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SYNTHESIS AND REACTIONS OF α- AND β-D-GLUCOPYRANOSYLURONIC ESTERS OF AMINO ACIDS*

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ABSTRACT

The synthesis of the fully benzylated α- and β-D-glucopyranosyluronic esters of 1-benzyl *N*-benzyloxycarbonyl-L-aspartic and -glutamic acids and *N*-(*tert*-butoxycarbonyl)-L-phenylalanine, followed by hydrogenolysis, afforded the respective anomers of the 1-*O*-acyl-D-glucopyranuronic acids **2**, **7**, and **12**. Esterification of both anomers of the *N*-acetylated derivatives of **2** and **7** by diazomethane was accompanied by glycosyl-bond cleavage, and, in the case of the α anomers, with concomitant 1→2 acyl migration to give, after *O*-acetylation, the 2-*O*-acyl *O*-acetyl methyl ester derivatives **5** and **10**, respectively. Similarly, **12α** yielded methyl 1,3,4-tri-*O*-acetyl-2-*O*-[*N*-(*tert*-butoxycarbonyl)-L-phenylalanyl]-D-glucopyranuronate and an analogue having a furanurono-6,3-lactone structure. Esterification of the C-5 carboxyl group in 1-*O*-acyl-α-D-glucopyranuronic acids by methanol in the presence of the BF_3–MeOH reagent (1–1.5 equiv.) proceeded without acyl migration. By using this procedure, followed by acetylation, the *N*-acetylated derivative of **7α** afforded methyl 2,3,4-tri-*O*-acetyl-1-*O*-(1-methyl *N*-acetyl-L-glutam-5-oyl)-α-D-glucopyranuronate, and **12α** gave methyl 2,3,4-tri-*O*-acetyl-1-*O*-(*N*-acetyl-L-phenylalanyl)-α-D-glucopyranuronate; the formation of the latter involved cleavage of the *tert*-butoxycarbonyl group by BF_3, followed by *N*-acetylation in the next step.

INTRODUCTION

D-Glucopyranosyl esters of amino acids, although readily hydrolysed, may undergo other reactions through nucleophilic attack at the 1-ester carbonyl group[1–4]. Diazomethane catalyses 1→2 acyl migration of 1-*O*-(*N*-acylaminoacyl)-α-D-glucopyranoses, and the reaction was rationalised in terms of a base-catalysed interchange in which diazomethane functions as a base[5,6]. Since the widely used derivatisation of glycopyranuronic acids to give per-*O*-acetylated methyl esters involves esterification of the C-5 carboxyl group with diazomethane, it was of interest to examine the effect of this reagent on the little known α anomers of D-glucopyranosyluronic esters.

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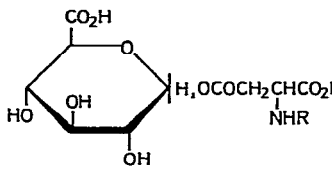
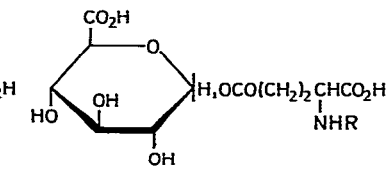
We now report on the synthesis and chemical reactions of 1-esterified D-glucopyranuronic acids having the side-chain carboxyl groups of L-aspartic and -glutamic acids and the carboxyl group of *N*-(*tert*-butoxycarbonyl)-L-phenylalanine, respectively, involved in the glycosyl ester linkage; compounds of this type may be useful models for studying the mechanism[7,8] and action of β-D-glucuronidase(s).

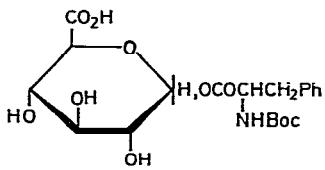
RESULTS AND DISCUSSION

The fully protected 1-esters of D-glucopyranuronic acid **1** and **6** were obtained (53 and 56% yield) as anomeric mixtures by the imidazole-promoted reaction of benzyl 2,3,4-tri-*O*-benzyl-D-glucopyranuronate[9,10] with 1-benzyl 4-pentachlorophenyl *N*-benzyloxycarbonyl-L-aspartate and 1-benzyl 5-pentachlorophenyl *N*-benzyloxycarbonyl-L-glutamate, respectively; the anomers were separated and characterised (see Experimental).

Catalytic hydrogenolysis of both anomers of **1**, performed in 2-methoxyethanol in the presence of catalytic amounts of acetic acid, afforded the respective anomers







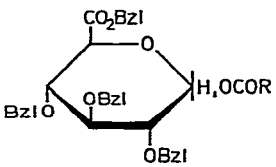
2 R = H

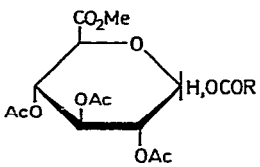
3 R = Ac

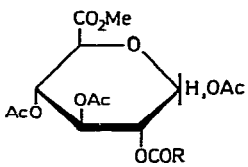
7 R = H_2^+ $CF_3CO_2^-$

8 R = Ac

12







1 R = CH_2CHCO_2Bzl (NHZ)

6 R = $CH_2CH_2CHCO_2Bzl$ (NHZ)

11 R = $CHCH_2Ph$ (NHBoc)

4 R = CH_2CHCO_2Me (NHAc)

9 R = $CH_2CH_2CHCO_2Me$ (NHAc)

13 R = $CHCH_2Ph$ (NHBoc)

14 R = $CHCH_2Ph$ (NHAc)

5 R = CH_2CHCO_2Me (NHAc)

10 R = $CH_2CH_2CHCO_2Me$ (NHAc)

15 R = $CHCH_2Ph$ (NHBoc)

Z = $PhCH_2OC(=O)$

Boc = $Me_3COC(=O)$

of 1-*O*-(L-β-aspartyl)-D-glucopyranuronic acid (**2**) as hygroscopic solids. Their structures were established from elemental analysis, optical rotation ($[\alpha]_D$ −22.5 and +73.6°), and p.m.r. ($J_{1,2}$ 7 and 3 Hz) data, and by their conversion into the corresponding *N*-acetyl derivatives **3β** and **3α**, respectively. Additional evidence that removal of the protecting groups from **1α** proceeded without acyl migration was provided by performing the hydrogenolysis in the presence of trifluoroacetic acid: the p.m.r. spectrum of the **2α** trifluoroacetate salt showed the signal for the anomeric proton at the same position and with the same coupling constant as that for the salt-free form. The i.r. spectrum of the former compound showed a band at 725 cm^{-1}, which was absent in the spectrum of the latter; since absorption in the 735–725 cm^{-1} region has been observed[1-5] in all of the spectra of D-glucopyranosyl esters of amino acid trifluoroacetate salts, but not in those of their salt-free forms, it might be considered diagnostic for trifluoroacetate ammonium salts.

In line with the results[2] obtained in the D-glucopyranosyl ester series, catalytic hydrogenolysis of both anomers of **6** in the absence of a strong acid (as used in the preparation of **2α** and **β**) led to intramolecular aminolysis with scission of the C-1 ester bond, to give pyroglutamic and D-glucuronic acids as the only isolable products. Similar treatment in the presence of trifluoroacetic acid proceeded without cleavage, to give the corresponding anomers of 1-*O*-(L-γ-glutamyl)-D-glucopyranuronic acid as hygroscopic, trifluoroacetate salts (**7β** and **7α**), from which the respective *N*-acetyl derivatives **8β** and **8α** were obtained.

In the dry state, the unprotected 1-esters **2** and **7** were stable for several months, but decomposition was much faster in water and practically instantaneous in slightly alkaline, aqueous media. T.l.c. indicated that aqueous solutions of the α anomers were more resistant towards hydrolysis than their β counterparts and that both anomers of **2**, **3**, **7**, and **8**, particularly those having aspartic acid as the aglycon, decomposed at a higher rate than the corresponding D-glucopyranosyl esters.

The *N*-acetylated 1-esters **3** and **8** were submitted to further esterification with ethereal diazomethane, followed by conventional acetylation. T.l.c. monitoring of the esterification step revealed considerable glycosyl-bond cleavage, the extent of which increased drastically with increasing amounts of the reagent. Thus, treatment of a solution of **3β** in *N*,*N*-dimethylformamide with four molar equivalents of diazomethane in ether at 0° for 1 h, followed by acetylation, gave (t.l.c.) mainly a mixture of methyl D-glucopyranuronate tetra-acetate and dimethyl *N*-acetylaspartate. Treatment of **3α** with 1.5 equivalents of diazomethane at −10° for 1 h revealed that, even with less than the theoretical amount of the reagent, esterification was accompanied by hydrolysis of the glycosyl bond and 1→2 acyl migration. Thus, the product isolated (28.6%) after acetylation was an ~3:1 mixture of methyl 2,3,4-tri-*O*-acetyl-1-*O*-(1-methyl *N*-acetyl-L-aspart-4-oyl)-α-D-glucopyranuronate (**4α**) and methyl 1,3,4-tri-*O*-acetyl-2-*O*-(1-methyl *N*-acetyl-L-aspart-4-oyl)-α-D-glucopyranuronate (**5α**) contaminated with its β anomer. The structure of **4α** was confirmed by comparison with an authentic sample prepared by the imidazole-promoted, dicyclohexylcarbodi-imide (DCC) condensation of methyl 2,3,4-tri-*O*-acetyl-D-glucopyranuronate and 1-methyl

N-acetylaspartic acid; the separation of the anomers was difficult, due to their decomposition on columns of silica gel, and only the α anomer was isolated. The structures of **5α** and its **β** anomer were deduced from elemental analysis, t.l.c. behaviour, and p.m.r. data of the product mixture; in the spectrum of the latter, the AcO-1 signal appeared as two singlets (τ 7.77 and 7.87, ratio *ax*:*eq* ~5:1) that integrated for one proton.

The reactions of both anomers of **8** with diazomethane were comparable to those described above, but much cleaner. With 2.2 equivalents of the reagent, **8β** afforded, after acetylation, crystalline methyl 2,3,4-tri-*O*-acetyl-1-*O*-(1-methyl *N*-acetyl-L-glutam-5-oyl)-β-D-glucopyranuronate (**9β**) in 25% yield. The product was indistinguishable from an authentic sample prepared by direct condensation. Similar treatment of **8α** with diazomethane led to parallel 1→2 acyl migration, to give (54.3%), after acetylation, methyl 1,3,4-tri-*O*-acetyl-2-*O*-(1-methyl *N*-acetyl-L-glutam-5-oyl)-D-glucopyranuronate (**10**) as an anomeric mixture (α:β ~3:1); no 1-*O*-acyl derivative **9α** could be detected (t.l.c.) in the reaction mixture. The p.m.r. and analytical data for **10** were fully consistent with the structure and anomeric ratio proposed.

The previously demonstrated resistance towards hydrolysis of the D-glucopyranosyl ester of *N*-(*tert*-butoxycarbonyl)-L-phenylalanine[6] prompted the synthesis of the corresponding D-glucopyranosyluronic ester as a potential model-compound for studying the above reactions. The starting material, benzyl 2,3,4-tri-*O*-benzyl-1-*O*-[*N*-(*tert*-butoxycarbonyl)-L-phenylalanyl]-D-glucopyranuronate (**11**) was prepared, by using *N*-(*tert*-butoxycarbonyl)-L-phenylalanine pentachlorophenyl ester in a manner analogous to that for the 1-esters **1** and **6**. Catalytic hydrogenolysis of **11β** (performed as in the preparation of **2β**) afforded 1-*O*-[*N*-(*tert*-butoxycarbonyl)-L-phenylalanyl]-β-D-glucopyranuronic acid (**12β**), which was characterised in the anhydrous form and as the crystalline monohydrate. Similarly, **11α** afforded the remarkably stable α-D-glucopyranosyluronic ester **12α**; a solution of this compound in D_2O was unchanged (monitoring by t.l.c. and p.m.r. spectroscopy) during 5 days at room temperature. Under similar conditions, the corresponding α-D-glucopyranosyl ester underwent[6] ~50% 1→2 acyl rearrangement and ~10% hydrolysis, and the greater stability of the glycosyl ester bond in **12α** may therefore be attributed to a stabilising effect of the uronic carboxyl group. The tendency[11,12] of the C-5 carboxyl group to impede the polarizability around the O–C-1–O bonds might also account, at least partially, for the longer survivals in the dry state of the unprotected 1-esters **2**, **7**, and **11**, as compared to those of the corresponding *O*-acetyl methyl ester derivatives **4**, **9**, and **13**.

Treatment of **12α** with 1.5 equivalents of ethereal diazomethane, followed by acetylation, yielded two major products that have similar R_F values and could be separated only after repeated chromatography on silica gel. The major component was assigned the structure methyl 1,3,4-tri-*O*-acetyl-2-*O*-[*N*-(*tert*-butoxycarbonyl)-L-phenylalanyl]-D-glucopyranuronate (**15**). The p.m.r. spectrum showed H-1 as two doublets (τ 3.57 and 4.21, $J_{1,2}$ 3 and 7 Hz) having a similar ratio as the two singlets (τ 7.81 and 7.88) for AcO-1 (ratio α:β ~3:1). The minor component, which was

obtained pure in only 8.3% yield, appeared to be the di-*O*-acetyl-*O*-[*N*-(*tert*-butoxycarbonyl)-L-phenylalanyl]-β-D-glucofuranurono-6,3-lactone **16** on the following grounds. The i.r. spectrum showed a strong carbonyl absorption at 1810 cm^{-1}, characteristic of a γ lactone, and there was no hydroxyl absorption. The p.m.r. spectrum showed the presence of only two acetyl groups (τ 7.87 and 7.92) whose ratios to the *N*-(*tert*-butoxycarbonyl)-L-phenylalanyl residue were 1:1:1, and the signal for H-1 was a singlet (τ 3.92), consistent with a furanoid ring having H-1,2 *trans*. Comparison of the p.m.r. spectra of **16** and 1,2,5-tri-*O*-acetyl-β-D-glucofuranurono-6,3-lactone[13,14] (OAc singlets: τ 7.83, 7.85, and 7.93) showed that the patterns of vicinal coupling for the ring protons in the two compounds were closely similar. Presumably, **16** was formed by the diazomethane-catalysed, nucleophilic attack of HO-3 on the carbonyl carbon of the C-5 ester group, followed by ring contraction and inversion of the anomeric configuration. However, the data available on **16** do not permit the positions of the two acetyl groups to be determined, and it is therefore not possible to deduce whether the above reactions occurred before or after the acyl-migration step.

In seeking a route to the methyl ester of α-D-glucopyranosyluronic esters, esterification by the boron trifluoride–methanol reagent[15–17] was examined. Preliminary experiments showed that treatment of methanolic solutions of 1-*O*-(*p*-methoxybenzoyl)-β-D-glucopyranuronic acid[18] for 5–10 h with 1–1.5 equivalents of the reagent at room temperature proceeds without appreciable glycosyl-bond cleavage, to give, after acetylation, methyl 2,3,4-tri-*O*-acetyl-1-*O*-(*p*-methoxybenzoyl)-β-D-glucopyranuronate[19] in 50–60% yields. In their study on dealkylation and deacylation of carbohydrate derivatives, Bonner *et al.*[20] observed that acetylated sugars are relatively resistant to the boron halide reagents.

When anhydrous **12β** was submitted to the sequence of reactions described above, the crystalline methyl 2,3,4-tri-*O*-acetyl-1-*O*-(*N*-acetyl-L-phenylalanyl)-β-D-glucopyranuronate (**14β**) was obtained (53.5%) as the only product. The formation of the *N*-acetyl derivative **14β** could have occurred only by the cleavage of the *tert*-butoxycarbonyl group by the boron halide, followed by acetylation in the next step. This result is not unexpected, since cleavage of *N*-(*tert*-butoxycarbonyl) peptides occurs[21] particularly easily by boron halides, anhydrous conditions being essential for completion of the reaction. Indeed, similar treatment of **12β** monohydrate gave a mixture of **14β** (48%) and methyl 2,3,4-tri-*O*-acetyl-1-*O*-[*N*-(*tert*-butoxycarbonyl)-L-phenylalanyl]-β-D-glucopyranuronate (**13β**, 10%); **14β** and **13β** were separated and their structures confirmed by comparison with authentic samples prepared by direct synthesis.

By using the BF_3–methanol procedure, followed by acetylation, the α-D-glucopyranosyl ester of *N*-acetyl-L-glutamic acid (**8α**) yielded (36%) crystalline methyl 2,3,4-tri-*O*-acetyl-1-*O*-(1-methyl *N*-acetyl-L-glutam-5-oyl)-α-D-glucopyranuronate (**9α**) prepared by direct synthesis; it is noteworthy that the corresponding 2-*O*-acyl isomer **10** was not detectable (t.l.c.) in the reaction mixture. Under the same conditions, **12α** furnished a mixture of the per-*O*-acetylated 1-*O*-acyl methyl ester deriva-

tives **13α** and **14α** (17.6 and 29%, respectively), which were separated and characterised by comparison with authentic samples. Thus, the above results indicate that the BF_3–methanol procedure esterifies the C-5 carboxyl group in α-D-glucopyranosyluronic esters without concomitant 1→2 acyl migration and provides a route to methyl (2,3,4-tri-*O*-acetyl-1-*O*-acyl-α-D-glucopyranosyl)uronates from the unprotected 1-esters of α-D-glucopyranuronic acid.

EXPERIMENTAL

General. — Melting points are uncorrected. Concentrations were carried out at diminished pressure on a rotary evaporator at <35°, if not stated otherwise, and solutions were dried with anhydrous sodium sulphate. Column chromatography was performed on silica gel (Merck, 0.05–0.2 mm), and t.l.c. on Kieselgel G (Merck) or cellulose (Microcrystalline, Merck). The solvents employed were: *A*, benzene–ethyl acetate (proportions are given in the text); *B*, acetonitrile–water (proportions are given in the text); *C*, 5:3:1 propan-2-ol–light petroleum–water; and *D*, 5:3 chloroform–ethyl acetate. Detection on t.l.c. plates was effected by charring with sulphuric acid, with ninhydrin reagent, with alkaline silver nitrate, or with the chlorine–starch–iodine reagent for peptides. Optical rotations were determined for 1% solutions in chloroform, unless otherwise stated. I.r. spectra were recorded with a Perkin–Elmer Model 297 spectrometer, and p.m.r. spectra with a Varian A-60A spectrometer for solutions in chloroform-*d* with tetramethylsilane as the internal standard, if not stated otherwise. The amount of diazomethane in ethereal solutions (redistilled before use) was determined by titration of an aliquot with benzoic acid, and the concentration of the reagent was adjusted to ~0.5 mmol/ml.

*Benzyl 2,3,4-tri-*O*-benzyl-1-*O*-[1-benzyl N-(benzyloxycarbonyl)-L-aspart-4-oyl]-D-glucopyranuronate* (**1**). — Benzyl 2,3,4-tri-*O*-benzyl-D-glucopyranuronate[9,10] (1.1 g), 1-benzyl 4-pentachlorophenyl *N*-benzyloxycarbonyl-L-aspartate[22] (1.3 g), and imidazole (0.68 g) were dissolved in dichloromethane (15 ml), and more (133 mg) of the amino acid component was added after 1 h; the mixture was kept at room temperature for 24 h, whereupon pentachlorophenol was filtered off. The filtrate was washed successively with water, 10% aqueous citric acid, water, aqueous sodium hydrogencarbonate, and water, dried, and concentrated. The residue was eluted from a column of silica gel with solvent *A* (10:1), to give chromatographically pure **1** (1.06 g, 59%) as an anomeric mixture. The solid left after evaporation of the slower-moving fractions was recrystallised from ethanol and then from di-(2-propyl) ether (2×), to give pure **1β** (130 mg), m.p. 103–104°, $[\alpha]_D$ +10°, ν_{max}^{KBr} 3330 (NH), 1760 and 1700 (C=O), 1600 and 1540 cm^{-1} (Amide I and II). P.m.r. data: τ 2.59–2.85 (m, 6 Ph), 4.28 (d, exchangeable with D_2O, NH), 4.41 (d, $J_{1,2}$ 7 Hz, H-1), 4.88 and 4.91 (2 s, 2 + 4 H, $PhCH_2OCON$, 2 CO_2CH_2Ph).

Anal. Calc. for $C_{53}H_{51}NO_{11}$: C, 71.21; H, 5.75; N, 1.57. Found: C, 71.16; H, 6.02; N, 1.66.

The faster-moving fractions were combined and re-chromatographed (2×)

in the same solvent system, to give pure **1α** (310 mg) as a syrup, $[\alpha]_D$ +27.5°. P.m.r. data: τ 2.59–2.85 (m, 6 Ph), 3.73 (d, $J_{1,2}$ 3 Hz, H-1), 4.18 (d, J 8 Hz, exchangeable with D_2O, NH), 4.88, 4.91, and 4.95 (3 s, $PhCH_2OCON$, 2 CO_2CH_2Ph) (Found: C, 70.97; H, 5.98; N, 1.55).

1-O-(L-β-*Aspartyl*)-β-D-*glucopyranuronic acid* (**2β**). — A solution of **1β** (180 mg) in 2-methoxyethanol (15 ml) was shaken with hydrogen at atmospheric pressure in the presence of 10% palladium-on-charcoal (180 mg) and a few drops of acetic acid (~0.3 ml) for ~24 h [monitoring by t.l.c.; cellulose, solvent *B* (3:1)]. After removal of the catalyst and concentration (0.1 torr) of the solvent (~3 ml), dry ether was added at 0°; the precipitated solid was centrifuged off, and a solution (1 ml) of the residue was lyophilised, to give **2β** (50 mg, 80%) as a hygroscopic, fluffy mass, m.p. 112–113.5° (dec.), $[\alpha]_D$ −22.5° (water). P.m.r. data: τ 4.34 (d, $J_{1,2}$ 7 Hz, H-1).

Anal. Calc. for $C_{10}H_{15}NO_{10}$: C, 38.84; H, 4.89; N, 4.53. Found: C, 38.72; H, 4.89; N, 4.44.

A sample (115 mg) of **2β** was treated with 10% acetic anhydride in 1:1 acetone–water (10 ml) at room temperature for ~5 h [monitoring by t.l.c.; cellulose, solvent *B* (3:1)]; after removal (0.1 torr) of the solvent, a solution of the residue in iced water (1 ml) was lyophilised to give a fluffy mass that was dissolved in anhydrous ethanol. Subsequent addition of dry ether precipitated 1-*O*-(*N*-acetyl-L-β-aspartyl)-β-D-glucopyranuronic acid (**3β**; 75 mg, 65%) as a hygroscopic solid, m.p. 83–86°, $[\alpha]_D$ −16° (water); ν_{max}^{KBr} 3500 (vs, broad; OH, NH), 1760 (C=O), 1650 and 1550 cm^{-1} (Amide I and II). P.m.r. data (D_2O): τ 4.30 (d, $J_{1,2}$ 7 Hz, H-1), 5.78 (d, J 10 Hz, H-5), and 7.93 (NAc).

Anal. Calc. for $C_{12}H_{17}NO_{11}$: C, 41.03; H, 4.87; N, 3.99. Found: C, 40.76; H, 5.13; N, 3.86.

1-O-(L-β-*Aspartyl*)-α-D-*glucopyranuronic acid* (**2α**). — (*a*) Catalytic hydrogenolysis of **1α** (292 mg), performed as described for **2β**, gave **2α** (72 mg, 72%) as a hygroscopic solid, $[\alpha]_D$ +73.6° (water). P.m.r. data (D_2O): τ 3.68 (d, $J_{1,2}$ 3 Hz, H-1).

Anal. Calc. for $C_{10}H_{15}NO_{10}$: C, 38.84; H, 4.89; N, 4.53. Found: C, 38.57; H, 4.84; N, 4.54.

N-Acetylation of a sample (60 mg) of **2α**, as described for **3β**, afforded 1-*O*-(*N*-acetyl-L-β-aspartyl)-α-D-glucopyranuronic acid (**3α**; 35 mg, 63%) as a hygroscopic solid, $[\alpha]_D$ +78° (water). P.m.r. data (D_2O): τ 3.80 (d, $J_{1,2}$ 3 Hz, H-1), 5.15 (d, J 10 Hz, H-5), and 8.02 (NAc); [$(CD_3)_2SO$]: τ 4.05 (d, $J_{1,2}$ 3 Hz, H-1) and 9.16 (NAc).

Anal. Calc. for $C_{12}H_{17}NO_{11}$: C, 41.03; H, 4.87; N, 3.99. Found: C, 40.76; H, 5.13; N, 4.22.

To a sample (30 mg) of **3α** in *N*,*N*-dimethylformamide (1 ml) were added 1.5 equiv. of ethereal diazomethane (0.5 mmol/ml, 0.25 ml), and the solution was kept at −10° for 1 h and then concentrated (0.1 torr). The residue was treated with 1:5 acetic anhydride–pyridine (3 ml) for 12 h at 0°, the solution was then concentrated (0.1 torr), and traces of anhydride were removed by co-distillation with water.

To a solution of the residue in chloroform were added a few drops of light petroleum to precipitate sugar-free aspartate contaminants, and, after filtration and evaporation of the solvent, the residual mass was extracted (3 × 1 ml) with cold ether to remove methyl D-glucopyranuronate tetra-acetate [t.l.c. in solvent *A* (1:2); R_F 0.74]. The final product was a solid foam (14 mg, 28.6% based on **3α**), $[\alpha]_D$ +73°, identified as a ~3:1 mixture of methyl 2,3,4-tri-*O*-acetyl-1-*O*-(1-methyl *N*-acetyl-L-aspart-4-oyl)-α-D-glucopyranuronate (**4α**) and 1,3,4-tri-*O*-acetyl-2-*O*-(1-methyl *N*-acetyl-L-aspart-4-oyl)-α-D-glucopyranuronate (**5α**) [t.l.c. in solvent *A* (1:2): R_F 0.27 and 0.34, respectively], containing traces of **5β** (not separated by t.l.c. from **5α**) as indicated by the p.m.r. data [(after D_2O exchange): τ 3.53 (d, ~0.9 H, $J_{1,2}$ 3 Hz, H-1), 5.57 (d, *J* 10 Hz, H-5), 6.21 and 6.25 (2 s, 2 CO_2Me), 7.77 + 7.87 (s, ~0.3 × 3 H, *ax* AcO-1 + s, ~0.1 × 3 H, *eq* AcO-1), and 7.97 (~11 H, OAc + NAc)] and elemental analysis (Calc. for $C_{20}H_{27}NO_{14}$: C, 47.52; H, 5.39; N, 2.77. Found: C, 47.38; H, 5.60; N, 2.94).

(*b*) *In the presence of trifluoroacetic acid.* A solution of **1α** (170 mg) in 2-methoxyethanol (10 ml) was catalytically debenzylated in the presence of 10% palladium-on-charcoal (170 mg) and trifluoroacetic acid (98%, 0.5 ml) until termination of hydrogen uptake (~24 h). After removal of the catalyst and solvent (0.3 torr), the residue was triturated with anhydrous ether, and a solution of the residue was lyophilised, to give **2α** trifluoroacetate salt (57 mg, 78%) as a hygroscopic solid, $[\alpha]_D$ +80.5° (water); ν_{max}^{KBr} 3490 (vs, broad; OH, NH), 1755 (C=O), 1630 and 1510 (Amino acid I and II), and 725 cm^{-1} ($CF_3CO_2^-$). P.m.r. data (D_2O): τ 3.67 (d, $J_{1,2}$ 3 Hz, H-1).

Anal. Calc. for $C_{10}H_{15}NO_{10} \cdot CF_3CO_2H$: C, 34.05; H, 3.81; N, 3.31. Found: C, 34.00; H, 4.07; N, 3.35.

Methyl 2,3,4-tri-O-acetyl-1-O-(1-methyl N-acetyl-L-aspart-4-oyl)-D-glucopyranuronate (**4**). — To a solution of methyl 2,3,4-tri-*O*-acetyl-D-glucopyranuronate[19] (1.34 g), 1-methyl *N*-acetylaspartic acid (833 mg), and imidazole (545 mg) in dichloromethane–*N*,*N*-dimethylformamide (4:1, 20 ml) was added DCC (824 mg) in dichloromethane (5 ml) at 0°; after 1 h, more (76 mg) of the amino acid component was added, and the mixture was kept at room temperature for 24 h. The precipitated *N*,*N*-dicyclohexylurea was filtered off, the filtrate was worked-up as described for **1**, and, after concentration, the residue was eluted from a column of silica gel with solvent *A* (1:2), to give an anomeric mixture of **4** (757 mg, 37.5%) as a syrup contaminated in each fraction with methyl 2,3,4-tri-*O*-acetyl-D-glucopyranuronate [t.l.c. in solvent *A* (1:2): R_F 0.26 and 0.59, respectively]. Rechromatography of this material afforded, in the first fractions, chromatographically homogeneous **4α** (40 mg) as a solid foam, $[\alpha]_D$ +78°. P.m.r. data: τ 3.37–3.54 (m, NH + H-1) [after deuteration→3.60 (d, $J_{1,2}$ 3 Hz, H-1)], 5.57 (d, *J* 10 Hz, H-5), 6.22 and 6.26 (2 s, 2 CO_2Me), and 7.97 (s, 3 OAc + NAc); [$(CD_3)_2SO$]: τ 3.76 (d, $J_{1,2}$ 3 Hz, H-1), 6.38 (s, 2 CO_2Me), 8.02 (3 OAc), and 8.10 (NAc).

Anal. Calc. for $C_{20}H_{27}NO_{14}$: C, 47.52; H, 5.39; N, 2.77. Found: C, 47.26; H, 5.55; N, 2.95.

A solution of the residue (50 mg), obtained after concentration of the slower-moving fractions, in ethyl acetate was fractionally precipitated with light petroleum, to give chromatographically homogeneous **4** (34 mg) as a solid that consisted mainly of the β anomer, as judged by p.m.r. data [τ 3.63 + 4.23 (d, ~0.2 H, $J_{1,2}$ 3 Hz + d, ~0.8 H, $J_{1,2}$ 7 Hz), H-1] (Found: C, 47.40; H, 5.37; N, 2.88).

*Benzyl 2,3,4-tri-*O*-benzyl-1-*O*-(1-benzyl* N*-benzyloxycarbonyl-*L*-glutam-5-oyl)-*D*-glucopyranuronate* (**6**). — Benzyl 2,3,4-tri-*O*-benzyl-D-glucopyranuronate (1.66 g) and 1-benzyl 5-pentachlorophenyl *N*-benzyloxycarbonyl-L-glutamate[2] (2.05 + 0.19 g) were treated in the presence of imidazole (1.02 g) as described for **1**. After work-up, the crude product was eluted from silica gel with solvent *A* (10:1), to give the anomeric mixture of **6** (1.53 g, 56.3%). Crystallisation (2×) from 96% ethanol afforded the β anomer (334 mg), m.p. 119–121°, $[\alpha]_D$ +3.2°, −14° (*c* 1.3, ethyl acetate); ν_{max}^{KBr} 3380 (NH), 1760 (C=O), 1700 and 1540 cm^{-1} (Amide I and II). P.m.r. data: τ 2.59–2.85 (m, 30 H, 6 Ph), 4.35 (d, $J_{1,2}$ 7 Hz, H-1), and 4.70 (d, *J* 8 Hz, NH).

Anal. Calc. for $C_{54}H_{53}NO_{12}$: C, 71.42; H, 5.88; N, 1.54. Found: C, 71.38; H, 6.04; N, 1.63.

The residue from the mother liquors was rechromatographed (2×), as just described, to give the pure α anomer (120 mg) as an oil, $[\alpha]_D$ +19.3°. P.m.r. data: τ 2.59–2.85 (m, 30 H, 6 Ph), 3.68 (d, $J_{1,2}$ 3 Hz, H-1), and 4.68 (d, *J* 8 Hz, NH) (Found: C, 71.36; H, 6.15; N, 1.53).

*1-*O*-(L-γ-Glutamyl)-β-D-glucopyranuronic acid* (**7β**) *trifluoroacetate salt.* — Catalytic hydrogenolysis of **6β** (305 mg) was performed as described for **2α** trifluoroacetate salt; after removal of the catalyst and concentration (0.1 torr, ~2 ml), the crude product was precipitated with dry ether, and a solution of this material in water (1 ml) was lyophilised. The resulting, fluffy mass was dissolved in warm propan-2-ol; on cooling and addition of a few drops of ether, the title product (115 mg, 78%) was deposited as a hygroscopic solid, m.p. 114–116°, $[\alpha]_D$ −9° (*c* 1.4, water); ν_{max}^{KBr} 3490 (broad, vs; OH), 1750 (C=O), 1630 and 1510 (Amino acid I and II), and 725 cm^{-1} ($CF_3CO_2^-$). P.m.r. data (D_2O): τ 4.34 (d, $J_{1,2}$ 7 Hz, H-1).

Anal. Calc. for $C_{11}H_{17}NO_{10} \cdot CF_3CO_2H$: C, 35.70; H, 4.15; N, 3.20. Found: C, 35.66; H, 4.29; N, 3.41.

A sample (120 mg) of the above salt was *N*-acetylated as described for **3β**, to give 1-*O*-(*N*-acetyl-L-γ-glutamyl)-β-D-glucopyranuronic acid (**8β**) as a hygroscopic solid (79 mg, 81%), m.p. 80–82°, $[\alpha]_D$ −14.4° (water). P.m.r. data (D_2O): τ 4.35 (d, $J_{1,2}$ 7 Hz, H-1) and 7.82 (NAc).

Anal. Calc. for $C_{13}H_{19}NO_{11}$: C, 42.74; H, 5.24; N, 3.83. Found: C, 42.64; H, 5.61; N, 3.69.

A sample (57 mg) of **8β** was treated with ethereal diazomethane (0.5 mmol/ml, 0.7 ml, 2.2 equiv.) and then with acetic anhydride–pyridine, as described for the esterification and *O*-acetylation of **3α**; after removal of acetic anhydride by co-distillation with water (0.1 torr), a solution of the residue in solvent *A* (1:2) was eluted from a column of silica gel with the same solvent, to give a product still contaminated with methyl D-glucopyranuronate tetra-acetate. Crystallisation of this material from

ethyl acetate–light petroleum gave methyl 2,3,4-tri-*O*-acetyl-1-*O*-(1-methyl *N*-acetyl-L-glutam-5-oyl)-β-D-glucopyranuronate (**9β**; 20 mg, 25% calc. on **8β**), whose m.p., mixture m.p., $[\alpha]_D$, and p.m.r. spectrum were indistinguishable from those of the sample prepared by direct synthesis (Found: C, 48.71; H, 5.83; N, 2.90).

1-O-(L-γ-Glutamyl)-α-D-glucopyranuronic acid (**7α**) *trifluoroacetate salt.* — Catalytic hydrogenolysis of **6α** (320 mg), as described for the β anomer, gave the title compound (130 mg, 84%) as a highly hygroscopic solid, $[\alpha]_D$ +90° (water). P.m.r. data (D_2O): τ 3.77 (d, $J_{1,2}$ 3 Hz, H-1).

Anal. Calc. for $C_{11}H_{17}NO_{10} \cdot CF_3CO_2H$: C, 35.70; H, 4.15; N, 3.20. Found: C, 35.79; H, 4.08; N, 3.36.

N-Acetylation of a sample (130 mg) of the above salt gave 1-*O*-(*N*-acetyl-L-γ-glutamyl)-α-D-glucopyranuronic acid (**8α**; 82 mg, 64%) as a very hygroscopic solid, $[\alpha]_D$ +63° (*c* 1.7, water). P.m.r. data (D_2O): τ 3.72 (d, $J_{1,2}$ 3 Hz, H-1) and 7.94 (s, NAc).

Anal. Calc. for $C_{13}H_{19}NO_{11} \cdot H_2O$: C, 40.73; H, 5.52; N, 3.65. Found: C, 40.92; H, 5.43; N, 3.34.

(*a*) *Esterification with diazomethane.* The reaction of a sample (24 mg) of **8α** with diazomethane was performed as described for **9β**, but at −10°, and the crude product was then submitted to conventional acetylation: the resulting syrup was applied to a column of silica gel. Fast elution (fractions: 1 ml/0.5 min) with solvent *A* (1:2) afforded a chromatographically homogeneous solid (18 mg, 55.4%), $[\alpha]_D$ +57.5°, which t.l.c. mobility [solvent *A* (1:2): R_F 0.23], spectral, and analytical data indicated to be methyl 1,3,4-tri-*O*-acetyl-2-*O*-(1-methyl *N*-acetyl-L-glutam-5-oyl)-D-glucopyranuronate (**10**; ratio α:β ~3:1). P.m.r. data: τ [3.63 + 4.23 (d, ~0.7 H, $J_{1,2}$ 3 Hz + d, ~0.3 H, $J_{1,2}$ 7 Hz), H-1], 3.77 (d, *J* 8 Hz, NH), 6.25 (s, 2 CO_2Me), [7.82 + 7.88 (s, 0.7 × 3 H, *ax* AcO-1 + s, 0.3 × 3 H, *eq* AcO-1)], and 7.93 (2 OAc + NAc).

Anal. Calc. for $C_{21}H_{29}NO_{14}$: C, 48.55; H, 5.63; N, 2.70. Found: C, 48.67; H, 5.82; N, 2.90.

(*b*) *Esterification catalyzed by BF_3–MeOH.* To a sample (16.5 mg) of **8α** in anhydrous methanol (8 ml) was added 10% BF_3–MeOH reagent (Fluka; 0.08 ml, 0.1 mmol). The solution was kept at room temperature overnight and then concentrated (traces of BF_3 being removed by repeated evaporations of MeOH from the product), and the residue was subjected to conventional acetylation. Fast elution of the crude product from silica gel with solvent *A* (1:2) afforded a chromatographically homogeneous solid (8 mg, 36% calc. on **8α**) indistinguishable (mixture m.p., $[\alpha]_D$, and p.m.r. spectrum) from **9α** prepared by direct synthesis (Found: C, 48.29; H, 4.93).

Methyl 2,3,4-tri-O-acetyl-1-O-(1-methyl N-acetyl-L-glutam-5-oyl)-D-glucopyranuronate (**9**). — Methyl 2,3,4-tri-*O*-acetyl-D-glucopyranuronate (668 mg) and 1-methyl *N*-acetyl-glutamic acid[23] (382 mg) were treated in the presence of DCC (412 mg) and imidazole (272 mg) as described for **4**. After work-up, the crude product was eluted from silica gel with solvent *A* (1:2) to give, in the slower-moving fractions,

pure α anomer (207 mg, 21%), which crystallised from 96% ethanol; m.p. 133–134°, $[\alpha]_D$ +101° (*c* 1.4). P.m.r. data: τ 3.55 (d, $J_{1,2}$ 3 Hz, H-1), 3.75 (d, *J* 8 Hz, NH), 5.58 (d, *J* 10 Hz, H-5), 6.25 and 6.27 (2 s, 2 CO_2Me), and 8.02 (3 OAc + NAc).

Anal. Calc. for $C_{21}H_{29}NO_{14}$: C, 48.55; H, 5.63; N, 2.70. Found: C, 48.52; H, 5.44; N, 2.87.

The material eluted in the faster-moving fractions was rechromatographed on silica gel with the same solvent, to give **9β** contaminated with its α anomer and the starting sugar component [t.l.c. in solvent *A* (1:2): R_F 0.25, 0.21, and 0.59, respectively]; crystallisation of the residue from ethyl acetate–light petroleum afforded the pure β anomer (31 mg), m.p. 71–73° (softening at 62–64°), $[\alpha]_D$ +21°. P.m.r. data: τ 3.75 (d, *J* 8 Hz, NH), 4.19 (d, $J_{1,2}$ 7 Hz, H-1), 6.25 and 6.27 (2 s, 2 CO_2Me), 7.95 and 7.98 (3 OAc + NAc) (Found: C, 48.46; H, 5.58; N, 2.68).

Benzyl 2,3,4-tri-O-benzyl-1-O-[N-(tert-butoxycarbonyl)-L-phenylalanyl]-D-glucopyranuronate (**11**). — Benzyl 2,3,4-tri-*O*-benzyl-D-glucopyranuronate (1.11 g) and *N*-(*tert*-butoxycarbonyl)-L-phenylalanine pentachlorophenyl ester[24] (1.2 g) were treated in the presence of imidazole (680 mg) as described for **1**. After work-up and elution of the crude product from silica gel with solvent *A* (10:1), chromatographically homogeneous **11** (1.25 g, 74%) was obtained as an anomeric mixture. Rechromatography of material eluted in the slower-moving fractions, followed by crystallisation from ethanol + some drops of water, gave the β anomer (300 mg), m.p. 103–104°, $[\alpha]_D$ −5.1°; ν_{max}^{KBr} 3370 (NH), 1758 and 1740 (C=O), 1690 and 1515 (Amide I and II), and 1365 cm^{-1} (Me_3C). P.m.r. data: τ 2.66–2.68 (m, 5 Ph), 4.35 (d, $J_{1,2}$ 7 Hz, H-1), and 8.60 (s, Me_3C).

Anal. Calc. for $C_{48}H_{51}NO_{10}$: C, 71.89; H, 6.41; N, 1.75. Found: C, 71.87; H, 6.29; N, 1.65.

Material eluted in the faster-moving fractions was rechromatographed (2×) on silica gel with chloroform, to give pure **11α** (185 mg) as a syrup, $[\alpha]_D$ +38.6°. P.m.r. data: τ 2.65–2.87 (m, 5 Ph), 3.62 (d, $J_{1,2}$ 3 Hz, H-1), and 8.60 (s, Me_3C) (Found: C, 72.05; H, 6.52; N, 1.91).

1-O-[N-(tert-Butoxycarbonyl)-L-phenylalanyl]-β-D-glucopyranuronic acid (**12β**). — Catalytic hydrogenolysis of **11β** (373 mg), as described for **2β**, gave, after removal of the catalyst and concentration (0.1 torr) of the filtrate, a syrupy residue that was dissolved in propan-2-ol; addition of ether precipitated the title compound (150 mg, 73%) as a hygroscopic solid, m.p. 77–80°, $[\alpha]_D$ −15° (MeOH). P.m.r. data [$(CD_3)_2CO$]: τ 2.72 (Ph), 3.67–3.96 (m, NH + 3 OH, disappeared on deuteration), 4.32 (d, $J_{1,2}$ 7 Hz, H-1), and 8.64 (s, Me_3C).

Anal. Calc. for $C_{20}H_{27}NO_{10}$: C, 54.41; H, 6.17; N, 3.17. Found: C, 54.55; H, 6.44; N, 3.04.

Addition of water (3 ml) to a solution of the above compound (200 mg) in acetone (0.5 ml) precipitated **12β** monohydrate as white crystals (123 mg, 60%), m.p. 118–123°, $[\alpha]_D$ −14.4°. P.m.r. data [$(CD_3)_2CO$]: τ 2.72 (Ph), 3.89 (d, *J* 8 Hz, NH, disappeared on deuteration), 4.36 (d, $J_{1,2}$ 7 Hz, H-1), 5.00 (m, 5 H, 3 HO + H_2O), and 8.65 (s, Me_3C).

Anal. Calc. for $C_{20}H_{27}NO_{10} \cdot H_2O$: C, 52.28; H, 6.36; N, 3.04. Found: C, 52.46; H, 6.48; N, 3.21.

(*a*) A sample (55 mg) of anhydrous **12β** in methanol (5 ml) was treated with 10% BF_3–MeOH reagent (0.15 ml, 1.5 equiv.) for 5.5 h at 25°, and the reaction mixture was further processed as described for **9α**. Conventional acetylation of the residue, followed by work-up (10% aqueous citric acid) and concentration of the solvent, left a residue that was eluted from silica gel with solvent *A* (1 : 1) and crystallised from chloroform–light petroleum, to give a product (35 mg, 53.5%) that was indistinguishable (mixture m.p., $[\alpha]_D$, p.m.r. spectrum) from methyl 2,3,4-tri-*O*-acetyl-1-*O*-(*N*-acetyl-L-phenylalanyl)-β-D-glucopyranuronate (**14β**) prepared by direct synthesis.

(*b*) Treatment of a sample (56 mg) of **12β** monohydrate with 10% BF_3–MeOH reagent (0.12 ml, 1.2 equiv.), as described above, followed by acetylation of the product and column chromatography, gave two products [t.l.c. in solvent *A* (1 : 1); R_F 0.36 (minor) and 0.78 (major)], identified as **14β** (6.4 mg, 10%) and methyl 2,3,4-tri-*O*-acetyl-1-*O*-[*N*-(*tert*-butoxycarbonyl)-L-phenylalanyl]-β-D-glucopyranuronate (**13β**; 34 mg, 48%), respectively, by comparison with the authentic samples prepared by direct condensation.

1-O-[N-(tert-*Butoxycarbonyl*)-L-*phenylalanyl*]-α-D-*glucopyranuronic acid* (**12α**). — Debenzylation of **11α** (180 mg), as described for **2β**, gave, after lyophilisation, the title compound (97 mg, 98%) as a fluffy mass that crystallised from propan-2-ol–ether; m.p. 140° (carbonisation, darkening at 115°), $[\alpha]_D$ +35.3° (methanol). P.m.r. data (D_2O): τ 2.73 (Ph), 3.78 (d, $J_{1,2}$ 3 Hz, H-1), and 8.66 (s, Me_3C); [$(CD_3)_2$-CO]: τ 2.70 (Ph), 3.79 (d, $J_{1,2}$ 3 Hz), and 8.65 (s, Me_3C).

Anal. Calc. for $C_{20}H_{27}NO_{10}$: C, 54.41; H, 6.17; N, 3.17. Found: C, 54.42; H, 6.34; N, 3.22.

(*a*) *Esterification with diazomethane.* Treatment of a sample (189 mg) of **12α** in *N*,*N*-dimethylformamide (2 ml) with ethereal diazomethane (0.43 mmol/ml; 1.1 ml, 1.1 equiv.) at 4° for 1 h, followed by evaporation (0.1 torr) of the solvent and conventional acetylation of the residue, gave a mixture containing two major components (t.l.c. in solvent *D*: R_F 0.76 and 0.73) which were separated on a column of silica gel from other minor (mainly decomposition) products; by combination and rechromatography of the appropriate fractions, the faster-moving component was obtained as a chromatographically homogeneous syrup (34 mg, 13.6%), $[\alpha]_D$ +66°. Its t.l.c. mobility in solvent *D* (not identical with that of **13α**), p.m.r. spectrum {τ 2.73 (Ph), [3.57 + 4.21 (d, ~0.7 H, $J_{1,2}$ 3 Hz + d, ~0.2 H, $J_{1,2}$ 7 Hz), H-1], 6.24 (s, CO_2Me), [7.81 + 7.88 (s, ~0.7 × 3 H, *ax* AcO-1 + s, ~0.3 × 3 H, *eq* AcO-1)], 8.00 (2 OAc + NAc), and 8.62 (s, Me_3C)}, and analytical data were indicative of the methyl 1,3,4-tri-*O*-acetyl-2-*O*-[*N*-(*tert*-butoxycarbonyl)-L-phenylalanyl]-D-glucopyranuronate (**15**, α : β ~3 : 1) structure.

Anal. Calc. for $C_{27}H_{35}NO_{13}$: C, 55.76; H, 6.07; N, 2.41. Found: C, 55.99; H, 6.28; N, 2.33.

The residue obtained upon concentration of the slower-moving fractions was

dissolved in chloroform; subsequent addition of light petroleum precipitated a chromatographically homogeneous solid (18 mg, 8.3%), whose i.r. [ν_{max}^{film} 3380 (NH), 1810 (C=O γ lactone), 1755 (C=O), 1715 and 1500 (Amide I and II), and 1370 cm^{-1} (Me_3C)], p.m.r. [τ 2.78 (Ph), 3.93 (s, 1 H, H-1), 6.95 (d, *J* 6 Hz, $CHCH_2Ph$), 7.87 and 7.97 (2 s, 2 OAc), and 8.58 (s, Me_3C)], and microanalytical data supported the di-*O*-acetyl-*O*-[*N*-(*tert*-butoxycarbonyl)-L-phenylalanyl]-β-D-glucofuranurono-6,3-lactone (**16**) structure.

Anal. Calc. for $C_{24}H_{29}NO_{11}$: C, 56.79; H, 5.76; N, 2.76. Found: C, 56.67; H, 6.03; N, 2.91.

(*b*) *Esterification catalysed by BF_3–MeOH.* A sample (69 mg) of **12α** in methanol (5 ml) was treated with 10% BF_3–MeOH reagent (0.15 ml, 1.2 equiv.) at 25°. for 2.5 h, the solution was then concentrated (0.1 torr), and the residue acetylated, as described for **12β**, to give two major products [t.l.c. solvent *A* (1:1): R_F 0.47 and 0.81] which were separated on silica gel with solvent *A* (1:1). The faster-moving product was obtained as a solid foam (16.4 mg, 17.6%), which was indistinguishable (by t.l.c., $[\alpha]_D$, and p.m.r. spectrum) from methyl 2,3,4-tri-*O*-acetyl-1-*O*-[*N*-(*tert*-butoxycarbonyl)-L-phenylalanyl]-α-D-glucopyranuronate (**13α**) prepared by direct synthesis (Found: C, 55.89; H, 6.15; N, 2.34.)

The second product to be eluted was a solid foam (24.3 mg, 29%) that was indistinguishable (by t.l.c., $[\alpha]_D$, and p.m.r. spectrum) from methyl 2,3,4-tri-*O*-acetyl-1-*O*-(*N*-acetyl-L-phenylalanyl)-α-D-glucopyranuronate (**14α**) prepared by direct synthesis (Found: C, 55.07; H, 5.93; N, 2.41).

*Methyl 2,3,4-tri-*O*-acetyl-1-*O*-[*N*-(tert-butoxycarbonyl)-L-phenylalanyl]-D-glucopyranuronate* (**13**). — Methyl 2,3,4-tri-*O*-acetyl-D-glucopyranuronate (334 mg) and *N*-*tert*-butoxycarbonyl-L-phenylalanine pentachlorophenyl ester (660 mg) were treated in the presence of imidazole (272 mg) as described for **1**. Elution of the crude product from silica gel with solvent *A* (1:1) afforded chromatographically homogeneous **13** (453 mg, 77.8%) as an anomeric mixture. Crystallisation from di-(2-propyl) ether (2×) gave **13β** (100 mg), m.p. 131–135°, $[\alpha]_D$ −6° (*c* 1.5). P.m.r. data: τ 2.70 (Ph), 4.09 (d, $J_{1,2}$ 7 Hz, H-1), 5.82 (m, H-5), 6.18 (s, CO_2Me), 6.80 (d, *J* 6 Hz, $CHCH_2Ph$), 7.86 (3 OAc), and 8.48 (s, Me_3C).

Anal. Calc. for $C_{27}H_{35}NO_{13}$: C, 55.76; H, 6.07; N, 2.41. Found: C, 55.50; H, 5.99; N, 2.57.

The mother liquor was evaporated, and the residue was passed through silica gel with the same solvent to give, in the slower-moving fractions, **13α** (50 mg) as a solid foam, $[\alpha]_D$ +61°. P.m.r. data: τ 2.63 (Ph), 3.50 (d, $J_{1,2}$ 3 Hz, H-1), 5.64 (d, *J* 10 Hz, H-5), 6.19 (s, CO_2Me), 6.80 (d, *J* 6 Hz, $CHCH_2Ph$), 7.86, 7.89 (3 OAc), and 8.48 (s, Me_3C) (Found: C, 55.58; H, 5.95; N, 2.55).

*Methyl 2,3,4-tri-*O*-acetyl-1-*O*-(N-acetyl-L-phenylalanyl)-D-glucopyranuronate* (**14**). — Condensation of methyl 2,3,4-tri-*O*-acetyl-D-glucopyranuronate (1 g) with *N*-acetyl-L-phenylalanine (621 mg) in the presence of DCC (618 mg) and imidazole (408 mg), as described for **4**, gave, after passing the crude product through silica gel with solvent *A* (1:1), chromatographically pure **14** (880 mg, 56%) as an anomeric

mixture. Crystallisation from chloroform–light petroleum afforded **14β** (315 mg), m.p. 167–168°, $[\alpha]_D$ +3.6°. P.m.r. data: τ 3.8 (s, Ph), 4.24 (d, $J_{1,2}$ 7 Hz, H-1), 6.3 (s, CO_2Me), 7.97, and 8.1 (3 OAc + NAc).

Anal. Calc. for $C_{24}H_{29}NO_{12}$: C, 55.06; H, 5.58; N, 2.68. Found: C, 55.21; H, 5.55; N, 2.59.

The residue left after concentration of the mother liquor was passed through silica gel with the same solvent, to give **14α** (50 mg) as a solid foam, $[\alpha]_D$ +75.8°. P.m.r. data: τ 3.77 (Ph), 3.64 (d, $J_{1,2}$ 3 Hz, H-1), 4.09 (d, J 8 Hz, NH), 6.27 (s, CO_2Me), 7.93, and 8.00 (3 OAc + NAc) (Found: C, 55.26; H, 5.69; N, 2.75).

*Conversion of 1-*O*-(*p*-methoxybenzoyl)-β-*D*-glucopyranuronic acid into the tri-*O*-acetyl methyl ester derivative.* — The unprotected 1-ester[18] (20 mg) in methanol (2 ml) was treated with 10% BF_3–MeOH reagent (0.05 ml, 1 equiv.) at room temperature for 6 h [monitoring by t.l.c. in solvent *B* (5:1)], the solution was then concentrated, and the residue conventionally acetylated at 4° overnight. The product was crystallised from hot ethanol, to give needles of methyl 2,3,4-tri-*O*-acetyl-1-*O*-(*p*-methoxybenzoyl)-β-D-glucopyranuronate (16.3 mg, 59%), m.p. 145–146°, $[\alpha]_D$ −15.9°. P.m.r. data: τ 2.0–3.2 (m, $-C_6H_4-$), 4.10 (d, $J_{1,2}$ 7 Hz, H-1), 5.77 (d, J 10 Hz, H-5), 6.17 (s, OMe), and 6.23 (s, CO_2Me); lit.[19] m.p. 144–145°, $[\alpha]_D$ −16° (chloroform).

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