Carbohydrate Research, 56 (1977) 183–187

Note

Peptide and aminoacyl derivatives of *p*-nitrophenyl 2-amino-2-deoxy-β-D-glucopyranoside

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(Received May 26th, 1976; accepted for publication, September 7th, 1976)

For investigations of the substrate specificity of a new β-D-hexosaminidase[1] from the mushroom *Hohenbuechelia serotina*, we have synthesised *p*-nitrophenyl-2-aroylamino-2-deoxy-β-D-glucopyranosides[2] and *p*-nitrophenyl 2-acylamino-2-deoxy β-D-glucopyranosides[3]. In extending these studies, we now describe the synthesis of some aminoacyl and peptide derivatives.

Condensation of protected amino acids and peptides with *p*-nitrophenyl 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranoside was effected in the presence of dicyclohexylcarbodiimide or by procedures involving mixed anhydrides. Deacetylation of the products (Table I) gave the compounds listed in Table. II. Some of these compounds were also obtained by a method previously used for synthesizing substituted *N*-aroyl and *N*-acyl derivatives of 2-amino-2-deoxyglucosides[2,3].

Only the *N*-benzoylglycyl (**10**) and *N*-benzyloxycarbonylglycyl (**13**) derivatives of *p*-nitrophenyl 2-amino-2-deoxy-β-D-glucopyranoside were cleaved at a significant rate by the β-D-hexosaminidase from *H. serotina*.

EXPERIMENTAL

General methods. — P.c. (1-butanol–water–acetic acid, 4:1:1) and electrophoresis (pyridine–acetate buffer, pH 4.3; 12 ml of pyridine and 4 ml of acetic acid made up to 1 litre with water) were performed on Whatman No. 1 paper. T.l.c. was performed on Siluphol with ethyl acetate. Melting points were determined on a Boethius table, and optical rotations were measured with a Perkin–Elmer Model 141 polarimeter.

Enzymic hydrolysis was effected by the addition of enzyme solution (0.1 ml) in 0.025M phosphate buffer (pH 4) to a 0.5mM solution (0.2 ml) of glycoside in 0.1M phosphate buffer (pH 4) containing M NaCl. After 30 min, the reaction was terminated by adding M sodium carbonate (1 ml), and the liberated *p*-nitrophenol was determined spectrophotometrically at 400 nm.

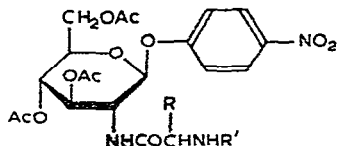

TABLE I

DATA FOR SOME *p*-NITROPHENYL 3,4,6-TRI-*O*-ACETYL-2-ACYLAMINO-2-DEOXY-β-D-GLUCOPYRANOSIDES

Compound	*R*	*R'*	*Formula*	*Method of synthesis*
4	H	Bz	$C_{27}H_{29}N_3O_{12}$	A B C
5	H	Ac	$C_{22}H_{27}N_3O_{12}\cdot H_2O$	A B
6	H	BzlOCO	$C_{28}H_{31}N_3O_{13}$	A B
7	H	$BzlOCONHCH_2CO$	$C_{30}H_{34}N_4O_{14}$	B
8	H	$AcNHCH_2CO$	$C_{24}H_{30}N_4O_{13}$	B
9	$CH_2CH_2CO_2Me$	BzlOCO	$C_{32}H_{37}N_3O_{15}$	B C

[a]In chloroform.

N-*Acylglycyl derivatives of 2-amino-2-deoxy*-D-*glucose.* — 0.01M Hippuric acid and 2-amino-2-deoxy-D-glucose were reacted[4] in the presence of dicyclohexylcarbodiimide. After deionising the reaction mixture with Amberlite IRC-50 (H^+) and IRA-45 (HO^-) resins, the eluate was concentrated *in vacuo*, and the residue was recrystallized from methanol to give 2-[(*N*-benzoylglycyl)amino]-2-deoxy-D-glucose (**1**, 22%), m.p. 202–203°, $[\alpha]_D^{20}$ +37° (*c* 0.5, pyridine–water, 1:1), +30° (*c* 0.66, *N*,*N*-dimethylformamide), R_F 0.6 (p.c.).

Anal. Calc. for $C_{15}H_{20}N_2O_7$: C, 52.92; H, 5.92; N, 8.23. Found: C, 52.88; H, 6.34; N, 7.87.

2-[(*N*-Acetylglycyl)amino]-2-deoxy-D-glucose (**2**, 60%), obtained by reactions similar to those for **1**, had m.p. 204° (from ethanol), $[\alpha]_D^{20}$ +56° (*c* 0.65, pyridine–water, 1:1), R_F 0.25 (p.c.).

Anal. Calc. for $C_{10}H_{17}N_2O_7$: C, 43.14; H, 6.52; N, 10.07. Found: C, 43.43; H, 6.53; N, 9.87.

3,4,6-Tri-O-acetyl-2-[(N-*benzyloxycarbonylglycyl)amino*]-*2-deoxy*-α-D-*glucopyranosyl chloride* (**3**). — A suspension of 2-(*N*-benzyloxycarbonylglycyl)amino-2-deoxy-D-glucose[4] (0.1 g) in freshly distilled acetyl chloride (2 ml) was saturated for 15 min with dry hydrogen chloride at −15°. The mixture was stored in a closed vessel

Yield (%)	M.p. (degrees)	$[\alpha]_D^{20}$ (degrees)	Analysis					
			Calc. (%)			Found (%)		
			C	H	N	C	H	N
68	240	−13	55.19	4.98	6.80	55.32	5.28	7.18
52								
73								
15	213	−20	48.62	5.37	7.73	48.86	5.18	7.46
61	216	−22						
35	199	−15	54.46	5.06	6.80	54.48	5.22	6.24
66								
68	168	−18	53.41	5.08	8.30	53.07	5.38	8.20
76	232	−12	49.48	5.19		49.75	5.24	
70	228	−26	54.62	5.30	5.97	54.94	5.55	5.74
78								

for 14 h at 20°, and then concentrated *in vacuo* at <40°. The residue was dried *in vacuo* over KOH and eluted from a column (0.5 × 4 cm) of silica gel with ether. The fractions containing the substance with R_F 0.7 (t.l.c.) were combined, and concentrated *in vacuo* to give **3** (85 mg, 60%), m.p. 89° (from ethyl acetate–ether), $[\alpha]_D^{20}$ +85° (*c* 1, acetone).

Anal. Calc. for $C_{22}H_{27}ClN_2O_{10}$: C, 51.60; H, 5.07; N, 5.10; Cl, 6.92. Found: C, 51.30; H, 5.28; N, 5.44; Cl, 6.90.

Preparation of p-*nitrophenyl 3,4,6-tri-*O-*acetyl-2-acylamino-2-deoxy-β-*D-*glucopyranosides.* — (*a*) *Method A.* A mixture of sodium *p*-nitrophenoxide (0.4 g) and a solution of unpurified **3** [obtained from 1.3 mmol of 2-(*N*-benzyloxycarbonylglycyl)amino-2-deoxy-D-glucose] in *N*,*N*-dimethylformamide (3 ml) was stirred for 16 h at room temperature, then poured into cold water (100 ml), and extracted with chloroform (5 × 10 ml). The extract was washed with aqueous 3% Na_2CO_3 until free from unreacted phenol, then dried, and concentrated *in vacuo*. The residue was eluted from silica gel with benzene, ether–benzene, and ether. The fractions containing the substance with R_F 0.54 (t.l.c.) were combined, and concentrated *in vacuo* to give *p*-nitrophenyl 3,4,6-tri-*O*-acetyl-2-[(*N*-benzyloxycarbonylglycyl)amino]-2-deoxy-β-D-glucopyranoside (**6**; 0.2 g, 35%), m.p. 199° (from ethanol), $[\alpha]_D^{20}$ −15° (*c* 1, chloroform).

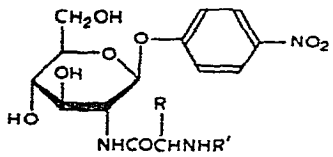

TABLE II

DATA FOR SOME *p*-NITROPHENYL 2-ACYLAMINO-2-DEOXY-β-D-GLUCOPYRANOSIDES

Compound	*R*	*R'*	*Formula*
10	H	Bz	$C_{21}H_{23}N_3O_9$
11	H	Ac	$C_{16}H_{21}N_3O_9$
12	H	BzlOCO	$C_{22}H_{25}N_3O_{10}\cdot C_2H_5OH$
13	H	$BzlOCONHCH_2CO$	$C_{24}H_{28}N_4O_{11}$
14	H	$AcNHCH_2CO$	$C_{18}H_{24}N_4O_{10}$
15	$CH_2CH_2CO_2Me$	BzlOCO	$C_{26}H_{31}N_3O_{12}$

[a] In *N,N*-dimethylformamide.

p-Nitrophenyl 3,4,6-tri-*O*-acetyl-2-[(*N*-benzoylglycyl)amino]-2-deoxy-β-D-glucopyranoside (**4**, 68%), obtained from the corresponding glycosyl chloride, had m.p. 240° (from ethanol), $[\alpha]_D^{20}$ −16.5° (*c* 0.66, pyridine–water, 1:1), −13° (*c* 1, chloroform).

p-Nitrophenyl 3,4,6-tri-*O*-acetyl-2-[(*N*-acetylglycyl)amino]-2-deoxy-β-D-glucopyranoside (**5**, 15%), obtained from the corresponding glycosyl chloride, had m.p. 213° (from methanol), $[\alpha]_D^{20}$ −20° (*c* 1, chloroform).

Analytical and other data for **4**–**6** are given in Table I.

(*b*) *Method B.* To 1 mmol of *p*-nitrophenyl 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranoside hydrochloride[5,6] in 90% aqueous tetrahydrofuran (10 ml), 1 mmol of triethylamine was added with vigorous stirring and cooling in ice. The solution was stirred in the cold for 20 min with subsequent addition of 1.2 mmol of *N*-acylamino acid and 1.2 mmol of dicyclohexylcarbodiimide in small portions. Stirring was continued for 2 h at 5° and then for 16 h at room temperature. *N,N'*-Dicyclohexylurea was collected, and washed with a small amount of tetrahydrofuran, and the filtrate was stirred with KU-2(H^+) resin (6 ml) for 10 min, then filtered, and concentrated at <40°. A solution of the residue in chloroform was washed successively with aqueous 3% Na_2CO_3 and water, dried (Na_2SO_4), filtered, and concentrated. The residue was recrystallized from ethanol. The yields of products obtained by this method are given in Table I.

(*c*) *Method C.* To a solution of 1 mmol of *N*-acylamino acid or peptide and 1 mmol of triethylamine in toluene (7 ml) at −5°, 1 mmol of methyl chloroformate

Yield (%)	M.p. (degrees)	$[\alpha]_D^{20}$ [a] (degrees)	Analysis					
			Calc. (%)			Found (%)		
			C	H	N	C	H	N
77	210	−21.5	54.66	5.02	9.11	54.38	5.17	9.08
83	208	−45	48.12	5.30	10.52	48.37	5.54	10.10
71	192	−21	53.63	5.81	7.82	53.83	5.70	7.95
75	200	−23	52.55	5.15	10.21	52.06	5.31	10.59
80	214	−22	47.37	5.30	12.28	47.22	5.25	12.01
78	198	−25	54.07	5.41	7.28	53.63	5.57	6.87

was added with stirring. The mixture was stirred vigorously for 25 min at −5° with subsequent addition, in small portions, of a cold solution of 1 mmol each of *p*-nitrophenyl 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranoside hydrochloride[5,6] and triethylamine in dry chloroform. The mixture was allowed to warm up to room temperature, kept thereat for 4 h, and then washed successively with M HCl (2 × 15 ml), aqueous 3% Na_2CO_3 (3 × 15 ml), and water. The organic layer was dried (Na_2SO_4), and concentrated *in vacuo*, and the residue was recrystallized from ethanol. The yields and properties of products obtained by this method are shown in Table I.

p-*Nitrophenyl 2-acylamino-2-deoxy-β-D-glucopyranosides.* — The triacetates in Table I were deacetylated with 10% triethylamine in methanol at room temperature for 8–10 h. Each reaction mixture was concentrated *in vacuo*, and the residue was dried *in vacuo* over P_2O_5 for 4–6 h and then recrystallized from ethanol. The properties of the products are shown in Table II.

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