Note

The synthesis of 2-acetamido-1-*N*-(4-L-aspartyl)-2-deoxy-β-D-galactopyranosylamine

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The amide linkage in 2-acetamido-1-*N*-(4-L-aspartyl)-2-deoxy-β-D-glucopyranosylamine[1,2] is hydrolysed by an *amido hydrolase*[3] to give aspartic acid and the glucopyranosylamine. A recent study[4] has indicated that the enzyme is highly specific for the 4-L-aspartyl group but not the carbohydrate moiety; the galactopyranosylamine derivative was synthesised in order to further investigate this specificity. A further interest in this compound arises from its possible occurrence in glycoproteins.

2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranosyl azide (**2**) was prepared from 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-galactose *via* the corresponding bromide **1**. A chloroformic solution of **1** was heated with sodium azide in dry formamide, and hydrogenation of the resulting azide **2**, using Adams' catalyst, gave the amine **3**. Coupling of **3** with 1-benzyl *N*-benzyloxycarbonyl-L-aspartate, using dicyclohexylcarbodiimide in dichloromethane at room temperature, gave the fully protected product **4**.

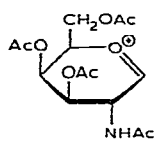
1

2 X = N_3, R = Ac
3 X = NH_2, R = Ac
4 X = $NHCOCH_2CH(COOCH_2Ph)NHOCOCH_2Ph$, R = Ac
5 X = $NHCOCH_2CH(COOH)NH_2$, R = H

Hydrogenolysis of **4** removed the benzyloxycarbonyl and benzyl ester protecting groups, and *O*-deacetylation with ammonia in methanol then gave the free aspartyl derivative **5**. The 100-MHz n.m.r. spectrum of the azide **2** in deuteriochloroform was consistent with a C_1^4 conformation; the signal for the anomeric proton appeared as a doublet at τ 5.18 with *J* 9.0 Hz indicating a *trans*-diaxial relationship for H-1 and H-2. The azide therefore has the β-D configuration, comparable to the analogous 2-amino-2-deoxyglucose derivative. Similarly, the spectrum of the free β-pyranosylamine derivative **5** in deuterium oxide at 100 MHz showed the anomeric H-1 signal as a doublet with *J* 9.0 Hz indicating the *trans*-diaxial relationship with H-2.

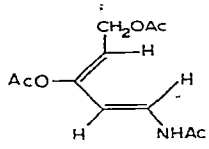
The mass spectrum of **4** was similar to that described for the analogous 2-amino-2-deoxyglucose derivative[5]. The molecular-ion peak was extremely weak, but the

carbohydrate moiety could be identified by a fragment at *m/e* 330 corresponding to the oxonium ion **6**. Further fragmentation of **6** resulted in a series of peaks at *m/e* 270, 210, and 150. The peak series reported for the 2-amino-2-deoxyglucose derivative, starting from *m/e* 241 (**7**), was also observed. Furthermore, a peak at *m/e* 373 was observed, showing the presence of fragment **8**.



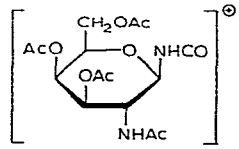
m/e 330

6



m/e 241

7



m/e 373

8

The c.d. spectrum of **5** had a maximum (212 nm, $\Delta\varepsilon$ +2.4), similar to the 2-amino-2-deoxyglucose derivative, in contrast to the 2-acetamido-2-deoxy-D-glucosides and -galactosides which have minima at this wavelength[6]. Compound **2** had a c.d. maximum (267 nm, $\Delta\varepsilon$ +0.11) attributable to the azide, which was approximately half the size of that observed with the 2-amino-2-deoxyglucose derivative (264 nm, $\Delta\varepsilon$ +0.21).

The free aspartyl compound **5** was hydrolysed by an *amido hydrolase* at one fifth of the rate for the corresponding 2-amino-2-deoxyglucose compound; **5** and the *gluco* analogue may be distinguished by t.l.c. and paper chromatography.

EXPERIMENTAL

General. — Melting points (uncorrected) were determined on a Reichert hot-stage, and optical rotations were determined on a Perkin–Elmer 141 polarimeter. All evaporations were conducted under reduced pressure. The mass spectrum was determined by PCMU Harwell, with an A.E.I. MS-9 instrument, using a source temperature of 140°. The c.d. spectrum was measured in aqueous solution on a Roussel–Jouan instrument. Silica gel G (Merck) was used for t.l.c.

2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-galactopyranosyl azide (**2**). — A chloroformic solution (120 ml) of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl bromide[7] (**1**), previously prepared from 2-amino-2-deoxy-D-galactose pentaacetate[8] (10 g), was added to a cold solution of sodium azide (6 g) in dry formamide (100 ml). After being stirred at room temperature overnight, the mixture was poured into ice–water. The chloroform layer was separated, the aqueous layer was extracted with chloroform, and the combined extracts were washed with water, dried, and evaporated. The residue was recrystallized from chloroform–ether to give the azide **2** (5.8 g, 61%), m.p. 164–166° (dec.), $[\alpha]_D^{22}$ −0.4° (*c* 0.95, chloroform), which was homogeneous on t.l.c. (chloroform–ether 3:1) (Found: C, 45.45; H, 5.5; N, 15.05. $C_{14}H_{20}N_4O_8$ calc.: C, 45.15; H, 5.4; N, 15.05%).

2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-galactopyranosylamine (**3**). — The

azide **2** (0.6 g) was dissolved in methanol and hydrogenated over Adams' catalyst at a pressure of 40 lb/sq.in. for 3 h. The catalyst was then filtered off and the methanol evaporated under reduced pressure at room temperature. Attempted recrystallization from cold chloroform–ether gave amorphous **3** (0.4 g, 71%), $[\alpha]_D^{22}$ −0.1° (*c* 0.93, chloroform), which was homogeneous on t.l.c. (chloroform–methanol, 10:1) (Found: C, 48.8; H, 6.2; N, 8.2. $C_{14}H_{22}N_2O_8$ calc.: C, 48.55; H, 6.4; N, 8.1%).

*2-Acetamido-3,4,6-tri-*O*-acetyl-1-*N*-(1-benzyl N-benzyloxycarbonyl-4-*L*-aspartyl)-2-deoxy-β-*D*-galactopyranosylamine* (**4**). — The β-D-amine **3** (0.40 g) and 1-benzyl *N*-benzyloxycarbonyl-L-aspartate[4] (0.42 g) were dissolved in dichloromethane. Dicyclohexylcarbodiimide (0.28 g) was added, and the mixture was stirred at room temperature for 6 h. Glacial acetic acid (0.5 ml) was added and, after 30 min, the precipitated *N,N'*-dicyclohexylurea was filtered off and washed with dichloromethane. The filtrate and washings were combined, washed with water, and dried, and the solvent was removed. Preparative-layer chromatography (Merck Silica Gel F254; chloroform–methanol, 20:1) gave **4** (0.42 g, 51%) which, on recrystallisation from chloroform–ether, had m.p. 148–149°, $[\alpha]_D^{22}$ +0.2° (*c* 0.26, chloroform) (Found: C, 58.0; H, 5.9; N, 6.1. $C_{33}H_{39}N_3O_{13}$ calc.: C, 57.8; H, 5.7; N, 6.1%).

*2-Acetamido-1-*N*-(4-L-aspartyl)-2-deoxy-β-D-galactopyranosylamine* (**5**). — The fully protected asparagine derivative **4** (1.0 g) was dissolved in ethanol–glacial acetic acid (100 ml, 10:1) and hydrogenated over 10% palladium–charcoal (0.1 g) at 45 lb/sq.in. for 3 h. After filtration and removal of the solvent at 40°, the residue was left *in vacuo* at room temperature over phosphorus pentaoxide–potassium hydroxide for 24 h. The amorphous solid was then dissolved in dry methanol (35 ml), and the cooled solution was saturated with ammonia. After refrigeration overnight, the solvent was removed and the remaining solid was carefully washed with acetone to remove acetamide. The residue was recrystallised from water–acetone to yield **5** (0.3 g, 61%), m.p. 214–215° (dec.), $[\alpha]_D^{22}$ +0.6° (*c* 5.0, water), which was homogeneous on t.l.c. (butyl alcohol–acetic acid–water, 10:20:5) (Found: C, 42.9; H, 6.4; N, 12.4. $C_{12}H_{21}N_3O_8$ calc.: C, 43.0, H, 6.3; N, 12.5%).

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