Conversion of 2-Acetamido-2-deoxy-β-D-glucopyranose (*N*-Acetylglucosamine) into 2-Acetamido-2-deoxy-β-D-galactopyranose (*N*-Acetylgalactosamine) Using a Biotransformation to Generate a Selectively Deprotected Substrate for S_N2 Inversion

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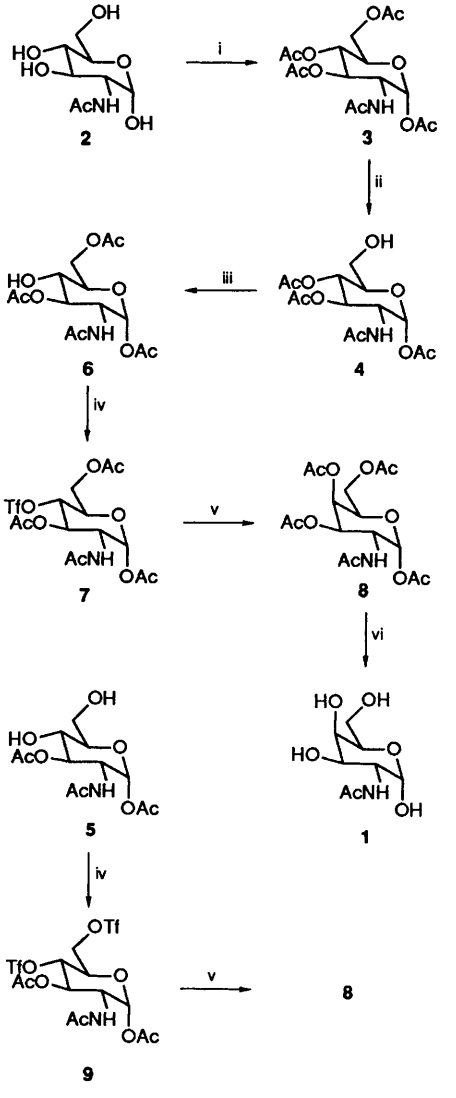
A procedure is described for the conversion of *N*-acetylglucosamine **2** into *N*-acetylgalactosamine **1**, in which use is made of a 4,6-acetyl migration in a precursor selectively deprotected by a biotransformation procedure.

N-Acetylgalactosamine **1** is a constituent of glycoproteins and polysaccharides. It is less readily available than *N*-acetylglucosamine **2**, which is readily accessible by hydrolysis of chitin, and more expensive. It was considered worthwhile therefore, to develop a method for the conversion of *N*-acetylglucosamine **1** into *N*-acetylgalactosamine **2**. Essentially, this requires epimerisation at C-4 in **1**, which must be selectively unmasked in a protected precursor. The strategy developed was to unmask the 6-hydroxy group in the peracetylated starting material **3** (Scheme 1) by regioselective enzymatic hydrolysis, and then to make use of the propensity for this derivative to undergo 4⟶6 acetyl migration to protect C-6 again and to unmask the 4-hydroxy group. The configuration at C-4 could then be inverted following standard procedures.

Accordingly, the tetraacetate **3** was subjected to enzymatic hydrolysis using an esterase from *Rhodosporidium toruloides*[1] to give the C-6 deprotected product **4**. This was accompanied by a small amount of the 1,3-di-*O*-acetyl derivative **5**, which was carried through the subsequent steps (see below). From a smaller scale hydrolysis, the triacetate **4** was isolated and characterised as the pure crystalline product. The triacetate **4** was rearranged, with acetyl migration, to the 1,3,6-tri-*O*-acetyl derivative **6**, by heating with a catalytic amount of acetic acid in toluene. The free hydroxy group was activated as the triflate **7** and the inversion step was carried out using caesium acetate to give the known tetraacetate **8**, which was hydrolysed nearly quantitatively to *N*-acetylgalactosamine **1**.

The small amount of 1,3-diacetate **5** was not removed since, during triflate formation, it was expected to give the 3,5-di-triflate **9** which, on displacement with caesium acetate, would give the tetraacetate **8**, thereby causing the side reaction to converge again with the major pathway.

A conversion of *N*-acetylglucosamine into *N*-acetylgalactosamine by an analogous inversion but using a quite different protection–deprotection strategy has recently been described.[2]



Scheme 1 *Reagents:* i, Ac_2O, pyridine; ii, yeast esterase; iii, H^+; iv, Tf_2O; v, CsOAc; vi, K_2CO_3–MeOH

Experimental

^{1}H NMR spectroscopy was carried out at 220 MHz using a Perkin-Elmer R34 spectrometer, or at 400 MHz using a Bruker WH400 spectrometer. ^{13}C NMR spectra were determined at 100.62 MHz using a Bruker WH400 spectrometer. *J* Values are in Hz. Mass spectra were determined using a Kratos MS 80 mass spectrometer. Unless otherwise stated, mass spectra were determined using chemical ionisation with ammonia as the ionising gas. Fast atom bombardment (FAB) spectra were determined using *m*-nitrobenzyl alcohol (NBA). The 'yeast esterase' was an acetone powder from *Rhodosporidium toruloides*[1] and was obtained from Glaxo Group Research. $[\alpha]_D$ Values in 10^{-1} deg cm^2 g^{-1}.

2-Acetamido-1,3,4,6-tetra-O-acetyl-2-deoxy-α-D-glucopyranose **3**.—2-Acetamido-2-deoxy-α-D-glucopyranose **2** (11 g,

50 mmol) was treated with acetic anhydride (47 ml, 0.50 mol) in pyridine (50 ml) at room temperature. After 12 h, TLC analysis showed the formation of one product (R_f 0.8, ethyl acetate–ethanol–water, 45:5:1). The mixture was evaporated under reduced pressure to give a syrup which was crystallised from ethanol to give 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-glucopyranose **3** (17.73 g, 92%), m.p. 136–137 °C (lit., m.p. 134–135 °C,[3] 137 °C,[4] 135–137 °C,[5] 139.5–140.5 °C,[6] 138–139 °C[7]); $[\alpha]_D$ +91 (*c* 0.6, MeOH) [lit. $[\alpha]_D$ +87.4 (*c* 1.07, $CHCl_3$),[7] +93 (*c* 1.0, $CHCl_3$),[6] +92 (*c* 1.0, $CHCl_3$),[4,8] +94 (*c* 1.0, $CHCl_3$)[3]]; δ_H(400 MHz; $CDCl_3$) 6.13 (1 H, d, *J* 3.6, 1-H), 5.63 (1 H, d, *J* 9.0, NH), 5.19 (2 H, m, 3-H, 4-H), 4.45 (1 H, ddd, *J* 10.5, 9.1, 3.6, 2-H), 4.21 (1 H, dd, *J* 4.0, 12.5, 6_a-H), 4.03 (1 H, dd, *J* 2.4, 12.5, 6_b-H), 3.96 (1 H, ddd, *J* 2.4, 4.0, 9.7, 5-H), 2.16 (3 H, s), 2.05 (3 H, s), 2.02 (3 H, s), 2.01 (3 H, s) and 1.90 (3 H, s); δ_C 171.5, 170.5, 169.7, 168.9, 168.4, 90.6, 70.6, 69.6, 67.5, 61.4, 51.0, 22.8, 20.7, 20.5 and 20.3.

2-*Actamido*-1,3,4-*tri*-O-*acetyl*-2-*deoxy*-α-D-*glucopyranoside* **4**.—2-Acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-glucopyranose **3** (33.15 g, 85 mmol) was suspended in citrate–phosphate buffer (pH 5.0, 50 mmol/100 mmol; 350 ml) at 30 °C. Yeast esterase (1.003 g) was added and the mixture was stirred for 24 h. The solution was evaporated under reduced pressure and the residue was extracted with ethyl acetate (2 × 100 ml) and ethanol (2 × 100 ml). The combined extracts were filtered and evaporated under reduced pressure to give a syrup, which was identified as 2-acetamido-1,3,4-tri-*O*-acetyl-2-deoxy-α-D-glucopyranose **4** (R_f 0.56, ethyl acetate–ethanol–water, 45:5:1). A minor product (R_f 0.33) present was identified by ^{1}H NMR as 2-acetamido-1,3-di-*O*-acetyl-2-deoxy-α-D-glucopyranose **5**. The mixture was used without further purification. This reaction was repeated on a smaller scale using 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-glucopyranose (8.005 g, 20 mmol). On this scale the compound was selectively and quantitatively hydrolysed to give, after crystallisation from ethanol, 2-acetamido-1,3,4-tri-*O*-acetyl-2-deoxy-α-D-glucopyranose **4** (5.74 g, 80%), m.p. 110 °C (from ethanol); $[\alpha]_D$ +57 (*c* 1.0, $CHCl_3$) (Found: C, 47.6; H, 6.2; N, 4.0. $C_{14}H_{21}NO_9$ requires C, 48.41; H, 6.09; N, 4.03%); *m/z* (CI, NH_3) $(M + 1)^+$ 348.1295 ($C_{14}H_{22}NO_9$ requires 348.1293); δ_H(400 MHz; $CDCl_3$) 6.15 (1 H, d, *J* 3.6, 1-H), 5.76 (1 H, d, *J* 9.0, NH), 5.25 (1 H, dd, *J* 9.6, 10.8, 3-H), 5.14 (1 H, t, *J* 9.7, 4-H), 4.43 (1 H, ddd, *J* 3.6, 9.0, 10.9, 2-H), 3.78 (1 H, ddd, *J* 2.2, 4.3, 10.1, 5-H), 3.66 (1 H, dd, *J* 2.2, 12.8, 6_a-H), 3.55 (1 H, dd, *J* 4.4, 12.8, 6_b-H), 2.16 (3 H, s), 2.04 (3 H, s) and 1.91 (3 H, s); δ_C 171.7, 170.0, 169.7, 168.7, 90.6, 71.9, 70.5, 67.8, 60.8, 51.1, 22.8, 20.7 20.6 and 20.4; *m/z* (relative abundance) FAB-EI (NBA), 370 ($M.Na^+$) (1.0), 348 (MH^+) (3.0), 331 (1.3), 330 (1.5), 3.07 (2.3), 288 (100), 168 (76.4), 154 (58.3), 138 (94.8), 137 (50.6) and 136 (66.3).

2-*Acetamido*-1,3-*di*-O-*acetyl*-2-*deoxy*-α-D-*glucopyranose* **5**.—This is the minor product from the previous reaction formed by the further deacetylation of the required product. It was isolated by flash chromatography using the system ethyl acetate–ethanol–water (45:5:1, v/v); δ_H(400 MHz; $CDCl_3$) 6.11 (1 H, d, *J* 3.6, 1-H), 5.71 (1 H, d, *J* 9.0, NH), 5.12 (1 H, dd, *J* 9.2, 11.0, 3-H), 4.3 (1 H, ddd, *J* 3.7, 9.0, 11.1, 2-H), 3.90 (1 H, dd, *J* 9.2, 9.8, 4-H), 3.84 (2 H, d, *J* 3.5, 6_a-H and 6_b-H), 3.7 (1 H, dt, *J* 9.8, 3.5, 5-H), 2.17 (3 H, s), 2.13 (3 H, s) and 1.93 (3 H, s).

2-*Acetamido*-1,3,6-tri-O-*acetyl*-2-*deoxy*-α-D-*glucopyranoside* **6**.—2-Acetamido-1,3,4-tri-*O*-acetyl-2-deoxy-α-D-glucopyranose **4** (16.046 g, 46 mmol) was added to toluene (300 ml) and heated to 80 °C. Glacial acetic acid (1% v/v; 3 ml) was added to the solution which was heated at 80 °C for 24 h. The solvent was removed under reduced pressure to give a brown syrup which was identified as the required product by ^{1}H NMR spectroscopy. The syrup was dissolved in ethyl acetate and filtered through silica. The solvent was removed under reduced pressure and the residue was crystallised from dichloromethane to yield 2-acetamido-1,3,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranose **6** (14.104 g, 87.9%), m.p. 160–161 °C; $[\alpha]_D$ +62 (*c* 1.0, $CHCl_3$) (Found: C, 48.3; H, 6.1; N, 4.0. $C_{14}H_{21}NO_9$ requires C, 48.41; H, 6.09; N, 4.03%); δ_H(400 MHz; $CDCl_3$) 6.12 (1 H, d, *J* 3.7, 1-H), 5.92 (1 H, d, *J* 8.9, NH), 5.08 (1 H, dd, *J* 9.2, 11.0 Hz, 3-H), 4.50 (1 H, dd, *J* 3.6, 12.4 Hz, 6_a-H), 4.31 (1 H, ddd, *J* 3.6, 8.9, 11.0, 2-H), 4.18 (1 H, dd, *J* 2.2, 12.4, 6_b-H), 3.82 (1 H, dt, *J* 9.5, 2.2, 3.6, 5-H), 3.6 (1 H, dd, *J* 9.2, 9.6, 4-H), 2.15 (3 H, s), 2.10 (3 H, s), 2.09 (3 H, s) and 1.90 (3 H, s); δ_C 172.0, 171.7, 170.3, 169.0, 90.8, 72.6, 67.8, 62.4, 51.1, 22.8, 20.8 and 20.6; *m/z* (relative abundance) FAB-EI (NBA) 370 $(M.Na)^+$ (12.8), 348 (MH^+) (6.7), 329 (1.4), 307 (10.3), 288 (43.0), 228 (18.5), 186 (7.3), 176 (11.8), 168 (22.1), 154 (100.0), 138 (35.3), 137 (64.9) and 136 (84.6).

2-*Acetamido*-1,3,6-*tri*-O-*acetyl*-4-*trifluoromethylsulfonyl*-2-*deoxy*-α-D-*glucopyranose* **7**.—2-Acetamido-1,3,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranose **6** (4.057 g, 11.7 mmol) was dissolved in dichloromethane (40 ml) and pyridine (4 ml) under an atmosphere of nitrogen. The solution was cooled to −40 °C using a dry ice–acetonitrile bath. Triflic anhydride (2.5 ml, 14.9 mmol) was added slowly to the solution with stirring. The reaction was followed by TLC (ethyl acetate–ethanol–water; 45:5:1). After 4 h there was one product (R_f 0.77) which was identified as the required compound **6** by NMR spectroscopy. On completion of the reaction, dichloromethane (60 ml) was added together with water–ice (100 ml). Sodium carbonate (0.2 g) was added to the mixture. The dichloromethane layer was removed and washed with dilute HCl (1 × 100 ml) and then saturated brine (1 × 100 ml). The dichloromethane was removed under reduced pressure to yield-2-acetamido-1,3,6-tri-*O*-acetyl-4-trifluoromethylsulfonyl-2-deoxy-α-D-glucopyranose **7** as a syrup/glass (5.171 g, 95%); δ_H(220 MHz; $CDCl_3$) 6.68 (1 H, d, *J* 9.8, N-H), 6.21 (1 H, d, *J* 3.7, 1-H), 5.51 (1 H, t, *J* 9.8, 3-H), 5.23 (1 H, t, *J* 9.8, 4-H), 4.64 (1 H, dt, *J* 3.7, 9.8, 2-H), 4.37 (1 H, dd, *J* 3.6, 12.4, 6_a-H), 4.25 (2 H, m, 5-H, 6_b-H), 2.18 (3 H, s), 2.16 (3 H, s), 2.14 (3 H, s) and 2.00 (3 H, s).

2-*Acetamido*-1,3,4,6-*tetra*-O-*acetyl*-2-*deoxy*-α-D-*galacto-pyranose* **8**.—2-Acetamido-1,3,6-tri-*O*-acetyl-4-trifluoromethylsulfonyl-2-deoxy-α-D-glucopyranose **7** (4.961 g, 10.6 mmol) was dissolved in dimethyl sulphoxide (40 ml) under an atmosphere of nitrogen. Caesium acetate (4.0 g, 20.8 mmol) was added and the mixture was stirred at room temperature for 14 h. The dimethyl sulphoxide was removed by distillation under reduced pressure. TLC [Kieselgel 60_{F254} (Merck), 0.25 mm, solvent system:ethyl acetate–ethanol–water, 45:5:1, v/v)] showed that one major product had been produced (R_f 0.62) together with some minor products with lower R_f. The syrup was dissolved in pyridine (10 ml), acetic anhydride (10 ml, 0.11 mol) was added and the solution was stirred at room temperature for 6 h. TLC (system as above) showed only one product (R_f 0.62). Ice–water (50 ml) and saturated brine (50 ml) were added, and the mixture was extracted with dichloromethane (3 × 100 ml) and ethyl acetate (2 × 50 ml). The organic fractions were combined, washed with dilute HCl (50 ml) and evaporated to dryness under reduced pressure. The crude syrup was crystallised from ethanol (3.68 g, 89%) to give compound **8**, m.p. 169–171 °C (lit.,[9] m.p. 178 °C); $[\alpha]_D$ +97 (*c* 1.0, $CHCl_3$) {lit. $[\alpha]_D$ +102 (*c* 1.6, $CHCl_3$)[9]}; δ_H(400 MHz; $CDCl_3$) 6.15 (1 H, d, *J* 3.6, 1-H), 5.82 (1 H, d, *J* 9.0, N-H), 5.36 (1 H, dd, *J* 0.7, 3.1, 4-H), 5.14 (1 H, dd, *J* 3.2, 11.7, 3-H), 4.63 (1 H, ddd, *J* 3.6, 9.0, 11.6, 2-H), 4.20 (1 H, ddd, *J* 0.9, 6.6, 6.8, 5-H), 4.04 (1 H, dd, *J* 6.8, 11.2, 6_a-H), 3.99 (1 H, dd, *J* 6.6, 11.2, 6_b-H), 2.10 (6 H, s), 1.97 (3 H, s), 1.96 (3 H, s) and 1.88 (3 H, s); δ_C 170.75, 170.10, 170.04, 169.95, 168.67, 91.06, 68.33, 67.57, 67.45, 66.54, 61.10, 46.75, 22.77, 20.62, 20.43 and 20.36.

2-Acetamido-2-deoxy-D-galactopyranose **1**.—2-Acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-galactopyranose **8** (1.17 g, 3.0 mmol) was dissolved in methanol (100 ml) and the solution cooled to −10 °C. Anhydrous potassium carbonate (0.83 g, 6.01 mmol) was added and the mixture was stirred for 75 min. TLC (ethyl acetate–ethanol–water, 45:5:1) showed one product (R_f 0.03). The mixture was dried, applied to a silica gel column (10 g, 10 mm diam.) and eluted with ethyl acetate–ethanol (4:1). The product was a syrup (0.617 g, 93%). A small amount was crystallised from dichloromethane–diethyl ether to give compound **1**, m.p. 156–158 °C [lit.,[10] m.p. 172–173 °C (*n.b.* the reported m.p. varies widely[9])]; $[\alpha]_D$ +83° (*c* 0.2, H_2O) (lit.,[10] $[\alpha]_D$ +86°) (*c* 1.0, H_2O); δ_H(400 MHz; $CDCl_3$) α-anomer 5.13 (1 H, d, *J* 3.7, 1-H), 4.04 (1 H, dd, *J* 3.7, 11.2, 2-H), 4.01 (1 H, t, *J* 6.2, 5-H), 3.90 (1 H, d, *J* 3.2, 4-H), 3.83 (1 H, dd, *J* 3.2, 11.1, 3-H), 3.65 (2 H, d, *J* 6.2, 6-H), 1.96 (3 H, s), β-anomer 4.55 (1 H, d, *J* 8.4, 1-H), 3.84 (1 H, d, *J* 3.2, 4-H), 3.78 (1 H, dd, *J* 8.4, 10.8, 2-H) and 3.67 (3 H, m, 3-H, 6-H); δ_C(α-anomer) 175, 91, 71, 69, 67, 61, 51 and 22.5; δ_C(β-anomer) 175, 96, 75, 71, 68, 61, 54 and 22.8; $[\alpha]_D$ +86 (*c* 1.0, H_2O).[10] The ^1H NMR spectrum was identical with the spectrum of an authentic mixture of the anomers of 2-acetamido-2-deoxy-D-galactopyranose, except that it represented a different anomeric ratio.

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