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Note

Synthesis of *O*-(2-amino-2-deoxy-β-D-glucosyl) derivatives of L-serine and L-threonine*

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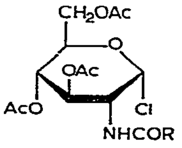
In many glycoproteins, the linkage between the carbohydrate and protein components involves the hydroxyl groups of L-serine and L-threonine. The development of methods of synthesis of compounds that contain this type of linkage is therefore important. We now report the synthesis of the model compounds **8–11**, in which the aglycon is the methyl ester or methylamide of *N*-benzyloxycarbonyl-L-serine and



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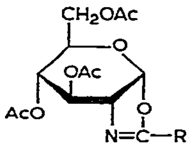

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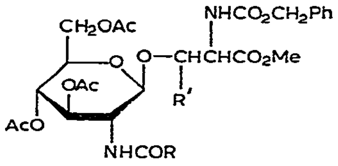

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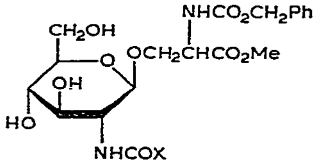
7 R = X, R' = Me



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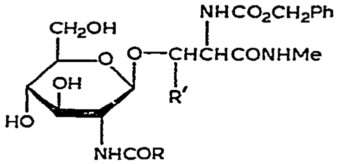
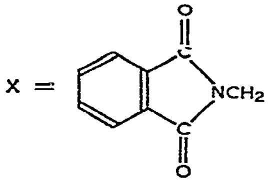


9 R = Me, R' = H
10 R = X, R' = H
11 R = X, R' = Me



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*Synthesis of Glycosides of *N*-Aminoacyl Derivatives of 2-Amino-2-deoxy-D-glucose. Part III.

-L-threonine, and the carbohydrate components are 2-acetamido-2-deoxy-D-glucose and 2-deoxy-2-(*N*-phthaloylglycylamino)-D-glucose.

The serine derivative **5** was obtained by (*a*) interaction of the oxazoline **3** with *N*-benzyloxycarbonyl-L-serine methyl ester (**12**) in the presence of toluene-*p*-sulphonic acid, (*b*) interaction of the acetochloro derivative **1** with **12** in the presence of $Hg(CN)_2$, and (*c*) the reaction of **1** and **12** in the presence of Ag_2CO_3. Methods (*a*) and (*c*) each gave a 40% yield of **5**, but the former was the most convenient procedure, because the latter was accompanied by darkening of the reaction mixture. The yield by method (*b*) was 16%.

The glycosides **6** and **7** were obtained in yields of ~30% by reaction of the oxazoline **4** (obtained from the acetochloro derivative **2**) with **12** and *N*-benzyloxycarbonyl-L-threonine methyl ester (**13**), respectively. Deacetylation of **6** with methanolic triethylamine gave **8**, and **9–11** were obtained from **5–7** by deacetylation with methanolic methylamine.

EXPERIMENTAL

General methods. — Melting points were determined on a Boethius table, and optical rotations with a Perkin–Elmer 141 polarimeter. T.l.c. was carried out on silica gel (Chemapol, Czechoslovakia) with *A*, ether; *B*, ether–ethyl acetate (4:1); *C*, ether–ethyl acetate (1:1); and *D*, ethyl acetate–ethanol (4:1). Detection was effected with Cl_2–benzidine. Column chromatography was performed on silica gel (Chemapol, Czechoslovakia, 100–160 μm) and alumina (Brockman IV). Pure solvents were used for the reactions, and dry solvents for the chromatography. The amino acid compositions of products were determined by using a JEOL amino acid analyser, after hydrolysis with 4M HCl at 100° for 18 h. N.m.r. spectra were recorded for solutions in $CDCl_3$ with a "ZKR"–60 instrument.

O-*(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-*N*-benzyloxycarbonyl-L-serine methyl ester* (**5**). — (*a*) Toluene-*p*-sulphonic acid was added to a boiling solution of 2-methyl-4,5-(3,4,6-tri-*O*-acetyl-2-deoxy-D-glucopyrano)-Δ^2-oxazoline [**3**; 0.61 g, 1.84 mmol; obtained[2] from 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride[1] (**1**), m.p. 131°, $[\alpha]_D$ +122° (*c* 1, chloroform)] and *N*-benzyloxycarbonyl-L-serine methyl ester (**12**; 0.5 g, 2 mmol) in toluene–nitromethane (1:1, 16 ml) until the solution was weakly acidic. The mixture was then boiled under reflux for 30 min, cooled to room temperature, and concentrated *in vacuo*. The residue was eluted from a column (32 × 3.2 cm) of silica gel with a gradient of benzene→ether→ethyl acetate. The fractions containing the component with R_F 0.35 (t.l.c., solvent *C*) were combined, and dried *in vacuo*, and the residue was crystallised from ethanol–ether (1:15) to give **5** (0.2 g), m.p. 168°, $[\alpha]_D^{20}$ $-5°$ (*c* 1, chloroform). N.m.r. data: δ 4.68 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1). More product was obtained from the mother liquors, to give a total of 0.4 g (40%). Hydrolysis of **5** gave serine and 2-amino-2-deoxyglucose in the ratio 1:0.84.

Anal. Calc. for $C_{26}H_{34}N_2O_{13}$: C, 53.60; H, 5.88; N, 4.80. Found: C, 53.44; H, 5.79; N, 4.60.

(*b*) To a solution of **1** (1.56 g, 6.2 mmol) in benzene (40 ml) was added $Hg(CN)_2$ (2.34 g), with stirring. The mixture was vigorously stirred and boiled, and a solution of **12** (2.27 g, 6.2 mmol) in benzene (100 ml) was added during 1 h. Benzene (400 ml) was then added with slow distillation. The cooled mixture (50 ml) was diluted with ethyl acetate (50 ml), decanted, washed with M NaCl (3 × 50 ml) and water (2 × 30 ml), dried (Na_2SO_4), and concentrated *in vacuo*. The residue was crystallised from ethanol–ether (1:15) to give **5** (195 mg, 6%). The mother liquor was concentrated, and the residue was subjected to chromatography as described in (*a*). The total yield of **5** was 16%, m.p. 165–166°, $[\alpha]_D^{20}$ $-3°$ (*c* 1, chloroform).

(*c*) To a solution of **12** (1.26 g, 5 mmol) in benzene (50 ml) was added freshly prepared Ag_2CO_3 (2.76 g). Benzene was evaporated, and a solution of **1** (3.5 g, 10 mmol) in benzene (100 ml) was added during 1.5 h, with simultaneous removal of benzene. Benzene was then added and distilled for 3 h. The mixture was concentrated *in vacuo*, and the residue was eluted from a column (25 × 2.8 cm) of silica gel by using a solvent gradient of benzene→ether→ethyl acetate. Fractions containing material with R_F 0.35 (t.l.c., solvent *C*) were combined, and concentrated *in vacuo*. The residue (1.39 g) was crystallized from ethanol–ether (1:15) to give **5**. Repeated chromatography of the mixed fractions gave more **5** (total yield, 40%), m.p. 166°, $[\alpha]_D^{20}$ $-4°$ (*c* 1, chloroform).

*2-Phthalimidomethyl-4,5-(3,4,6-tri-*O*-acetyl-2-deoxy-*D*-glucopyrano)-Δ^2-oxazoline* (**4**). — A solution of 3,4,6-tri-*O*-acetyl-2-deoxy-2-(*N*-phthaloylglycylamino)-α-D-glucopyranosyl chloride[3] (**2**, 2.8 g) in acetone (100 ml) was stirred overnight with $AgNO_3$ (1.5 g) and collidine (5 ml). Ether (100 ml) was added, and the solution was filtered through a thin layer of alumina and then concentrated *in vacuo*. The residue was triturated with hexane and dried *in vacuo* over P_2O_5 to give chromatographically homogeneous **4** (1.8 g, 78%), R_F 0.5 (t.l.c., solvent *B*). Traces of collidine were removed from the product by chromatography on alumina (elution with hexane) until the eluate was free of collidine (Dragendorf reaction). Subsequent elution with ether, followed by combination and concentration (<40°) of the fractions containing the component of R_F 0.5 (t.l.c., solvent *B*), gave **4** (50%), m.p. 118–119°, $[\alpha]_D^{20}$ $-9°$ (c 1, acetone), ν_{max} 1680 cm^{-1} (-N=C-O-). Oxazoline **4** was completely hydrolysed by 0.05M H_2SO_4 in 1 h at room temperature.

Anal. Calc. for $C_{22}H_{22}N_2O_{10}$: C, 55.58; H, 4.67; H, 5.90. Found: C, 56.16; H, 4.70; N, 6.10.

N*-Benzyloxycarbonyl-*O*-[3,4,6-Tri-*O*-acetyl-2-deoxy-2-(*N*-phthaloylglycylamino)-β-*D*-glucopyranosyl]-*L*-serine methyl ester* (**6**). — Toluene-*p*-sulphonic acid was added to a mixture of the oxazoline **4** (1.5 g, 3.6 mmol) and **12** (1 g, 4 mmol) in boiling toluene (20 ml) until the solution was weakly acidic. The mixture was then boiled under reflux for 30 min and concentrated to dryness, and the residue was eluted from silica gel with a gradient of benzene→ether→ethyl acetate. The fractions containing the component having R_F 0.35 (t.l.c., solvent *B*) were combined, and concentrated *in vacuo*, and the residue was crystallised from ethanol–ether (1:15) to give **6** (0.75 g, 35%), m.p. 199–200°, $[\alpha]_D^{20}$ $-7°$ (*c* 1, chloroform). N.m.r. data: δ 4.68 (d, 1 H, $J_{1,2}$ 8 Hz, H-1). Hydrolysis of **6** gave serine, glycine, and 2-amino-2-deoxyglucose in the ratios 1:1:0.83.

Anal. Calc. for $C_{34}H_{37}N_3O_{15}$: C, 56.12; H, 5.13; N, 5.77. Found: C, 56.33; H, 5.37; N, 5.92.

N-*Benzyloxycarbonyl*-O-*[3,4,6-tri*-O-*acetyl-2-deoxy-2-(*N-*phthaloylglycylamino)-β-*D*-glucopyranosyl]-*L*-threonine methyl ester* (**7**). — By a procedure similar to that described for the synthesis of **6**, the oxazoline **4** and *N*-benzyloxycarbonyl-L-threonine methyl ester (**13**) were allowed to react. Chromatography of the product mixture on silica gel gave a fraction, R_F 0.25 (t.l.c., solvent *B*), which was crystallised from ethanol–ether to give **7** (30%), m.p. 221°, $[\alpha]_D^{20}$ $-8°$ (*c* 1, chloroform). N.m.r. data: δ 4.67 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1).

Anal. Calc. for $C_{35}H_{39}N_3O_{15}$: C, 56.68; H, 5.30; N, 5.67. Found: C, 56.37; H, 5.55; N, 5.94.

Hydrolysis of **7** gave threonine, glycine, and 2-amino-2-deoxyglucose in the ratios 1.09:1:0.84.

O-*(2-Acetamido-2-deoxy-β-*D*-glucopyranosyl)*-N-*benzyloxycarbonyl-*L*-serine methylamide* (**9**). — To a solution of **5** (128 mg, 0.22 mmol) in methanol (15 ml) was added methanolic 4M methylamine (4 ml). The mixture was kept at room temperature for 12 h and then concentrated *in vacuo*, and the residue was kept overnight in a vacuum desiccator over P_2O_5 and then crystallised from ethanol to give **9** (47 mg), m.p. 250°. The product from the mother liquor was eluted from silica gel with a gradient of ether→ethyl acetate→ethanol, to give more **9** (total yield, 60%), $[\alpha]_D^{20}$ $-21°$ (*c* 1, *N*,*N*-dimethylformamide), R_F 0.4 (t.l.c., solvent *D*).

Anal. Calc. for $C_{20}H_{29}N_3O_9$: C, 52.74; H, 6.42; N, 9.22. Found: C, 52.90; H, 6.32; N, 9.37.

N-*Benzyloxycarbonyl*-O-*[2-deoxy-2-(*N-*phthaloylglycylamino)-β-*D*-glucopyranosyl]-*L*-serine methylamide* (**10**). — By the procedure described for **5**, **6** was converted into **10** (58%), m.p. 207°, $[\alpha]_D^{20}$ $-2°$ (*c* 1, *N*,*N*-dimethylformamide).

Anal. Calc. for $C_{28}H_{32}N_4O_{11}$: C, 56.00; H, 5.37; N, 9.33. Found: C, 56.12; H, 5.42; N, 9.64.

N-*Benzyloxycarbonyl*-O-*[2-deoxy-2-(*N-*phthaloylglycylamino)-β-*D*-glucopyranosyl]-*L*-threonine methylamide* (**11**). — By the procedure described for **5**, **7** was converted into **11** (62%), m.p. 201° (from ethanol), $[\alpha]_D^{20}$ $-1°$ (*c* 1, *N*,*N*-dimethylformamide).

Anal. Calc. for $C_{29}H_{34}N_4O_{11}$: C, 56.67; H, 5.58; N, 9.12. Found: C, 56.63; H, 5.55; N, 8.88.

N-*Benzyloxycarbonyl*-O-*[2-deoxy-2-(*N-*phthaloylglycylamino)-β-*D*-glucopyranosyl-*L*-serine* (**8**). — A solution of **6** (0.22 g) in methanolic 4% triethylamine (20 ml) was kept overnight at room temperature, and then concentrated *in vacuo*. The residue was kept for 24 h in a vacuum desiccator over P_2O_5, and then crystallized from ethanol–methanol to give **8** (115 mg, 63%), m.p. 231–232°, R_F 0.37 (t.l.c., solvent *D*).

The product from the mother liquor was eluted from silica gel with a gradient of ether→ethyl acetate→ethanol, to give more **8** (40 mg; total yield, 84%), m.p. 232° (from methanol), $[\alpha]_D^{20}$ $-22°$ (*c* 1, *N*,*N*-dimethylformamide).

Anal. Calc. for $C_{28}H_{31}N_3O_{12}$: C, 55.90; H, 5.19; N, 6.98. Found: C, 55.44; H, 5.40; N, 7.25.

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