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Note

The use of the *o*-nitrophenyl group as a protecting/activating group for 2-acetamido-2-deoxyglucose

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Abstract

The *o*-nitrophenyl group, a protecting group with latent activation potential, was used as a protecting group for the glycosidic position. It is stable to common conditions used in synthesis and can be activated for displacement and glycoside formation by an alcohol, using zinc chloride as a catalyst. Good to excellent yields of β-glycosides of the important amino sugar *N*-acetylglucosamine were obtained. A mechanism for the reaction is proposed. © 2003 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Since the first glycoside synthesis performed by Fischer,[1] numerous glycosyl donors have been designed and used. Some of them require direct activation of the anomeric center, such as glycosyl halides,[2–6] thioglycosides,[7–9] glycosyl phosphates,[10–12] glycosyl phosphites[13,14] and glycosyl 2-propenyl carbonates.[15] Other glycosidic leaving groups can be activated remotely by the interaction of a promoter with a site on the leaving group that is not directly attached to the anomeric center. This is the case with glycosyl trichloroacetimidates,[16] *n*-pentenyl glycosides,[17] 2-pyridylthioglycosides,[18] and 3-methoxy-2-pyridyloxy glycosides.[19–21] The vast majority of the activating groups described above cannot survive most of the typical transformations carried out on carbohydrate molecules. These include acylations, alkylations, oxidations, reductions, epoxidations, sulfations, phosphorylations, halogenations and hydroxylations. The development of activating groups that are stable to these conditions but that can still readily promote coupling is therefore of extreme interest.

Glycosides of 2-acetamido-2-deoxy-D-glucose (GlcNAc) are widely distributed in living organisms. They are found in human milk, in blood group substances, in bacterial lipopolysaccharide antigens where they constitute the dominant epitopes, and in plants.[22] Of special interest is the occurrence of amino sugars in a variety of antibiotics, such as kanamycin C, paromomycin, hydroxymycin, trehalosamine and zygomycin A.[23] The preparation of glycosides of GlcNAc is problematic. 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl bromide is too reactive to be of much practical use. The corresponding chloro derivative is also reactive and tends to give oxazolines as a major product. The use of non-participating protecting groups on the amino nitrogen is often necessary in order to avoid this. Because of their chemical lability, the use of halo groups for activation of the anomeric position precludes transformations elsewhere on the pyranose ring before the coupling step. This is a major obstacle to be overcome in the design of synthetic strategies.

The *o*-nitrophenyl group is a very inert structure susceptible only to reductions with hydrogen, ferric chloride, and strong metal hydride reductants such as lithium aluminum hydride. It is resistant to acids, bases, oxidizing agents, acylating agents and alkylating agents, and such commonly used reductants as sodium borohydride. Because the strongly electron-withdrawing nitro group is in direct conjugation with the glycosidic oxy-

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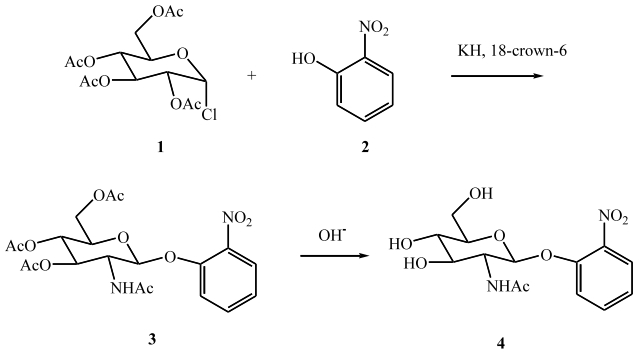
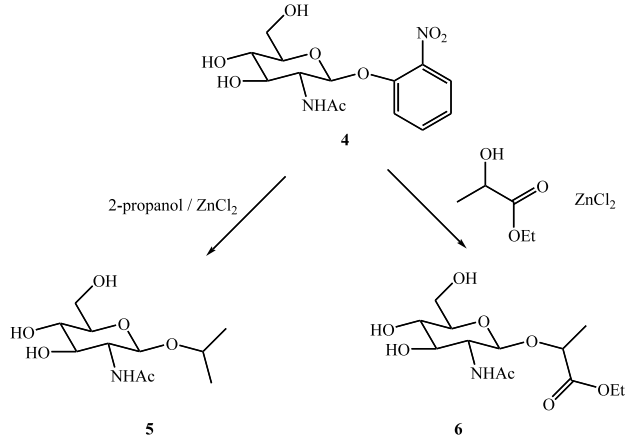
gen, the *o*-nitrophenyl group can, in principle, function as a latent activating group for glycosides. This would allow the use of this group in vast majority of routine transformations typical in carbohydrate chemistry, but would make the coupling of another group to the anomeric position possible without installing a different activating group. Here we present the preparation of this stable new glycosyl donor and its use in glycosylation reactions

2. Results and discussion

The *o*-nitrophenyl glycoside of GlcNAc, **3**, was readily synthesized by reacting 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl chloride (**1**) with *o*-nitrophenol in the presence of KH and 18-crown-6 (Scheme 1). The facile displacement of the chloro group of **1** under these conditions has previously been described.[24] Under standard deacetylation conditions, **3** was converted into *o*-nitrophenyl 2-acetamido-2-deoxy-D-glucoside (**4**). This product is stable at room temperature for indefinite time. The suitability of the *o*-nitrophenyl group as a leaving group was evaluated by reacting glycoside **4** with two secondary alcohols: 2-propanol and ethyl lactate (Scheme 2) at 70 °C in the presence of anhydrous zinc chloride. This afforded high yields of isopropyl 2-acetamido-2-deoxy-β-D-glucoside (**5**) and 1-(ethoxycarbonyl)ethyl 2-acetamido-2-deoxy-β-D-glucoside (**6**). The β anomer was formed exclusively. Because of the acidic nature of the reaction conditions, no racemization of the ethyl lactyl group was observed.



Scheme 1.



Scheme 2.

The proposed mechanism for the transglycosylation reaction is thought to involve the coordination of zinc to both the nitro group and the glycosidic oxygen atom, mediating the elimination of the *o*-nitrophenyl group and the generation of the positive charge at C-1. This can be stabilized by the acetamido group and by the ring oxygen. An oxonium ion is therefore produced through a concerted process. Since the reaction conditions are acidic, no oxazoline byproducts were formed. The participation of the acetamido group in glycosylation reactions involving GlcNAc shields the bottom face of the oxonium intermediate, permitting attack of the alcohol from the equatorial position only, thus affording exclusively the trans glycoside in this case.

In summary, we have developed a new glycosylation procedure for *N*-acetylglucosamine, using the *o*-nitrophenyl group as a protecting/activating function. This aglycon provides good protection of the anomeric center against a variety of agents and can be activated with $ZnCl_2$ in the presence of alcohols to give β-glycosides of *N*-acetylglucosamine in high overall yields and total stereoselectivity. The common and unwanted formation of oxazoline by-products is avoided, as is the necessity to use other protecting groups on the amino nitrogen to avoid this. One major drawback of the present method is that relatively high temperatures are needed to effect reaction in a reasonable time. This is an especially serious shortcoming when complex glycosides or oligosaccharides are to be prepared.

3. Experimental

3.1. General methods

Melting points were measured on a Fisher–Johns melting point apparatus. Optical rotations were measured ($\lambda = 589$ nm) at room temperature (rt) using a Perkin–Elmer 341 polarimeter in a 1 mL cell. The ^{1}H and ^{13}C NMR spectra were recorded at 500 MHz on a Varian VXR spectrometer. The HRMS FAB mass spectra were obtained using a Jeol HX-110 double focusing mass spectrometer operating in positive ion mode. All solvents were distilled in order to remove traces of water. The solid reactants were dried in the vacuum oven at rt. Potassium hydride was washed with hexane before weighing.

3.2. Preparation of *o*-nitrophenyl 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-β-D-glucopyranoside (3)

In a two-necked, round-bottomed flask equipped with a dropping funnel and purged with N_2, KH (12.85 g, 0.2 mol) was added to CH_2Cl_2 (80 mL) at 0 °C. To this, a soln of 18-crown-6 (26.5 g, 0.1 mol) and *o*-nitrophenol (28 g, 0.2 mol) in CH_2Cl_2 (100 mL) was added through a dropping funnel during 1 h. The suspension was stirred for another 2 h, after which time 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride (36.6 g, 0.1 mol) was added and stirring was continued at rt for a further 2 h. The reaction was quenched with glacial AcOH (12 mL). The soln was washed with 5% aq $NaHCO_3$ (1 L) and then with water. The combined organic layers were dried with Na_2SO_4 and then rotary evaporated, to afford crude product **3** (45 g), which was used in the next step without further purification

3.3. Preparation of *o*-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside (4)

To a soln of **3** (45 g) in MeOH (1050 mL) and water (70 mL) K_2CO_3 (55 g, 0.4 mol) was added and the suspension was stirred overnight at rt. After filtration of the salts, the soln was rotary evaporated. More MeOH was added to precipitate the excess salt, which was then filtered off. Water was added to the soln and *o*-nitrophenol removed by rotary evaporation at 40 °C. Recrystallization from hot EtOH gave **4** (18.4 g) in 54% overall yield starting from **1**; mp 176–177 °C; $[\alpha]_D^{23}$ + 30.4° (*c* 0.77, CH_3OH); ^1H NMR (CD_3OD): δ 1.99 (s, 3 H, NHAc), 3.40 (dd, 1 H, $J_{3,4}$ 8.6, $J_{4,5}$ 9.9 Hz, H-4), 3.48 (ddd, 1 H, $J_{4,5}$ 9.7, $J_{5,6b}$ 5.9, $J_{5,6a}$ 2.2 Hz, H-5), 3.57 (dd, 1 H, $J_{2,3}$ 10.3, $J_{3,4}$ 8.61 Hz, H-3), 3.72 (dd, 1 H, $J_{6a,6b}$ 12.1, $J_{6b,5}$ 5.9 Hz, H-6b), 3.88 (dd, 1 H, $J_{2,3}$, $J_{1,2}$ 8.4 Hz, H-2), 3.93 (dd, 1 H, $J_{6a,5}$ 2.2 Hz, H-6a), 5.14 (d, 1 H, H-1), 7.17 (ddd, 1 H, $J_{a,b}$ 8.1, $J_{b,c}$ 7.2, $J_{b,d}$ 1.3 Hz, H–Ar_b), 7.50 (dd, 1 H, $J_{c,d}$ 8.3 Hz, H–Ar_d), 7.56 (ddd, 1 H, $J_{a,c}$ 1.5 Hz, H–Ar_c), 7.70 (dd, 1 H, H–Ar_a); ^{13}C NMR (CD_3OD): δ 22.9, 57.2, 62.6, 71.8, 75.6, 78.7, 101.7, 119.6, 123.8, 125.3, 134.6, 142.7, 151.1, 174.0; IR (film): 3279 (b), 1642 (s), 1527 (s), 1351 (m), 1351 (m), 1068 (s) cm^{-1}; HRFABMS [M + H^+] calcd 343.1141, found 343.1139.

3.4. Isopropyl 2-acetamido-2-deoxy-β-D-glucopyranoside (5)

A suspension of compound **4** (0.1 g, 0.29 mmol) and $ZnCl_2$ (0.1 g, 0.7 mmol) in dry 2-propanol (5 mL) was heated at 70 °C for 22 h. The solvent was removed by evaporation and the crude reaction mixture dissolved in water and rotary evaporated to distill off the *o*-nitrophenol. In order to remove the traces of $ZnCl_2$, column chromatography with 6:1 $CHCl_3$–MeOH was performed. The product **5** (0.068 g) was recovered in 95% yield: $[\alpha]_D^{23}$ − 5.0° (*c* 0.48, CH_3OH); ^1H NMR (D_2O): δ 1.03 (d, 3 H, *J* 6.1 Hz, CH_3), 1.09 (d, 3 H, CH_3), 1.94 (s, 3 H, NHAc), 3.33 (m, 1 H, H-5), 3.34 (m, 1 H, H-2), 3.45 (dd, 1 H, $J_{6a,6b}$ 10.3, $J_{6b,5}$ 8.1 Hz, H-6b), 3.52 (dd, 1 H, $J_{6a,5}$ 8.3 Hz, H-6a), 3.163 (dd, 1 H, $J_{2,3}$ 5.52, $J_{3,4}$ 12.3 Hz, H-3), 3.82 (dd, 1 H, $J_{3,4}$ 12.3, $J_{4,5}$ 1.9 Hz, H-4), 3.92 (m, 1 H, *J* 6.1, *i*-propyl), 4.49 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1); ^{13}C NMR (D_2O): δ 21.3, 22.3, 22.4, 56.06, 61.0, 70.1, 73.8, 74.1, 76.0, 99.8, 174.7; IR (film): 3323 (b), 1648 (s), 1374 (m), 1071 (s) cm^{-1}; HRFABMS [M + H^+] calcd 264.1447, found 264.1451.

3.5. 1-(Ethoxycarbonyl)ethyl 2-acetamido-2-deoxy-β-D-glucopyranoside (6)

A suspension of compound **4** (0.1 g, 0.29 mmol) and $ZnCl_2$ (0.1 g, 0.7 mmol) in dry ethyl lactate (2 mL) was heated at 70 °C for 20 h. The solvent was removed and the crude reaction mixture was dissolved in water and rotary evaporated to distill off the *o*-nitrophenol. In order to remove the traces of $ZnCl_2$ and separate the product from the ethyl lactate dimer, column chromatography with a 7:1 $CHCl_3$–MeOH eluent was performed. The product **6** (0.06 g) was recovered in 63% yield: mp 199–200 °C, $[\alpha]_D^{23}$ − 50.1° (*c* 0.46, CH_3OH); ^1H NMR (D_2O): δ 1.05 (t, 3 H, *J* 7.0 Hz, CH_3–ethyl), 1.14 (d, 3 H, *J* 6.8 Hz, CH_3), 1.78 (s, 3 H, NHAc), 3.03 (m, 1 H, H-5), 3.08, (m, 1 H, $J_{2,1}$ 8.06, $J_{NH,2}$ 1.71 Hz, H-2), 3.26 (dd, 1 H, $J_{2,3}$ 8.06, $J_{3,4}$ 10.25 Hz, H-3), 3.37 (dd, 1 H, H-4), 3.43 (dd, 1 H, $J_{6a,b}$ 11.97, $J_{6a,5}$ 5.61 Hz, H-6a), 3.65 (dd, 1 H, H-6b), 3.96 (d, 2 H, *J* 7.0 Hz, CH_2–ethyl), 4.26 (d, 1 H, H-1), 4.28 (d, 1 H, *J* 6.8 Hz, CH–lactyl); ^{13}C NMR (D_2O): δ 8.7, 13.3, 17.7, 50.9, 56.0, 57.8, 65.2, 68.8, 69.0, 71.3, 95.6, 169.9, 170.0; IR (film): 3439 (m), 3367 (s), 1740 (s), 1666 (s), 1138 (m) cm^{-1}; HRFABMS [M + H^+] calcd 322.1502, found 322.1493.

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