Use of the *p*-nitrobenzyloxycarbonyl group as an orthogonal amine protecting group in the synthesis of β-GlcNAc terminating glycosides

Xiangping Qian and Ole Hindsgaul*

Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2G2

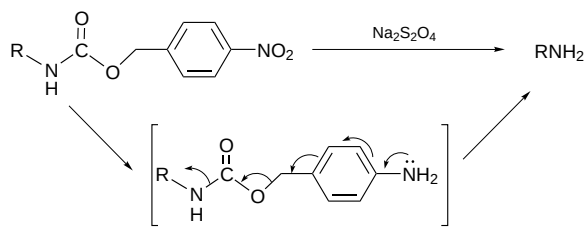
The *p*-nitrobenzyloxycarbonyl group serves as a participating group in the stereoselective formation of 2-amino-β-glucosides and as an N-protecting group which can be readily removed either by hydrogenolysis or by reaction with sodium dithionite under neutral conditions.

β-D-Glycosides of *N*-acetyl-2-amino-2-deoxy sugars are widely distributed in living organisms where they constitute building blocks of glycoconjugates.[1] The synthesis of such glycosides requires glycosyl donors where the amino group is protected. The phthalimido (Phth) and azido groups have been the most widely used.[2,3]

More recently, the carbamate functionality has been used for protection of the amino group in peptide, protein and carbohydrate synthesis.[4–7] Boullanger *et al*. conducted a systematic study on the glycosylation of simple acceptor alcohols with various *N*-alkoxycarbonyl derivatives of glucosamine, including the *p*-nitrobenzyloxycarbonyl (PNZ) group.[8] It was found that β-glycosides were obtained stereoselectively in good yield without the formation of an oxazolidinone when the β-acetate of these carbamate derivatives was used as glycosyl donor in the presence of a Lewis acid. The 2,2,2-trichloroethyl and allyl carbamates have since been effectively applied in the synthesis of glycoconjugates containing 2-acetamido glycosides.[7,9] These can be deprotected with zinc dust in acetic acid[7] and with Pd^0 complexes,[9] respectively.

We chose to investigate the potential of the *p*-nitrobenzyl carbamates of 2-amino sugars since this group should be selectively cleavable by the reduction of the electron-withdrawing nitro group to the electron-donating amine substituent followed by 1,6-elimination[4] (Scheme 1). As there are many methods for reducing the aromatic nitro group, mild and chemoselective deprotection methods should be readily available. Furthermore, unlike the 2,2,2-trichloroethyl or allyl carbamates, the *p*-nitrobenzyl carbamate can be removed by hydrogenolysis at the end of a synthesis along with the standard *O*-benzyl protecting groups.

As shown in Scheme 2, treatment of glucosamine hydrochloride **1** with 1 equiv. of NaOMe in MeOH, followed by *p*-nitrobenzyl chloroformate–Et_3N and *O*-acetylation gave **2**. The trichloroacetimidate **3**† was formed by regioselective deacetylation at O-1 with benzylamine followed by treatment of the reducing sugar with CCl_3CN in the presence of K_2CO_3.[10] Imidate **3** was evaluated as a glycosyl donor in CH_2Cl_2 using $BF_3{\cdot}Et_2O$ as a promoter.‡§ The octyl β-glycoside **4** was formed in 82% yield.¶ Glycosylation of **5** gave the β-(1 → 6) linked disaccharide **6** in 91% yield (Scheme 3).†,¶ The reaction of **3** with the less reactive 4-OH groups of **10** and **11** also gave β-(1 → 4) linked disaccharides **12**† and **13** in good yields (79 and 75%,¶ respectively) (Scheme 4). These results indicate that the combination of the PNZ moiety with anomeric trichloroacetimidate activation can afford β-glycosides in high yield with high stereoselectivity.

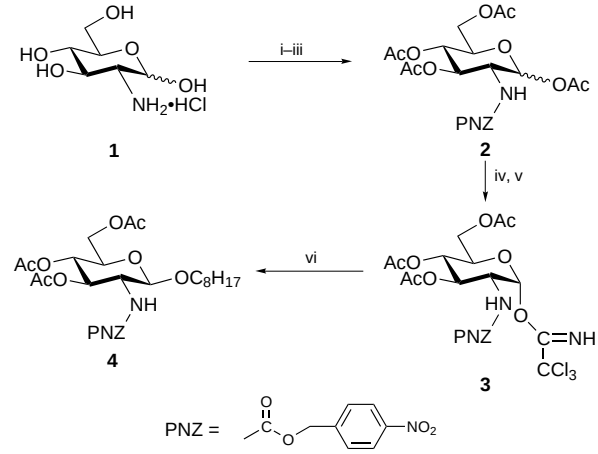


Scheme 1

A major disadvantage of using the *N*-phthalimido group in oligosaccharide synthesis is that its deprotection requires vigorous conditions, and esters usually do not survive.[11] Recently, the more reactive tetrachlorophthaloyl (TCP) group was applied as an amine protecting group.[12,13] The TCP group can be removed under milder conditions than the Phth group, either by a slight excess of ethylenediamine at 60 °C or by $NaBH_4$ reduction. Although the extent of cleavage of carboxylate ester groups is significantly reduced under these conditions, yields are still moderate especially in the presence of 3-OAc and 6-OAc groups when using ethylenediamine.[12*b*] Ketone or aldehyde functionalities may also be reduced by $NaBH_4$ during the removal of the TCP group. Clearly, chemoselective removal of the PNZ group under conditions where esters are inert would enhance its utility.

Methods for the removal of the PNZ group were examined using the simple glycoside **4** as a model compound. Removal of the PNZ group and acetylation of the resulting amine can be directly achieved in one step by hydrogenolysis using 10% Pd–C under H_2 flow in the presence of Ac_2O, yielding **9** in 83% yield.¶ As shown in Scheme 3, the free amine **8** could also be isolated (76%¶) when the hydrogenolysis was performed in the absence of Ac_2O.

Attempted reduction with stannous chloride[14] in non-acidic and non-aqueous media (EtOH or EtOAc) was ineffective. *p*-Nitrobenzyl esters can, however, be reductively cleaved by sodium dithionite ($Na_2S_2O_4$) under neutral or slightly alkaline conditions.[15] We found that sodium dithionite was a very



Scheme 2 *Reagents and conditions*: i, NaOMe (1 equiv.), MeOH, 10 min; ii, *p*-nitrobenzyl chloroformate (1 equiv.), Et_3N (1 equiv.), 0 °C to room temp; iii, Ac_2O, pyridine, 16 h, 78% (3 steps); iv, $BnNH_2$ (1.1 equiv.), THF, 16 h; v, CCl_3CN (10 equiv.), K_2CO_3, CH_2Cl_2, 7 h, 75% (2 steps); vi, $BF_3{\cdot}Et_2O$ (0.5 equiv.), octanol (0.7 equiv.), 4 Å molecular sieves, CH_2Cl_2, −30 to 0 °C, 2 h, 82% (based on octanol)

effective reducing agent even in the absence of base. The PNZ group can be readily removed quickly in quantitative yield. As shown in Scheme 4, deacetylation of **12** using NaOMe in MeOH followed by cleavage of PNZ and re-acetylation with Ac_2O–pyridine gave **16**.† When disaccharide **12** was treated directly with sodium dithionite in MeCN–EtOH–H_2O solution, followed by *N*-acetylation using Ac_2O in MeOH, disaccharide **16** was obtained, demonstrating the stability of the three *O*-acetate groups to the reduction conditions. The free amine group can also be transformed into the acetamido group in 'one pot' by simply adding Ac_2O directly to the reaction mixture. Disaccharide **16** was thus formed in a 'one pot' reaction in 86% yield.¶

In summary, we have demonstrated that the PNZ group functions as a good participating group for the formation of 2-amino-β-glucosides. This N-protecting group can be conveniently removed either by hydrogenolysis along with *O*-benzyl ethers or selectively by sodium dithionite under neutral conditions where carboxylate esters remain stable. Since *O*-acetyl groups can be removed by treatment with NaOMe–MeOH in the presence of the PNZ group, this group is effectively an orthogonal protecting group and should thus find unique application in oligosaccharide synthesis.

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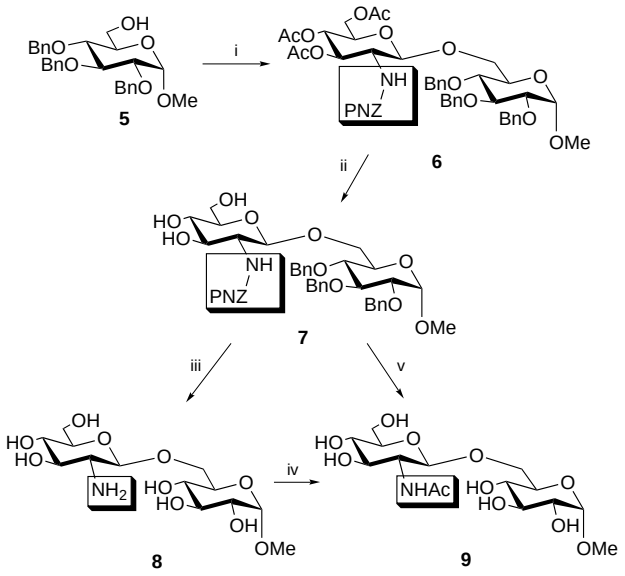
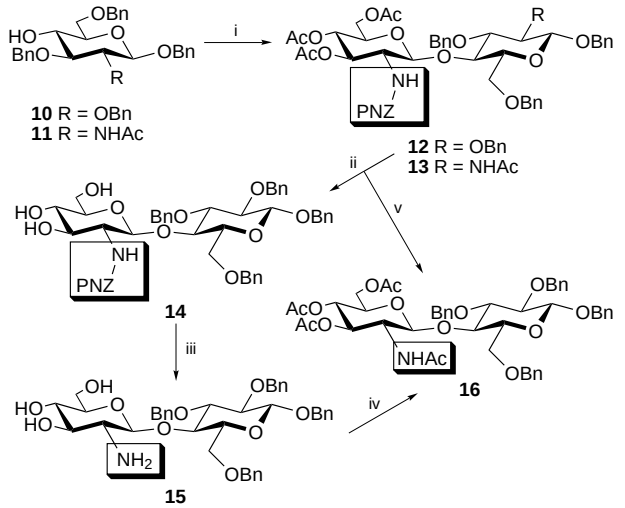


Scheme 3 *Reagents and conditions*: i, $BF_3{\cdot}Et_2O$ (0.5 equiv.), **3** (1.5 equiv.), 4 Å molecular sieves, CH_2Cl_2, −30 to 0 °C, 2 h, 91%; ii, NaOMe, MeOH, 2.5 h, 93%; iii, H_2, 10% Pd–C, MeOH, 76%; iv, Ac_2O, EtOH, 1 h, 87%; v, H_2, 10% Pd–C, MeOH, Ac_2O, 83%



Scheme 4 *Reagents and conditions*: i, $BF_3{\cdot}Et_2O$ (0.5 equiv.), **3** (1.5 equiv.), 4 Å molecular sieves, CH_2Cl_2, −30 to 0 °C, 2 h, 79% for **12**, 75% for **13**; ii, NaOMe, MeOH, 3.5 h, quant.; iii, $Na_2S_2O_4$ (8 equiv.), EtOH–H_2O, 10 min, quant.; iv, Ac_2O, pyridine, 16 h, 93%; v, $Na_2S_2O_4$ (8 equiv.), MeCN–EtOH–H_2O, 1 h; then Ac_2O, 10 min, 86%

Footnotes

* E-mail: ole.hindsgaul@ualberta.ca

† *Selected data* for **3**: ^{1}H NMR ($CDCl_3$) δ 8.75 (s, 1 H, C=NH), 6.39 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1). For **6**: J_{C1H1} 168.5 Hz, $J_{C1'H1'}$ 160.4 Hz; HR-ESMS for $C_{48}H_{54}N_2O_{17}Na$ (M + Na^+): calc. 953.3320, found 953.3348. For **12**: J_{C1H1} 160.0 Hz, $J_{C1'H1'}$ 158.1 Hz; HR-ESMS for $C_{54}H_{58}N_2O_{17}Na$ (M + Na^+): calc. 1029.3633, found 1029.3634. For **15**: HR-ESMS for $C_{40}H_{47}NO_{10}Na$ (M + Na^+): calc. 724.3098, found 724.3119. For **16**: ^{1}H NMR ($CDCl_3$) δ 4.55 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 4.45 (d, 1 H, $J_{1',2'}$ 7.6 Hz, H-1′); J_{C1H1} 161.8 Hz, $J_{C1'H'}$ 159.2 Hz.

‡ Typical procedure of glycosylation: compound **3** (236 mg, 0.375 mmol), **5** (116 mg, 0.25 mmol) and powdered 4 Å molecular sieves (250 mg) in dry CH_2Cl_2 (5 ml) were stirred for 10 min at −30 °C under nitrogen. Then $BF_3{\cdot}Et_2O$ (15 μl, 0.125 mmol) in dry CH_2Cl_2 (0.75 ml) was added, and the reaction mixture was stirred for a further 2 h below 0 °C. After neutralization with Et_3N, the reaction mixture was filtered through Celite, washed with CH_2Cl_2 and concentrated. Column chromatography (3 : 2, hexane–EtOAc) gave disaccharide **6** (211 mg, 91%).

§ The ^{1}H NMR spectra of most disaccharides containing the PNZ moiety gave broad peaks which make assignments difficult. The β-linkage of the disaccharides was confirmed by ^{13}C–^{1}H HMQC experiments and NMR data for the *N*-acetyl disaccharide obtained by N-acetylation after the reductive cleavage step.

¶ Yields were calculated based on the weight of pure compounds isolated by column chromatography.

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