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SYNTHESES AND REARRANGEMENTS OF D-GLUCOSYL ESTERS OF ASPARTIC ACID LINKED THROUGH THE 1- OR 4-CARBOXYL GROUP*

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ABSTRACT

Catalytic hydrogenation of 2,3,4,6-tetra-*O*-benzyl-1-*O*-[1-benzyl *N*-(benzyloxycarbonyl)-L-aspart-4-oyl]-α-D-glucopyranose (**1α**) in acetic acid–2-methoxyethanol gave 1-*O*-(L-β-aspartyl)-α-D-glucopyranose (**2α**) contaminated with 2-*O*-(L-α-aspartyl)-D-glucopyranose (**8**). Evidence that **8** was formed from the 1-oyl isomer of **1α**, namely 2,3,4,6-tetra-*O*-benzyl-1-*O*-[4-benzyl *N*-(benzyloxycarbonyl)-L-aspart-1-oyl]-α-D-glucopyranose (**7α**), *via* 1→2 acyl migration, was obtained by submitting the deprotected D-glucosyl ester to successive *N*-acetylation, esterification, and *O*-acetylation; the final product was identified as a ~4:1 mixture of 2,3,4,6-tetra-*O*-acetyl-1-*O*-[1-methyl *N*-(acetyl)-L-aspart-4-oyl]-α-D-glucopyranose (**4α**) and 1,3,4,6-tetra-*O*-acetyl-2-*O*-[4-methyl *N*-(acetyl)-L-aspart-1-oyl]-D-glucopyranose (**6**) which were also prepared by definitive methods. On the other hand, deprotection of **1β** gave isomerically pure **2β** which was converted into the peracetylated ester derivative **4β**; an explanation for the differences in aglycon isomeric purity of **2α** and **2β** is given. Hydrogenolysis of **7β** under the above conditions led to intermolecular transesterification with scission of the C-1 ester bond to give 1-(2-methoxyethyl) L-aspartic acid and D-glucose. Catalytic hydrogenation of **7α** and **7β**, performed in the presence of trifluoroacetic acid, afforded 1-*O*-(L-α-aspartyl)-α- and -β-D-glucopyranoside trifluoroacetate salts (**11α** and **11β**), respectively. The structure of **11β** was established by successive conversion into 2,3,4,6-tetra-*O*-acetyl-1-*O*-[4-methyl *N*-(acetyl)-L-aspart-1-oyl]-β-D-glucopyranose (**5β**) which was also prepared by definitive methods. Analogous treatment of **11α** gave the *N*-acetyl derivative **12** which underwent 1→2 acyl migration during esterification with diazomethane to give the *N*-acetyl methyl ester derivative **10**; acetylation of **10** afforded **6**.

INTRODUCTION

The side-chain carboxyl groups of aspartic and glutamic acids are involved in the active sites of several glycosidases, as shown by the effect of chemical modification

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*Glycosyl Esters of Amino Acids: Part VII. For Part VI, see Ref. 14.

on enzymic activity[1]. For almond glucosidase, the involvment of a carboxyl group has been demonstrated in the reaction with conduritol B epoxide which binds stoichiometrically to the enzyme; the inhibitor was recovered as (+)-inositol after the action of hydroxylamine, suggesting an ester bond with the enzyme[2,3]. In the enzymic process, two possible mechanistic roles have been assigned to an ionized carboxyl group: (*a*) electrostatic stabilization of the developing oxonium ion derived from the the sugar moiety, or (*b*) direct nucleophilic assistance, resulting in the formation of a covalent glycosyl–enzyme intermediate of inverted configuration[4]. However, corroborating evidence for a covalent intermediate has not yet been obtained for any glycosidase-catalyzed hydrolysis of natural substrates[4]. On the other hand, covalent catalysis has been demonstrated for a related enzyme, sucrose phosphorylase[5], and recently the existence of a covalent intermediate was suggested in the β-D-glucosidase and methyl α-D-glucosidase-catalyzed hydrolysis of *p*-nitrophenyl β-D-glucopyranoside[6] and methyl-^{14}C α-D-glucopyranoside[7], respectively.

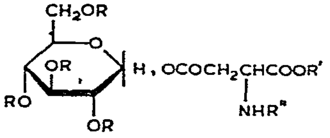
Thus, glycosyl esters of aspartic and glutamic acid may serve as useful models for studying the mechanism and action of glycosidases. As part of our synthetic work on C-1 sugar–amino acid esters, we have already reported[8] the preparation of 1-*O*-(L-β-aspartyl)-β-D-glucopyranose by catalytic hydrogenation of the corresponding, fully benzylated C-1 ester; the latter compound was obtained by the imidazole-promoted reaction[9] of tetra-*O*-benzyl-α-D-glucopyranose and 1-benzyl 4-pentachlorophenyl *N*-benzyloxycarbonyl-L-aspartate, followed by fractionation of the resulting mixture of anomers.

We now report on the synthesis, characterization, and chemical reactivity of the isomeric D-glucose–aspartic acid esters and comment on the rearrangement reactions which take place during the formation of some isomeric forms.

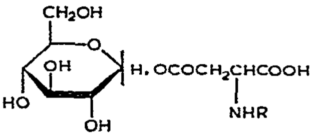
RESULTS AND DISCUSSION

Catalytic hydrogenation of the oily 2,3,4,6-tetra-*O*-benzyl-1-*O*-[1-benzyl *N*-(benzyloxycarbonyl)-L-aspart-4-oyl]-α-D-glucopyranose[8] (**1α**) in acetic acid–2-methoxyethanol afforded a chromatographically homogeneous solid, whose elemental analysis, optical rotation, and spectral data supported the structure 1-*O*-(L-β-aspartyl)-α-D-glucopyranose (**2α**); hydrolysis of a sample in 2M HCl (100°, 2 h) gave 75% optically pure L-aspartic acid. Treatment of **2α** with 10% acetic anhydride in acetone–water gave, after chromatography on a cellulose column, a hygroscopic product whose analytical and optical data were consistent with the structure 1-*O*-(*N*-acetyl-L-β-aspartyl)-α-D-glucopyranose (**3α**). The n.m.r. spectrum of **3α** in deuterium oxide clearly indicated the anomeric proton at τ 3.86 ($J_{1,2}$ 3 Hz) and the *N*-acetyl singlet at τ 7.99; however, the ratio of their peak intensities was indicative of the presence of a contaminant with HO-1 unsubstituted.

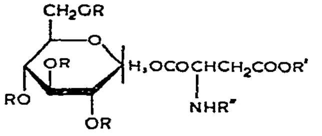
For further characterization, **2α** was submitted in turn to (*a*) selective *N*-acetylation, (*b*) esterification of the aglycon carboxyl group with diazomethane, and (*c*) *O*-acetylation of the sugar moiety with acetic anhydride–pyridine. The final prod-



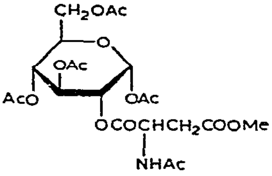
1 R = R′ = $PhCH_2$; R″ = $PhCH_2OCO$
4 R = R″ = Ac; R′ = Me



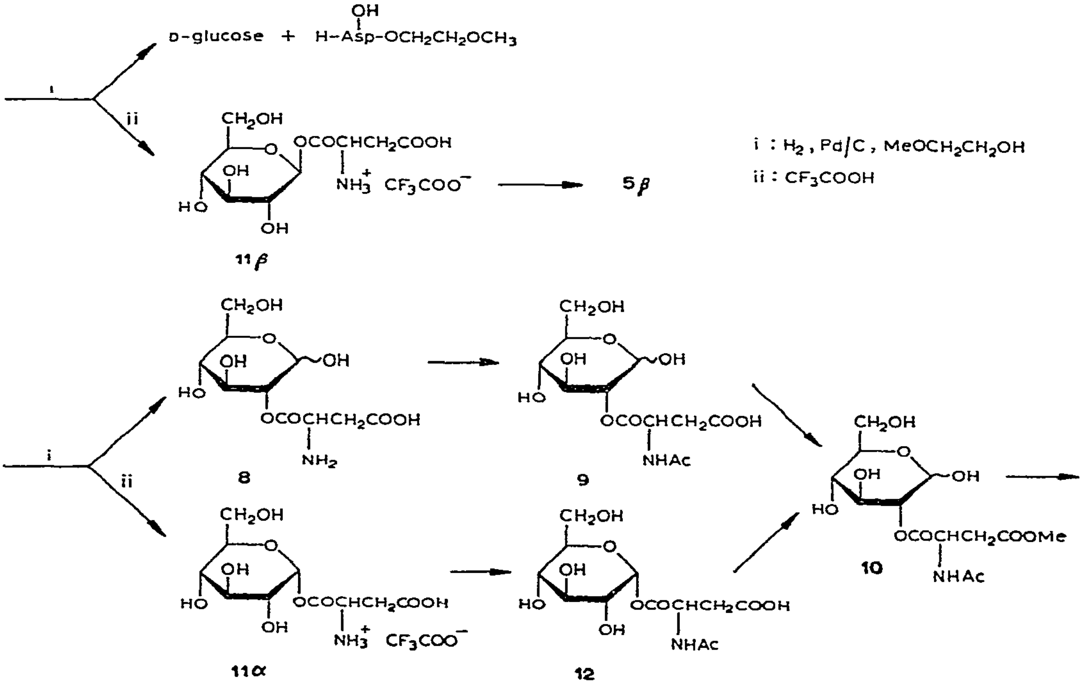
2 R = H
3 R = Ac



5 R = R″ = Ac; R′ = Me
7 R = R′ = $PhCH_2$; R″ = $PhCH_2OCO$



6α



i : H_2, Pd/C, $MeOCH_2CH_2OH$
ii : CF_3COOH

uct was purified on a column of silica gel to give a chromatographically homogeneous solid, whose analytical data, optical rotation ($[\alpha]_D$ +76.8° in chloroform), and i.r. spectrum were consistent with the structure 2,3,4,6-tetra-*O*-acetyl-1-*O*-[1-methyl *N*-(acetyl)-L-aspart-4-oyl]-α-D-glucopyranose (**4α**). The n.m.r. spectrum of **4α** in chloroform-*d* revealed the anomeric proton as one doublet at τ 3.68 ($J_{1,2}$ 3 Hz) and the five acetyl groups as unresolved singlets in a narrow range at τ 7.85–8.03. However, the rest of the data were not compatible with the structure assigned: the ester methyl group of the aspartate moiety was observed as two singlets (τ 6.25 and 6.31) of different intensities (ratio ~4:1), and a weak singlet at τ 7.77, indicative of an *ax* 1-*O*-acetyl group, was also discernible. A comparison with the n.m.r. spectra of synthetically prepared 1-methyl *N*-acetylaspartic acid and 1,4-dimethyl *N*-acetylaspartate showed that the resonances at τ 6.25 and 6.31 coincided with those of the aspartate ester methyl groups at positions 1 and 4, respectively.

The above observations suggested that the product obtained from **2α** was contaminated with component(s) isomeric to **4α**, namely 2,3,4,6-tetra-*O*-acetyl-1-*O*-[4-methyl *N*-(acetyl)-L-aspart-1-oyl]-α-D-glucopyranose (**5α**) and/or 1,3,4,6-tetra-*O*-acetyl-2-*O*-[4-methyl *N*-(acetyl)-L-aspart-1-oyl]-α-D-glucopyranose (**6α**).

The direct synthesis of the protected ester **4** was achieved by the imidazole-promoted dicyclohexylcarbodi-imide (DDC) condensation[9] of 2,3,4,6-tetra-*O*-acetyl-D-glucopyranose and 1-methyl *N*-acetyl-L-aspartic acid. The product was obtained as an anomeric mixture which was resolved on silica gel. The n.m.r. spectra of both anomers indicated that traces of the 1-oyl isomer **5α** and **5β**, respectively, were present, the intensity ratio of the singlets assigned to the 1- and 4-methyl aspartate group being ~7:1. The n.m.r. spectrum of the crystalline α anomer (m.p. 42–44°) showed the signals assigned to H-1 and to the five acetyl groups at positions identical to those observed in the spectrum of the product obtained from **2α**, but in the former the singlet at τ 7.77 (*ax* 1-OAc) was absent.

Conversion of the known[8] β anomer of **2** into the fully protected ester **4** was performed in the same manner as with **2α**. In the n.m.r. spectrum of the product, there was no signal for a 4-methyl aspartate group, and the other signals were superimposable upon those observed in the spectrum of **4β** prepared by direct synthesis.

In order to determine the isomeric purity of the aspartate aglycon in the unprotected esters **2α** and **2β**, a procedure was elaborated based on the observation[10] that the 1- and 4-esters of aspartic acid can be distinguished by their reduction products. Treatment of **2β** with lithium borohydride at 0°, followed by elution of the mixture through a column of Dowex-50(H^+) resin, yielded 2-amino-4-hydroxybutyric acid together with some aspartic acid as the only ninhydrin-positive components. Hence, in the sample of **2β**, only the carboxyl group at position 4 of aspartic acid was involved in the sugar–aglycon ester bond. By contrast, reduction of **2α** gave a mixture (~4:1) of 2-amino- and 3-amino-4-hydroxybutyric acid which was resolved by paper chromatography; the components were estimated spectrophotometrically. From these data, it is reasonable to assume that partial isomerization of the aspartyl residue had occurred prior to the formation of the glycosidic ester bond, presumably at the stage of the imidazole-catalyzed transfer of the acyl group to the sugar receptor; it is known that the aspartyl residue readily undergoes rearrangements of the transpeptidation type. Apparently, the contaminant 1-oyl isomer present in **1β** was readily removed by crystallisation, whereas this was not possible with the oily α anomer of **1** whose chromatographic mobility was indistinguishable from that of its 1-oyl isomer, *i.e.*, 2,3,4,6-tetra-*O*-benzyl-1-*O*-[4-benzyl *N*-(benzyloxycarbonyl)-L-aspart-1-oyl]-α-D-glucopyranose[9] (**7α**). Accordingly, the isomeric contaminant(s) in **2α** and its derivatives **3α** and **4α** should have arisen from **7α**.

Each anomer of **7**[9] was then hydrogenolysed under conditions used for the deprotection of the 4-oyl isomer **1**. Hydrogenolysis of **7β** in acetic acid–2-methoxyethanol led to transfer of the acyl group to the alcoholic solvent: 1-(2-methoxyethyl) aspartic acid and D-glucose were identified as the major products, together with small proportions (t.l.c.) of a ninhydrin- and silver nitrate-positive compound (presumably the unprotected D-glucosyl ester), and traces of aspartic acid. The mixture was fractionated on a cellulose column, and 1-(2-methoxyethyl) aspartic acid was isolated pure in 63% yield, thus indicating that intermolecular transesterification of the aglycon, with cleavage of the glycosidic ester bond, was the principal reaction. The fact that the spot ascribed to the unprotected D-glucosyl ester of the 1-yl isomer of aspartic acid disappeared during work-up of the hydrogenation mixture indicates that, under the above conditions, a sample of **1β** contaminated with its 1-oyl isomer **7β** would yield exclusively the isomerically pure **2β**.

Similar hydrogenolysis of **7α** led to a mixture containing a major ninhydrin- and silver nitrate-positive component, together with D-glucose and aspartic acid as the minor components. The main product was isolated as a highly hygroscopic solid (50% yield) which was tentatively identified as 2-*O*-(L-α-aspartyl)-D-glucopyranose (**8**). The n.m.r. spectrum in deuterium oxide contained a doublet for H-1 at relatively high field (τ 4.54), with a coupling constant of 4 Hz, suggesting that HO-1 of D-glucose was unsubstituted. The formation of **8** would require intramolecular transfer of the aspartyl residue to the *cis*-related HO-2 group of D-glucose, *i.e.*, direct nucleophilic attack of the adjacent hydroxyl on the carbonyl carbon involved in the glycosidic ester bond. Facile O → O shifts of this nature are known for 2′(3′)-*O*-aminoacyl derivatives of ribonucleosides[11], the simplest model compounds of aminoacyl t-RNA. These compounds behave[12,13] as activated esters in peptide syntheses, and the reactivity of the ester linkage has been partly ascribed to the presence of the *cis*-diol system. The behaviour of these compounds is similar to that observed for the anomers of **7**, although, for the hydrogenation product of **7β**, the reactivity of the glycosidic ester bond cannot be ascribed to participation by a *cis*-diol system.

Further support for the structure of **8** was obtained by *N*-acetylation; the product (75% yield) gave analytical and spectral data consistent with the structure 2-*O*-[*N*-(acetyl)-L-α-aspartyl]-D-glucopyranose (**9**). Treatment of **9** with diazomethane, followed by acetylation of the hydroxyl groups, afforded a homogeneous, stable solid, to which the structure of 1,3,4,6-tetra-*O*-acetyl-2-*O*-[4-methyl *N*-(acetyl)-L-aspart-1-oyl]-D-glucopyranose (**6**) was assigned. The n.m.r. spectrum in chloroform-*d* contained two anomeric doublets (τ 3.64, $J_{1,2}$ 3 Hz and τ 4.24, $J_{1,2}$ 7 Hz) of equal intensities, and two singlets for AcO-1 (τ 7.81 and 7.84, ratio ~1:1) appearing at lower field than those for the remaining acetyl groups. Proof that the aspartyl residue in **6** is linked to HO-2 of D-glucose was provided by comparison with an authentic sample of **6α**, prepared by the imidazole-promoted DCC condensation of 1,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose and 4-methyl *N*-acetyl-L-aspartic acid. Hence, on the basis of this evidence, the contaminant present in the protected D-glucosyl ester **4α**, prepared from **2α**, is identified as **6**, *i.e.*, the fully protected derivative of the rearrangement product of the 1-yl isomer **7α**.

We have shown[14] that catalytic hydrogenation of the fully benzylated β-D-glucopyranuronic ester of *N*-benzyloxycarbonyl-L-alanine, in the presence of trifluoroacetic acid, gives the unprotected ester as a labile trifluoroacetate salt. When similar reaction conditions were applied to the D-glucosyl ester **7β**, the major product (61%) was a crystallisable, unstable compound whose elemental analysis, and i.r. and n.m.r. spectra suggested the structure 1-*O*-(L-α-aspartyl)-β-D-glucopyranose trifluoroacetate salt (**11β**). Further characterization of **11β** was achieved by submitting it successively to *N*-acetylation, esterification, and *O*-acetylation; the final product (11% yield) was indistinguishable from an authentic sample of 2,3,4,6-tetra-*O*-acetyl-1-*O*-[4-methyl *N*-(acetyl)-L-aspart-1-oyl]-β-D-glucopyranose (**5β**), synthesized by the imidazole-promoted DCC condensation.

Similar treatment of **7α** afforded the unprotected D-glucosyl ester trifluoroacetate salt **11α**. The n.m.r. spectrum of **11α** in deuterium oxide contained an anomeric doublet (τ 3.75, $J_{1,2}$ 2.5 Hz) having chemical shift and coupling constant clearly different from those for the 2-*O*-substituted ester **8**. Selective *N*-acetylation of **11α** gave 1-*O*-(*N*-acetyl-L-α-aspartyl)-α-D-glucopyranose (**12**), whose optical rotation, and n.m.r. and i.r. spectra again showed distinct differences from those of the *N*-acetylated, 2-*O*-substituted ester **9**. Esterification of **12** with diazomethane, followed by acetylation of the hydroxyl groups, gave a stable, crystalline compound whose physical data were consistent with the structure 1,3,4,6-tetra-*O*-acetyl-2-*O*-[4-methyl *N*-(acetyl)-L-aspart-1-oyl]-D-glucopyranose (**6**) and not with that of 2,3,4,6-tetra-*O*-acetyl-1-*O*-[4-methyl *N*-(acetyl)-L-aspart-1-oyl]-α-D-glucopyranose (**5α**).

In order to find out whether the 1→2 migration of the aspartyl residue had occurred during the esterification of the 4-carboxyl group of the aspartate moiety, or during the next step involving acetylation of the sugar hydroxyl groups, the products formed by the action of diazomethane on the *N*-acetyl derivatives **9** and **12** were examined. After purification of the reaction mixtures on cellulose, the chromatographically homogeneous products were shown to be identical, and their physical data fully supported the structure 2-*O*-[4-methyl *N*-(acetyl)-L-aspart-1-oyl]-D-glucopyranose (**10**). The formation of the 2-*O*-substituted derivative **10** from the D-glucosyl ester **12** may be explained by the enhanced nucleophilicity of HO-2 of D-glucose caused by the loss of the free aglycon carboxyl group; presumably, the rearrangement proceeds by way of an intermediate 1,2-cyclic orthoester which then decomposes into the 2-*O*-substituted derivative **10**.

All of the D-glucosyl esters described are extremely alkali-labile compounds and undergo rapid cleavage of the ester bond, even in slightly alkaline, aqueous media. However, their stability in aqueous solution were significantly different, depending on the mutual positions of the aglycon functions as well as on the anomeric configuration of the sugar moiety. Thus, the extent of glycosidic cleavage of 1-*O*-(L-β-aspartyl)-α-D-glucopyranose (**2α**) in water (~10% solution, pH 3.12) was not more than ~10% (t.l.c.) after one week at room temperature, whereas 1-*O*-(L-α-aspartyl)-β-D-glucopyranose trifluoroacetate salt (**11β**), under the same conditions, underwent complete cleavage in less than 24 h.

EXPERIMENTAL

General. — Evaporations were performed in a rotary evaporator *in vacuo* at bath temperature below 35°, if not stated otherwise. Column chromatography was performed on silica gel (Merck, 0.05–0.2 mm); or cellulose powder (Whatman standard grade), packed as a slurry by using a plunger. The solvent systems used were: *A* acetonitrile–water (proportions are given in the text); *B* 9:1:1 methanol–acetic acid–water; *C* benzene–ethyl acetate (proportions are given in the text); *D* 60:15:25 1-butanol–acetic acid–water. T.l.c. was performed on Kieselgel G (Merck) or cellulose (microcrystalline, Merck); detection was effected with 10% sulphuric acid and heating, with ninhydrin reagent, or with alkaline silver nitrate. Melting points are uncorrected. Optical rotations were determined for 1% solutions in chloroform unless otherwise stated. I.r. spectra were recorded on a Perkin–Elmer Model 137 spectrometer. N.m.r. spectra were recorded on solutions in chloroform-*d* unless otherwise stated, with tetramethylsilane as internal standard, using a Varian A-60 spectrometer.

Aspartic acid derivatives. — 1-Methyl *N*-acetyl-L-aspartic acid was prepared by the method of Shalitin and Bernhard[15]. 4-Methyl *N*-acetyl-L-aspartic acid was prepared as follows. An aqueous solution of 4-methyl L-aspartic acid hydrochloride[16] was neutralized with sodium hydrogen carbonate at 0°, and to the mixture acetic anhydride and sodium hydrogen carbonate (1.2 equiv. each) were added with stirring at 0°. After 4 h, the solution was shaken with Dowex-50 x8(H^+) resin, filtered, and evaporated to dryness. To a solution of the residue in ethanol, 1 equiv. of dicyclohexylamine in ether was added. Storage at 0° afforded the dicyclohexylamine salt of 4-methyl *N*-acetyl-L-aspartic acid (57%), m.p. 170–171° (from ethanol–ether), $[\alpha]_D$ +21.1° (ethanol).

Anal. Calc. for $C_{19}H_{34}N_2O_5$: C, 61.60; H, 9.25; N, 7.56. Found: C, 61.67; H, 9.08; N, 7.75.

Shaking of the above salt with Dowex-50 x8(H^+) in water afforded 4-methyl *N*-acetyl-L-aspartic acid[17] as a syrup. N.m.r. data: τ 6.32 (s, 3 H, 4-COO*Me*), 7.99 (s, N*Ac*).

1,4-Dimethyl *N*-acetyl-L-aspartate was prepared by treating an aqueous solution of 1,4-dimethyl L-aspartate hydrochloride[18] with acetic anhydride–sodium hydrogen carbonate as described above. The reaction mixture, after shaking with Dowex-50 x8(H^+) resin, was evaporated to dryness, and the residue eluted from silica gel with solvent *C* (1:3) to give the title compound (57%) as a viscous oil, $[\alpha]_D$ −21.4°. N.m.r. data: τ 6.26 (s, 1-COO*Me*), 6.31 (s, 4-COO*Me*), 7.99 (s, N*Ac*).

Anal. Calc. for $C_8H_{13}NO_5$: C, 47.29; H, 6.45; N, 6.89. Found: C, 47.42; H, 6.62; N, 6.82.

4-Hydroxy-2-amino-L-butyric acid was prepared by reduction of 4-methyl L-aspartic acid hydrochloride[16] with lithium borohydride (10-fold excess) in tetrahydrofuran at room temperature for 5 h. After work-up, the product was eluted from a column of Dowex-50 x8(H^+) resin with M NH_4OH. Crystallisation from methanol plus some drops of water gave the pure product (30%), m.p. 192–194°, $[\alpha]_D$ $-7.0°$ (water); lit.[19] m.p. 187°; lit.[20] m.p. 203°, $[\alpha]_D$ $-8.0°$ (water).

4-Hydroxy-3-amino-L-butyric acid was prepared as follows. The amino function of methyl *N*-benzyloxycarbonyl-L-asparaginate[21] was deprotected by conventional catalytic hydrogenation, and the crude product was immediately treated with lithium borohydride as described above, but for 18 h. After work-up and separation of the reaction mixture on a column of Dowex-50 x8(H^+) resin, the material displaced by M NH_4OH was hydrolysed in an evacuated, sealed tube with 6M HCl (100°, 8 h) to give the title compound. After crystallisation from methanol plus some drops of water, the product (35% yield) had m.p. 239–240°, $[\alpha]_D$ $-16.7°$ (water); lit.[10] m.p. 232–233.5° (Found: C, 40.31; H, 7.87; N, 11.66. $C_4H_9NO_3$ calc.: C, 40.33; H, 7.62; N, 11.76%).

1-(2-Methoxyethyl) 4-benzyl *N*-benzyloxycarbonyl-L-aspartate was prepared by the DCC condensation of 4-benzyl *N*-benzyloxycarbonyl-L-aspartic acid and 2-methoxyethanol in ethyl acetate. After removal of *N*,*N'*-dicyclohexylurea, the crude product was eluted from silica gel with solvent *C* (1:1) to give the title compound as a viscous oil (50%), $[\alpha]_D$ $+8.1°$.

Anal. Calc. for $C_{22}H_{25}NO_7$: C, 63.60; H, 6.07; N, 3.37. Found: C, 63.47; H, 5.91; N, 3.58.

Catalytic hydrogenation (10% Pd/C) of the foregoing compound in acetic acid–2-methoxyethanol (2:1) gave a solid, which was dissolved in ethanol containing a few drops of water. Addition of ether then precipitated 1-(2-methoxyethyl) L-aspartic acid (52%) as a hygroscopic solid which decomposed (t.l.c.; solvent *A*, 2:1) on standing into a less-mobile, ninhydrin-positive component (presumably the 4-isomer) and aspartic acid.

Anal. Calc. for $C_7H_{13}NO_5$: C, 43.98; H, 6.85; N, 7.32. Found: C, 44.20; H, 6.86; N, 7.41.

Treatments with lithium borohydride. — A suspension (0.2 mmole) of each D-glucosyl ester in tetrahydrofuran was treated with a tenfold excess of 0.1M lithium borohydride in tetrahydrofuran at 0° for 2 h and then at room temperature for 24 h. The excess of borohydride was decomposed with methanolic HCl to pH 1, and boric acid was removed by repeated evaporation with methanol. The residue was eluted from a column (3 x 2 cm) of Dowex-50 x8(H^+) resin with water followed by M NH_4OH. Ninhydrin-positive fractions were combined and evaporated to dryness, and aliquots were submitted to paper chromatography (solvent *D*) together with standards of 2-amino- and 3-amino-4-hydroxy-L-butyric acid. The chromatograms were developed with ninhydrin, the spots were eluted with ethanol–water (3.5:1, plus 5 mg of $CuSO_4 \cdot 5H_2O$/100 ml), and the absorption was measured at 510 nm.

Hydrolysis of D-*glucosyl esters.* — This was performed with samples

(~0.6 mmol) in evacuated, sealed tubes, using 2M HCl (10 ml) at 105° for 2 h. After evaporation, the residue was dissolved in water (2 ml) and applied to a column (6×2 cm) of Dowex-50 x8(H^+) resin which was eluted with water and then with 2M HCl. The latter solvent displaced aspartic acid which was checked for optical purity.

1-O-(L-β-Aspartyl)-α-D-glucopyranose (**2α**). — To a solution of 2,3,4,6-tetra-*O*-benzyl-1-*O*-[1-benzyl *N*-(benzyloxycarbonyl)-L-aspart-4-oyl]-α-D-glucopyranose[8] (**1α**, 446 mg) in acetic acid–2-methoxyethanol (2:1, 15 ml) was added 10% palladium-on-charcoal (400 mg), and hydrogen was passed through the stirred suspension until evolution of carbon dioxide [$Ba(OH)_2$ solution] ceased (~1 h). The mixture was then shaken with hydrogen at atmospheric pressure and room temperature until termination of hydrogen uptake (~18 h). The catalyst was centrifuged off, the supernatant was evaporated to dryness (0.1 torr), and the remaining solid was shaken with dry ether (3 x 5 ml) and dried over phosphorus pentaoxide. Dissolution of the residue in hot methanol followed by subsequent addition of ether at 0° afforded **2α** as hygroscopic crystals (87 mg, 58%), m.p. 120–124° (dec.), $[\alpha]_D$ +91.7° (water), +73.3° (methanol); ν_{max}^{KBr} 3450 broad vs (OH), 1740 vs (C=O), 1620 and 1500 (amino acid I and II). N.m.r. data (D_2O): τ 3.79 (d, $J_{1,2}$ 3 Hz, H-1).

Anal. Calc. for $C_{10}H_{17}NO_9$: C, 40.68; H, 5.80; N, 4.75. Found: C, 40.50; H, 5.91; N, 4.72.

N-Acetylation of compound **2α** (129 mg) in water (25 ml) was performed with a 20% solution of acetic anhydride in acetone (25 ml) at 0° for 3 h (monitoring by t.l.c., solvent *A*, 3:1). The solution was concentrated (0.1 torr) to ~10 ml and then freeze-dried. The residue was chromatographed on a column (60 × 1.2 cm) of cellulose (25 g) with solvent *B* to give chromatographically homogeneous 1-*O*-(*N*-acetyl-L-β-aspartyl)-α-D-glucopyranose (**3α**) (110 mg, 76%). Crystallisation from methanol–ether afforded analytically pure **3α** as hygroscopic crystals with no definite melting-point, $[\alpha]_D$ +65.0° (water), +70.0° (methanol). N.m.r. data (D_2O): τ 3.86 (d, $J_{1,2}$ 3 Hz, H-1), 7.99 (s, N*Ac*).

Anal. Calc. for $C_{12}H_{19}NO_{10}$: C, 42.73; H, 5.67; N, 4.15. Found: C, 42.70; H, 5.67; N, 4.36.

To a sample (56 mg) of freeze-dried **3α** in methanol (1 ml), an ethereal solution of diazomethane (~20 ml, freshly distilled) was added at 0°. After ~2 h, the reaction was complete (t.l.c., solvent *A*, 3:1), ether was removed, and a precooled mixture of dry pyridine–acetic anhydride (5:1, 6 ml) was added to the oily residue. After storage for 24 h at 0°, the solution was concentrated (0.1 torr), and the residue was eluted from a silica gel column with solvent *C* (1:2) to give a chromatographically homogeneous solid (41 mg, 66.7%), $[\alpha]_D$ +76.8°, which gave an elemental analysis appropriate for 2,3,4,6-tetra-*O*-acetyl-1-*O*-[1-methyl *N*-(acetyl)-L-aspart-4-oyl]-α-D-glucopyranose (**4α**) and showed chromatographic mobility and i.r. spectrum indistinguishable from those of an authentic sample described below; the n.m.r. data are given in the Results section.

Conversion of **3β** *into* **4β**. — A sample (260 mg) of **3β**[8] was esterified and *O*-

acetylated as described above; the crude product was purified on silica gel (solvent *C*, 1:1) to give a hygroscopic solid (137 mg, 34.2%), which was indistinguishable (t.l.c., $[\alpha]_D$, and i.r. and n.m.r. spectra) from authentic 2,3,4,6-tetra-*O*-acetyl-1-*O*-[1-methyl *N*-(acetyl)-L-aspart-4-oyl]-β-D-glucopyranose (**4β**) described below.

*2,3,4,6-Tetra-*O*-acetyl-1-*O*-[1-methyl* N-*(acetyl)*-L-*aspart-4-oyl*]-D-*glucopyranose* (**4**). — To a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose (348 mg), 1-methyl *N*-acetyl-L-aspartic acid (189 mg), and imidazole (136 mg) in dichloromethane (20 ml) was added DCC (206 mg) in dichloromethane (10 ml) at 0°. The mixture was mechanically stirred at room temperature for 24 h, *N,N'*-dicyclohexylurea was filtered off, and the combined filtrate and washings were treated successively with water, 10% citric acid, water, aqueous sodium hydrogen carbonate, and water, dried (Na_2SO_4), and concentrated. The residue was eluted from a silica gel column with solvent *C* (1:2); combination and concentration of the appropriate fractions, followed by rechromatography with the same solvent, resulted in the complete separation of the anomers; total yield: 246 mg, 47.4%. The faster-moving fractions afforded **4β** as a hygroscopic foam, $[\alpha]_D$ +2.4°. N.m.r. data: τ 3.31–3.72 broad (1 H, NH), 4.28 (d, $J_{1,2}$ 7 Hz, H-1), 6.24 and 6.31 (s, 3H, 1- and 4-COO*Me*, ratio ~7:1), 7.90–8.05 (15H, unresolved, 4*Ac*O+N*Ac*).

Anal. Calc. for $C_{21}H_{29}NO_{14}$: C, 48.56; H, 5.63; N, 2.69. Found: C, 48.72; H, 5.80; N, 2.90.

The slower-moving fractions gave the α-D anomer of **4** which, after recrystallisation from ether–light petroleum, had m.p. 42–44°, $[\alpha]_D$ +77.2°. N.m.r. data: τ 3.30–3.58 (broad, NH), 3.68 (d, $J_{1,2}$ 3 Hz, H-1), 6.24 and 6.31 (s, 1- and 4-COO*Me*, ratio ~7:1), 7.89–8.03 (15H, unresolved, 4*Ac*O+N*Ac*) (Found: C, 48.50; H, 5.48; N, 2.84).

*2,3,4,6-Tetra-*O*-acetyl-1-*O*-[4-methyl* N-*(acetyl)*-L-*aspart-1-oyl*]-D-*glucopyranose* (**5**). — The reaction was performed with 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose, 4-methyl *N*-acetyl-L-aspartic acid, DCC (2 mmol of each), and imidazole (4 mmol), as described for **4**, except that ethyl acetate was used as the solvent. After work-up, the residue was eluted from silica gel (solvent *C*, 1:2) to give **5** (200 mg, 19.3%). The faster-moving fractions afforded **5β**, m.p. 153–155° (from ether–light petroleum), $[\alpha]_D$ +3.8°; ν_{max}^{KBr} 3450 (NH), 1770 vs and 1755 vs (C=O), 1670 and 1540 cm^{-1} (amide I and II). N.m.r. data: τ 3.37–3.61 (m, NH), 4.25 (d, $J_{1,2}$ 7 Hz, H-1), 6.32 (s, 4-COO*Me*), 7.90, 7.95, 8.05 (unresolved, 15H, 4*Ac*O+N*Ac*).

Anal. Calc. for $C_{21}H_{29}NO_{14}$: C, 48.56; H, 5.63; N, 2.69. Found: C, 48.71; H, 5.51; N, 2.79.

Material from the slower-moving fractions was rechromatographed to give **5α** as a hygroscopic foam, $[\alpha]_D$ +76.9°; ν_{max}^{KBr} 3550 (NH), 1780 vs (C=O), 1680 and 1550 cm^{-1} (amide I and II). N.m.r. data: τ 3.37–3.61 (m, NH), 3.63 (d, $J_{1,2}$ 3.5 Hz, H-1), 6.30 (s, 4-COO*Me*), 7.94, 7.96, 7.97, 8.00 (unresolved, 15H, 4*Ac*O+N*Ac*) (Found: C, 48.38; H, 5.93; N, 2.85).

*1,3,4,6-Tetra-*O*-acetyl-2-*O*-[4-methyl* N-*(acetyl)*-L-*aspart-1-oyl*]-α-D-*glucopyranose* (**6α**). — This compound was prepared as described for **5** by using 1,3,4,6-

tetra-*O*-acetyl-α-D-glucopyranose. After working-up, the residue was eluted from silica gel with solvent *C* (3:1) to give chromatographically pure **6α** (65%) which, after one recrystallisation from ethyl acetate–light petroleum, had m.p. 132–133°, $[\alpha]_D$ +89.5°; ν_{max}^{KBr} 3400 (NH), 1770 vs and 1750 vs (C=O), 1680 and 1540 (amide I and II), 1070 cm^{-1} (C–O–C). N.m.r. data: τ 3.51–3.75 (broad, NH), 3.64 (d, $J_{1,2}$ 3.5 Hz, H-1), 6.32 (s, 4-COO*Me*), 7.81 (s, 1-*Ac*O), 7.95, 8.00, 8.01 (unresolved, 12H, 3*Ac*O+N*Ac*).

Anal. Calc. for $C_{21}H_{29}NO_{14}$: C, 48.56; H, 5.63; N, 2.69. Found: C, 48.63; H, 5.76; N, 2.63.

*Catalytic hydrogenation of 2,3,4,6-tetra-*O*-benzyl-1-*O*-[4-benzyl *N*-(benzyloxycarbonyl)-*L*-aspart-1-oyl]-β-*D*-glucopyranose* [9] (**7β**). — (*a*) *Without trifluoroacetic acid.* A solution of **7β** (360 mg) in acetic acid–2-methoxyethanol (2:1, 15 ml) was hydrogenated as described for **2α**; t.l.c. (solvent *A*, 2:1) of the reaction mixture revealed two major spots [D-glucose and 1-(2-methoxyethyl) aspartic acid], a minor ninhydrin- and silver nitrate-positive spot, and traces of aspartic acid. Fractionation of the mixture on a cellulose column with solvent *A* (2:1) afforded a solid foam (48 mg, 63%) which was identical with authentic 1-(2-methoxyethyl) aspartic acid.

(*b*) *In the presence of trifluoroacetic acid.* To a solution of **7β** (200 mg) in 2-methoxyethanol (15 ml), 10% palladium-on-charcoal (200 mg) and trifluoroacetic acid (98%, 0.50 ml) were added. Hydrogen was passed through the suspension until evolution of carbon dioxide ceased, and the mixture was then shaken with hydrogen at atmospheric pressure until termination of hydrogen uptake. After removal of the catalyst and solvent (0.1 torr), the residue was triturated with dry ether to give a solid which was dissolved in methanol; subsequent addition of dry ether at 0° precipitated 1-*O*-(L-α-aspartyl)-β-D-glucopyranose trifluoroacetate (**11β**; 57 mg, 61%) as hygroscopic crystals with no definite m.p., $[\alpha]_D$ +9.4° (methanol); ν_{max}^{KBr} 3420 broad vs (OH), 1760 s (C=O), 1650 sh and 1500 (NH_3^+ deformations), 1630 s (ionized trifluoroacetic carboxyl), 1070 cm^{-1} (C–O–C). N.m.r. data (D_2O): τ 4.44 (d, $J_{1,2}$ 7 Hz, H-1).

Anal. Calc. for $C_{12}H_{18}F_3NO_{11}$: C, 35.21; H, 4.43; N, 3.42. Found: C, 35.18; H, 4.58; N, 3.55.

N-Acetylation of compound **11β** (200 mg) was performed as described for **3α**, and the crude product was eluted from a cellulose column with solvent *A* (2:1) to give the *N*-acetyl derivative of **11β** as a chromatographically homogeneous, hygroscopic foam (76%) which decomposed on standing into D-glucose and aspartic acid. N.m.r. data (D_2O): τ 4.44 (d, $J_{1,2}$ 7 Hz, H-1), 8.00 (s, N*Ac*).

The above compound was immediately treated with diazomethane and then with acetic anhydride–pyridine, as described for the conversion of **3α** into **4α**. Fractionation of the product gave a mixture of penta- and tetra-*O*-acetyl-D-glucopyranose and a chromatographically homogeneous solid (11.5%, calc. on **11β**) which gave analytical data appropriate for 2,3,4,6-tetra-*O*-acetyl-1-*O*-[4-methyl *N*-(acetyl)-L-aspart-1-oyl]-β-D-glucopyranose (**5β**) and was indistinguishable (t.l.c., mixture m.p., i.r. and n.m.r. spectra) from the authentic sample described above.

*Catalytic hydrogenation of 2,3,4,6-tetra-*O*-benzyl-1-*O*-[4-benzyl *N*-(benzyloxycarbonyl)-*L*-aspart-1-oyl]-α-*D*-glucopyranose*[9] (**7α**). — (*a*) *Without trifluoroacetic acid.*

A solution of **7α** (193 mg) in acetic acid–2-methoxyethanol was hydrogenated as described for **2α**; the catalyst was removed by centrifugation, and dry ether was added to the supernatant at 0°. On cooling, chromatographically homogeneous 2-*O*-(L-α-aspartyl)-D-glucopyranose (**8**) (32 mg, 50%) was deposited as deliquescent crystals, $[\alpha]_D$ +43.0° (methanol), +32.0° (water); ν_{max}^{KBr} 3450 broad vs (OH), 1760 vs (C=O), 1620 s (ionized COO^-), 1550 sh and 1650 sh (NH_3^+ deformations), and 1070 cm^{-1} (C–O–C). N.m.r. data (D_2O): τ 4.54 (d, $J_{1,2}$ 4 Hz, H-1). The compound was highly hygroscopic, and a satisfactory elemental analysis could not be obtained.

Thermogravimetric (t.g.) analysis of the analytical sample kept at room temperature for 4 days gave 10.70 ±0.3% of H_2O; $C_{10}H_{17}NO_9 \cdot 2H_2O$ calc.: 10.88%.

N-Acetylation of the above compound (68 mg) was performed as described for **3α**; the residue was eluted from a cellulose column with solvent *A* (2:1) to give chromatographically homogeneous 2-*O*-[*N*-(acetyl)-L-α-aspartyl]-D-glucopyranose (**9**) (41 mg, 53%) as a hygroscopic, solid foam. After dissolution in water (1 ml) and freeze-drying, the product was obtained as a fluffy mass, $[\alpha]_D$ +8.3° (*c* 2.0, methanol), +6.8° (*c* 1.9, water); ν_{max}^{KBr} 3450 broad vs (OH), 1740 vs (C=O), 1650 and 1560 (amide I and II), and 1080 cm^{-1} (C–O–C). N.m.r. data (D_2O): τ 4.54 (d, $J_{1,2}$ 4 Hz, H-1), 7.98 (s, N*Ac*).

Anal. Calc. for $C_{12}H_{19}NO_{10}$: C, 42.73; H, 5.67; N, 4.15. Found: C, 42.49; H, 5.86; N, 4.39.

A solution of **9** (73 mg) in *N*,*N*-dimethylformamide (1 ml) was treated with diazomethane in ether (~10 ml) at −10° for 2 h. After removal of the solvent (0.1 torr), the residue was eluted from a cellulose column with solvent *A* (2:1) to give 2-*O*-[4-methyl *N*-(acetyl)-L-aspart-1-oyl]-D-glucopyranose (**10**) as a chromatographically homogeneous syrup (64 mg, 74%); dissolution in methanol and subsequent precipitation with ether afforded **10** as a solid, hygroscopic foam; $[\alpha]_D$ +14.4° (*c* 1.6, methanol), +15.4° (*c* 1.5, water); ν_{max}^{KBr} 3450 vs (OH), 1730 vs (C=O), 1640 and 1540 (amide I and II), and 1080 cm^{-1} (C–O–C). N.m.r. data: τ 4.54–4.78 (m, 1H, H-1), 6.30 (s, 4-COO*Me*), 7.98 (s, N*Ac*).

Immediate treatment of crude **10** with acetic anhydride–pyridine, followed by purification of the product on silica gel (solvent *C*, 1:2), afforded 1,3,4,6-tetra-*O*-acetyl-2-*O*-[4-methyl *N*-(acetyl)-L-aspart-1-oyl]-D-glucopyranose (**6**) as a chromatographically homogeneous solid foam (45 mg, 47.6%), $[\alpha]_D$ +45.3° (*c* 1.4). N.m.r. data: τ 3.51–3.62 (m, NH), 3.64 and 4.24 (2d, $J_{1,2}$ 3 Hz, $J_{1,2}$ 7 Hz, ratio ~1:1, H-1), 6.32 (s, 4-COO*Me*), 7.81, 7.84, 7.88, 7.94, 8.01 (15H, unresolved, 4*Ac*O+N*Ac*). The i.r. spectrum was indistinguishable from that of **6** derived from **11α** and very similar to that of an authentic sample of **6α** described above.

(*b*) *In the presence of trifluoroacetic acid.* A solution of **7α** (239 mg) in 2-methoxyethanol (15 ml) was hydrogenated in the presence of trifluoroacetic acid (0.53 ml), as described for **2α**. After working-up, the residue was triturated with dry ether to give highly hygroscopic crystals (100 mg, 90%) of 1-*O*-(L-α-aspartyl)-α-D-glucopyranose trifluoroacetate salt (**11α**), $[\alpha]_D$ +55.2° (*c* 3.9, methanol), +61.7° (*c* 2.2, water); ν_{max}^{KBr} 3440 broad vs (OH), 1770 vs (C=O), 1680 and 1520 (NH_3^+ deformations), 1650 sh (ionized trifluoroacetic carboxyl), and 1080 cm^{-1} (C–O–C). N.m.r. data (D_2O): τ 3.75 (d, $J_{1,2}$ 2.5 Hz, H-1).

Anal. Calc. for $C_{12}H_{18}F_3NO_{11}\cdot H_2O$: C, 33.72; H, 4.72; N, 3.28. Found: C, 33.60; H, 4.99; N, 3.30.

T.g. analysis of the above sample gave 4.12 ±0.3% of H_2O; $C_{12}H_{18}F_3NO_{11}\cdot H_2O$ calc.: 4.23%.

N-Acetylation of the above compound (200 mg), performed as described for **3α**, afforded, after chromatography of the reaction mixture on a cellulose column with solvent *A* (2:1), chromatographically homogeneous 1-*O*-(*N*-acetyl-L-α-aspartyl)-α-D-glucopyranose (**12**; 122 mg, 74.5%). Crystallisation from methanol–ether gave the analytically pure sample as hygroscopic crystals with no definite m.p., $[\alpha]_D$ +69.0° (methanol), +64.7° (water); ν_{max}^{KBr} 3500 broad vs (OH), 1755 vs (C=O), 1660 and 1560 (amide I and II), and 1080 cm^{-1} (C–O–C). N.m.r. data (D_2O): τ 3.80 (d, $J_{1,2}$ 2.5 Hz, H-1), 7.95 (s, N*Ac*).

Anal. Calc. for $C_{12}H_{19}NO_{10}$: C, 42.73; H, 5.67; N, 4.15. Found: C, 42.82; H, 5.64; N, 4.40.

A sample (110 mg) of **12** was esterified as described for the conversion of **9** into **10**, and part of the crude product was eluted from a cellulose column. Crystallisation from methanol–ether afforded a solid foam (26 mg, 45%); $[\alpha]_D$ +19.4° (*c* 0.7, methanol), +13.3° (*c* 0.7, water); which was indistinguishable from the methyl ester derivative **10** derived from **8**, as judged by t.l.c. mobility, and i.r. and n.m.r. spectra.

The second portion of crude **10** was immediately treated with acetic anhydride–pyridine, and the product was eluted from a silica gel column with solvent *C* (1:2) to give 1,3,4,6-tetra-*O*-acetyl-2-*O*-[4-methyl *N*-(acetyl)-L-aspart-1-oyl]-D-glucopyranose (**6**) as an anomeric mixture (49 mg, 58%), $[\alpha]_D$ +40.0°, whose n.m.r. spectrum was indistinguishable from that of **6** derived from **8**, except that the α:β anomeric ratio was ~3:1. Repeated chromatography on silica gel led to partial separation of the anomers: the slower-moving fractions afforded pure **6α**, m.p. and mixture m.p. 133–135°, $[\alpha]_D$ +86.0°.

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