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Note

The synthesis of *N*-(benzyloxycarbonyl)-3-*O*-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-β-D-glucopyranosyl]-L-serine methyl ester, and its condensation with activated esters of amino acids*

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The sequence Asn-X-Ser (or Thr) has been detected in most glycoproteins containing the 2-acetamido-1-*N*-(L-aspart-4-oyl)-2-deoxy-D-glucopyranosylamine protein–carbohydrate linkage[1], and it has been assumed that the L-serine (or L-threonine) residue is necessary for the biosynthesis of the glycosyl-Asn linkage. Migration of a glycosyl group from an O to an N atom is known in the pyrimidine nucleoside series[2,3], and glycosylation of L-serine residues can occur under biological conditions[4]. Thus, it was of interest to prepare a tripeptide of the type Asn-X-Ser in which a 2-acetamido-2-deoxy-α-D-glucopyranosyl group is linked to O-3 of the L-serine residue, and to attempt to transpose by chemical reaction the glycosyl group from O-3 of the L-serine residue to N-4 of the L-asparagine residue, in order to test whether such a pathway would be chemically possible. In the glycoproteins of the "mucin type", the protein–carbohydrate linkage consists of a 2-acetamido-2-deoxy-α-D-galactopyranosyl-L-serine residue[5], and synthesis of the D-glucosyl analog could serve as a model for the synthesis of the mucin type of protein–carbohydrate linkage. The synthesis of 3-*O*-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-L-serine was attempted, in order to prepare the glycosyl-tripeptides just mentioned and as a model for the synthesis of the 2-acetamido-2-deoxy-α-D-galactopyranosyl compound.

Treatment of 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-glucopyranosyl bromide[6] (**1**) with alcohols[6–10] or bases[11–13] has been reported to give either the α or β anomer, or both, of the glycosides, as **1** contains a nonparticipating

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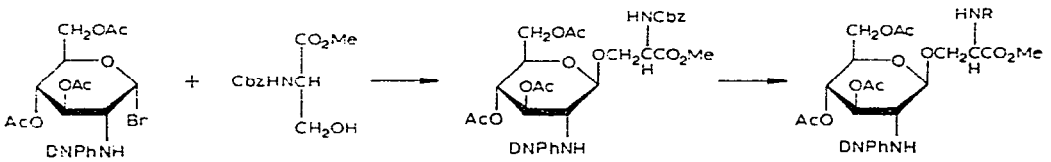
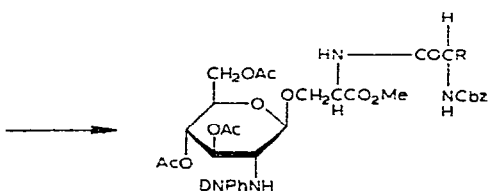
group at C-2 and this has no stereochemical control over the anomeric configuration of the condensation products. Consequently, the condensation of **1** with *N*-(benzyloxycarbonyl)-L-serine methyl ester[14] (**2**) was performed in solution in benzene containing mercuric cyanide, in the hope of obtaining a large proportion of the α anomer. After removal of mercuric cyanide and benzene, the amorphous residue showed several spots on analysis by t.l.c. The only material that could be obtained in crystalline form (yield 44%) showed in the i.r. spectrum a sharp band at 1700 cm^{-1} corresponding to the CO of the benzyloxycarbonyl group, and it gave an elemental analysis agreeing with that calculated for a triacetyl-(benzyloxycarbonyl)-deoxydinitroanilino-glucopyranosyl-serine methyl ester (**3**). Evidence for the β-D configuration of the glycoside was based on the n.m.r. data, which showed a one-proton doublet at high field (δ 5.9, $J_{1,2}$ 8.5 Hz), characteristic of the axial orientation[15].

Removal of the *N*-benzyloxycarbonyl group of **3** with hydrogen bromide in acetic acid gave the hydrobromide of 3-*O*-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-β-D-glucopyranosyl]-L-serine methyl ester (**4**) which, on treatment with triethylamine, gave **5**. The yield of compound **5** is lowered by the formation of bromide **1** by the action of hydrobromic acid on **3** (see ref. 16). The protected *p*-nitrophenyl esters of glycine[17], L-alanine[18], phenyl-L-alanine[18], and L-leucine[19] were condensed with **5**, to give the corresponding *N*-(benzyloxycarbonylamino acid)-3-*O*-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-β-D-glucopyranosyl]-L-serine methyl esters (**6**–**9**) in yields of 12–34%. The condensation of *p*-nitrophenyl esters with amino acids and peptides has previously been reported[20] to give, occasionally, unsatisfactory



Cbz = $PhCH_2OCO$
DNPh = 2,4-dinitrophenyl



6 R = H
7 R = Me
8 R = CH_2CHMe_2
9 R = $PhCH_2$

yields (~20%). These glycodipeptides were synthesized in order to test the stability of the *N*-(2,4-dinitrophenyl) derivative **5** under the conditions of synthesis of the peptide linkage.

Attempts to remove the *N*-(2,4-dinitrophenyl) group of **3** with an excess of AG-1 X-4 (OH^-) ion-exchange resin under the conditions described earlier[12], and to acetylate the resulting compound to give the known[21] 3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-*N*-(benzyloxycarbonyl)-L-serine methyl ester were unsuccessful, as the mildly basic conditions caused β-elimination of the glycosyl residue. Thus, it was not possible to verify the β-D-linkage by direct comparison with the *N*-acetyl analog[21]. In conclusion, we were unable either to obtain the desired α-D-linkage, or to replace the *N*-(2,4-dinitrophenyl) group by an *N*-acetyl group.

EXPERIMENTAL

General methods. — See ref. 21.

N-(Benzyloxycarbonyl)-3-O-[3,4,6-tri-O-acetyl-2-deoxy-2-(2,4-dinitroanilino)-β-D-glucopyranosyl]-L-serine methyl ester (**3**). — From a solution of *N*-(benzyloxycarbonyl)-L-serine methyl ester[14] (**2**, 2.25 g) in benzene (150 ml) was distilled off ~50 ml of solvent, and mercuric cyanide [$Hg(CN)_2$] (2.25 g) and benzene (30 ml) were added. Benzene (~30 ml) was distilled off, and 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-glucopyranosyl bromide[6] (4.5 g) was added. The mixture was boiled under reflux for 2 h, kept overnight at room temperature, and evaporated; the char-like residue was mixed with chloroform. The suspension was filtered, and the filtrate was successively washed with water, 20% aqueous KI, and water, dried (Na_2SO_4), and evaporated. A solution of the residue in a small volume of chloroform was diluted with anhydrous ether, to give a yellow powder (3.5 g) that crystallized from hot methanol as needles (1.8 g, 30%), m.p. 176–177°, $[\alpha]_D^{20}$ +45° (*c* 1.0, chloroform); t.l.c. in 4:1 (v/v) chloroform–ethyl acetate: R_F 0.40; ν_{max}^{KBr} 3350 (NH), 1740 (OAc), 1700 (Cbz group CO), and 1610–1520 cm^{-1} (peptide Amide I, NH, aryl C=C, and NO_2); n.m.r. data ($CDCl_3$): δ 5.90 (d, 1 H, $J_{1,2}$ 8.5 Hz), 3.80 (s, 3 H, OMe), and 2.17, 2.06, and 1.84 (3 s, 9 H, 3 $COCH_3$).

Anal. Calc. for $C_{30}H_{34}N_4O_{16}$: C, 50.99; H, 4.85; N, 7.93; O, 36.22. Found: C, 50.92; H, 4.84; N, 7.59; O, 36.08.

The total material (1.6 g) obtained by evaporation of the mother liquor was applied to a column of silica gel. Elution with a linear gradient of chloroform–ethyl acetate [1:0 to 9:1 (v/v)] gave additional **3** (0.85 g) after crystallization from methanol (total yield 2.65 g, 44%).

General procedure for the condensation of p*-nitrophenyl esters of amino acids with 3-O-[3,4,6-tri-O-acetyl-2-deoxy-2-(2,4-dinitroanilino)-β-D-glucopyranosyl]-L-serine methyl ester* (**6–9**). — To a solution of **3** (0.25 mmole) in glacial acetic acid (1 ml) was added 30% hydrogen bromide in acetic acid (1 ml). The mixture was kept for 1 h at room temperature, and the resulting hydrobromide (**4**) was precipitated by addition of

TABLE I

PROPERTIES AND ANALYTICAL DATA OF
N-(BENZYLOXYCARBONYLAMINO ACID)-3-*O*-[3,4,6-TRI-*O*-ACETYL-2-DEOXY-2-(2,4-DINITROANILINO)-β-D-GLUCOPYRANOSYL]-L-SERINE METHYL ESTERS

Compound	*M.p.* (*degrees*)	$[\alpha]_D^{25}$ [a] (*degrees*)	R_F [b]	*Yield* (%)	*Formula*	*Anal.*			ν_{max}^{KBr} (cm^{-1})
						Calc.	*Found*	*Amino acid*[c]	
6	140–141	+56 (0.5)	0.63	47	$C_{32}H_{37}N_5O_{17}$	C, 50.30 H, 4.88 N, 9.17 O, 35.59	50.16 4.81 9.11 35.70	Ser 1.00 Gly 1.08	3320 (NH), 3150 (aryl CH), 1730 (OAc), 1690 (Cbz group CO), 1650–1525 (peptide Amide I, NH, aryl C=C, and NO_2)
7	176–179	+72 (1.2)	0.67	39	$C_{33}H_{39}N_5O_{17}$	C, 50.96 H, 5.06 N, 9.01 O, 34.98	50.82 5.03 8.91 34.86	Ser 1.00 Ala 1.03	3340 (NH), 1730 (OAc), 1680 (Cbz group CO), 1650–1525 (peptide Amide I, NH, aryl C=C, and NO_2)
8	201–203	+77 (0.3)	0.98	12	$C_{36}H_{45}N_5O_{17}$	C, 52.75 H, 5.53 N, 8.54 O, 33.18	52.73 5.54 8.46 33.20	Ser 1.00 Leu 0.89	3340 (NH), 1740 (OAc), 1680 (Cbz group CO), 1650–1525 (peptide Amide I, NH, aryl C=C, and NO_2)
9	181–182 (soft. 177)	+71 (0.45)	0.86	23	$C_{39}H_{43}N_5O_{17}$	C, 54.86 H, 5.08 N, 8.20 O, 31.86	54.74 5.09 8.19 31.93	Ser 1.00 Phe 0.85	3325 (NH), 1730 (OAc), 1680 (Cbz group CO), 1650–1525 (peptide Amide I, NH, aryl C=C, and NO_2)

[a]In *N,N*-dimethylformamide; concentration in parentheses. [b]By ascending t.l.c. on precoated plates of silica gel (Merck) with 14:1 (v/v) chloroform–methanol. [c]The relative proportion of the amino acids in the hydrolyzates was determined by g.l.c. of the *N*-trifluoroacetylated butyl esters with a Perkin–Elmer model 900 chromatograph on a column of Tabsorb (Regis Chemical Co., Chicago, IL 60610) programmed for a rise of 4°/min from 75 to 225°: The glycopeptide (~4 mg) was hydrolyzed by heating with constant boiling-point HCl (0.5 ml, ~5.8M) for 24 h at 110°, followed by evaporation of the solution under high vacuum in the presence of NaOH pellets; the dry residue was heated with 3M HCl in butanol (0.5 ml) for 1 h at 100°, followed by treatment with a 25% solution of trifluoroacetic anhydride in dichloromethane (0.1 ml) for 1 h at 100°.

anhydrous ether, and rapidly filtered off. Treatment of **4** with triethylamine (35 μl) in *N,N*-dimethylformamide (2 ml) gave the free amine **5**. The *p*-nitrophenyl ester of *N*-(benzyloxycarbonyl)-glycine[17], -L-alanine[18], -phenyl-L-alanine[18], or -L-leucine[19] (0.25 mmole) was added to **5**, followed by the addition of chloroform (10 ml). The mixture was stirred overnight, the solvents were removed *in vacuo*, the residue was dissolved in chloroform, and the solution was successively washed with M HCl and water, dried (Na_2SO_4), and evaporated. The syrup was crystallized from ethanol; the characteristics of the resulting components are reported in Table I.

Attempt to remove the N-*(2,4-dinitrophenyl) group of* **3**. — A solution of **3** (30 mg) in acetone (10 ml) and water (3 ml) was stirred with an excess of AG-1 X-4 (OH^-) ion-exchange resin (200–400 mesh) at 45–50° until it became colorless. The resin was filtered off, and washed thoroughly with water. The filtrate and washings were combined, and evaporated *in vacuo*. The last trace of moisture was removed by drying the amorphous powder *in vacuo* in the presence of P_2O_5. The residue was treated with pyridine (1 ml) and acetic anhydride (1 ml), and the solution kept overnight. The mixture was poured into iced water (10 ml), and extracted with chloroform. The extract was dried (Na_2SO_4), and evaporated. On examination by t.l.c., spots that migrated at the same rate as those of 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-D-glucopyranose [R_F 0.37 in 19:1 (v/v) chloroform–ethanol] and *N*-(benzyloxycarbonyl)-L-serine, [R_F 0.50 in 1:1 (v/v) chloroform–ethanol] were detected, along with decomposed material that gave a streak.

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