Carbohydrate Research, 36 (1974) 241–245

GLYCOSIDASES. LIGANDS FOR AFFINITY CHROMATOGRAPHY: III. SYNTHESES OF *p*-AMINOPHENYL 2-ACETAMIDO-2-DEOXY-1-THIO-β-D-GLUCOPYRANOSIDE AND -GALACTOPYRANOSIDE*

CHRISTOPHER S. JONES, RAMESH H. SHAH, DANIEL J. KOSMAN, AND OM P. BAHL
Department of Biochemistry, Faculty of Health Sciences, State University of New York at Buffalo, Buffalo, New York 14214 (U. S. A.)
(Received December 18th, 1973; accepted in revised form, March 19th, 1974)

ABSTRACT

p-Aminophenyl 2-acetamido-2-deoxy-1-thio-β-D-glucopyranoside and -galactopyranoside were synthesized for use as ligands in the purification of 2-acetamido-2-deoxy-β-D-glucosidase, from *Aspergillus niger* and *Phaseolus vulgaris*, by affinity chromatography. The condensation of 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-glucose and -β-D-galactose with *p*-nitrothiophenol in the presence of zinc chloride afforded *p*-nitrophenyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-β-D-glucopyranoside and -galactopyranoside, respectively. The former was also obtained by the reaction of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride with *p*-nitrothiophenol in the presence of potassium hydroxide. *O*-Deacetylation followed by reduction with hydrogen over palladium on barium sulfate gave the *p*-aminophenyl 2-acetamido-2-deoxy-1-thio-β-D-glucopyranoside and -galactopyranoside. Inhibition constants (K_i) of the *p*-nitrophenyl and *p*-aminophenyl 2-acetamido-2-deoxy-1-thio-β-D-glucosides and -galactosides for *A. niger* 2-acetamido-2-deoxy-β-D-glucosidase were determined by using *p*-nitrophenyl 2-acetamido-2-deoxy-β-D glucopyranoside and -galactopyranoside as substrates.

INTRODUCTION

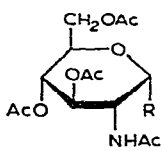
Glycosidases such as α-L-fucosidase, α- and β-D-galactosidases, 2-acetamido-2-deoxy-β-D-glucosidase, α- and β-D-mannosidases, and 2-acetamido-2-deoxy-α-D-glucosidase are involved in the degradation of glycoproteins. Their purification by affinity chromatography necessitated the availability of suitable ligands. Therefore, the syntheses of *p*-aminophenyl 1-thioglycosides derived from β-D-galactose, β-D-fucose, α-L-fucose, and α-D-mannose were undertaken and have been reported earlier[1,2]. This communication describes the syntheses of *p*-aminophenyl 2-acetamido-2-deoxy-1-thio-β-D-glucopyranoside (**5**) and -β-D-galactopyranoside (**9**) as inhibitors of the enzyme 2-acetamido-2-deoxy-β-D-glucosidase, one of the key enzymes

*Supported by grants from the United States Public Health Service AM-10273 and NS-10273-02.

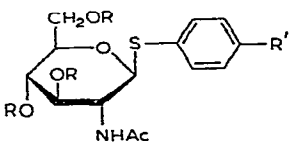
associated with the metabolism of glycoproteins. This enzyme from *Aspergillus niger* and *Phaseolus vulgaris*, used in the present studies, has been found to exhibit both 2-acetamido-2-deoxy-β-D-glucosidase and 2-acetamido-2-deoxy-β-D-galactosidase activities[3,4].

RESULTS AND DISCUSSION

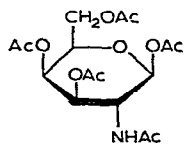
The reaction of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride (**1**) with *p*-nitrothiophenol, under conditions[5] similar to those used for the preparation of the 1-oxy analogue of **3**, yielded *p*-nitrophenyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-β-D-glucopyranoside (**3**) in 52% yield. The condensation of 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-glucose (**2**) with *p*-nitrothiophenol in the presence of anhydrous zinc chloride also afforded **3** in 56% yield. Similarly, 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-galactose (**6**) yielded *p*-nitrophenyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-β-D-galactoside (**7**) in 67% yield.



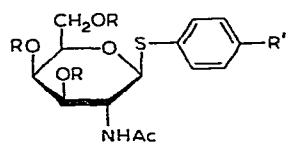
1 R = Cl
2 R = OAc



3 R = Ac, R' = NO_2
4 R = H, R' = NO_2
5 R = H, R' = NH_2



6



7 R = Ac, R' = NO_2
8 R = H, R' = NO_2
9 R = H, R' = NH_2

The glycosides **3** and **7** were *O*-deacetylated[6] with catalytic amounts of sodium methoxide to afford *p*-nitrophenyl 2-acetamido-2-deoxy-1-thio-β-D-glucoside (**4**) and

TABLE I

INHIBITION CONSTANTS (K_i) OF *p*-NITROPHENYL AND *p*-AMINOPHENYL 2-ACETAMIDO-2-DEOXY-1-THIO-β-D-GLUCOPYRANOSIDES AND -GALACTOPYRANOSIDES FOR *A. niger* 2-ACETAMIDO-2-DEOXY-β-D-GLUCOSIDASE

Inhibitor	*Inhibition constant* (K_i)[a]	
	2-Acetamido-2-deoxy-β-D-glucosidase activity[b] (mM)	*2-Acetamido-2-deoxy-β-D-galactosidase activity*[c] (mM)
4	1.4	4.0
5	5.2	15.6
8	8.4	3.1
9	n.d.[d]	10.0

[a]Calculated from Lineweaver–Burk plots obtained by determining enzyme-reaction velocities at 30° for five substrate concentrations ranging from 0.4mM to 3.5mM and two inhibitor concentrations (between 1mM and 3mM). Substrate: [b]*p*-Nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside (K_m 0.6mM); [c]*p*-Nitrophenyl 2-acetamido-2-deoxy-β-D-galactopyranoside (K_m 0.7mM). [d]Not determined because of lack of inhibition.

-galactoside (**8**). Reduction of **4** and **8** over palladium on barium sulfate with hydrogen under pressure afforded *p*-aminophenyl 2-acetamido-2-deoxy-1-thio-β-D-glucoside (**5**) and -galactoside (**9**), respectively.

The K_i values of compounds **4**, **5**, **8**, and **9** for *A. niger* 2-acetamido-2-deoxy-β-D-glucosidase[4], determined from their respective Lineweaver–Burk plots, are given in Table I. With the exception of **9**, which failed to inhibit 2-acetamido-2-deoxy-β-D-glucosidase activity, all of the other compounds reported here competitively inhibited both the 2-acetamido-2-deoxy-β-D-glucosidase and 2-acetamido-2-deoxy-β-D-galactosidase activities to varying extents. Most effective inhibition of these two activities was caused by *p*-nitrophenyl 2-acetamido-2-deoxy-1-thio-β-D-glucoside (**4**) and -galactoside (**8**), respectively. In this respect, the *p*-aminophenyl glycosides **5** and **9** were less effective than their *p*-nitrophenyl analogues **4** and **8**.

EXPERIMENTAL

General methods. — Melting points were determined with a Fisher–Johns apparatus and are uncorrected. Optical rotations were measured in a 1-dm cell with a Perkin–Elmer Model 141 automatic polarimeter. Unless otherwise mentioned, *N*,*N*-dimethylformamide was employed as solvent. I.r. spectra were recorded with a Beckman IR-33 spectrophotometer. The R_F values were determined by t.l.c. on plates coated with silica gel G containing a fluorescent indicator. The solvents employed for acetylated and deacetylated compounds were 50:1 chloroform–methanol and 3:1:1 ethyl acetate–acetic acid–water, respectively. The spots were detected under a short-wave u.v. lamp. *p*-Nitrothiophenol was obtained from Aldrich Chemical Co. (Cedar Knolls, N. J.), and was of 80+% purity. Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, Tennessee.

Enzyme studies. — Inhibition studies of *A. niger* 2-acetamido-2-deoxy-β-D-glucosidase[4] were carried out in 50mM citrate buffer, pH 4.6, by using *p*-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside and -galactopyranoside as substrates for 2-acetamido-2-deoxy-β-D-glucosidase and 2-acetamido-2-deoxy-β-D-galactosidase activities, respectively. The assay conditions have been described elsewhere[7].

p-*Nitrophenyl 2-acetamido-3,4,6-tri*-O-*acetyl-2-deoxy-1-thio-β-*D-*glucopyranoside* (**3**). — *A. From 2-acetamido-3,4,6-tri*-O-*acetyl-2-deoxy-α-*D-*glucopyranosyl chloride* (**1**). To a partial solution of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride[8] (**1**, 6.3 g) in acetone (140 ml) was added, under an atmosphere of nitrogen, *p*-nitrothiophenol (7.8 g) and 3% aqueous sodium hydroxide solution (57 ml). The mixture was stirred under nitrogen for 2.5 h at room temperature, after which time the product was filtered off, washed with water and acetone, and recrystallized twice from 1:1 chloroform–methanol; yield, 4.3 g (52%), m.p. 282–283.5° dec. An additional recrystallization from the same solvent provided analytically pure **3**; m.p. 285–286° dec., $[\alpha]_D^{25}$ −26.0° (*c* 0.89); R_F 0.60; ν_{max}^{KBr} 3340 (NH), 1750 (ester C=O), 1670 (amide, type I band), 1605, 1590, and 1490 (aromatic), 1540 (amide, type II band), 1520 and 1350 (NO_2), 1248 and 1233 (acetate C–O–C), 920, 858 (C–N), 832, 750 (C–N–O), and 690 cm^{-1}.

Anal. Calc. for $C_{20}H_{24}N_2O_{10}S$: C, 49.58; H, 4.99; N, 5.78. Found: C, 49.47; H, 4.93; N, 5.82.

*B. From 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-*D*-glucopyranose* (**2**). To an intimate mixture of 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-glucopyranose[9] (**2**, 0.50 g) and *p*-nitrothiophenol (0.60 g) was added 0.55 ml of a solution of anhydrous zinc chloride (0.27 g) in 1 ml of 1:19 acetic anhydride–acetic acid. The mixture was heated for 3 min at 125° at atmospheric pressure, and then under diminished pressure for 15 min. After cooling to room temperature, the syrupy mixture was crystallized from acetone. The light-yellow crystals of **3** were filtered off and washed with acetone and water; yield, 0.48 g (77%), m.p. 270–273° dec. Two recrystallizations from chloroform–methanol afforded 0.35 g (56%) of **3**; m.p. and mixed m.p. with **3** from method *A*, 281–283° dec., $[\alpha]_D^{25}$ −25.5° (*c* 0.26). Its i.r. spectrum and mobility on t.l.c. (silica gel) were identical with that of **3** from method *A*.

p-*Nitrophenyl 2-acetamido-2-deoxy-1-thio-β-*D*-glucopyranoside* (**4**). — To a suspension of **3** (3.8 g) in dry methanol (80 ml) was added[6] 2M sodium methoxide in methanol (5 ml). The mixture was heated at 50° until dissolution had occurred (9 min), and the solution was then kept for 20 min at room temperature. The crystallized product was filtered off and washed with ether; yield, 2.1 g (75%), m.p. 225–228° dec. One recrystallization from 1:1 methanol–acetone afforded the analytical sample; m.p. 230–232° dec., $[\alpha]_D^{25}$ −48.2° (*c* 1.03); R_F 0.63; ν_{max}^{KBr} 3500 (shoulder), 3400, and 3320 (OH, NH), 1655 (amide, type I band), 1600, 1590, and 1490 (aromatic), 1560 and 1542 (NH), 1520 and 1355 (NO_2), 1510, 855, and 742 cm^{-1}.

Anal. Calc. for $C_{14}H_{18}N_2O_7S$: C, 46.92; H, 5.06; N, 7.82. Found: C, 46.79; H, 5.14; N, 7.72.

p-*Aminophenyl 2-acetamido-2-deoxy-1-thio-β-*D*-glucopyranoside* (**5**). — *p*-Nitrophenyl 2-acetamido-2-deoxy-1-thio-β-D-glucopyranoside (**4**, 0.82 g) was dissolved in methanol (200 ml) and hydrogenated over 5% palladium on barium sulfate (0.35 g) at an initial pressure of 50 lb.in.$^{-2}$ for 20 h. After removal of the catalyst by filtration through Celite, the filtrate was evaporated to dryness, and the residue was crystallized from 1:1 methanol–propyl alcohol; yield, 0.50 g (67%); m.p. 246–248° dec., $[\alpha]_D^{25}$ +3.9° (*c* 1.06, methanol); R_F 0.41; ν_{max}^{KBr} 3520, 3460, 3365, and 4180 (OH, NH), 1650 (amide, type I band), 1615 (NH_2), 1600 and 1500 (aromatic), 1560 (amide, type II band), 1275 (C–N), and 880 cm^{-1}.

Anal. Calc. for $C_{14}H_{20}N_2O_5S$: C, 51.20; H, 6.14; N, 8.53. Found: C, 51.31; H, 6.21; N, 8.29.

A methanol-solvate of **5** was obtained, from an earlier preparation, in 76% yield; m.p. 226–228° dec., $[\alpha]_D^{25}$ +2.8° (*c* 1.00, methanol); R_F 0.41.

Anal. Calc. for $C_{14}H_{20}N_2O_5S \cdot 1.25\ CH_3OH$: C, 49.71; H, 6.84; N, 7.60; S, 8.70. Found: C, 49.41; H, 6.66; N, 7.65; S, 8.73.

p-*Nitrophenyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-β-*D*-galactopyranoside* (**7**). — Fusion of 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-galactose[10] (**6**, 0.50 g) with *p*-nitrothiophenol (0.62 g) and processing as described for **3**, gave 0.42 g (67%) of **7**, m.p. 215–220°. Two recrystallizations from chloroform–

methanol afforded the analytical sample; m.p. 225–227°, $[\alpha]_D^{25}$ −2.0° (*c* 0.30); R_F 0.51; ν_{max}^{KBr} 3410 (NH), 1745 (ester C=O), 1665 (amide, type I band), 1595, 1580, and 1482 (aromatic), 1540 (shoulder, amide, type II band), 1520 and 1345 (NO_2), 1258, 1238, and 1225 (acetate C–O–C), 912, 850 (C–N), 842, (*p*-disubstituted phenyl), and 740 cm^{-1} (C–N–O).

Anal. Calc. for $C_{20}H_{24}N_2O_{10}S$: C, 49.58; H, 4.99; N, 5.78. Found: C, 49.86; H, 5.02; N, 5.69.

p-*Nitrophenyl 2-acetamido-2-deoxy-1-thio-β-*D*-galactopyranoside* (**8**). — To a suspension of **7** (0.35 g) in dry methanol (15 ml) was added[6] M sodium methoxide in methanol (1.5 ml). The mixture was refluxed for 30 min, cooled to room temperature, diluted with methanol (25 ml), and neutralized by stirring with methanol-washed Dowex-50 (H^+) resin. The resin was filtered off and washed thoroughly with methanol. The filtrate was evaporated to dryness and the residual light-yellow solid was filtered with the aid of acetone; yield, 0.19 g (73%); m.p. 223–227° dec. An analytical sample, m.p. 225–227° dec., was obtained from another preparation by recrystallization from methanol–acetone; $[\alpha]_D^{25}$ −43.1° (*c* 0.29); R_F 0.68; ν_{max}^{KBr} 3500 (broad) and 3300 (OH, NH), 1650 (amide, type I band), 1600, 1585, and 1485 (aromatic), 1560 and 1540 (NH), 1520 and 1345 (NO_2), 1510, 855, and 740 cm^{-1}.

Anal. Calc. for $C_{14}H_{18}N_2O_7S \cdot 0.75H_2O$: C, 45.21; H, 5.29; N, 7.53. Found: C, 45.03; H, 5.24; N, 7.48.

p-*Aminophenyl 2-acetamido-2-deoxy-1-thio-β-*D*-galactopyranoside* (**9**). — A mixture of *p*-nitrophenyl 2-acetamido-2-deoxy-1-thio-β-D-galactopyranoside (**8**, 0.18 g), 5% palladium on barium sulfate (0.10 g), and methanol (50 ml) was shaken under hydrogen for 24 h at an initial pressure of 50 $lb.in^{-2}$. The catalyst was removed by filtration through Celite, and the filtrate was evaporated to dryness. The solid residue was triturated with a small amount of methanol and filtered to give **9** as pale-yellow crystals; yield, 0.14 g (82%); m.p. 224–226° dec., $[\alpha]_D^{25}$ +5.8° (*c* 0.36); R_F 0.46; ν_{max}^{KBr} 3480 (shoulder), 3380, and 3310 (OH, NH), 1650 (shoulder) and 1635 (amide, type I band), 1600 (NH_2), 1580 (shoulder), 1570 (shoulder), 1550 (amide, type II band), 1500 (aromatic), 1265 (C–N), 865, and 820 cm^{-1} (*p*-disubstituted phenyl).

Anal. Calc. for $C_{14}H_{20}N_2O_5S$: C, 51.20; H, 6.14; N, 8.53. Found: C, 50.94; H, 6.16; N, 8.99.

REFERENCES

1 R. H. Shah and O. P. Bahl, *Carbohyd. Res.*, 32 (1974) 15.
2 M. L. Chawla and O. P. Bahl, *Carbohyd. Res.*, 32 (1974) 25.
3 K. M. L. Agrawal and O. P. Bahl, *J. Biol. Chem.*, 243 (1968) 103.
4 O. P. Bahl and K. M. L. Agrawal, *J. Biol. Chem.*, 244 (1969) 2970.
5 D. H. Leaback and P. G. Walker, *J. Chem. Soc.*, (1957) 4754.
6 A. Thompson, M. L. Wolfrom, and E. Pacsu, *Methods Carbohyd. Chem.*, 2 (1963) 215.
7 O. P. Bahl and K. M. L. Agrawal, *J. Biol. Chem.*, 243 (1968) 98.
8 D. Horton, *Methods Carbohyd. Chem.*, 6 (1972) 282.
9 L. Hough and M. I. Taha, *J. Chem. Soc.*, (1956) 2042.
10 B. Weissmann, *J. Org. Chem.*, 31 (1966) 2505.