_2$ (10mM). The reaction mixture was incubated at 30℃ for 12 h with shaking (120 rpm). After the reaction is completed monitored by TLC and DEPT135, the mixture was boiled and insoluble material was removed by centrifugation. β -galactosidase and β-glucosidase were added to the supernatant. After 2h, the mixture was boiled and insoluble material was removed by centrifugation. The supernatant was directly loaded on the SPE column and eluted by selected solution. The Fmoc tag was removed in 10% piperidine in water. After centrifugation, the supernatant was concentrated to dry under vacumm.

Compound **3**

Acceptor **2** 200mg 0.33mmol
Donor compound **1** 300mg 0.50mmol
Total volume 25ml
Elution solution 20% methanol in water
Lgt C 5U
GalE 2U
Yield 151mg 85%

$^1$H NMR (400 MHz, $D_2O$) δ 4.79 (d, *J* = 3.7 Hz, 1H), 4.39 (d, *J* = 8.0 Hz, 1H), 4.35 (d, *J* = 7.7 Hz, 1H), 4.20 (dd, *J* = 6.3 Hz, 1H), 4.02 – 3.93 (m, 1H), 3.91 – 3.85 (m, 2H), 3.83 – 3.72 (m, 3H), 3.72 – 3.65 (m, 3H), 3.65 – 3.59 (m, 1H), 3.60 – 3.49 (m, 6H), 3.49 – 3.39 (m, 2H), 3.21 (dd, *J* = 8.4 Hz, 1H), 3.11 (t, *J* = 5.0 Hz, 2H).

$^{13}$C NMR (101 MHz, $D_2O$) δ 103.28, 101.95, 100.33, 78.56, 77.38, 75.45, 74.82, 74.28, 72.81, 72.19, 70.93, 70.85, 69.14, 68.98, 68.56, 65.84, 60.55, 60.40, 59.48, 39.42.

$M+H^+$ 548.2178 calc.548.2191

Compound **5**

Acceptor **2** 80mg 0.13mmol
Donor compound **4** 116mg 0.20mmol
Total volume 10ml
Elution solution 20% methanol in water
Wbs J 10U
Yield 47mg 70%

$^1$H NMR (400 MHz, $D_2O$) δ 5.22 – 5.14 (m, 1H), 4.45 – 4.33 (m, 2H), 4.15 – 4.04 (m, J = 6.4 Hz, 1H), 4.04 – 3.94 (m, 1H), 3.90 – 3.79 (m, 2H), 3.78 – 3.70 (m, J = 10.1 Hz, 2H), 3.70 – 3.53 (m, 9H), 3.52 – 3.44 (m, 1H), 3.40 – 3.31 (m, 1H), 3.25 (dd, J = 8.7 Hz, 2H), 3.14 (t, J = 5.1 Hz, 1H), 1.09 (d, J = 6.3 Hz, 3H).

$^{13}$C NMR (101 MHz, $D_2O$) δ 102.05, 100.26, 99.34, 76.33, 75.74, 75.27, 75.21, 74.07, 73.52, 72.78, 71.64, 69.59, 69.07, 68.14, 65.78, 61.08, 60.01, 59.35, 39.35, 15.26.

$M+H^+$ 532.2228 calc.548.2241

Compound **7**

Acceptor **2** 300mg 0.5mmol
Donor Sialic acid 230mg 0.74mmol, CTP 390mg 0.74mmol
Total volume 25ml
Load solution containing 200mM NaCl
Elution solution water
Nm CSS 5U
Pd 2,6SiaT 2U
Yield 274mg 80%

$^{1}$H NMR (400 MHz, $D_2O$) δ 4.46 (d, J = 8.0 Hz, 1H), 4.34 (d, J = 7.8 Hz, 1H), 4.25 (d, J = 3.3 Hz, 1H), 4.04 (ddd, J = 19.9, 7.2 Hz, 1H), 3.95 – 3.69 (m, 8H), 3.67 – 3.40 (m, 9H), 3.32 (t, J = 8.4 Hz, 1H), 3.18 (t, J = 4.9 Hz, 2H), 2.62 (dd, J = 12.4, 4.6 Hz, 1H), 2.62 (dd, J = 12.4, 4.6 Hz, 1H), 1.94 (s, 3H), 1.65 (dd, J = 12.1 Hz, 1H).

$^{13}$C NMR (101 MHz, $D_2O$) δ 174.90, 173.45, 103.12, 101.78, 100.24, 79.39, 74.62, 74.41, 73.70, 72.60, 72.48, 72.33, 71.77, 70.73, 68.47, 68.34, 65.73, 63.61, 62.60, 60.07, 51.73, 40.04, 39.36, 22.01.

$M+H^+$ 677.2611 calc. 677.2611

**General Procedure to prepare P1 trisaccharide**

P1 trisaccharide was performed in a reaction buffer (100mM Tris pH=7.5, monitored by PB-10), compound **8** (50mg, 0.13mmol), 10 mM $MnCl_2$, UDP-Glc (115mg, 0.2mmol) and GalE (5U), Lgt B(5U). Synthesis was carried out at 30℃. After 12h, the mixture was boiled and insoluble material was removed by centrifugation. HexA was added. After 2h, another portion of UDP-Glc (115mg, 0.2mmol) ,Lgt C (5U) and GalE(2U) was added. The reaction mixture was incubated at 30°C and monitored by TLC. After 10h, the mixture was boiled and insoluble material was removed by centrifugation. β-galactosidase and Hex A were added and allowed to react for 2h. The mixture was boiled and insoluble material was removed by centrifugation. The supernatant was directly loaded on the SPE column and eluted by 20% methanol in water. After Cbz group was removed by catalytic hydrogenation, compound 10 was obtained in a yield of 45% (two steps).

$^{1}$H NMR (400 MHz, $D_2O$) δ 4.82 (d, J = 3.7 Hz, 1H), 4.43 (d, J = 8.4 Hz, 1H), 4.41 (d, J = 8.8 Hz, 1H), 3.90 (dd, J = 3.4 Hz, 1H), 3.87 – 3.78 (m, 4H), 3.75 (dd, J = 9.3, 5.1 Hz, 1H), 3.73 – 3.68 (m, 1H), 3.68 – 3.52 (m, 10H), 3.51 – 3.42 (m, 2H), 3.42 – 3.37 (m, 1H), 3.36 – 3.30 (m, 2H), 1.92 (s, 3H).

$^{13}$C NMR (101 MHz, $D_2O$) δ 174.74, 102.83, 101.24, 100.23, 78.62, 78.30, 77.23, 75.78, 75.32, 74.67, 73.64, 72.45, 72.27, 72.09, 70.91, 69.80, 68.49, 60.98, 60.60, 55.49, 39.79, 22.09.

$M+H^+$ 689.2478 calc. 589.2456

**The procedure of synthesis of Gb3 and BSA conjugate**

Dissolve the BSA at a concentration of 2mg/ml in 0.1M Sodium phosphate, pH 7.4. Dissolve the

Compound **3** be coupled in the same buffer and add them to the reaction in at least a 10-fold molar excess over the amount of BSA. Add the solution prepared to the BSA solution to obtain at least a 10-fold molar excess of small molecule to protein. Add EDC to the above solution to obtain at least a 10-fold molar excess of EDC over the amount of protein present. Also, add sulfo-NHS to the reaction to bring its final concentration to 5 mM. React for 2 hours at room temperature. Purify the conjugate by dialysis.