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A new one-pot synthesis of Gb_3 and $isoGb_3$ trisaccharide analogues

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Abstract—Gb_3 and $isoGb_3$ are both biologically important oligosaccharides. A new efficient synthesis of Gb_3 and $isoGb_3$ trisaccharide analogues has been achieved by one-pot sequential glycosylation strategy starting from simple monosaccharide building blocks promoted by *N*-(phenylthio)-ε-caprolactam at room temperature.

1. Introduction

Glycosphingolipids[1] are a class of naturally occurring bioactive compounds usually embedded in the membrane of all animal cells and some plant cells. The clinically important blood group antigens and the immunologically relevant tumor-associated antigens are examples of glycosphingolipids. Gb_3 glycolipid [α-D-Gal-(1→4)-β-D-Gal(1→4)-β-D-Glc-1→*O*-ceramide] (Fig. 1) is highly enriched in Burkitt lymphoma cell-lines, human teratocarcinoma, human embryonal carcinoma, and other types of tumor cells, and Gb_3 glycolipid is also closely related to Fabry's disease, a lysosomal storage disorder caused by a deficiency of human lysosomal α-galactosidase A.[2]

Gb_3 also serves as a cell-surface receptor for *Shiga*-like toxins (SLTs).[3] *Shiga*-like toxins belong to a small but clinically significant family of AB_5 bacterial toxins. They consist of a cytotoxic enzyme A subunit noncovalently linked to a homopentameric cell-adhesion carrier, B_5. The entry of the enzymatic A subunit into the host cell relies on adhesion of the B_5 subunit to the cell membrane, a recognition that is highly specific and mediated by multiple terminal trisaccharide of Gb_3, known as P^k-trisaccharide. Inhibition of interactions between toxin B pentamer and its cell-surface receptors hold a potential treatment for bacterial infections.

Another glycosphingolipid, $isoGb_3$, also plays an important role in many events.[4] It was found to be associated with

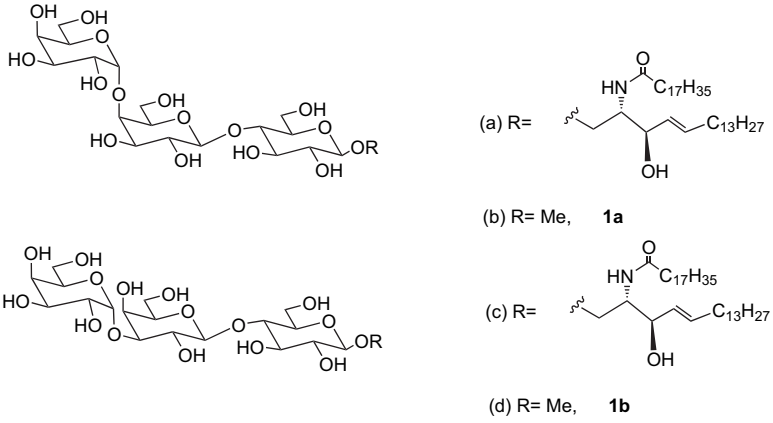

Figure 1. The structures of Gb_3, $isoGb_3$, and their methyl glycoside analogues **1a** and **1b**.

Keywords: Gb_3; $isoGb_3$; Thioglycoside; One-pot synthesis; Glycosylation.
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malignant neoplasms[4a,b] and shown that the Gal(αl-3)Gal segment of many glycosphingolipids can act as an attachment site for bacteria, bacterial toxins, and viruses.[4c,d] Recently, isoGb$_3$ was disclosed as an endogenous ligand for human NKT cells.[4f,g]

Because of their biological significance as well as common structures shared by various *globo*-series glycosphingolipids, a number of Gb$_3$/isoGb$_3$ tri- and di-saccharide analogues have been synthesized.[5] However, in the reported chemical syntheses of Gb$_3$ and isoGb$_3$, the trisaccharide donors are normally prepared in 17–19 steps due to the tedious functional group manipulations and glycosylations, more efficient synthetic routes to the trisaccharide analogues are still needed. Herein we wish to report a new synthesis of the methyl glycosides **1a** and **1b** (Fig. 1), using one-pot sequential glycosylation strategy.

2. Results and discussion

Oligosaccharide synthesis by one-pot sequential glycosylation strategy[6] is one of the efficient routes for saccharide assembly. Recently many important oligosaccharides such as fucosyl GM$_1$,[7] Globo-H,[8] Lewis Y,[9] antigen N3 minor glycoside,[10] and α-Gal epitopes[11] have been synthesized using one-pot method. Promoters play an important role in the one-pot synthesis. There are many promoters available including dimethyl-(thiomethyl)sulfonium triflate (DMTST),[12] *N*-iodosuccinimide/triflic acid (NIS/TfOH),[13] phenylsulfenyl chloride and silver triflate (PhSCl/AgOTf),[14] and 1-benzenesulfinylpiperidine and triflic anhydride (BSP/Tf$_2$O),[15] but all of them have to be employed under low temperature (−78 °C to 0 °C) conditions. Very recently, a new promoter, *N*-(phenylthio)-ε-caprolactam (Fig. 2), has been reported by Wong's group.[16] This reagent can be used at room temperature to promote the glycosyl coupling reactions. We decided to make the trisaccharides **1a** and **1b** with this new promoter by one-pot glycosylation procedure.

For the synthesis of the target trisaccharides, we chose thioglycosides as glycosyl donors because they are stable enough in storage and can be activated by a variety of promoters including *N*-(phenylthio)-ε-caprolactam. The retrosynthetic analysis of **1a** and **1b** is shown in Figure 3. For the synthesis of **1a**, the perbenzylated thiogalactoside **2** and 2,3-*O*-benzoylated-6-*O*-benzylated thiogalactoside **3** are chosen as the first and second components, and the methyl glucoside **4** is selected as the third component. For the synthesis of **1b**, thiogalactoside **5** is used as the second building block instead of **3**. All the designed building blocks are very simple with the widely used benzyl/benzylidene and benzoyl functionalities as the hydroxyl protecting groups.

The galactosyl donor **2**[6a] was prepared from galactose pentaacetate in a three-step process as shown in Scheme 1.

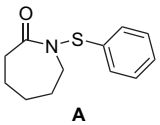

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Figure 2. The structure of *N*-(phenylthio)-ε-caprolactam.

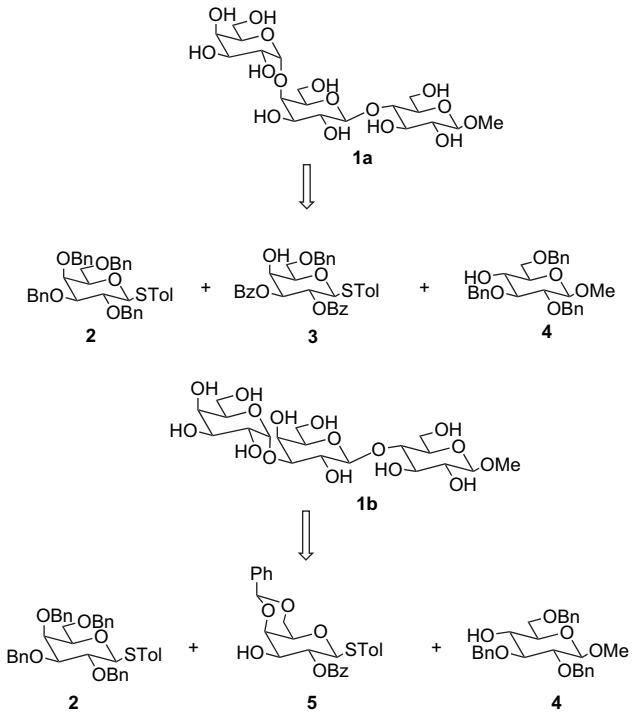
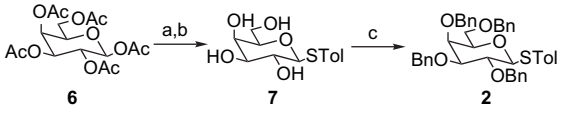


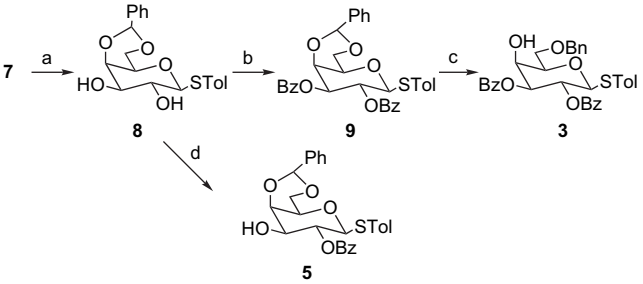
Figure 3. The retrosynthesis of trisaccharides **1a** and **1b**.

Treatment of the peracetylated galactose **6** with thiocresol in the presence of BF$_3$·Et$_2$O followed by O-deacetylation with NaOMe afforded compound **7**.[17] O-Benzylation of **7** provided the desired galactosyl donor **2** in 84% isolated yield.

The synthesis of galactosyl building block **3**[6a] began from compound **7** in three steps as shown in Scheme 2. Treatment of **7** with benzaldehyde dimethyl acetal and a catalytic amount of DL-10-camphorsulfonic acid (CSA) in acetonitrile yielded the 4,6-*O*-benzylidene derivative **8**.[18] The remaining free hydroxyl groups of **8** were benzoylated to afford fully protected saccharide **9**,[18] which was subjected



Scheme 1. Synthesis of galactosyl donor **2**. Reagents and conditions: (a) MePhSH, BF$_3$·Et$_2$O, CH$_2$Cl$_2$, 80%; (b) NaOMe, MeOH, 99%; (c) BnBr, NaH, DMF, 84%.



Scheme 2. Synthesis of galactosyl donor–acceptors **3** and **5**. Reagents and conditions: (a) benzaldehyde dimethyl acetal, CSA, MeCN, 81%; (b) BzCl, pyridine, 74%; (c) NaCNBH$_3$, HCl·Et$_2$O, THF, 93%; (d) BzCl, Ag$_2$O, KI, DCM, 50%.

to regioselective reductive cleavage by treatment with $NaCNBH_3$ in the presence of etheral hydrochloride, providing the acceptor–donor **3** with the 4-OH exposed. On the other hand, diol **8** was regioselectively benzoylated to obtain the desired building block **5**[19,6a] with the 3-OH free in 50% isolated yield.

The synthesis of acceptor **4** (Scheme 3) started from methyl glucoside **10**. After 4,6-*O*-benzylidenation of **10**, the remaining two OH groups of **11**[20] were benzylated to provide **12**.[21] Compound **12** was then transformed into the 4-OH free derivative **4**[22] with $NaCNBH_3/HCl\cdot Et_2O$ in 90% isolated yield.

With all these building blocks in hand, glycosylations between the donors and acceptors were explored. The coupling reaction of donor **2** and acceptor **3** at room temperature with *N*-(phenylthio)-ε-caprolactam/Tf_2O as the promoter was firstly tried. Although it was often thought that the 4-OH of galactose moiety is not easy to couple with glycosyl donors, we successfully obtained the desired disaccharide **13** in 87% isolated yield (Scheme 4). It is also noteworthy that only the desired α-anomer was obtained in the glycosylation of **2** with **3**. The configuration of the newly formed glycosidic linkage was confirmed by the ^1H NMR coupling constant of the anomeric proton H-1 (3.5 Hz). The successful preparation of disaccharide **13** indicated that *N*-(phenylthio)-ε-caprolactam/Tf_2O can serve as the promoter for stereoselective glycosylation. We next focused on applying this promoter to the one-pot synthesis of trisaccharide **14**. Consecutive coupling of *p*-tolyl thiogalactosides **2** and **3**, and methyl glucoside **4** in dichloromethane at room temperature produced trisaccharide **14** smoothly in 47% isolated yield (Scheme 4). Finally, global deprotection of **14** was performed in two steps: the benzoyl group was removed with NaOMe in methanol, and the benzyl functionality was cleaved with Pd/C catalyzed hydrogenolysis. The target trisaccharide **1a** was obtained in 80% yield from **14**.

Similarly, for the synthesis of $isoGb_3$ trisaccharide **1b**, we also checked the two-component coupling reaction of **2** and **5**, and we obtained disaccharide **15** in 90% isolated yield (Scheme 5). After successful preparation of disaccharide **15**, the one-pot sequential glycosylations of building blocks **2** and **5**, and methyl glucoside **4** were performed at room temperature, leading to trisaccharide **16** in 50% isolated yield (Scheme 5). Global deprotection of **16** was carried out in the same way as above-mentioned to provide the target trisaccharide **1b** in 90% yield.

The structures of **1a** and **1b**, as well as the correct anomeric configuration of each glycosidic linkage were confirmed by their ^1H NMR, ^{13}C NMR, and HRMS analyses.

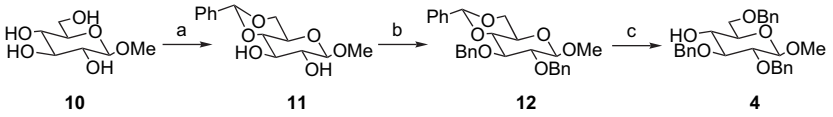
3. Conclusion

In conclusion, the trisaccharide analogues of Gb_3 and $isoGb_3$ were synthesized in an efficient way with high stereoselectivity by one-pot sequential glycosylation strategy using very common building blocks. Importantly, all the glycosyl coupling reactions were carried out at room temperature in the presence of *N*-(phenylthio)-ε-caprolactam/Tf_2O.

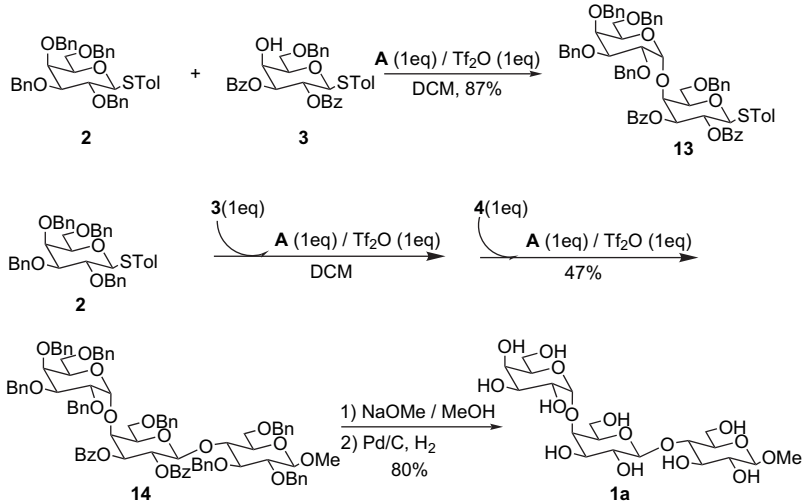
4. Experimental

4.1. General methods

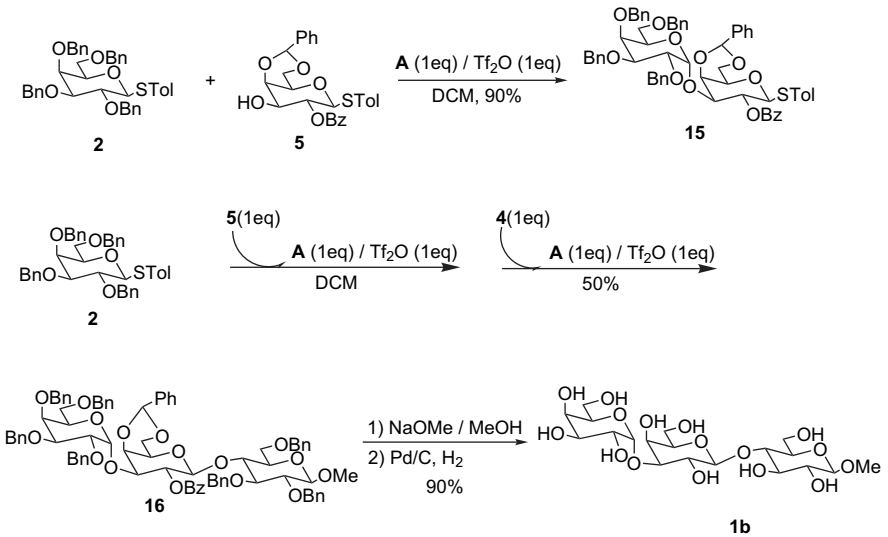
All reactions were carried out under an atmosphere of nitrogen unless noted otherwise. All solvents were dried and distilled by standard techniques. Analytical thin-layer



Scheme 3. Synthesis of glucosyl acceptor **4**. Reagents and conditions: (a) benzaldehyde dimethyl acetal, CSA, MeCN, 80%; (b) BnBr, NaH, DMF, 74%; (c) $NaCNBH_3$, $HCl\cdot Et_2O$, THF, 90%.



Scheme 4. Synthesis of Gb_3 trisaccharide **1a**.



Scheme 5. Synthesis of $isoGb_3$ trisaccharide **1b**.

chromatography (TLC) was performed on silica gel 60 F_{254} (E. Merck Co.). Compounds spots were visualized by UV light and/or by staining with an ethanol solution of phosphomolybdic acid and cerium sulfate. Column chromatography was performed on silica gel (200–300 mesh, Qingdao Chemical Industry). NMR spectra were recorded with Varian INOVA-500 spectrometer. Chemical shifts were referenced to the internal TMS. Mass spectra were recorded on a PE SCLEX QSTAR spectrometer.

Optical rotations were measured with an AA-10R automatic polarimeter.

4.2. Synthesis

4.2.1. *p*-Methylphenyl 2,3-di-*O*-benzoyl-6-*O*-benzyl-4-*O*-(2′,3′,4′,6′-tetra-*O*-benzyl-α-D-galactopyranosyl)-1-thio-β-D-galactopyranoside (13). To a solution of **2** (100.0 mg, 0.155 mmol), **3** (90.5 mg, 0.155 mmol), and *N*-(phenylthio)-ε-caprolactam (34.0 mg, 0.155 mmol) in CH_2Cl_2 (45 mL) was added 4 Å molecular sieves (600.0 mg). The mixture was stirred for 2 h at room temperature, and then Tf_2O (43.7 mg, 0.155 mmol) was added. The resulting mixture was stirred at room temperature for 0.5 h. The course of the reaction was monitored by TLC. Et_3N (0.5 mL) was then added to the mixture. The precipitate was filtered off and the filtrate was concentrated. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate 5:1) to give **13** (137.1 mg, 87%) as a thick oil. ^1H NMR (500 MHz, $CDCl_3$): δ 2.19 (s, 3H), 2.92–2.95 (m, 1H), 3.33 (t, 1H, J=9.0 Hz), 3.71–3.74 (m, 1H), 3.87–3.95 (m, 2H), 3.98–4.09 (m, 6H), 4.25–4.30 (m, 2H), 4.39 (d, 1H, J=2.5 Hz), 4.46 (d, 1H, J=11.0 Hz), 4.66 (d, 1H, J=12.0 Hz), 4.75 (d, 1H, J=11.5 Hz), 4.79 (d, 1H, J=11.5 Hz), 4.83 (d, 1H, J=11.0 Hz), 4.88 (d, 1H, J=3.5 Hz, H-1′), 4.90 (d, 1H, J=11.5 Hz), 5.23 (dd, 1H, J=2.5 Hz, 10.0 Hz), 5.69 (t, 1H, J=10.0 Hz), 6.96 (d, 2H, J=8.0 Hz), 7.18–7.45 (m, 32H, Ar-H), 7.48–7.51 (m, 1H), 7.88–7.90 (m, 2H), 7.94–7.96 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 21.38, 67.66, 68.18, 69.53, 69.63, 72.64, 73.15, 73.33, 74.28, 74.95, 75.18, 75.70, 76.91, 78.54, 78.96, 86.15, 100.21, 127.31, 127.36, 127.45, 127.54, 127.63, 127.94, 128.07, 128.19, 128.34, 128.08, 129.50, 129.68, 129.91, 130.07, 133.16, 133.67, 133.98, 138.30, 138.69, 138.87, 165.10, 166.28. HRMS (ESI) Anal. Calcd for $C_{68}H_{66}NaO_{12}S$ $[M+Na]^+$: 1129.4167; found: 1129.4168.

4.2.2. Methyl 2,3,6-tri-*O*-benzyl-4-*O*-[2′,3′-di-*O*-benzoyl-6′-*O*-benzyl-4′-*O*-(2″,3″,4″,6″-tetra-*O*-benzyl-α-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (14). After a mixture of donor **2** (100.0 mg, 0.155 mmol), acceptor **3** (90.5 mg, 0.155 mmol), *N*-(phenylthio)-ε-caprolactam (34.0 mg, 0.155 mmol) and 4 Å molecular sieves (600.0 mg) in CH_2Cl_2 (45 mL) was stirred for 2 h at room temperature, Tf_2O (43.7 mg, 0.155 mmol) was added via a syringe. After 0.5 h, TLC indicated that both the donor and acceptor were consumed completely, **4** (72.0 mg, 0.155 mmol) and *N*-(phenylthio)-ε-caprolactam (34.0 mg, 0.155 mmol) were then added into the flask. The reaction mixture was stirred for another 10 min, and Tf_2O (43.7 mg, 0.155 mmol) was added. After stirring for 1 h, the reaction was quenched with Et_3N (0.5 mL) and all insoluble materials were removed by filtration. The filtrate was concentrated and the residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate 5:1 to 2:1) to provide **14** (104.9 mg, 47%) as a thick oil. $[\alpha]_D^{20}$ 8.0 (c 0.0025, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 2.93–2.96 (m, 1H), 3.26–3.29 (m, 1H), 3.33–3.37 (m, 2H), 3.48 (s, 3H), 3.52–3.62 (m, 4H), 3.68–3.70 (m, 1H), 3.93–4.01 (m, 3H), 4.05–4.07 (m, 3H), 4.10–4.12 (m, 1H), 4.19 (d, 1H, J=8.0 Hz, H-1), 4.20–4.43 (m, 7H), 4.51–4.59 (m, 3H), 4.62–4.65 (m, 2H), 4.73 (d, 1H, J=11.5 Hz), 4.78–4.85 (m, 3H), 4.91 (d, 1H, J=3.5 Hz, H-1″), 4.94 (d, 1H, J=8.0 Hz, H-1′), 5.03–5.07 (m, 2H), 5.74 (dd, 1H, J=7.5 Hz, 10.5 Hz), 7.14–7.38 (m, 46H), 7.46–7.49 (m, 1H), 7.86–7.89 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 56.94, 67.40, 67.80, 68.18, 69.48, 70.68, 72.33, 72.87, 73.00, 73.35, 73.74, 74.09, 74.34, 74.72, 74.88, 75.64, 76.24, 79.10, 81.86, 82.33, 100.66, 100.84, 104.44, 127.22, 127.35, 127.44, 127.50, 127.76, 127.89, 127.96, 128.02, 128.12, 128.19, 128.30, 128.42, 129.30, 129.56, 129.83, 133.06, 133.15, 138.19, 138.40, 138.61, 138.76, 138.92, 139.18, 165.15, 166.44. HRMS (ESI) Anal. Calcd for $C_{89}H_{90}NaO_{18}$ $[M+Na]^+$: 1469.6019; found: 1469.6013.

4.2.3. Methyl 4-*O*-[4′-*O*-(α-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (1a). To a solution of compound **14** (45.0 mg, 0.03 mmol) in MeOH (10 mL) was added NaOMe/MeOH (30 wt %, 0.4 mL), and the reaction mixture was stirred for 8 h at room temperature. The mixture was neutralized (H^+ resin, weak acid). The resin was filtered off and the filtrate was concentrated. The resulting residue was dissolved in THF/AcOH/H_2O (v/v 2:2:1, 10 mL) and then Pd/C (10.0 mg) was added. The reaction mixture was stirred under H_2 atmosphere for 24 h. The Pd/C was filtered off and the filtrate was concentrated. The product was purified by C-18 reverse-phase column chromatography to give **1a** (12.9 mg, 80%) as an oil. $[\alpha]_D^{20}$ 17.7 (*c* 0.00338, H_2O/CH_3OH 1:2); ^1H NMR (500 MHz, D_2O): δ 3.27–3.31 (m, 1H), 3.56 (s, 3H), 3.57–3.60 (m, 2H), 3.60–3.64 (m, 2H), 3.67–3.73 (m, 3H), 3.75 (d, 1H, *J*=3.0 Hz), 3.77–3.79 (m, 1H), 3.79–3.87 (m, 4H), 3.87–3.94 (m, 2H), 4.00 (d, 1H, *J*=7.5 Hz), 4.02–4.04 (m, 2H), 4.35 (t, 1H, *J*=6.0 Hz), 4.40 (d, 1H, *J*=8.0 Hz, H-1′), 4.50 (d, 1H, *J*=7.5 Hz, H-1), 4.94 (d, 1H, *J*=4.0 Hz, H-1″); ^{13}C NMR (125 MHz, D_2O): δ 57.95, 60.76, 61.12, 61.27, 69.31, 69.70, 69.86, 71.57, 71.66, 72.91, 73.62, 75.19, 75.55, 76.17, 78.10, 79.40, 101.05, 103.77, 104.10. HRMS (ESI) Anal. Calcd for $C_{19}H_{34}NaO_{16}$ $[M+Na]^+$: 541.1739; found: 541.1728.

4.2.4. *p*-Methylphenyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(2′,3′,4′,6′-tetra-*O*-benzyl-α-D-galactopyranosyl)-1-thio-β-D-galactopyranoside (15). To a solution of **2** (100.0 mg, 0.155 mmol), **5** (74.1 mg, 0.155 mmol), and *N*-(phenylthio)-ε-caprolactam (34.0 mg, 0.155 mmol) in CH_2Cl_2 (45 mL) was added 4 Å molecular sieves (600.0 mg), and the mixture was stirred for 2 h at room temperature. Tf_2O (43.7 mg, 0.155 mmol) was then added, and the reaction mixture was stirred at room temperature for 0.5 h. The course of the reaction was monitored by TLC. Et_3N (0.5 mL) was then added to the mixture. The precipitate was filtered off and the filtrate was concentrated. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate 5:1) to give **15** (134.9 mg, 90%) as an oil. ^1H NMR (500 MHz, $CDCl_3$): δ 2.32 (s, 3H), 3.17–3.25 (m, 2H), 3.41 (s, 2H), 3.63 (dd, 1H, *J*=3.0 Hz, 10.0 Hz), 3.82 (t, 1H, *J*=6.5 Hz), 3.92–3.96 (m, 2H), 4.00 (dd, 1H, *J*=3.0 Hz, 10.0 Hz), 4.25 (d, 1H, *J*=10.5 Hz), 4.31–4.47 (m, 6H), 4.56 (d, 1H, *J*=11.5 Hz), 4.62 (d, 1H, *J*=12.0 Hz), 4.72 (d, 1H, *J*=10.0 Hz, H-1), 4.77 (d, 1H, *J*=11.5 Hz), 5.05 (d, 1H, *J*=3.5 Hz, H-1′), 5.38 (s, 1H), 5.60 (t, 1H, *J*=9.5 Hz), 6.96–7.42 (m, 32H, Ar-H), 8.02 (d, 2H, *J*=8.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 21.25, 68.66, 68.98, 69.34, 69.84, 69.91, 71.80, 72.17, 73.11, 73.23, 74.57, 74.94, 75.53, 75.80, 78.59, 85.44, 94.64, 101.04, 126.55, 127.25, 127.30, 127.38, 127.46, 127.55, 127.66, 127.80, 128.06, 128.10, 128.14, 128.22, 128.29, 128.89, 129.46, 129.81, 130.07, 132.92, 134.16, 137.66, 138.08, 138.28, 138.54, 138.66, 138.80, 164.63. MS (ESI) Anal. Calcd for $C_{61}H_{64}NO_{11}S$ $[M+NH_4]^+$: 1018.4; found: 1018.7.

4.2.5. Methyl 2,3,6-tri-*O*-benzyl-4-*O*-[2′-*O*-benzoyl-4′,6′-*O*-benzylidene-3′-*O*-(2″,3″,4″,6″-tetra-*O*-benzyl-α-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (16). After a mixture of donor **2** (100.0 mg, 0.155 mmol), acceptor **5** (74.1 mg, 0.155 mmol), *N*-(phenylthio)-ε-caprolactam (34.0 mg, 0.155 mmol) and 4 Å molecular sieves (600.0 mg) in CH_2Cl_2 (45 mL) was stirred for 2 h at room temperature, Tf_2O (43.7 mg, 0.155 mmol) was added via a syringe. After 0.5 h, TLC indicated that both the donor and acceptor were consumed completely, **4** (72.0 mg, 0.155 mmol) and *N*-(phenylthio)-ε-caprolactam (34.0 mg, 0.155 mmol) were then added. After stirring for another 10 min, Tf_2O (43.7 mg, 0.155 mmol) was added. After stirring for 1 h, the reaction was quenched with Et_3N (0.5 mL) and all insoluble materials were removed by filtration. The filtrate was concentrated and the residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate 3:1) to give **16** (103.9 mg, 50%) as a thick oil. $[\alpha]_D^{20}$ 6.2 (*c* 0.0030, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 2.97 (s, 1H), 3.16 (dd, 1H, *J*=6.0 Hz, 9.0 Hz), 3.22–3.28 (m, 2H), 3.39 (t, 1H, *J*=7.5 Hz), 3.46–3.48 (m, 5H), 3.60–3.68 (m, 4H), 3.73 (d, 1H, *J*=11.0 Hz), 3.79 (dd, 1H, *J*=3.5 Hz, 10.0 Hz), 3.84 (t, 1H, *J*=6.5 Hz), 3.88–3.91 (m, 1H), 3.94–3.97 (m, 1H), 4.11–4.26 (m, 6H), 4.35 (t, 2H, *J*=12.0 Hz), 4.42–4.53 (m, 4H), 4.58–4.63 (m, 2H), 4.69 (d, 1H, *J*=6.0 Hz), 4.77–4.86 (m, 4H), 5.04 (d, 1H, *J*=3.5 Hz, H-1″), 5.10 (d, 1H, *J*=11.0 Hz), 5.32 (s, 1H), 5.61 (dd, 1H, *J*=8.5 Hz, 10.0 Hz), 7.06–7.38 (m, 40H), 7.48–7.50 (m, 3H), 7.97–7.99 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 56.95, 66.60, 68.36, 68.73, 68.80, 69.84, 71.37, 72.13, 72.26, 73.09, 73.14, 73.22, 74.41, 74.64, 74.68, 74.92, 75.26, 75.48, 75.84, 77.64, 78.58, 81.96, 83.03, 95.55, 100.95, 101.20, 104.48, 126.39, 127.09, 127.29, 127.37, 127.45, 127.49, 127.58, 127.65, 127.81, 127.91, 127.99, 128.09, 128.15, 128.23, 128.34, 128.41, 128.70, 129.76, 129.85, 133.01, 137.77, 138.16, 138.46, 138.60, 138.65, 138.78, 139.07, 164.69. HRMS (ESI) Anal. Calcd for $C_{82}H_{88}NO_{17}$ $[M+NH_4]^+$: 1358.6047; found: 1358.6053.

4.2.6. Methyl 4-*O*-[3′-*O*-(α-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (1b). To a solution of compound **16** (40.2 mg, 0.03 mmol) in MeOH (10 mL) was added NaOMe/MeOH (30 wt %, 0.4 mL), and the reaction mixture was stirred for 8 h at room temperature. The mixture was neutralized (H^+ resin, weak acid). The resin was filtered off and the filtrate was evaporated in vacuum. The resulting residue was dissolved in THF/AcOH/H_2O (v/v 2:2:1, 10 mL) and then Pd/C (10.0 mg) was added. The reaction mixture was stirred under H_2 atmosphere for 24 h. The Pd/C was filtered off and the filtrate was concentrated. The product was purified by C-18 reverse-phase column chromatography to give **1b** (13.9 mg, 90%) as an oil. $[\alpha]_D^{20}$ 20.1 (*c* 0.00579, H_2O/CH_3OH 1:2); ^1H NMR (500 MHz, D_2O): δ 3.29–3.30 (m, 1H), 3.58 (s, 3H), 3.61–3.63 (m, 1H), 3.65–3.68 (m, 3H), 3.71–3.87 (m, 8H), 3.94–4.02 (m, 3H), 4.18–4.21 (m, 2H), 4.41 (d, 1H, *J*=8.0 Hz, H-1), 4.52 (d, 1H, *J*=7.5 Hz, H-1′), 5.14 (d, 1H, *J*=4.0 Hz, H-1″); ^{13}C NMR (125 MHz, D_2O): δ 57.98, 60.88, 61.68, 61.74, 65.56, 68.95, 69.89, 70.03, 70.33, 71.59, 73.52, 75.21, 75.49, 75.80, 77.96, 79.38, 96.19, 103.60, 103.83. HRMS (ESI) Anal. Calcd for $C_{19}H_{34}O_{16}Na$ $[M+Na]^+$: 541.1739; found: 541.1748.

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