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N-*p*-Nitrobenzyloxycarbonyl Galactosamine Imidate as a Glycosyl Donor for the Efficient Synthesis of Mucin Core-2 Analogue

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Abstract—An efficient synthesis of the mucin core-2 analogue **1a** was accomplished using *N*-*p*-nitrobenzyloxycarbonyl(PNZ)-protected trichloroacetimidate **4** as a novel glycosyl donor.

For the last several years we have been involved in the study of glycosyltransferases, specifically α-L-fucosyltransferase, α(2,3)-sialyltransferase, and sulfotransferase responsible for the synthesis of various mucin core 2-linked carbohydrate chains. Our recent study indicates that the core-2 branched structure Galβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα is a preferred acceptor for 3-*O*-sulfotransferase[1a] and α(2,3)-sialyltransferase[1b] in colon tumor tissues. We have already described the association of the novel α(1,2)-L-fucosylating activity with the Lewis type α(1,3/4)-L-fucosyltransferase, which can convert Galβ1,3(Fucα1,4)GlcNAc (Le^a) to Fucα1,2-Galβ1,3(Fucα1,4)GlcNAc (Le^b).[2] We are presently investigating an unusual α(1,2)-L-fucosyltransferase capable of converting Galβ1,4(Fucα1,3)GlcNAc (Le^x) to Fucα1,2Galβ1,4(Fucα1,3)GlcNAc (Le^y) structure. As we plan to use branched saccharides for this study, molecules **1a** and **1b** become the target acceptors for this enzyme (Fig. 1). Both acceptors can be used to identify this unique enzyme in the presence of the commonly occurring α(1,2)-L-fucosyltransferase. The present communication describes the chemical synthesis of **1a**.

Synthesis of **1a** involves the construction of the GalNAcβ1,3GalNAc linkage. However, synthesis of this linkage has not attracted much attention.[3] Various galactosamine donors have become available for the synthesis of GalNAcβ-linked compounds.[4] The 1,2-*trans*-glycosylation of amino sugars requires glycosyl donors containing participating protective groups in the C2-position. It is important to use a protecting group that can be selectively removed under mild conditions without degradation of the resulting product and other protecting groups. We have investigated various glycosyl donors and finally selected the *N*-*p*-nitrobenzyloxycarbonyl(PNZ)[5]-protected trichloroacetimidate **4**, which can be readily obtained from galactosamine hydrochloride **2** by five successive high yield conversions. Removal of the PNZ group is also convenient. Simple treatment of *N*-PNZ-protected saccharides with sodium hydrosulphite under neutral conditions provides the free amines. The employment of PNZ avoids the vigorous conditions required for removal of *N*-phthalimido (NPhth) protection,[6] which is widely used in the synthesis of amino sugars.

Thus, treatment of **2** with one equivalent of NaOMe in MeOH, followed by one equivalent of *p*-nitrobenzyl chloroformate–Et_3N (Scheme 1) gave the *p*-nitrobenzyl carbamate **3**, which was fully *O*-acetylated (Ac_2O–pyridine, DMAP). Selective removal of anomeric *O*-acetyl group (hydrazine acetate, DMF) followed by treatment with Cl_3CCN–DBU afforded the key donor **4**, exclusively as α-isomer.

Compound **4** showed high reactivity when used as a glycosyl donor in the presence of either triethylsilyl triflate (Et_3SiOTf) or boron trifluoride diethyl etherate ($BF_3{\cdot}Et_2O$) as a promotor (Scheme 2). Disaccharide **6** was obtained in high yield (89%) from the condensation of **4** with **5** in CH_2Cl_2 employing the Et_3SiOTf catalyst. The newly formed β-linkage in **6** was confirmed by ^{13}C NMR data which included two signals for C-1_B and C-1_A at δ 101.01 ($^1J_{C,H}$ = 158.5 Hz) and 99.43 ($^1J_{C,H}$ = 168.7 Hz), respectively. Glycosylation by **4** of the diol **11** (Et_3SiOTf, −65 °C) afforded the β-(1→4) linked disaccharide **12** in 78% yield. The glycosylation of **16** ($BF_3{\cdot}Et_2O$, −60 °C) gave the β-(1→3) linked disaccharide

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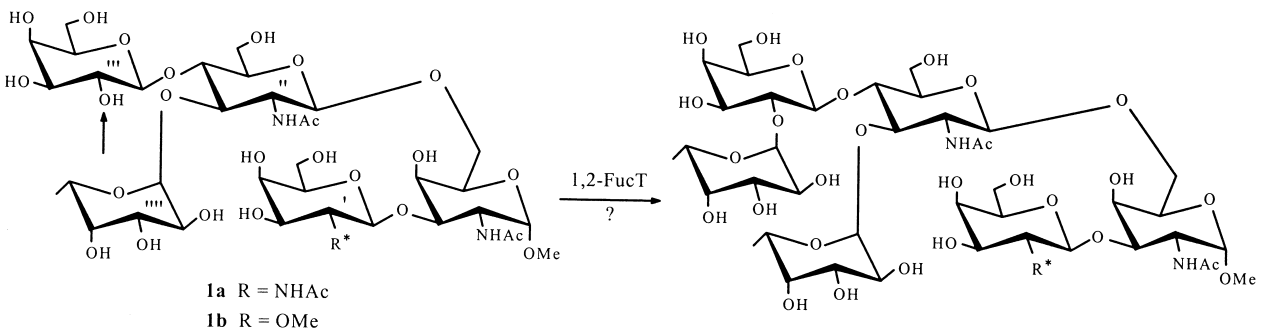
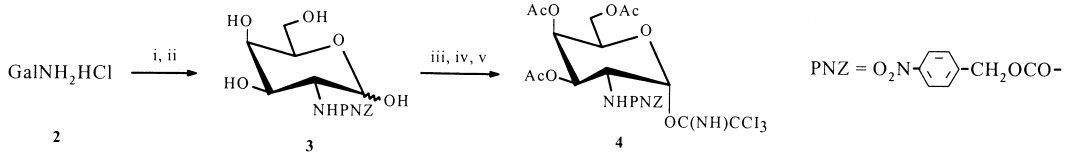
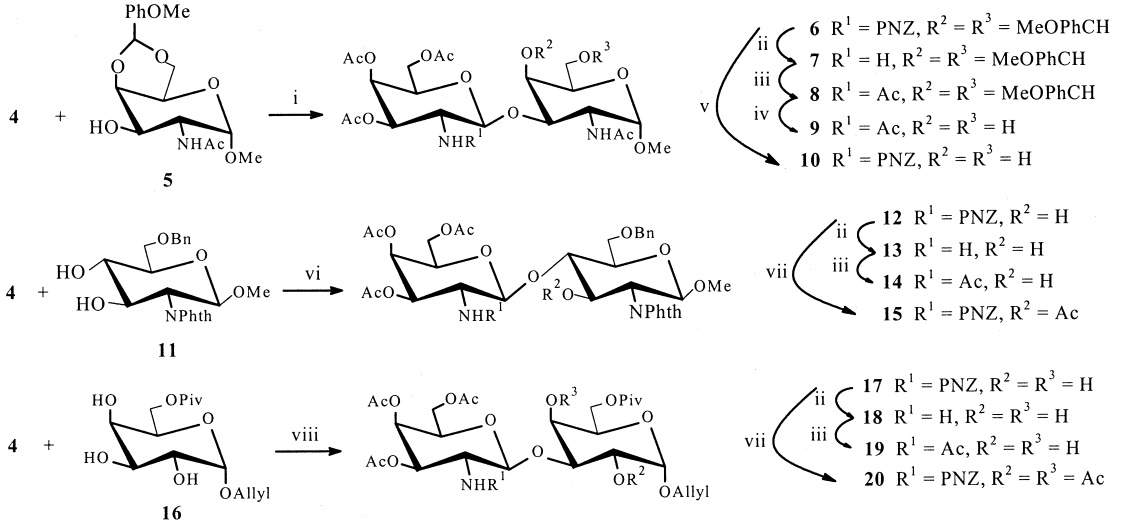


Figure 1. This C-2 position is blocked for commonly occurring α(1,2)-L-fucosyltransferase.



Scheme 1. Reagents and conditions: (i) NaOMe–MeOH (1 equiv), rt; (ii) *p*-nitrobenzyl chloroformate–Et_3N (1 equiv), 0 °C; (iii) pyridine–Ac_2O, DMAP, 95%; (iv) hydrazine acetate, DMF, 0 °C, 2 h, 87%; (v), CCl_3CN (3 equiv)–DBU, CH_2Cl_2, −10 °C, 90%.

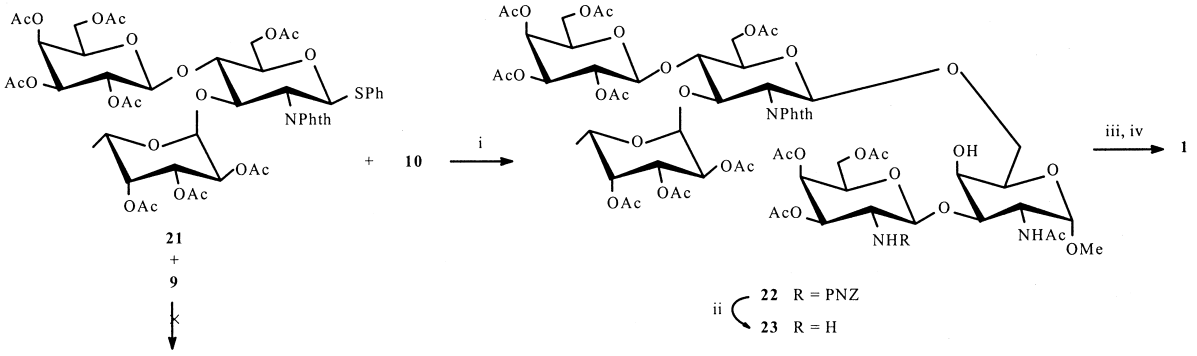


Scheme 2. Reagents and conditions: (i) **4** (1.5 equiv), TESOTf (0.24 equiv), CH_2Cl_2, 4 Å molecular sieves, rt, 15 min, 89%; (ii) $Na_2S_2O_4$ (8 equiv), MeCN:EtOH:H_2O (v/v/v 1:1:1), 10 min; (iii) Ac_2O–MeOH; (iv) 75% acetic acid, 45 °C, 2 h, 90%; (v) 80% acetic acid, 40 °C, 2 h, 92%; (vi) **4** (1.2 equiv), TESOTf, CH_2Cl_2, −65 °C, 1 h, 78%; (vii) pyridine–Ac_2O, DMAP; (viii) **4** (1.2 equiv), $BF_3{\cdot}Et_2O$ (0.5 equiv), CH_2Cl_2, −60 °C, 30 min, 82%.

17 in 82% yield. Removal of PNZ from compounds **6**, **12** and **17** under mild condition by sodium hydrosulphite[7] gave **7**, **13** and **18** in 80, 77 and 78% yields, respectively. Ensuing *N*-acetylation afforded **8**, **14** and **19** in quantitative yields. The above results indicate that a combination of the *N*-PNZ moiety with anomeric trichloroacetimidate activation[8] in **4** affords an efficient donor for galactosamine β-glycosylation.

With the methodology of effectively constructing GalNAcβ1,3GalNAc linkage in hand, we turned our attention to the synthesis of our target compound **1a** (Scheme 3). Initially we tried to use the diol **9**, obtained by hydrolysis of disaccharide **8** in 75% acetic acid at 45 °C, as an acceptor. However, condensation with the Lewis^x donor **21**[9] under *N*-iodosuccinimide (NIS)–triflic acid (TfOH) condition[10] did not proceed, possibly owing to the poor solubility of **9** in CH_2Cl_2.

In order to increase solubility, the diol **10**, which was directly obtained by treatment of **6** with 80% acetic acid (40 °C, 2 h), was attempted as an acceptor and found to be successful. Thus, glycosylation of **21** with **10** under NIS–TMSOTf condition at −50 °C in CH_2Cl_2 provided the fully protected pentasaccharide **22** in 46% yield. The ^{13}C NMR DEPT135 spectra showed a downshift of C-6 in the terminal GalNAcα residue to δ 69.1 ppm, confirming this position as the site of glycosylation. The PNZ group in **22** was reductively cleaved with sodium hydrosulphite. Removal of both the phthalimido and acetate groups from **23** was accomplished by treatment with hydrazine hydrate:ethanol (v/v, 1:4) at 90 °C,



Scheme 3. Reagents and conditions: (i) **21** (1.5 equiv), NIS–TMSOTf, CH_2Cl_2, 4 Å molecular sieves, −50 °C, overnight, 46%; (ii) $Na_2S_2O_4$ (8 equiv), MeCN:EtOH:H_2O, 10 min, 65%; (iii) hydrazine hydrate:MeOH (1:4 v/v), 90 °C, 6 h, 90%; (iv) MeOH:Ac_2O (1:1, v/v), 0 °C, quantitative.

followed by *N*-acetylation (Ac_2O–MeOH) to furnish the final product **1a**. The structure of **1a** was confirmed by ^1H, ^{13}C NMR and FABMS spectroscopy.[11]

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7. Typical procedure for removing PNZ group and *N*-acetylation: **6** (0.1 mmol) was dissolved in 4.5 mL CH_3CN:EtOH:H_2O (v/v/v 1:1:1). Sodium hydrosulphite (140 mg) was added and the stirring was continued for 10 min at room temperature. TLC (4:1 CH_2Cl_2:MeOH) indicated that the reaction was complete. The solvent was concentrated and dried over vacuum. Dry MeOH (2.5 mL) and Ac_2O (2 mL) were added successively. After 1 h, the solvent was removed and the residue was purified by column chloromatography (12:1 CH_2Cl_2:MeOH) to afford compound **8** (55 mg, 80%).
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11. Selected data for **4**: ^1H NMR ($CDCl_3$, 400 MHz) δ 8.78 (s, 1H, C=N*H*), 8.20 (d, 2H, J=9.2 Hz, arom H), 7.47 (d, 2H, J=8.4 Hz, arom H), 6.45 (d, 1H, J=4.0 Hz, H-1), 5.50 (d, 1H, J=2.4 Hz, H-4), 5.26 (dd, 1H, J=3.0, J=11.8 Hz, H-3), 5.19 (s, 2H, OCOCH_2PhNO_2), 4.94 (d, 1H, J=10.0 Hz, N*H*PNZ), 4.57 (m, 1H, H-2), 4.20–4.04 (m, 3H, H-5, H-6), 2.18, 2.01, 1.99 (3 s, 9H, 3 OAc); for **6**: ^1H NMR ($CDCl_3$, 400 MHz) δ 8.14 (d, 2H, J=7.2 Hz, arom H), 7.45 (d, 2H, J=8.8 Hz, arom H), 7.44 (d, 2H, J=8.8 Hz, arom H), 6.85 (d, 2H, J=8.4 Hz, arom H), 5.74 (d, 1H, J=8.8 Hz, H-1′), 5.49 (s, 1H, MeO PhC*H*), 5.35 (d, 1H, J=2.8 Hz, H-4′), 4.85 (d, 1H, J=3.2 Hz, H-1), 4.28 (d, 1H, J=2.8 Hz, H-4), 3.78, 3.41 (2 s, 6H, 2 OCH_3), 2.17, 2.05, 1.94, 1.92 (4 s, 12H, 4 COCH_3); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 170.63, 170.46, 170.31, 170.24 (4 *C*O), 101.01 ($J_{C1'H1'}$=158.5 Hz, C-1′), 99.43 (J_{C1H1}=168.7 Hz, C-1), 69.34 (C-6), 61.74 (C-6′), 55.48, 55.33 (2 OCH_3), 23.31, 20.75, 20.70, 20.60 (4 *C*OCH_3); for **1**: FABMS m/z 972.7 $[M+Na]^+$; ^1H NMR (D_2O, 400 MHz) δ 5.13 (d, 1H, J=3.6 Hz, H-1‴), 4.81 (d, 1H, J=3.7 Hz, H-1), 4.58 (d, 1H, J=8.0 Hz, H-1″), 4.53 (d, 1H, J=8.0 Hz, H-1‴), 4.48 (d, 1H, J=7.6 Hz, H-1′), 3.36 (s, 3H, OCH_3), 2.06, 2.04, and 2.02 (each s, 9H, 3×NHAc), 1.20 (d, 3H, J=6.4 Hz, CMe); ^{13}C NMR (D_2O, 100.6 MHz) δ 173.53, 173.10, 172.63 (3 *C*O), 101.68 (C-1‴), 100.82 (C-1′), 100.34 (C-1″), 97.60 (C-1‴), 97.12 (C-1), 54.69 (OMe), 21.29, 21.26, 21.04 (3×NHAc), 14.27 (C-6‴).