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Note

Synthesis of *N*-acetyl-lactosamine via ozonolysis of a nitro derivative

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N-Acetyl-lactosamine (2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-D-glucose, **10**) occurs widely in glycoproteins and glycolipids[1,2] as a component of their oligosaccharide moieties and as a constituent of oligosaccharides[3] found in the milk of mammals. The procedures for the isolation of **10** from these natural sources are complicated and the yields low[4]. An enzymic method[5] for the production of **10** on a preparative scale has been reported. These enzymic procedures are complicated and require several enzymes and nucleoside diphosphate sugars. Increasing needs for **10** for synthesis and medicinal uses led us to attempts to develop a chemical synthesis simpler than those published[6–8].

The sequence adopted was the nitromethane synthesis starting from 3-*O*-β-D-galactopyranosyl-D-arabinose[6] (**1**). Purification of the resulting 1-deoxy-1-nitroalditols **2** and **3** by chromatography on Dowex 1-X8 (HCO_3^-) resin was a significant improvement over the original procedure[7]. Due to different pK_a values of common reducing sugars (~ 12.5) and 1-deoxy-1-nitroalditols (~ 9), **2** and **3** were retarded on the resin and could be separated from **1**.

A modified acetylation procedure for sensitive nitroalditols[9] converted the mixture of **2** and **3** into a mixture (95%) of the octa-acetates **4** and **5**.

The treatment of the mixture of **4** and **5** with methanolic ammonia gave a 2.3 : 1 mixture of the *O*-deacetylated 2-acetamido-2-deoxy derivatives **7** and **8**. Ozonolysis[10] of the mixture of **7** and **8** as the sodium nitronates gave a mixture of *N*-acetylepilactosamine (2-acetamido-2-deoxy-β-D-galactopyranosyl-D-mannose, **9**) and **10**, from which **9** was crystallised (15% from **1**). Treatment of the mixture of **9** and **10** with aqueous ammonia turned the original ratio of 2.5 : 1 to 1 : 4 (^{13}C NMR data) and **10** could then be crystallised (32% from **1**).

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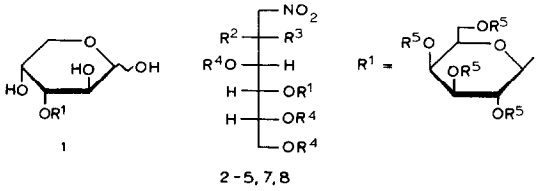
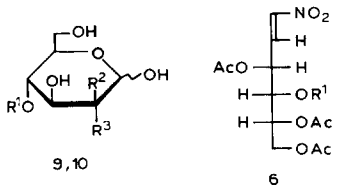


1 2–5, 7, 8



9, 10 6

1 $R^5 = H$
2 $R^2 = OH$, $R^3 = R^4 = R^5 = H$
3 $R^2 = R^4 = R^5 = H$, $R^3 = OH$
4 $R^2 = OAc$, $R^3 = H$, $R^4 = R^5 = Ac$
5 $R^2 = H$, $R^3 = OAc$, $R^4 = R^5 = Ac$
6 $R^5 = Ac$
7 $R^2 = NHAc$, $R^3 = R^4 = R^5 = H$
8 $R^2 = R^4 = R^5 = H$, $R^3 = NHAc$
9 $R^2 = NHAc$, $R^3 = R^5 = H$
10 $R^2 = R^5 = H$, $R^3 = NHAc$

When the above procedure was applied to L-arabinose, a mixture of 2-acetamido-2-deoxy-L-mannose and 2-acetamido-2-deoxy-L-glucose was obtained from which the former (36%) and the latter (19%, both yields are from 3,4,5,6-tetra-*O*-acetyl-1,2-dideoxy-1-nitro-L-*arabino*-hex-1-enitol) were obtained via the crystalline phenylhydrazone and by chromatography on Dowex 50W-X8 (Ba^{2+}), respectively.

Oxidative decomposition[11] of 2-acetamido-1,2-dideoxy-1-nitro-L-mannitol with hydrogen peroxide gave 2-acetamido-2-deoxy-L-mannose, but the isolation procedure was complicated and the yield was somewhat lower than with ozonolysis.

EXPERIMENTAL

General methods.—Melting points were measured on a Kofler hot-stage and are uncorrected. Microanalyses were obtained with a Perkin–Elmer 240 instrument and optical rotations at 20° using a Perkin–Elmer 141 polarimeter. ^{13}C NMR spectra (75.46 MHz) were recorded at 20° with a Bruker AM-300 spectrometer (internal MeOH, δ 50.15). Chromatography was performed on columns C_1 (1.2 × 95 cm) of Dowex 1-X8 (HCO_3^-) resin (100–200 mesh) and C_2 (1.2 × 95 cm) of Dowex 50W-X8 (Ba^{2+}) resin (200–400 mesh) by elution with water, and the eluates were monitored with a Knauer 5100 differential refractometer and by descending PC using *A*, 1-butanol–EtOH–water (5:1:4); *B*, EtOAc–pyridine–water (12:5:4); and detection with alkaline silver nitrate[12]. A Fischer 502 ozone generator was used for the preparation of ozone from gaseous oxygen. Solvents were evaporated under diminished pressure at < 40°.

2-Acetamido-2-deoxy-4-O-β-D-galactopyranosyl-D-mannose (**9**) *and* -D-*glucose* (**10**).—A solution of **1**[6] (2 g) in Me_2SO (25 mL) was mixed with nitromethane (17 mL) and methanolic 2 M NaOMe (4 mL), and the mixture was stirred for 3.5 h at room temperature. Ether (200 mL) was added, stirring was continued for 2 h, and the precipitate was collected by filtration, thoroughly washed with ether, and added to a stirred suspension of Dowex 50W (H^+) resin (10 mL) in water (100 mL) at ~0°. The decationised solution was concentrated and passed through column C_1 to give **1** (0.2 g) and then a mixture (2.0 g, 83%) of **2** and **3**. The retention volume relative to that of **1** was 2.7; PC (solvent *A*): R_1 1.4 (**2**) and 1.9 (**3**).

A solution of the mixture (2.0 g) of **2** and **3** in MeOH (15 mL) was added dropwise to a stirred solution of concd H_2SO_4 (0.5 mL) in acetic anhydride (71 mL) so that the temperature did not exceed 40°. After 4 h, the mixture was poured into a water–ice mixture and extracted with $CHCl_3$. The extract was neutralised with aq $NaHCO_3$, dried (Na_2SO_4), and concentrated to a syrupy mixture (3.1 g) of **4** and **5**. A solution of the mixture in dry MeOH (30 mL) was saturated with gaseous ammonia, stored at 25° for 24 h, then concentrated with the addition of water (3×) to give a syrupy 2.3:1 mixture (3.6 g) of **7** and **8** [PC (solvent *B*), R_1 1.9 and 2.0] and acetamide. ^{13}C NMR data (D_2O): δ 177.6 (CH_3CONH_2), 175.0, 174.7 (C=O), 106.2, 106.0 (C-1′), 77.77, 77.5 (C-1), 64.8, 64.4 (C-6), 64.1, 63.8 (C-6′), 54.2, 53.0 (C-2), 24.1, 23.9 (CH_3), 23.4 (CH_3CONH_2), 80.5, 80.3, 78.0, 75.7, 73.7, 73.3, 73.1, 71.5, 70.2.

Ozone (40 mg/min) was passed for 40 min into a solution of the mixture **7** and **8** in water (15 mL) and M NaOH (6 mL). The neutral mixture was concentrated and the residue was subjected to chromatography on column C_2 to give sodium nitrate (0.4 g), then **1** (0.2 g), and a mixture (1.1 g, 44%) of **9** and **10**. Crystallisation from MeOH afforded **9** (0.38 g, 15%), mp 230–235°, $[\alpha]_D$ +31° (*c* 1, H_2O); lit.[7] mp 232–235°, $[\alpha]_D$ +28.2° (H_2O). ^{13}C NMR data (D_2O): δ 176.8, 175.8 (C=O), 104.2 (C-1′), 94.2 (C-1α), 94.0 (C-1β), 77.3 (C-4α), 76.9 (C-4β), 76.6 (C-5′), 76.4 (C-5β), 73.7 (C-3′), 72.2 (C-2′), 72.0 (C-3β), 71.9 (C-5α), 69.8 (C-4′), 68.8 (C-3α), 62.4 (C-6′), 61.1 (C-6), 54.7 (C-2β), 54.0 (C-2α), 23.4, 23.1 (CH_3).

A solution of the syrupy mixture (1.1 g) of **9** and **10** in water (25 mL) was saturated with gaseous ammonia, then stored at room temperature for 20 h. The solvent was evaporated together with added water (3×) and the residue was crystallised from MeOH to give **10** (0.54 g, 22%), mp 168–170°, $[\alpha]_D$ +26° (*c* 1, H_2O); lit.[7] mp 168–169°, $[\alpha]_D$ +24.7° (H_2O).

Repeated epimerisation of the mother liquor gave a further crop (0.25 g, 10%) of **10**.

2-Acetamido-2-deoxy-L-mannose and -L-*glucose.*—3,4,5,6-Tetra-*O*-acetyl-1,2-dideoxy-1-nitro-L-*arabino*-hex-1-enitol[13] was converted[14] into a mixture (2.3 g) of 2-acetamido-1,2-dideoxy-1-nitro-L-mannitol and -L-glucitol to a solution of which in water (5 mL) was added M NaOH (9 mL). The solution was treated with ozone for 20 min as described above, then flushed with nitrogen for 5 min, and phenylhydrazine (1.1 mL) and EtOH (3.2 mL) were added. The mixture was stored

for 24 h at room temperature, and the crystalline precipitate was collected and washed with water to give 2-acetamido-2-deoxy-L-mannose phenylhydrazone (1.12 g, 37%), mp 180–182°, $[\alpha]_D$ +74° (*c* 1.9, MeOH). ^{13}C NMR data (MeOD): δ 139.2 (C-1), 55.2 (C-2), 73.5 (C-3/5), 72.8 (C-4), 66.1 (C-6), 148.2, 114.8, 131.0, 121.0 (C–Ph), 23.8 (CH_3), 174.6 (C=O).

Anal. Calcd for $C_{14}H_{21}N_3O_5$: C, 53.84; H, 6.73; N, 13.46. Found: C, 53.63; H, 6.84; N, 13.25.

To the filtrate obtained after removal of the phenylhydrazone were added benzaldehyde (0.3 mL), pyridine (0.17 mL), and EtOH (0.4 mL). The mixture was heated at 100° for 3 h, cooled, filtered, washed with EtOAc, and decolourised with charcoal, and the solvent was evaporated. Column (C_2) chromatography of the residue and crystallisation from MeOH afforded 2-acetamido-2-deoxy-L-glucose (0.36 g, 17%), mp 210° (dec), $[\alpha]_D$ −44° (*c* 1.1, H_2O); lit.[15] for the D enantiomer, mp 210° (dec), $[\alpha]_D$ +41° (H_2O).

Treatment of the above phenylhydrazone (1.12 g) with benzaldehyde (0.55 mL), EtOH (0.9 mL), pyridine (0.3 mL), and water (6 mL), work-up as for the *L-gluco* isomer, and crystallisation from MeOH afforded 2-acetamido-2-deoxy-L-mannose (0.75 g, 36%), mp 122–124°, $[\alpha]_D$ −9.0° (*c* 1.5, H_2O); lit.[16] for the D enantiomer, mp 128–129°, $[\alpha]_D$ +9.7° (H_2O).

Oxidation of 2-acetamido-1,2-dideoxy-1-nitro-L-mannitol.—(*a*) Ozone was introduced into a solution of the title compound (1 g) in water (6 mL) and M NaOH (5 mL) at room temperature for 10 min. Evaporation of the solvent and column (C_2) chromatography of the residue afforded 2-acetamido-2-deoxy-L-mannose (0.68 g, 72%).

(*b*) To a solution of the title compound (1 g) and sodium molybdate (0.1 g) in 0.5 M NaOH (10 mL) was added aq 30% H_2O_2 (2.4 mL) at such a rate that the temperature did not exceed 30°. The mixture was stored at room temperature for 20 h, then 5% Pd/C (0.05 g) was added. After 20 h, acetic acid (0.3 mL) was added, and air was bubbled through the mixture for 4 h, which was then filtered, deionised with Dowex 50W (H^+) and Dowex 1 (HCO_3^-) resins, and concentrated. Column (C_2) chromatography of the residue afforded 2-acetamido-2-deoxy-L-mannose (0.57 g, 60%).

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