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An efficient glycosylation reaction for the synthesis of asialo GM2 analogues

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Abstract—We investigated the coupling reaction of glycosyl donors *N*-trichloroethoxycarbonyl-galactosamine-*O*-trichloroacetimidate (**2a**) and *N*-*p*-nitrobenzyloxycarbonyl-galactosamine-*O*-trichloroacetimidate (**2b**) with the 4′-OH of lactose derivatives (**3a–d**) to synthesize key intermediates of asialo GM2 analogues, and found that the glycosylation yield with **2a** was 90% or more in all investigated cases.

Oligosaccharides containing β-D-glycosides of *N*-acetyl-2-amino-2-deoxy units are widely distributed in living organisms.[1] In this respect the biologically active gangliosides GM1 and GM2, which both contain an *N*-acetyl-2-amino-2-deoxy-D-galactopyranosyl residue β-linked to O-4′ of lactose,[2] are typical examples.

Asialo GM2 (GA2) has been widely studied as a tumor-associated marker and a monoclonal antibody,[3] and various synthetic efforts to produce asialo GM2 have been reported with moderate yields.[4] However, the glycosylation step required for its synthesis has only been reported with low yields (<50%). Given its interesting biological activity, we therefore set out to develop a more efficient synthesis of asialo GM2 analogues **1** (see Fig. 1).

As indicated in Figure 1, the retrosynthesis of asialo GM2 involves assembly of two key building blocks **2** and **3**. Compound **2** is an efficient glycosyl donor, in which the NH_2 group needs to be protected. The phthalimido (Phth) and azido groups have been the most widely used.[5] Recently, *p*-nitrobenzyloxycarbonyl[6] (PNZ) and trichloroethoxycarbonyl (Teoc) groups were applied in the synthesis of glycoconjugates containing 2-acetamido glycoside units.[7] However, the linkage of *N*-phthalimido or azido halide donors and OH-4′ lactose derivatives have been reported to yield asialo GM2 in low to moderate yields (20–54%).[4] In our hands, Teoc and PNZ[6] proved to be stable under the glycosylation conditions (−20 °C, in diethyl ether and TMSOTf (0.10 equiv) as catalyst), and both could be easily and selectively cleaved.[7] On the other hand, since *O*-glycosyl trichloroacetimidate was shown to be superior compared to the glycosyl donors containing halides (Cl or Br) at the anomeric centre,[8] we selected *N*-trichloroethoxycarbonyl-galactosamine-*O*-trichloroacetimidate (**2a**) and *N*-*p*-nitrobenzyloxycarbonyl-galactosamine-*O*-trichloroacetimidate (**2b**) as glycosyl donors to investigate their coupling efficiency with 4′-OH lactose derivatives.

Scheme 1 outlines the syntheses of these donor units. Treatment of galactosamine hydrochloride **4** with 1 equiv of NaOMe in MeOH, followed by the addition of trichloroethyl chloroformate or *p*-nitrobenzyl chloroformate (1 equiv)–TEA (1 equiv) and *O*-acetylation (Ac_2O–pyridine) afforded compounds **5a** (92%) or **5b** (92%) in good yields. Finally, regioselective deacetylation at *O*-1 with hydrazine acetate in DMF followed by treatment of the reducing sugar with trichloroacetonitrile in the presence of DBU, produced the Teoc-trichloroacetimidate **2a** (79%; overall yield from **4** is 73%).[9] The PNZ analogue **2b** was obtained in 75% yield (overall yield from **4** is 69%).

As stated above, the 4′-OH lactose derivatives showed a very low reactivity as glycosyl acceptors. As benzyl ethers are able to enhance the reactivity of neighbouring hydroxyl groups in glycosylation reactions,[10] we selected the benzyl group as the protecting group for the lactose units of **3a–d** (Scheme 2).

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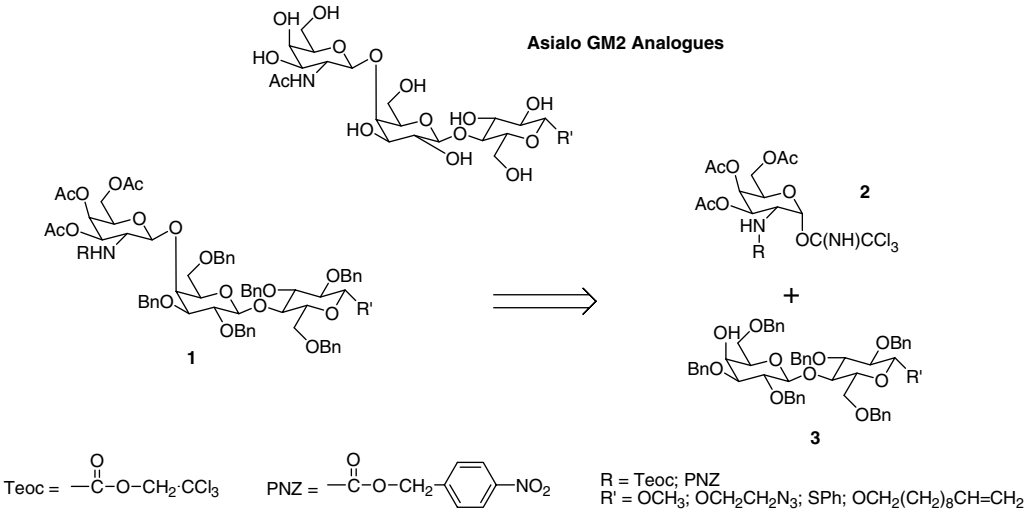
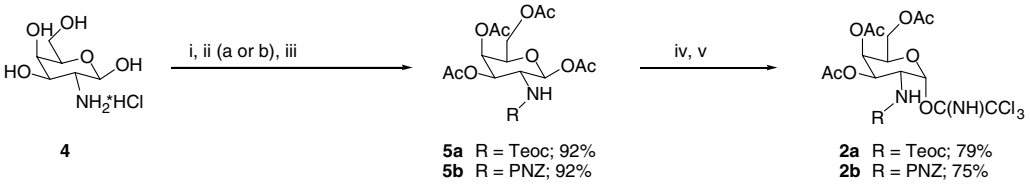
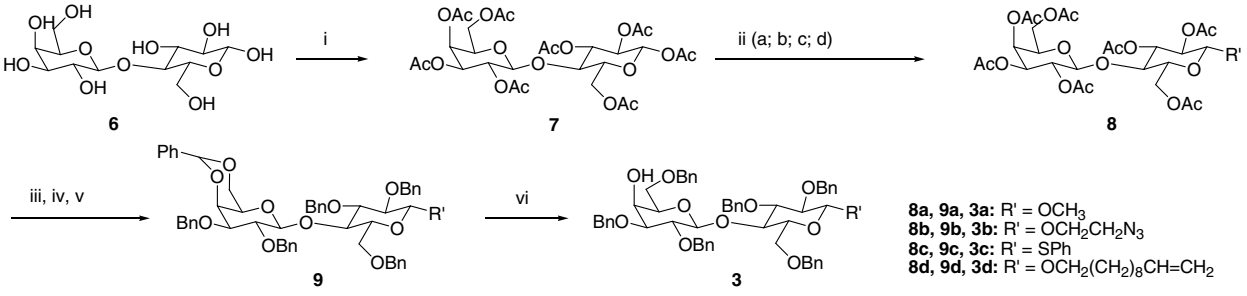


Figure 1. Retrosynthesis of the key intermediates of asialo GM2 analogues.



Scheme 1. Reagents and conditions: (i) NaOMe (1 equiv)/MeOH, room temperature, 30 min; (ii) a. trichloroethyl chloroformate (1 equiv), Et_3N (1 equiv), room temperature, 2 h; b. *p*-nitrobenzyl chloroformate (1 equiv), Et_3N (1 equiv, room temperature, 2 h); (iii) Ac_2O/Py, room temperature, overnight; (iv) hydrazine acetate, DMF, 0 °C, 2 h; (v) CCl_3CN (10 equiv)/DBU, CH_2Cl_2, −10 °C, 5 h.

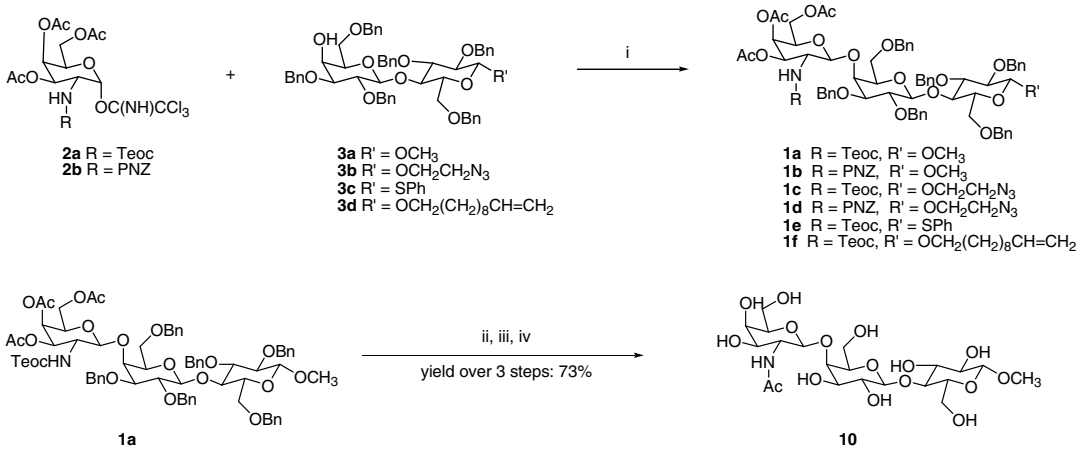


Scheme 2. Reagents and conditions: (i) Ac_2O/NaOAc, reflux, 2 h, 77%; (ii) a. 24% $NH_2NH_2·H_2O$, CH_3CN, 24 h, room temperature, 86%; CH_3I/Ag_2O/CH_3CN, room temperature, 24 h, **8a**, 84%; b. 2-azidoethanol (2.5 equiv), $BF_3·C_2H_5OC_2H_5$, CH_2Cl_2, 0 °C to room temperature, 24 h, **8b**, 80%; c. thiophenol (2.5 equiv), $BF_3·C_2H_5OC_2H_5$, CH_2Cl_2, 0 °C to room temperature, 24 h, **8c**, 75%; d. 33% HBr in AcOH, 0 °C–rt, 5 h, 72%; undec-10-en-1-ol, AgOTf, dry toluene, −78 °C to room temperature, overnight, **8d**, 84%; (iii) NaOMe/MeOH, IR resin (H^+); (iv) $PhCH(OCH_3)_2$/CSA/THF, reflux, 3 h; (v) NaH/BnBr/DMF, overnight, room temperature, **9a** (50%); **9b** (57%), **9c** (54%), **9d** (60%) (three steps); (vi) $NaCNBH_3$/HCl in Et_2O/dry THF, 30 min, **3a** (78%), **3b** (78%), **3c** (76%), **3d** (77%).

In Scheme 2, the synthetic route to the lactose acceptors is depicted. Heating a mixture of lactose **6**, acetic anhydride and anhydrous sodium acetate afforded peracetyllactose **7** (yield: 77%).[11] After treatment with hydrazine, the anomeric acetyl group in **7** was selectively deprotected, and the product was treated with CH_3I and silver oxide to give **8a** (72%).[12] Alternatively, compound **7** was reacted with 2-azidoethanol or thiophenol in the presence of BF_3-diethyl etherate to afford **8b** (80%) and **8c** (75%), respectively. Bromination of **7** with hydrogen bromide 33% (w/w) in acetic acid at 0 °C and subsequent coupling with undec-10-en-1-ol gave

Table 1. Glycosylation yields of glycosyl donors **2a**–**b** and acceptors **3a**–**d**

Glycosyl donor	Lactose derivative	Molar ratio of **2**:**3**	Product	Isolated yield, %
2a	**3a**	1.5	**1a**	91
2b	**3a**	1.5	**1b**	69
2a	**3b**	1.5	**1c**	90
2b	**3b**	1.5	**1d**	71
2a	**3c**	1.5	**1e**	90
2a	**3d**	1.5	**1f**	90



Scheme 3. Reagents and conditions: (i) TMSOTf/dry Et_2O, −20 °C, 5 h; (ii) active Zn powder/Ac_2O, room temperature, 5 h; (iii) NaOMe/MeOH, room temperature; (iv) H_2 (50 psi), Pd (10%)/C, room temperature, 6 h.

8d (84%). Compounds **8a**–**d** were characterized by NMR and LC–MS. Compounds **8a**–**d** were deacetylated using NaOMe in MeOH, followed by selective protection of OH-4′ and OH-6′ with α,α-dimethoxytoluene under mild acidic conditions in anhydrous THF. The remaining OH-groups were benzylated with NaH and benzyl bromide in DMF to give **9a**–**d** (50%; 57%; 54%; 60%). Selective cleavage[13] of the benzaldehyde acetal in **9a**–**d** with $NaBH_3CN$–HCl in dry THF afforded acceptor compounds **3a**–**d** in good yields (76–78%).[14]

Finally, glycosyl donors **2a**–**b** were reacted with acceptors **3a**–**d** at −20 °C in dry diethyl ether in the presence of TMSOTf (0.11 equiv) to give GM2 analogues **1a**–**f**.[15] The glycosylation yields are listed in Table 1.

The results in Table 1 clearly show that the glycosylation efficiency of glycosyl donor *N*-trichloroethoxycarbonyl-galactosamine-*O*-trichloroacetimidate (**2a**) was better than that of *N*-*p*-nitrobenzyloxycarbonyl-galactosamine-*O*-trichloroacetimidate (**2b**). Replacement of the *N*-Teoc group in **1a** by an *N*-acetyl group with active Zn powder in acetic anhydride[7] followed by deacetylation with NaOMe in MeOH and debenzylation with H_2 (50 psi) and Pd/C (10%) in MeOH at room temperature gave β-D-GalNAc-(1 → 4)-β-D-Gal-(1 → 4)-β-D-Glc-OMe (methyl asialo GM2, **10**)[16] in 73% yield (three steps), see Scheme 3.

In conclusion, we have prepared and investigated two glycosyl donors **2a** and **2b**, which could be linked efficiently to lactose acceptors **3a**–**d**, and showed unambiguously that *N*-trichloroethoxycarbonyl-galactosamine-*O*-trichloroacetimidate was an efficient donor with glycosylation yields of 90% or more.

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9. Compound **2a**. ^1H NMR (300 MHz, $CDCl_3$), all couplings in Hz. δ (ppm): 8.74 (s, 1H, C=NH), 6.40 (d, 1H, $J = 3.6$, H-1), 5.46 (d, 1H, $J = 2.6$, H-4), 5.22 (dd, 1H, $J_1 = 3.2$, $J_2 = 11.4$, H-3), 5.01 (d, 1H, $J = 9$, NHTeoc), 4.70 and 4.64 (ABq, $J = 9.5$, 1H each, Cl_3CCH_2OCO), 4.46–4.47 (m, 1H, H-2), 4.01–4.32 (m, 3H, H-5, H-6), 2.11 (s, 3H, $COCH_3$), 1.96 (s, 6H, 2 $COCH_3$). HRMS $[C_{17}H_{20}{}^{35}Cl_5{}^{37}Cl_1N_2O_{10}+Na]^+$ 646.91582 (calcd 646.91173, Δ ppm = 6.33).
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14. Compound **3a**. HRMS $[M+Na]^+$ 919.40567 (calcd 919.40333, Δ ppm = 2.54).
15. Typical glycosylation procedure: a mixture of compound **2a** (238 mg, 0.33 mmol), **3a** (200 mg, 0.22 mmol) and active powdered 4 Å molecular sieves (1.0 g) in dry diethyl ether (6 mL) was stirred for 1 h at room temperature under N_2. After cooling to −20 °C, 150 μL of TMSOTf solution (50 μL TMSOTf dissolved in 2.0 mL dry diethyl ether) (0.0237 mmol) was injected into the reaction, and the mixture was stirred for about 4 h at −20 °C. The reaction was monitored by TLC (3:7, ethyl acetate:petroleum ether (40–60)). When acceptor **3a** had almost disappeared, the reaction was quenched with triethylamine and was filtered through Celite, washed with CH_2Cl_2 and concentrated. Column chromatography (3:7, ethyl acetate:petroleum ether) gave trisaccharide **1a** (280 mg, 91%). ^{13}C NMR δ (C_6D_6, ppm): 170.3, 170.0, 154.6, 140.0, 139.6, 139.3, 129.5, 129.2, 129.1, 129.0, 128.9, 128.8, 128.7, 128.2, 127.9, 127.8, 105.3, 103.1, 102.3, 83.2, 83.1, 82.6, 81.3, 76.2, 76.0, 75.3, 74.7, 73.9, 73.6, 71.4, 68.8, 67.2, 61.6, 60.3, 56.3, 53.8, 20.8, 20.6, 20.4.
16. Compound **10**. NMR (400 MHz, D_2O), all couplings in Hz. ^1H δ (ppm): 4.54 (d, $J = 8$, 1H), 4.35 (d, $J = 8$, 1H), 4.33 (d, $J = 8$, 1H), 4.01 (d, $J = 2.8$, 1H), 3.90 (d, $J = 12$, 1H), 3.84–3.78 (m, 2H), 3.77–3.46 (m, 15 H), 3.26–3.29 (m, 1H), 3.24–3.18 (m, 1H), 1.97 (s, 3H). ^{13}C δ (ppm): 175.34, 103.43, 103.35, 103.05, 78.83, 76.51, 75.20, 75.15, 74.73, 74.70, 73.09, 72.78, 71.43, 71.37, 68.17, 61.42, 61.05, 60.37, 57.58, 53.03, 22.77. HRMS $[M+Na]^+$ 582.20233 (calcd 582.20100, Δ ppm = 2.28).