A Simple General Synthesis of 2,4-Dinitrophenyl Glycopyranosides

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The acetyl groups of the *O*-acetyl derivatives of 2.4-dinitrophenyl glycopyranosides can readily be removed by hydrogen chloride in methanol or methanol–chloroform, without concomitant removal of the aglycone.

2,4-DINITROPHENYL glycosides are useful substrates for glycosidases, since the good 2,4-dinitrophenolate leaving group may make the hydrolysis of any glycosyl-enzyme intermediate kinetically accessible.[1] However, it has been difficult to synthesise these compounds since dinitrophenyl glycosides. The fully acetylated derivatives themselves can be synthesised readily by condensation of 2,4-dinitrophenol with the acetohalogenosugar under basic conditions. In our hands the conventional use of sodium 2,4-dinitrophenolate in aqueous

TABLE 1

2,4-Dinitrophenyl per-*O*-acetylglycopyranosides

Sugar residue	Yield (%)	Method	M.p. (°C)	Lit.m.p. (°C)	$[\alpha]_D^{25}$(°) (*c* 1 in $CHCl_3$)	Lit.[α] D(°)	Formula	Required (%) C	H	N	Found (%) C	H	N
β-D-Xylopyranosyl	7·7	A	151—153	—	−89	—	$C_{17}H_{18}N_2O_{12}$	46·15	4·1	6·35	46·6	4·2	56·2
	28	B											
α-L-Arabinopyranosyl	55	B	128—131	—	−16	—					46·2	4·15	6·25
β-D-Glucopyranosyl	14·4	A	177—179	173—177 [a]	+33	+34·5 (*c* 4 in $CHCl_3$) [a]	$C_{20}H_{22}N_2O_{14}$	46·7	4·3	5·45	46·95	4·4	5·2
β-D-Glactopyranosyl	15	A	180—182	174—176 [b]	+68	+74 (*c* 1·44 [b] in $CHCl_3$)							
6-Deoxy-β-D-glucopyranosyl	19 [c]	B	152—156		+235		$C_{18}H_{20}N_2O_{12}$	47·35	4·4	6·15	47·65	4·5	6·0
6-Deoxy-β-D-galactopyranosyl	20 [c]	B	169—172		+74						47·6	4·5	5·95
6-Chloro-6-deoxy-β-D-glucopyranosyl	31	B	215—216		+20		$C_{18}H_{19}N_2O_{12}Cl$	44·05	3·85	5·7	44·1	3·8	5·55
2-Acetamido-2-deoxy-β-D-glucopyranosyl	17	A	161—162		+19		$C_{20}H_{23}N_3O_{13}$	46·8	4·5	8·2	47·15	4·5	8·45
β-Chitobiosyl (NAG_2)	11	A	171—172	191—192 [d]	(*c* 0·1, MeOH) −8·3 (*c* 0·1 in MeOH)	−7·5 [d] (*c* 0·1 in MeOH)	$C_{32}H_{40}N_4O_{20}$	48·0	5·05	7·0	47·95	5·15	6·8

[a] W. W. Pigman, *J. Res. Natl. Bureau Stds.*, 1944, **33**, 129. [b] Ref. 2. [c] From the sugar. [d] G. Lowe, G. Sheppard, M. L. Sinnott, and A. Williams, *Biochem. J.*, 1967, **104**, 893.

TABLE 2

2,4-Dinitrophenyl glycopyranosides

Sugar residue	Yield (%)	Method	M.p. (°C)	Lit.m.p. (°C)	$[\alpha]_D^{25}$(°)	Lit.[α] D^{25}(°)	Formula	Required (%) C	H	N	Found (%) C	H	N
β-D-Xylopyranosyl [e]	51	1	148—150	158—159 [a]	−121 (*c* 1 in MeOH)	−105 [a] (*c* 1·1 in MeOH)	$C_{11}H_{12}N_2O_9$	41·75	3·8	8·85	41·6	4·05	8·45
α-L-Arabinopyranosyl [d]	21	1	158—160	167 [a]	−94 (*c* 1 in Me_2N·CHO)	−103 [a] (*c* 1·05 in Me_2N·CHO)					41·65	3·9	8·55
β-D-Glucopyranosyl [e]	67	1	98—100	100—101 [a]	−102 (*c* 1 in MeOH	−93 [a] (*c* 1·06 in MeOH)	$C_{12}H_{14}N_2O_{10}$	41·6	4·05	8·1	43·65	4·9	7·1
							$C_{12}H_{14}N_2O_{10},C_2H_6O$ [a]	44·55	4·95	6·95			
β-D-Galactopyranosyl [d]	52	1	161—163 [b]	150—151 [a]	−97 (*c* 1 in	−105 (*c* 1 in	$C_{12}H_{14}N_2O_{10}$	41·6	4·05	8·1	40·8 [b]	4·1 [b]	7·7 [b]
	70	2			MeOH)	MeOH)							
6-Deoxy-β-D-Glucopyranosyl	60	1	142—145		−150 (*c* 1 in MeOH)		$C_{12}H_{14}N_2O_9$	43·65	4·25	8·5	43·15	4·15	8·0
6-Deoxy-β-D-Galactopyranosyl [d]	83	1	135—136		−87 (*c* 1 in MeOH)						44·2	4·55	8·3
6-Chloro-6-deoxy-β-D-glucopyranosyl [e]	27	1	86—88		−133 (*c* 1 in MeOH)		$C_{12}H_{13}N_2O_9Cl$	41·3	3·7	8·0			
							$C_{12}H_{13}N_2O_9Cl,H_2O$	37·65	3·9	7·3	37·65	3·9	7·2
2-Acetamido-2-deoxy-β-D-glucopyranosyl	38	2	124—125		+23 (*c* 1 in MeOH)		$C_{14}H_{17}N_3O_{10}$	43·4	4·6	10·85	41·65	4·35	10·45
							$C_{14}H_{17}N_3O_{10},H_2O$	41·5	4·7	10·35			
β-Chitobiosyl (NAG_2) [e,f]	62	2	183—185	191—192° [g] (decomp.)									

[a] Ref. 2. [b] Taken from ref. 1*b*. [c] Substrate for β-glucosidase of sweet almond meal (B.D.H.). [d] Substrate for β-galactosidase of *E. coli* (Boehringer) and *E. coli* K40 (Worthington). [e] Substrate for hens' egg white lysozyme. [f] This compound was not analysed, but released 98% of the theoretical quantity of 2,4-dinitrophenol on treatment with N-sodium hydroxide. The i.r. spectrum showed the presence of an amide group (1650 and 1560 cm^{-1}) and the absence of *O*-acetyl group. On t.l.c. on silica gel G the compound gave a single spot, R_F 0·31 (1:1:1 methanol–ethyl acetate–benzene); $\lambda_{max.}$ 275 nm (methanol). [g] Table 1, ref.

attempted Zemplén deacetylation of the corresponding *O*-acetyl compounds removes the aglycone. So far the only reported general synthesis of the deprotected glycosides is that of Hengstenberg and Wallenfels, from the corresponding ethyl 1-thioglycosides using the trimethylsilyl protecting group.[2]

We now report that deacetylation of the *O*-acetyl derivatives in methanol or methanol–chloroform containing hydrogen chloride leads smoothly to the 2,4-acetone (Method A) tended to give lower yields than the use of anhydrous potassium carbonate in refluxing anhydrous acetone, as recommended by Latham *et al.*[3] (Method B), except with the 2-acetamido-2-deoxy-sugars when migration of the *N*-acetyl group to the 1-position took place to the exclusion of glycoside formation. Yields and characterisation data of the protected and deprotected glycosides are given in Tables 1 and 2.

[1] (*a*) J. D. G. Sutherland, Ph.D. Thesis, Glasgow, 1973; (*b*) M. L. Sinnott and O. M. Viratelle, *Biochem. J.*, 1973, **133**, 89.

[2] W. Hengstenberg and K. Wallenfels, *Carbohydrate Res.*, 1969, **11**, 85.

[3] H. G. Latham, E. L. May, and E. Mosettig, *J. Org. Chem.*, 1950, **15**, 884.

EXPERIMENTAL

Acetohalogeno-sugars.— 2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl bromide, m.p. 90—91° (lit.,[4] 87—88°), its C-4 epimer, m.p. 81—83° (lit.,[4] 83—84°), 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide, m.p. 96—99° (lit.,[4] 100—101°), and its C-4 epimer, m.p. 139—141° (lit.,[4] 138—139°), were made without difficulty by the method of Bárczai-Marcos and Körösy;[5] 2,3,4-tri-*O*-acetyl-6-deoxy-α(?)-D-glucopyranosyl bromide and its C-4 epimer were obtained by the same method, and were used immediately without further purification. 2,3,4-Tri-*O*-acetyl-6-chloro-6-deoxy-α-D-glucopyranosyl bromide, m.p. 159—165° (lit.,[6] 165—166°) was made according to the method of Helferich and Bredereck.[6]

Treatment of 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-D-glucopyranose and octa-*O*-acetylchitobiose with acetyl chloride and hydrogen chloride in dry ether [7] for 30 h at room temperature, followed by evaporation under reduced pressure, removal of residual solvent by threefold co-distillation with dry benzene, and crystallisation of the residue from chloroform–ether yielded the crude α-glycopyranosyl chlorides, which were used without further purification.

Acetylated 2,4-Dinitrophenyl Glycopyranosides.—Examples of both methods are given.

Method A. 2,4-Dinitrophenyl 2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranoside. To a solution of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride (17·3 g) and 2,4-dinitrophenol (26·2 g) in acetone (500 ml) was added aqueous N-sodium hydroxide (142 ml). After 20 h at room temperature, the solution was partly evaporated under reduced pressure. The resulting aqueous slurry was filtered and the residue was washed thoroughly with cold saturated sodium hydrogen carbonate solution and then with distilled water. Recrystallisation from methanol afforded the *product* (4·2 g, 17%).

Method B. 2,4-Dinitrophenyl 2,3,4-Tri-O-acetyl-α-L-arabinopyranoside.—To a solution of 2,4-dinitrophenol (5 g) and 2,3,4-tri-*O*-acetyl-β-L-arabinopyranosyl bromide (10 g) in anhydrous acetone (100 ml) was added anhydrous potassium carbonate (5 g). The suspension was heated for 20 h under reflux, cooled, and diluted with water (100 ml). The resulting crystals were filtered off, and the mother liquor was further diluted with water (300 ml) and saturated aqueous sodium hydrogen carbonate (20 ml). After 20 min agitation, the second crop of crystals was filtered off, and the combined crops were recrystallised from methanol, yielding the *product* (7·2 g, 55%).

2,4-Dinitrophenyl Glycopyranosides.—Method A: Use of methanol alone. The acetyl glycoside (16 mg ml^{-1}) was suspended in 3—5% (w/v) hydrogen chloride in absolute methanol, and the suspension was agitated at 22° until homogeneous. After 48 h at 0° this solution was evaporated under reduced pressure (bath temp. <20°). The resulting usually crystalline residue was triturated with cold ether to remove hydrogen chloride. The glycosides were dissolved in the cold in the least polar possible of the solvents methanol, acetone, and ether; the solutions were filtered, and the products were obtained crystalline by the addition of ether to acetone or methanol solutions, or petroleum to acetone or ether solutions, at or below room temperature. Trituration with dichloromethane was also found to be useful in inducing recalcitrant members of the series to crystallise.

Method B. Use of methanol–chloroform. Example 1. 2,4-Dinitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside. 2,4-Dinitrophenyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (0·5 g) was dissolved in a mixture of dry methanol (30 ml) and chloroform (20 ml) and a 16% (w/w) solution of hydrogen chloride in methanol (10 ml) was added. The reaction (at room temperature) was monitored by t.l.c. [silica gel G; ethyl acetate–methanol–benzene (1 : 1 : 1) as eluant]. After 3 h the mixture was evaporated under reduced pressure (bath temp. 30°), and the resultant oil was triturated with chloroform, yielding crystals which on careful recrystallisation from aqueous methanol gave the *product* (0·14 g, 38%).

Example 2. 2,4-Dinitrophenyl β-D-galactopyranoside. To a solution of the tetra-*O*-acetyl derivative (0·5 g) in chloroform (5 ml) was added a solution of hydrogen chloride (3% w/w) in methanol (3 ml). The resultant solution was kept at room temperature for 18—24 h (crystallisation of the product had then begun) and then at 0° for a further 24 h; premature cooling caused the crystallisation of partially deacetylated material. The product was filtered off and recrystallised from methanol.

Note on recrystallisation. Although the deprotected 2,4-dinitrophenyl glycosides can be recrystallised from methanol, the analytically pure products still contain sufficient 2,4-dinitrophenol for inconveniently high optical densities of solutions used in enzyme studies to be observed. Recrystallisation from both protic and aprotic solvents often yields solvates,[2] but the products from aprotic solvents contain no 2,4-dinitrophenol.

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[4] B. Capon, P. M. Collins, A. A. Levy, and W. G. Overend, *J. Chem. Soc.*, 1964, 3242.

[5] M. Bárczai-Marcos and F. Körösy, *Nature*, 1950, **165**, 369.

[6] B. Helferich and H. Bredereck, *Ber.*, 1927, **60**, 1995.

[7] F. Ballardie and B. Capon, *J.C.S. Chem. Comm.*, 1972, 828.