SYNTHESES OF SOME AMINOACYL DERIVATIVES OF 2-ACETAMIDO-2-DEOXY-β-D-GLUCOPYRANOSYLAMINE AND OF 2-AMINO-1-*N*-(4-L-ASPARTYL)-2-DEOXY-β-D-GLUCOPYRANOSYLAMINE

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ABSTRACT

Syntheses of the 1-*N*-glycyl, -L-alanyl, -L-valyl, -5-L-glutamyl, and -L-seryl derivatives of 2-acetamido-2-deoxy-β-D-glucopyranosylamine are described. 2-Amino-1-*N*-(4-L-aspartyl)-2-deoxy-β-D-glucopyranosylamine was synthesized by making use of the base-labile *N*-trifluoroacetyl group. Only the aspartyl derivative was hydrolyzed by an *amido hydrolase*. The ^{1}H n.m.r. spectra of the glycopeptides indicate the C_1^4 conformation in each case. The mass spectra of the fully protected glycyl, L-alanyl, L-valyl, and L-seryl derivatives are described. The o.r.d. and c.d. spectra of the 1,2-di-*N*-acyl derivatives showed the presence of a strong, positive Cotton effect near 200 nm, and a weaker, positive Cotton effect near 220 nm.

INTRODUCTION

An *amido hydrolase* which hydrolyzes the glycopeptide 2-acetamido-1-*N*-(4-L-aspartyl)-2-deoxy-β-D-glucopyranosylamine[1,2] (**1**), a linkage known to occur in various glycoproteins, has been isolated from a variety of sources[3]. For use in determining the specificity of the enzyme, and in order to examine the properties of glycopeptides of this type, the following compounds were prepared by synthetic routes similar to that previously used[2] for the preparation of **1**. 2-Acetamido-2-deoxy-1-*N*-glycyl-β-D-glucopyranosylamine (**2**), 2-acetamido-1-*N*-L-alanyl-2-deoxy-β-D-glucopyranosylamine (**3**), 2-acetamido-2-deoxy-1-*N*-L-valyl-β-D-glucopyranosylamine (**4**), 2-acetamido-2-deoxy-1-*N*-(5-L-glutamyl)-β-D-glucopyranosylamine (**5**), and 2-acetamido-2-deoxy-1-*N*-L-seryl-β-D-glucopyranosylamine (**6**). The *N*-deacetylated derivative of **1**, namely 2-amino-1-*N*-(4-L-aspartyl)-2-deoxy-β-D-glucopyranosylamine (**7**), was also prepared for hydrolysis studies by *amido hydrolase* and acid. The glycopeptides **2**, **3**, and **5** have been prepared previously by different methods[4–6], but our physical constants are not in good agreement with the literature values.

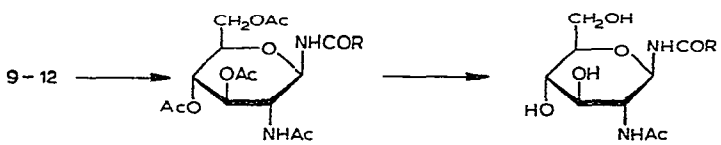
DISCUSSION

Synthesis of 1-aminoacyl derivatives of 2-acetamido-2-deoxy-β-D-glucopyranosylamine. — 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl azide was prepared from the corresponding α-D bromide by treatment at room temperature

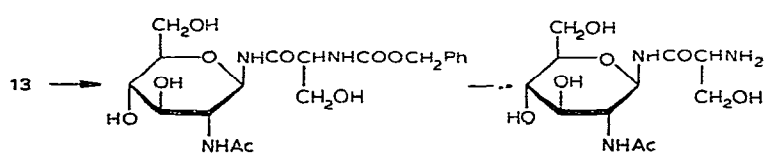
with sodium azide in formamide followed by hydrogenation to give the amine **8**. Coupling of **8** with the protected amino acids (*N*-benzyloxycarbonylglycine, *N*-benzyloxycarbonyl-L-alanine, *N*-benzyloxycarbonyl-L-valine, 1-benzyl *N*-benzyloxycarbonyl-L-glutamate, and *N*-benzyloxycarbonyl-L-serine) by treatment with dicyclohexylcarbodiimide in dichloromethane gave the fully protected glycopeptide derivatives **9–13** (40–64%). As in the preparation[2] of the glycopeptide **1**, column chromatography on silica gel was essential for the purification of these compounds. The glycyl, alanyl, valyl, and glutamyl derivatives (**9–12**) were hydrogenated to remove the amino acid protecting-groups, giving the tri-*O*-acetyl derivatives **14–17** (63–84%), and finally *O*-deacetylated by treatment with ammonia in methanol to give the free glycopeptides (57–90%). In the case of the seryl derivative **13**, hydrogenation gave an impure product, but *O*-deacetylation, followed by hydrogenation, proceeded smoothly giving a pure product (**18** and **6**) in each case.

8

9 R = $CH_2NHCOOCH_2Ph$
10 R = $CH(Me)NHCOOCH_2Ph$
11 R = $CH(i\text{-}Pr)NHCOOCH_2Ph$
12 R = $CH_2CH_2CH(COOCH_2Ph)NHCOOCH_2Ph$
13 R = $CH(CH_2OH)NHCOOCH_2Ph$



14	R = CH_2NH_2	2
15	R = $CH(Me)NH_2$	3
16	R = $CH(i\text{-}Pr)NH_2$	4
17	R = $CH_2CH_2CH(NH_2)COOH$	5
	R = $CH_2CH(NH_2)COOH$	1



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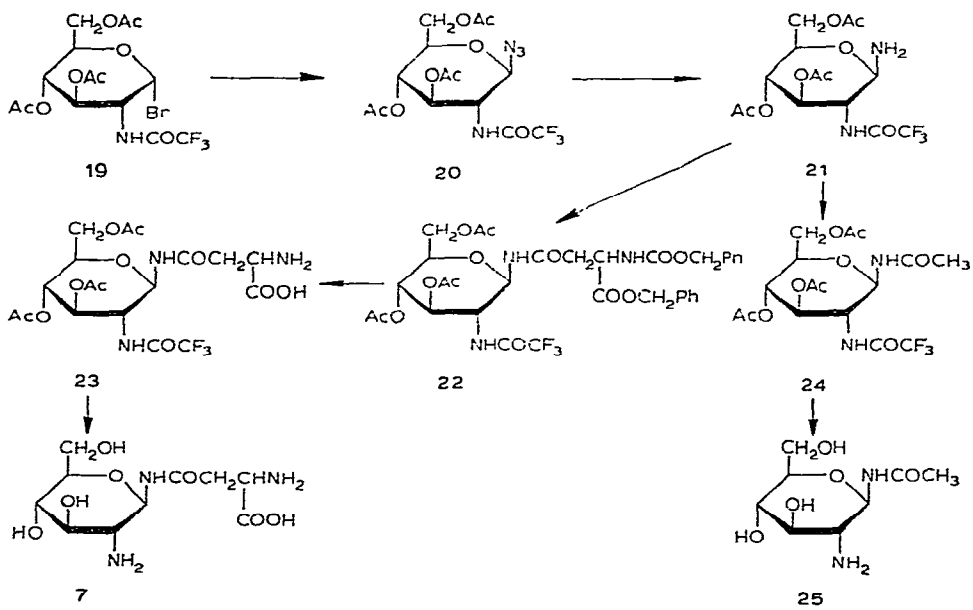
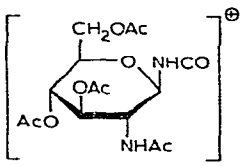
*Synthesis of 2-amino-1-*N*-(4-L-aspartyl)-2-deoxy-β-D-glucopyranosylamine* (**7**). — 3,4,6-Tri-*O*-acetyl-2-deoxy-2-trifluoroacetamido-β-D-glucopyranosyl azide (**20**) was prepared from the known α-D bromide[7] **19** by treatment with sodium azide in formmide, and its β-D-configuration was established by n.m.r. spectroscopy. Hydrogenation over Adams' catalyst gave the amine **21**. 1-Benzyl *N*-benzyloxycarbonyl-L-aspartate was most conveniently prepared by the method used for the corresponding glutamate derivative[8], involving treatment of the *N*-benzyloxycarbonylamino acid anhydride with benzyl alcohol and dicyclohexylamine in ether. The 1-benzyl *N*-benzyloxycarbonyl-L-aspartate was isolated as the crystalline dicyclohexylammonium salt and then converted by acidification into the free acid. The aspartate was coupled with the amine **21** by treatment with dicyclohexylcarbodiimide in dichloromethane, the benzyl and benzyloxycarbonyl groups were removed by hydrogenation, and the acetyl and trifluoroacetyl groups by treatment with ammonia in methanol, eight days being required for complete removal of the trifluoroacetyl group. 1-*N*-acetyl-2-amino-2-deoxy-β-D-glucopyranosylamine (**25**) was also prepared via the 1-acetamido-2-trifluoroacetyl compound **24**; **25** has also been prepared by a different route[9].



Enzyme hydrolyses. — Each of the free glycopeptides prepared was treated with an *amido hydrolase* preparation from rat liver[3]. The enzyme failed to hydrolyze the glycyl, alanyl, valyl, seryl, and glutamyl derivatives, but there was considerable hydrolysis of 2-amino-1-(4-L-aspartyl)-2-deoxy-β-D-glucopyranosylamine (**7**). These results indicate that the enzyme is highly specific for the 4-L-aspartyl group, but not for the sugar moiety.

^1H N.m.r spectra. — The identity and structure of all of the free glycopeptides were confirmed by ^1H n.m.r. spectroscopy for solutions in deuterium oxide (at 60 MHz for **1**, **3**, **4**, **6**, and **7**, and 100 MHz for **2** and **5**). In each case, the anomeric proton appeared as a doublet having J 8.5–9.5 Hz, indicating a *trans*-diaxial relationship for H-1 and H-2, as expected for the C_1^4 conformation. The chemical shift of the anomeric proton was higher for **7** (τ 5.03), which has a free 2-amino group, than for the 2-*N*-acetyl compounds **1**, **3**, **4**, and **6** (τ 4.92–4.95); the signals for the anomeric protons of **2** and **5** appeared at τ 4.49.

Mass spectra of fully protected glycopeptides. — Mass spectra of 2-acetamido-3,4,6-tri-*O*-acetyl-1-*N*-(1-benzyl *N*-benzyloxycarbonyl-4-L-aspartyl)-2-deoxy-β-D-glucopyranosylamine and of the corresponding glutamyl derivative have been described[10]. We have expanded these studies to the fully protected glycyl (**9**), alanyl (**10**), valyl (**11**), and seryl (**13**) derivatives. Each compound gave a molecular ion, but that for **11** was extremely weak. Each compound gave a peak at *m/e* 373, indicating the formation of the fragment **26**. The seryl derivative (**13**) gave a peak at *m/e* 536, much stronger than that for the molecular ion, corresponding to a fragment in which the $-CH_2OH$ group is lost, and allowing a positive identification of this amino acid moiety. As reported for the aspartyl and glutamyl derivatives[9], the amino-sugar moiety of each compound could be identified by a fragment at *m/e* 330, corresponding to the oxonium ion **27**. The peak series starting from an ion at *m/e* 241 (**28**), also reported for the aspartyl and glutamyl derivatives[9], was given by **9**, **10**, **11**, and **13**. In the spectra of **9** and **10**, a metastable peak at *m/e* 292, corresponding to the fragmentation of **26** → **27**, was located.



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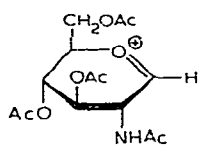

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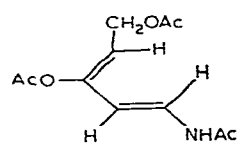

m/e 241

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Fragments arising from the stepwise removal of *O*-acetyl groups from the molecular ion were observed in the spectra of **9** (*m/e* 478, 418, 358, 366*, 307), **10** (491, 432, 380*), and **13** (508, 448). Other peaks recorded in the higher mass regions of the spectra are given in the experimental.

O.r.d. and c.d. spectra. — O.r.d. and c.d. spectra were obtained for **1**, **2**, **3**, **4**, **5**, **6**, **25**, and 2-acetamido-1-*N*-acetyl-2-deoxy-β-D-glucopyranosylamine (**30**). The data are summarized in Table I. It is of interest to compare these results with those reported for 2-acetamido-2-deoxy-D-glucose and its methyl glycosides, which all give negative Cotton effects[11,12]. For the methyl β-D-glycoside (the compound most closely analogous to those in the present study), the trough of the o.r.d. curve falls near 220 nm with a molecular rotation of −2690° at the lowest point. Its c.d. curve has a minimum at 215 nm. Application of the octant rule to the results for 2-acetam-

ido-2-deoxy-D-glucose and -D-galactose derivatives has indicated[11] that the preferred orientation of the amide group is such that its plane includes also the C-5 substituents with the carbonyl group *cis* to the axial H-2.

TABLE I

O.R.D. AND C.D. DATA[a] FOR THE AMINO ACID DERIVATIVES **1–6**, **25**, AND **30**

Compound	*O.r.d. spectra, [ϕ] (degrees)*	*C.d. spectra*
25	λ_{max} 228 nm, +1825; λ_{min} 203 nm −24,200	λ_{max} 213 nm, $\Delta\varepsilon$ +3.01
30	At 233 nm +7910 (sh); λ_{max} below 200 nm; at 210 nm +11,950	At 200 nm, $^{sh}\Delta\varepsilon$ +9.79 λ_{max} 194 nm, $\Delta\varepsilon$ +15.6
1	At 225 nm +6800 (sh); λ_{max} 206 nm +10,880	At 218 nm, $^{sh}\Delta\varepsilon$ +1.63 λ_{max} 197 nm, $\Delta\varepsilon$ +10.88
2	λ_{max^1} 227 nm +4820; λ_{min^1} 220 nm +4690 λ_{max^2} below 200 nm; at 207 nm; +13,600	At 217 nm, $^{sh}\Delta\varepsilon$ +1.48 λ_{max} 197 nm, $\Delta\varepsilon$ +11.15
3	λ_{max^1} 234 nm +2700; λ_{min^1} 220 nm +2610 λ_{max^2} 204 nm +16,200	λ_{max^1} 222 nm, $\Delta\varepsilon$ +1.19 λ_{max^2} below 200 nm; at 201 nm, $\Delta\varepsilon$ +9.70
4	λ_{max^1} 233 nm +5350; λ_{min^1} 223 nm +4860 λ_{max^2} 204 nm +21,490	λ_{max^1} 225 nm, $\Delta\varepsilon$ +0.865 λ_{max^2} 197 nm, $\Delta\varepsilon$ +18.55
5	λ_{max^1} 238 nm +8720; λ_{min^1} 220 nm +7300 λ_{max^2} 205 nm +16,000	λ_{max} below 200 nm; at 200 nm, $\Delta\varepsilon$ +11.97
6	λ_{max^1} 237.5 nm +3550; λ_{min^1} 224 nm +2240 λ_{max^2} 205.5 nm +19,200	λ_{max^1} 225 nm, $\Delta\varepsilon$ +0.96 λ_{max^2} 196 nm, $\Delta\varepsilon$ +17.6

[a]sh = shoulder.

In contrast to 2-acetamido-2-deoxy-D-glucosides, the 1-*N*-acyl compounds all gave positive Cotton effects. 1-*N*-Acetyl-2-amino-2-deoxy-β-D-glucopyranosylamine gave a single, positive Cotton effect, with the trough much more pronounced than the peak. The c.d. maximum was at 213 nm. For the 1,2-di-*N*-acyl derivatives, a much stronger, positive Cotton effect appeared at lower wavelength (with the c.d. maximum near 200 nm), while there was a weaker positive Cotton effect clearly discernible as a separate maximum in the c.d. spectra of the alanyl, valyl, and seryl derivatives (and indicated by shoulders in the spectra of the others) near 220 nm. The lower wavelength Cotton effect must be associated with the 1,2-di-*N*-acyl system, whereas the weaker Cotton effect at longer wavelengths may be associated with the 1-*N*-acyl group only.

The differential, dichroic absorption for the valyl derivative **4** was exceptionally high ($\Delta\varepsilon$+18.55), and was of the order of magnitude usually associated with an inherently dissymmetric, chromophoric system. It has been suggested that this may indicate that the 1β and 2α amide chromophores are fairly rigidly fixed in their con-

formation with respect to each other. Models indicate that there would be considerable restriction of free rotation of the amido groups in all the 1,2-di-*N*-acyl compounds. In the least-hindered orientation of the amido groups, the carbonyl groups of the 2-amido and 1-amido substituents are *cis* to H-2 and H-1, respectively.

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. N.m.r. spectra were measured for solutions in D_2O at 60 MHz (Perkin–Elmer R-10) with sodium 4,4-dimethyl-4-silapentane-1-sulphonate as an internal standard, and at 100 MHz (Varian HA-100) with tetramethylsilane as an external standard. Mass spectra were determined at 70 eV with an A.E.I. MS-9 instrument. Source temperatures were 200° for **9**, 210° for **10**, 240° for **11**, and 235° for **13**. The peak at *m/e* 79 was taken as the base peak in each case. O.r.d. and c.d. spectra were measured on aqueous solutions, using a Cary instrument. Enzyme hydrolyses were carried out at 37° in phosphate buffer at pH 7. Merck Silica gel G was used for t.l.c.

N-Benzyloxycarbonylamino acid derivatives. — The *N*-benzyloxycarbonyl derivatives of glycine, L-alanine, L-valine, and L-serine were prepared by standard methods[13], and 1-benzyl *N*-benzyloxycarbonyl-L-glutamate by the method of Klieger and Gibian[8].

A solution of *N*-benzyloxycarbonyl-L-aspartic acid[14] (10.6 g) in acetic anhydride (40 ml) was left at room temperature overnight. Evaporation of the solvent left the crystalline *N*-benzyloxycarbonyl-L-aspartic anhydride which was washed with dry ether and, without further purification, suspended in dry ether (45 ml). Benzyl alcohol (12.5 ml) and dicyclohexylamine (8.1 ml) were added simultaneously, and the reaction mixture was left at room temperature for 24 h and then at 0° for 1 week. The dicyclohexylammonium salt of 1-benzyl *N*-benzyloxycarbonyl-L-aspartate crystallized slowly. Recrystallization from ethanol gave material (8.1 g, 38%), m.p. 110–112°, $[\alpha]_D^{24}$ +2.65° (*c* 1.5, methanol) (Found: C, 68.9; H, 7.7; N, 5.7. $C_{30}H_{40}N_2O_6$ calc.: C, 68.7; H, 7.7; N, 5.35%).

The dicyclohexylammonium salt (8 g) was suspended in ethyl acetate (60 ml and 10M hydrochloric acid (3 ml) in ethyl acetate (3 ml) was added. After stirring for 20 min, the dicyclohexylammonium chloride was removed, and the filtrate was washed with water, dried, and evaporated. The residue was recrystallized from ethyl acetate–light petroleum to give 1-benzyl *N*-benzyloxycarbonyl-L-asparate (3.4 g, 63%), m.p. 83–84°, $[\alpha]_D^{20}$ −10.2° (*c* 3.6, glacial acetic acid); lit.[16] m.p. 84–85°, $[\alpha]_D^{18}$ −9.7° (glacial acetic acid).

2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl azide. — A chloroformic solution (240 ml) of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl bromide, prepared[15] from 2-amino-2-deoxy-D-glucose penta-acetate (20 g), was added to a cold solution of sodium azide (12 g) in dry formamide (240 ml). After

being stirred at room temperature for 4 h, the mixture was poured into ice–water. The chloroform was separated, the aqueous layer was extracted with chloroform, and the combined extracts were washed with ice–water, dried, and evaporated. The residue was recrystallized from ethyl acetate–light petroleum to give the title azide (10.7 g, 56%), m.p. 169–170°, $[\alpha]_D^{23}$ −43.8° (*c* 2, chloroform); lit.[1] m.p. 166–168°, $[\alpha]_D^{23}$ −50° (chloroform).

Synthesis of glycopeptides **2**, **3**, **4**, *and* **5**. — (*a*) *Coupling reactions.* The β-D-amine[2] **8** (11 mmoles) and the fully protected amino acid (11 mmoles) were dissolved in dichloromethane (75 ml). Dicyclohexylcarbodiimide (12 mmoles) was added, and the mixture stirred at room temperature for 6 h. Glacial acetic acid (1 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, dried, and concentrated at 40°. The residue was eluted from silica gel (120 g), with the solvents specified below, to give *N*,*N*′-dicyclohexylurea followed by the required product. The following compounds were thus obtained.

2-Acetamido-3,4,6-tri-*O*-acetyl-1-*N*-(*N*-benzyloxycarbonylglycyl)-2-deoxy-β-D-glucopyranosylamine (**9**, 40%), eluted with chloroform–methanol (40:1) and crystallized from chloroform–light petroleum, had m.p. 205° and $[\alpha]_D^{25}$ −0.61° (*c* 0.3, chloroform), and was homogeneous in t.l.c. (chloroform–methanol, 14:1) (Found: C, 53.7; H, 5.75; N, 7.8. $C_{24}H_{31}N_3O_{11}$ calc.: C, 53.6; H, 5.8; N, 7.8%). Mass spectrum: *m/e* 537 (M^+, 15.4%), 478 (6.0), 430 (2.6), 418 (8), 387 (2), 373 (37), 358 (6.7), 330 (55).

2-Acetamido-3,4,6-tri-*O*-acetyl-1-*N*-(*N*-benzyloxycarbonyl-L-alanyl)-2-deoxy-β-D-glucopyranosylamine (**10**, 64%), eluted with chloroform–methanol (75:1) and recrystallized from chloroform–light petroleum, had m.p. 209° and $[\alpha]_D^{22}$ −5.63° (*c* 2, chloroform), and was homogeneous in t.l.c. (chloroform–methanol, 20:1) (Found: C, 54.4; H, 6.0; N, 7.6. $C_{25}H_{33}N_3O_{11}$ calc.: C, 54.4; H, 6.0; N, 7.6%). Mass spectrum: *m/e* 551 (M^+, 2%), 491 (2.2), 444 (0.8), 432 (9.0), 416 (3.4), 401 (0.9), 373 (28), 330 (24).

2-Acetamido-3,4,6-tri-*O*-acetyl-1-*N*-(*N*-benzyloxycarbonyl-L-valyl)-2-deoxy-β-D-glucopyranosylamine (**11**, 44%), eluted with chloroform–methanol (100:1) and recrystallized from chloroform–light petroleum, had m.p. 239–240° and $[\alpha]_D^{22}$ −9.67° (*c* 0.7, chloroform), and was homogeneous in t.l.c. (chloroform–methanol, 14:1) (Found: C, 55.8; H, 6.5; N, 7.2. $C_{27}H_{37}N_3O_{11}$ calc.: C, 55.9; H, 6.4; N, 7.3%). Mass spectrum: *m/e* 579 (M^+, 0.1%), 472 (1.7), 429 (2.6), 373 (1.5), 330 (9%).

2-Acetamido-3,4,6-tri-*O*-acetyl-1-*N*-(1-benzyl *N*-benzyloxycarbonyl-5-L-glutamyl)-2-deoxy-β-D-glucopyranosylamine (**12**, 63%), eluted with chloroform–acetone (8:1) and recrystallized from dichloromethane–ethanol, had m.p. 217–218° and $[\alpha]_D^{23}$ −4.31° (*c* 1.2, chloroform), and was homogeneous in t.l.c. (chloroform–methanol, 20:1); lit.[6] m.p. 210–212°, $[\alpha]_D$ +12.9° (Found: C, 58.1; H, 5.65; N, 6.15. $C_{34}H_{41}N_3O_{13}$ calc.: C, 58.4; H, 5.9; N, 6.0%).

(*b*) *Hydrogenation.* The fully protected glycopeptides (6.2 mmoles) were hydrogenated in glacial acetic acid–water (70 ml, 9:1) over 10% palladium–charcoal (0.6 g)

at a pressure of 35 lb/sq. in. for 2 h, with the exception of the glutamyl derivative which required 50 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 at least 24 h. The following compounds were thus obtained.

2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-*N*-glycyl-β-D-glucopyranosylamine (**14**, 84%), m.p. 204.5–205.5° (from methanol–ether), $[\alpha]_D^{25}$ −0.94° (*c* 0.4, water), which was homogeneous in t.l.c. (chloroform–methanol, 4:1) (Found: C, 47.6; H. 6.15; N, 10.1. $C_{16}H_{25}N_3O_9$ calc.: C, 47.6; H, 6.2; N, 10.4%).

2-Acetamido-3,4,6-tri-*O*-acetyl-1-*N*-L-alanyl-2-deoxy-β-D-glucopyranosylamine, (**15**, 83%), m.p. 187–188° (from ethanol–light petroleum), $[\alpha]_D^{23}$ +2.93° (*c* 0.6, water), homogeneous in t.l.c. (chloroform–methanol, 4:1) (Found: 48.4; H, 6.3; N, 10.1. $C_{17}H_{27}N_3O_9$ calc.: C, 48.9; H, 6.5; N, 10.1%).

2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-*N*-L-valyl-β-D-glucopyranosylamine (**16**, 63%), m.p. 209–210° (from ethanol–light petroleum), $[\alpha]_D^{22}$ +47.6° (*c* 1, water), homogeneous in t.l.c. (chloroform–methanol, 6:1) (Found: C, 51.5; H, 7.15; N, 9.35. $C_{19}H_{31}N_3O_9$ calc.: C, 51.2; H, 7.0; N, 9.4%).

2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-*N*-(5-L-glutamyl)-β-D-glucopyranosylamine (**17**, 65%), m.p. 235° (decomp.) (from aqueous methanol–ether), $[\alpha]_D^{23}$ +2.03° (*c* 1, water), homogeneous in t.l.c. (chloroform–methanol 1:1) (Found: C, 48.0; H, 6.1; N, 8.8. $C_{19}H_{29}N_3O_{11}$ calc.: C, 48.0; H, 6.15; N, 8.85%).

(*c*) O-*Deacetylation*. The acetylated glycopeptides (3.4 mmoles) were dissolved in dry methanol (30 ml), and ammonia was passed through the cooled solution to saturation. After refrigeration overnight, the solvent was removed at 40° and the residue was washed with acetone to remove acetamide. The following compounds were thus obtained.

2-Acetamido-2-deoxy-1-*N*-glycyl-β-D-glucopyranosylamine (**2**, 65%), m.p. 236–237° (from ethanol–water), $[\alpha]_D^{23}$ +23.54° (*c* 0.8, water), homogeneous in t.l.c. (butanol–acetic acid–water, 10:20:5); lit.[4] m.p. 187–192° $[\alpha]_D$ +30.5° (water) for the product containing 2.5 H_2O (Found: C, 41.9; H, 7.35; N, 14.7. $C_{10}H_{19}N_3O_6 \cdot H_2O$ calc.: C, 41.9; H, 7.0; N, 14.7%). N.m.r. data (100 MHz, D_2O): τ 7.57 (3-proton singlet, NAc), 6.25 (2-proton singlet, glycyl CH_2), 5.60–6.18 (6-proton multiplet, H-2–H-6), 4.49 (1-proton doublet, *J* 9 Hz, H-1).

2-Acetamido-1-*N*-L-alanyl-2-deoxy-β-D-glucopyranosylamine (**3**, 57%), m.p. 234–235° (decomp.) (from methanol–ether), $[\alpha]_D^{23}$ +27° (*c* 0.5, water), homogeneous in t.l.c. (butyl alcohol–pyridine–water, 6:4:3, and butyl alcohol–acetic acid–water, 10:20:5); lit.[5] monohydrate, m.p. 200°, $[\alpha]_D$ +43.8° (water) (Found: C, 45.0; H, 7.5; N, 14.1. $C_{11}H_{21}N_3O_6$ calc.: C, 45.5; H, 7.2; N, 14.4%). N.m.r. data (60 MHz, D_2O): τ 8.75 (6-proton doublet, *J* 7 Hz, alanyl CH_3), 8.0 (3-proton singlet, NAc), 6.47 (1-proton triplet, *J* 7 Hz, alanyl CH) overlapping with 5.90–6.50 (6-proton multiplet, H-2–H-6), 4.90 (1-proton doublet, $J_{1,2}$ 8.5 Hz, H-1).

2-Acetamido-2-deoxy-1-*N*-L-valyl-β-D-glucopyranosylamine (**4**, 66%), m.p. 246° (decomp.) (from methanol), $[\alpha]_D^{23}$ +18.1° (*c* 0.4, water), homogeneous in t.l.c. (butyl alcohol–pyridine–water, 6:4:3, and in butyl alcohol–acetic acid–water, 10:20:5)

(Found: C, 48.9; H, 7.85; N, 13.1 ($C_{13}H_{25}N_3O_6$ calc.: C, 48.9; H, 7.85; N, 13.2%). N.m.r. data (60 MHz, D_2O): τ 9.12 (6-proton triplet, J 6 Hz, probably two overlapping doublets at 9.07 and 9.17, valyl Me_2), 8.0 (singlet, NAc, overlapping with multiplet, valyl H-3', 4 protons), 6.80 (1-proton doublet, J 5.1 Hz, valyl H-2'), 6.0–6.60 (6-proton multiplet, H-2–H-6), 4.95 (1-proton doublet, $J_{1,2}$ 8.7 Hz, H-1).

2-Acetamido-2-deoxy-1-*N*-(5-L-glutamyl)-β-D-glucopyranosylamine (**5**, 90%), m.p. 214° (from water–methanol), $[\alpha]_D^{22}$ +20.4° (*c* 2, water), homogeneous in t.l.c. (butyl alcohol–acetic acid–water, 10:20:5, on silica gel and on 0.1N boric acid-impregnated silica gel); lit.[6] trihydrate m.p. 185–190°, $[\alpha]_D$ +18° (water). N.m.r. data (100 MHz, D_2O): τ 7.55 [singlet, NAc, overlapping with multiplet, glutamyl (C-3)H_2, 5 protons], 7.05–7.25 [2-proton multiplet, glutamyl (C-4)H_2], 5.50–6.05 [7-proton multiplet, H-2–H-6 and glutamyl (C-2)H], 4.49 (1-proton doublet, $J_{1,2}$ 9 Hz, H-1).

Synthesis of glycopeptide **6**. — The β-D-amine **8** (3 g) and *N*-benzyloxycarbonyl-L-serine (2.1 g) were dissolved in dichloromethane (95 ml), dicyclohexylcarbodiimide (2.5 g) was added, and the reaction was carried out as described above. The product was eluted from silica gel (115 g) with chloroform–methanol (40:1) to give, first, *N*,*N*'-dicyclohexylurea, followed by some unidentified material, and then (after recrystallization from chloroform–light petroleum) 2-acetamido-3,4,6-tri-*O*-acetyl-1-*N*-(*N*-benzyloxycarbonyl-L-seryl)-2-deoxy-β-D-glucopyranosylamine (**13**, 2.7 g, 53%), m.p. 196–197°, $[\alpha]_D^{22}$ −8.06° (*c* 1, chloroform), which was homogeneous in t.l.c. (chloroform–methanol, 12:1) (Found: C, 52.6; H, 5.85; N, 7.15. $C_{25}H_{33}N_3O_{12}$ calc.: C, 52.9; H, 5.8; N, 7.4%).

A solution of **13** (1 g) in dry ethanol (70 ml) was cooled in ice and saturated with ammonia. After cooling overnight and removal of the solvent, the residue was washed with acetone and recrystallized from aqueous methanol–ether to give 2-acetamido-1-*N*-(*N*-benzyloxycarbonyl-L-seryl)-2-deoxy-β-D-glucopyranosylamine (**18**, 0.6 g, 77%), m.p. 238–240°, $[\alpha]_D^{22}$ +10.8° (*c* 0.8, water), which was homogeneous in t.l.c. (chloroform–methanol, 2:1) (Found: C, 51.8; H, 6.1; N, 9.7. $C_{19}H_{27}N_3O_9$ calc.: C, 51.7; H, 6.1; N, 9.5%).

A solution of **18** (1.4 g) in distilled water (100 ml) was hydrogenated over Adams' catalyst (0.3 g) at 50 lb/sq. in. for 8 h. After filtration, the solvent was evaporated at 35°, and the residue was recrystallized from aqueous methanol–ether to give 2-acetamido-2-deoxy-1-*N*-L-seryl-β-D-glucopyranosylamine (**6**, 0.7 g, 72%), m.p. 208–209° (decomp.), $[\alpha]_D^{22}$ +21.1° (*c* 0.6, water) which was homogeneous in t.l.c. (butyl alcohol–pyridine–water, 6:4:3, and butyl alcohol–acetic acid–water, 10:20:5) (Found: C, 40.7; H, 7.3; N, 12.6. $C_{11}H_{21}N_3O_7 \cdot H_2O$ calc.: C, 40.6; H, 7.1; N, 12.9%). N.m.r. data (60 MHz, D_2O): τ 8.0 (3-proton singlet, NAc), 6.0–6.7 [9-proton multiplet, H-2–H-6 and seryl protons, including a doublet at 6.47, J 9 Hz, for seryl (C-3)H_2], 4.95 (1-proton doublet, $J_{1,2}$ 8.5 Hz, H-1).

3,4,6-Tri-O-acetyl-2-deoxy-2-trifluoroacetamido-β-D-glucopyranosyl azide (**20**). — The α-D bromide[7] **19** (1 g) was added to a solution of sodium azide (1 g) in dry formamide (20 ml). After 1.5 h at room temperature, the reaction was complete (t.l.c.). The solution was poured into ice–water and extracted with chloroform. The

chloroform layer was washed with cold water, dried, and evaporated, and the residue was recrystallized from chloroform–light petroleum to give **20** (0.7 g, 70%), m.p. 145°, $[\alpha]_D^{18}$ −52.2° (*c* 1.2, chloroform), which was homogeneous in t.l.c. (chloroform–methanol, 14:1) (Found: C, 39.5; H, 4.0; N, 12.9. $C_{14}H_{16}F_3N_4O_8$ calc.: C, 39.5; H, 3.75; N, 13.2%). N.m.r. data (60 MHz, $CDCl_3$, internal Me_4Si): τ 7.95 (6-proton singlet, 2 OAc), 7.89 (3-proton singlet, OAc), 5.6–6.2 (6-proton multiplet, H-2,5,6,6'), 5.1 (1-proton doublet, $J_{1,2}$ 9 Hz, H-1), 4.7 (2-proton quintet, 1:2:4:2;1, *J* 9.9 Hz, H-3,4), 2.42 (1-proton doublet, *J* 9.6 Hz, N-H).

*3,4,6-Tri-*O*-acetyl-2-deoxy-2-trifluoroacetamido-β-*D*-glucopyranosylamine* (**21**). — A solution of **20** (3.2 g) in methanol (100 ml) was hydrogenated over Adams' catalyst (0.03 g) for 2.5 h at 45 lb/sq. in. After filtration and removal of the solvent at room temperature, the residue was recrystallized from chloroform–light petroleum to give **21** (2.4 g, 82%), m.p. 158°, $[\alpha]_D^{22}$ −14.4° (*c* 0.6, methanol) (Found: C, 42.0; H, 4.6; N, 6.8. $C_{14}H_{19}F_3N_2O_8$ calc.: C, 42.0; H, 4.75; N, 7.0%).

*2-Amino-1-*N*-(4-*L*-aspartyl)-2-deoxy-β-*D*-glucopyranosylamine* (**7**). — The amine **21** (4 g) and 1-benzyl *N*-benzyloxycarbonyl-L-aspartate (3.6 g) were dissolved in dichloromethane (150 ml), dicyclohexylcarbodiimide (2.2 g) was added, and the coupling reaction was carried out as described for the 2-acetamido derivatives. The product was eluted from silica gel (150 g) with dichloromethane–acetone (8:1). Crystallization of the major fraction from methanol gave 3,4,6-tri-*O*-acetyl-1-*N*-(*N*-benzyloxycarbonyl-4-L-aspartyl)-2-deoxy-2-trifluoroacetamido-β-D-glucopyranosylamine (**22**, 2.7 g, 35%), m.p. 203–204°, $[\alpha]_D^{20}$ +18.1° (*c*, 0.4, chloroform), which was homogeneous in t.l.c. (chloroform–methanol, 40:1); a satisfactory microanalysis was not obtained.

A solution of **22** (2.1 g) in 9:1 glacial acetic–water (80 ml) was hydrogenated over 10% palladium–charcoal (0.3 g) at 43 lb/sq. in. for 2 h. The solution was filtered, and the solvent was removed at 40°. The residue was dried *in vacuo* over potassium hydroxide–phosphorus pentaoxide to give the triacetate **23** which was used without further purification.

A solution of **23** (0.6 g) in dry methanol (30 ml) was cooled to 0° and saturated with ammonia. The solution was cooled, and the reaction was followed by t.l.c. (butyl alcohol–acetic acid–water, 10:20:5). The starting material disappeared within 24 h, giving two products. After 8 days, only the product of lower R_F value remained. The solvent was removed, and the residue was washed with acetone and recrystallized from aqueous methanol to give **7** (0.3 g, 94%) which decomposed above 200° without melting (after losing water at 150–200°) and had $[\alpha]_D^{23}$ +25.1° (*c* 0.1, water) (Found: C, 38.3; H, 6.5; N, 13.1. $C_{10}H_{19}N_3O_7 \cdot H_2O$ calc.: C, 38.6; H, 6.8; N, 13.5%). N.m.r. data (60 MHz, D_2O): τ 7.05 [2-proton doublet, *J* 5.1 Hz, aspartyl (C-3)H_2], (6.1–6.8 6-proton multiplet, H-2–H-6), 5.95 [1-proton triplet, *J* 5.1 Hz, aspartyl (C-2)H], 5.03 (1-proton doublet, $J_{1,2}$ 9.5 Hz, H-1).

*1-*N*-Acetyl-3,4,6-tri-*O*-acetyl-2-deoxy-2-trifluoroacetamido-β-*D*-glucopyranosylamine* (**24**). — A mixture of the amine **21** (0.6 g), acetic anhydride (5 ml), and dry methanol (5 ml) was stored at room temperature for 18 h. The solution was evapo-

rated at 40°, and the product was recrystallized from ethyl acetate to give **24** (0.3 g, 45%), m.p. 237–238°, $[\alpha]_D^{22}$ −1.19° (*c* 0.3, ethanol), which was homogeneous in t.l.c. (chloroform–methanol, 14:1) (Found: C, 43.2; H, 4.85; N, 6.25. $C_{16}H_{21}F_3N_2O_9$ calc.: C, 43.4; H, 4.75; N, 6.35%).

1-N-Acetyl-2-amino-2-deoxy-β-D-glucopyranosylamine (**25**). — A solution of **24** (0.9 g) in dry methanol (100 ml) was cooled to 0° and saturated with ammonia. The solution was cooled, and the reaction was followed by t.l.c. (chloroform–methanol, 1:1); after two days, all of the starting material had disappeared, with the formation of two products. After 10 days, only a trace of the product of higher R_F value remained, and after 2 weeks no further reaction was observed. The solvent was removed and the residue was recrystallized from aqueous methanol–ether to give **25** (0.32 g, 70%), m.p. 233–234° (decomp., commencing at 227°), $[\alpha]_D^{22}$ −38.1° (*c* 0.7, water), which was homogeneous in t.l.c. (chloroform–methanol, 1:1); lit.[9] m.p. 224–225° (decomp.), $[\alpha]_D^{23}$ −4.3° (water).

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