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Note

p-Nitrophenyl glycosides of some 2-acylamido-2-deoxy-D-glucoses

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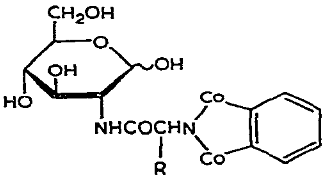
The effect of *N*-acetyl-β-D-glucosaminidase from pig epididymis on various *N*-acyl derivatives of methyl 2-amino-2-deoxy-β-D-glucopyranoside has been investigated by Leaback and Walker[1]. We now report on the synthesis of two *p*-nitrophenyl 2-acylamido-2-deoxy-β-D-glucosides, which are potential substrates for this enzyme.

The reaction of 2-amino-2-deoxy-D-glucose with *N*-phthaloylglycine or *N*-phthaloyl-DL-alanine in the presence of dicyclohexylcarbodiimide in aqueous pyridine[2,3] gave the *N*-acyl derivatives **1** and **2**. Derivatives of this type are also obtainable in small yield by the reaction of 2-amino-2-deoxy-D-glucose with the acyl chloride of the *N*-protected amino acid in aqueous *p*-dioxane in the presence of magnesium oxide.



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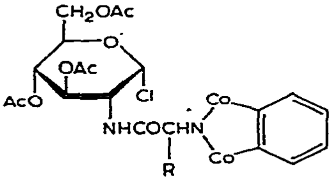
2 R = Me



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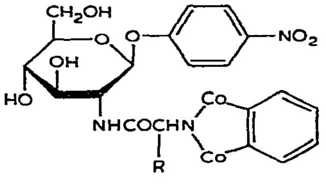

8 R = H
9 R = Me

Treatment of the phthalimido derivatives **1** and **2** with dry hydrogen chloride in acetyl chloride gave ~60% of the corresponding acetochloro derivatives **3** and **4**. Reaction of the glycosyl chloride **3** in aqueous acetone with lead carbonate gave 3,4,6-tri-*O*-acetyl-2-deoxy-2-(*N*-phthaloylglycylamido)-D-glucopyranose (**5**), from which **1** was regenerated by deacetylation.

The glycosyl chlorides **3** and **4** reacted[4] with sodium or potassium *p*-nitrophenolate to yield ~75% of the corresponding acetylated *p*-nitrophenyl β-D-glycosides (**6** and **7**), saponification of which with methanolic triethylamine gave *p*-nitrophenyl 2-deoxy-2-(*N*-phthaloylglycylamido)-β-D-glucopyranoside (**8**) and the alanine analogue **9**, respectively.

The α-D configuration of **3** and **4** was indicated by the n.m.r. data ($J_{1,2}$ 3.5 Hz) for H-1. Likewise, the β-D configuration of **6** and **7** was established ($J_{1,2}$ 8.2–8.3 Hz).

EXPERIMENTAL

General methods. — Chromatography (p.c.) was performed on paper C (middle), Leningrad Factory No. 2, by the ascending method with 1-butanol–water–acetic acid (4:1:1). Column chromatography was performed on silica gel KSK (0.125–0.068 mm) pre-activated at 150° for 3 h. Thin-layer chromatography (t.l.c.) was performed on silica gel KSK. Detection was effected with alkaline silver nitrate, 0.2% of ninhydrin in acetone (at 105°), or chlorine–benzidine. M.p.s. were determined on a Kofler hot-stage, and optical rotations were obtained by using a Perkin–Elmer 141 polarimeter.

2-Deoxy-2-(N-phthaloylglycylamido)-D-glucose (**1**). — (*a*) To a solution of 2-amino-2-deoxy-D-glucose hydrochloride (2.2 g) in 2M sodium hydroxide (5 ml) at 0°, a solution of *N*-phthaloylglycine (2.1 g) in pyridine (15 ml) was added followed by a solution of dicyclohexylcarbodiimide (3 g) also in pyridine (30 ml). The mixture was then stirred overnight at room temperature. Water (100 ml) was added, and *N,N'*-dicyclohexylurea was collected and washed with boiling water. The combined filtrate and washings were evaporated *in vacuo* at 40° to a small volume. The product that separated was collected, washed with ethanol, and crystallized from water to give **1** (60%), m.p. 229°; $[\alpha]_D^{20}$ +40° (*c* 0.5, water–pyridine), +45° (*c* 0.68, *N,N*-dimethylformamide); R_F 0.5 (p.c.).

Anal. Calc. for $C_{16}H_{18}N_2O_8 \cdot H_2O$: C, 50.00; H, 5.24. Found: C, 50.15; H, 5.28.

A water-free compound was obtained after drying **1** *in vacuo* at 60° for 20 h over P_2O_5.

Anal. Calc. for $C_{16}H_{18}N_2O_8$: C, 52.45; H, 5.39; N, 7.64. Found: C, 52.12; H, 5.38; N, 7.28.

(*b*) A solution of *N*-phthaloylglycyl chloride (prepared by the treatment of 0.42 g of *N*-phthaloylglycine with an excess of thionyl chloride in benzene) in *p*-dioxane (5 ml) was added dropwise during 10 min to a mixture of 2-amino-2-deoxy-D-glucose hydrochloride (0.22 g) and magnesium oxide (0.2 g) in water (8 ml) at ~0°. The mixture was stirred overnight and then filtered. The insoluble material was recrystallised from water to yield **1** (0.16 g, 45%).

2-Deoxy-2-(N-phthaloyl-DL-alanylamido)-D-glucose (**2**). — *N*-Phthaloylalanine (0.01 mole) and 2-amino-2-deoxy-D-glucose (from 0.01 mole of the hydrochloride) were treated with dicyclohexylcarbodiimide as described in (*a*) above. After removal of the *N,N'*-dicyclohexylurea, the filtrate was evaporated *in vacuo* and the residue was extracted with hot ethanol (2×5 ml). The combined extracts were evaporated and the residue was eluted from a column (2.4×20 cm) of silica gel by ethyl acetate containing an increasing concentration of ethanol. Concentration of the appropriate fractions gave **2** (1.5 g, 42%), m.p. 199–201° (from ethanol); $[\alpha]_D^{20}$ +43° (*c* 1, water–pyridine), +45° (*c* 0.66, *N,N*-dimethylformamide); R_F 0.6 (p.c.).

Anal. Calc. for $C_{17}H_{20}N_2O_8$: C, 53.68; H, 5.30; N, 7.37. Found: C, 53.64; H, 5.59; N, 7.21.

*3,4,6-Tri-*O*-acetyl-2-deoxy-2-(*N*-phthaloylglycylamido)-α-*D*-glucopyranosyl chloride* (**3**). — A suspension of **1** (0.366 g) in freshly distilled acetyl chloride (10 ml) was saturated with dry hydrogen chloride at −20°. The mixture was stored in a closed vessel for 16 h at 20° and then evaporated *in vacuo*. Absolute ethyl acetate was twice distilled from the residue, which was then dried and crystallised from benzene to give **3** (0.28 g). The mother liquor was evaporated and the residue was eluted from silica gel with ether to give an additional amount of **3** (total yield 59%), m.p. 165–166° (from ethyl acetate–ether), $[\alpha]_D^{20}$ +102° (*c* 0.68, acetone), R_F 0.8 (t.l.c., ether).

Anal. Calc. for $C_{22}H_{23}ClN_2O_{10}$: C, 51.72; H, 4.53; Cl, 6.93; N, 5.48. Found: C, 51.63; H, 4.80; Cl, 6.49; N, 5.75.

Also obtained in the above chromatographic separation was 3,4,6-tri-*O*-acetyl-2-deoxy-2-(*N*-phthaloylglycylamido)-D-glucose (**5**, 150 mg), m.p. 207° (from ethyl acetate–ether), $[\alpha]_D^{20}$ +56° (*c* 0.5, acetone), R_F 0.5.

Anal. Calc. for $C_{22}H_{24}N_2O_{11}$: C, 53.66; H, 4.91; N, 5.69. Found: C, 53.65; H, 5.21; N, 5.62.

A solution of **3** (0.1 g) in 75% aqueous acetone (10 ml) was stirred with lead carbonate (0.15 g) for 16 h. Insoluble material was collected and washed twice with acetone (5 ml), and the combined filtrate and washings were evaporated *in vacuo*. The residue was crystallized from ethyl acetate–ether to give **5** (40 mg), m.p. 207°.

*3,4,6-Tri-*O*-acetyl-2-deoxy-2-(*N*-phthaloyl-*DL*-alanylamido)-α-*D*-glucopyranosyl chloride* (**4**). — Treatment of **2** with acetyl chloride, as described above for **1**, gave **4** (60%), m.p. 164° (from ether), $[\alpha]_D^{20}$ +90° (*c* 1, acetone), R_F 0.75 (t.l.c., ether). N.m.r. data (60 MHz): δ 6.28 (*d*, $J_{1,2}$ 3.5 Hz, H-1).

Anal. Calc. for $C_{23}H_{25}ClN_2O_{10}$: C, 52.63; H, 4.80; Cl, 6.75; N, 5.34. Found: C, 52.72; H, 4.95; Cl, 7.12; N, 4.94.

p-*Nitrophenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-(*N*-phthaloylglycylamido)-β-*D*-glucopyranoside* (**6**). — A mixture of **3** (0.58 g), sodium *p*-nitrophenolate (0.332 g), and *N,N*-dimethylformamide was stirred at 20° for 16 h, and then diluted with cold water (50 ml). After 1 h, the product was collected, washed with water, alcohol, and ether, and crystallized from ethanol to give **6** (75%), m.p. 246–247°; $[\alpha]_D^{20}$ −4° (*c* 1, pyridine), +7° (*c* 0.66, *N,N*-dimethylformamide). N.m.r. data: δ 5.51 (*d*, $J_{1,2}$ 8.3 Hz, H-1).

Anal. Calc. for $C_{28}H_{27}N_3O_{13} \cdot 0.5H_2O$: C, 54.10; H, 4.50; N, 6.76. Found: C, 53.94; H, 4.51; N, 6.71.

p-*Nitrophenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-(*N*-phthaloyl-*DL*-alanylamido)-β-*D*-glucopyranoside* (**7**). — Treatment of **4**, essentially as described above for **3**, gave **7** (45%), m.p. 234°, $[\alpha]_D^{20}$ −65° (*c* 0.7, *N,N*-dimethylformamide). N.m.r. data: δ 5.41 (*d*, $J_{1,2}$ 8.2 Hz, H-1).

Anal. Calc. for $C_{29}H_{29}N_3O_{13}$: C, 55.50; H, 4.63; N, 6.71. Found: C, 55.55; H, 4.65; N, 6.69.

p-*Nitrophenyl 2-deoxy-2-(*N*-phthaloylglycylamido)-β-*D*-glucopyranoside* (**8**). —

Compound **6** (0.6 g) was stirred with 10% methanolic triethylamine (40 ml) at 20° for 16 h. The solution was then evaporated *in vacuo*, and methanol was evaporated three times from the residue which was then crystallized from aqueous ethanol to give **8** (0.33 g). Additional amounts of **8** were obtained from the mother liquor (total yield 85%), m.p. 237°, $[\alpha]_D^{20}$ +42° (*c* 1, water–pyridine), R_F 0.65 (p.c.).

Anal. Calc. for $C_{22}H_{21}N_3O_{10}$: C, 54.21; H, 4.44; N, 8.62. Found: C, 53.90; H, 4.55; N, 8.49.

p-*Nitrophenyl 2-deoxy-2-(*N*-phthaloyl-*DL*-alanylamido)-β-*D*-glucopyranoside* (**9**). — Treatment of **7**, as described above for **6**, gave **9** (70%), m.p. 216° (from water–ethanol), $[\alpha]_D^{20}$ −60° (*c* 0.68, *N*,*N*-dimethylformamide).

Anal. Calc. for $C_{23}H_{23}N_3O_{10}$: C, 55.08; H, 4.61; N, 8.38. Found: C, 54.46; H, 4.62; N, 8.38.

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