Carbohydrate Research, 32 (1974) 25–29

GLYCOSIDASES. LIGANDS FOR AFFINITY CHROMATOGRAPHY: II. SYNTHESES OF *p*-AMINOPHENYL 1-THIO-L-FUCOPYRANOSIDES*

MANOHAR L. CHAWLA† 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 May 11th, 1973; accepted in revised form June 30th, 1973)

ABSTRACT

Syntheses of *p*-aminophenyl 1-thio-α-L- and β-L-fucopyranosides are described. 1,2,3,4-Tetra-*O*-acetyl-α-L-fucopyranose, on heating with *p*-nitrothiophenol in the presence of *p*-toluenesulfonic acid under diminished pressure, gave a mixture of *p*-nitrophenyl 2,3,4-tri-*O*-acetyl-1-thio-α- and β-L-fucopyranosides, which was separated by chromatography on silica gel. When the reaction was carried out in the presence of zinc chloride at atmospheric pressure, the β-anomer was the exclusive product. Deacetylation of the aryl α-L- and β-L-thiofucopyranosides with sodium methoxide, followed by catalytic hydrogenation in the presence of palladium on barium sulfate, afforded the respective aminophenyl 1-thiofucopyranosides. The aryl thiofucopyranosides thus synthesized were tested for their inhibitory activity toward clam α-L-fucosidase. The *p*-aminophenyl 1-thio α-L-fucopyranoside showed a competitive-type inhibition, with a K_i of 0.71mM.

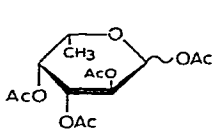
INTRODUCTION

In the preceding paper are described syntheses of the 1,2-*trans* *p*-aminophenyl 1-thio-D-glycopyranosides to be used as ligands for the affinity chromatography of β-D-galactosidase and α-D-mannosidase[1]. These syntheses were achieved by condensation of the appropriate acylglycosyl bromides with *p*-nitrothiophenol in the presence of potassium hydroxide, followed by deacetylation and catalytic hydrogenation[1]. This method gives exclusively the 1,2-*trans* glycosides, and, therefore, was not suitable for synthesis of the 1,2-*cis* *p*-aminophenyl 1-thio-α-L-fucopyranoside (**4a**). In order to prepare the 1,2-*cis* anomer (**2a**), acid-catalyzed condensation of L-fucopyranose tetraacetate (**1**) with *p*-nitrothiophenol was performed. This type of reaction is known to give both the α- and the β-anomers, whose proportions may vary with the nature of the acid catalyst, aglycon, anomeric linkage of the acetyl sugar, temperature, and pressure[2–8]. Consequently, the prediction of ratios of the two anomeric glycosides in such reactions is not possible[1–3].

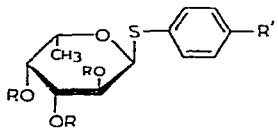
*Supported by National Institutes of Health. Grant No. 10273, and Population Council of New York. Grant No. M-72.39.

†Supported by a Clinical Training Grant No. GM-01960 from the National Institutes of Health. This paper is a part of a thesis to be submitted by M.L.C. in partial fulfillment of the requirements for a Ph. D. degree.

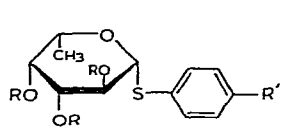
The present communication describes details of the syntheses of 1,2-*cis* (α) and -*trans* (β) *p*-aminophenyl 1-thio-L-fucopyranosides. Also, the inhibitory activity of the thiofucopyranosides for clam α-L-fucosidase is described.



1



2a R = Ac, R' = NO_2
3a R = H, R' = NO_2
4a R = H, R' = NH_2



2b R = Ac, R' = NO_2
3b R = H, R' = NO_2
4b R = H, R' = NH_2

RESULTS AND DISCUSSION

Tetra-*O*-acetyl-α-L-fucopyranose[9] (**1**) was heated for 45 min at 130° with *p*-nitrothiophenol in 4:1 acetic acid–acetic anhydride under diminished pressure in the presence of *p*-toluenesulfonic acid, and the two *p*-nitrophenyl 1-thio-L-fucopyranosides, α- (**2a**) and β- (**2b**), were obtained in 70% yield in a 1:1 ratio. The two anomers were separated by chromatography on a silica gel column with subsequent preparative t.l.c. on silica gel. The β-anomer (**2b**) resembled its D-enantiomer[1] with respect to its m.p., specific rotation, i.r. and n.m.r. spectra, and chromatographic properties. The two products **2a** and **2b** conformed to Hudson's isorotation rules[10] in that the α-anomer **2a** had the more negative rotation of the two.

Reaction of the fucose tetraacetate **1** with *p*-nitrothiophenol at atmospheric pressure, with zinc chloride in lieu of *p*-toluenesulfonic acid as the catalyst, gave the thio-β-fucopyranoside (**2b**) in a yield of 70% as the sole product.

The two anomers **2a** and **2b** were deacetylated with catalytic amounts of sodium methoxide in methanol. Catalytic reduction of **3a** and **3b**, thus obtained,

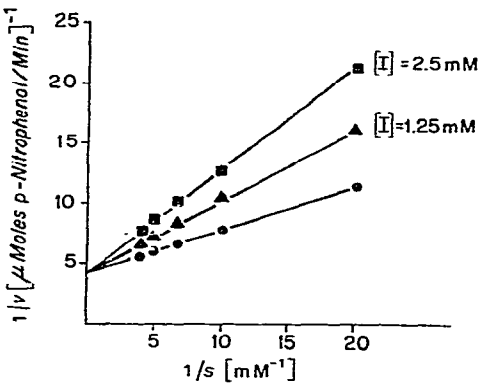


Fig. 1. Competitive inhibition of clam α-L-fucosidase by *p*-aminophenyl 1-thio-α-L-fucopyranoside (**4a**). Under the standard assay-conditions of the enzyme (see Experimental) the inhibitor concentration was either 1.25 or 2.50 mM. Each tube contained 0.023 units of α-L-fucosidase in 24.1 μg of the protein.

with hydrogen over palladium on barium sulfate, afforded the *p*-aminophenyl 1-thio-L-fucopyranosides **4a** and **4b** in 75–85% yields from **2a** and **2b**.

Inhibition of clam α-L-fucosidase by various concentrations of *p*-aminophenyl 1-thio-α-L-fucopyranoside was studied under standard assay-conditions for the enzyme. The Lineweaver–Burke plot of $1/v$ versus $1/s$ shown in Fig. 1, indicated a competitive-type inhibition. The values for K_m and K_i calculated from the plot are 0.085 and 0.71mM, respectively. Neither *p*-nitrophenyl 1-thio-α-L (**2a**) and β-L-(**2b**) fucopyranosides, nor *p*-aminophenyl 1-thio-β-L-fucopyranoside (**4b**), inhibited the enzyme. Preliminary studies indicate that *p*-aminophenyl 1-thio-α-L-fucopyranoside (**4a**) is a suitable ligand for the affinity chromatography of clam α-L-fucosidase.

EXPERIMENTAL

General. — Melting points were determined with a Fisher–Johns apparatus and are uncorrected. Specific rotations were measured in a 1-dm cell with a Perkin–Elmer Model 141 automatic polarimeter. N.m.r. spectra were recorded at 100 MHz with chloroform-*d* as solvent and tetramethylsilane as internal reference. I.r. spectra were recorded with a Perkin–Elmer Model 237 i.r. spectrophotometer. Thin-layer chromatography was performed on plates precoated with silica gel H (Analtech Inc., Newark, Delaware) with the following solvents: (*A*, 4:1 benzene–ethyl acetate; *B*, 3:1:1 ethyl acetate–acetic acid–water. Solvent *A* was employed for the t.l.c. of the acetylated and solvent *B* of the deacetylated derivatives. The spots were detected either by exposure to iodine vapor or under short-wave u.v. light.

Silica gel, Davison, grade 923, 100–200 mesh, was used for column chromatography without pretreatment. The fractions were monitored by t.l.c. on microscope slides precoated with silica gel H, with solvent *A*. Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, Tennesee. *p*-Nitrothiophenol (Aldrich Chemical Co., Inc., Cedar Knolls, N.J.) was of 80+% purity and was used without purification. *p*-Nitrophenyl α-L-fucopyranoside was obtained from Pierce Chemical Company, Rockford, Ill. A Perkin–Elmer double-beam spectrophotometer Model 124 was used for enzyme assays.

p-*Nitrophenyl 2,3,4-tri-O-acetyl-1-thio-*α-L *and* β-L-*fucopyranosides* (**2a**, **2b**). — A. *By using* p-*toluenesulfonic acid.* 1,2,3,4-Tetra-*O*-acetyl-L-fucopyranose[9] (**1**, 1.0 g) and *p*-nitrothiophenol (2.0 g) were added to a mixture of acetic anhydride (0.5 ml), acetic acid (2.0 ml), and *p*-toluenesulfonic acid monohydrate (0.5 g). The mixture was stirred for 45 min at 130° under diminished pressure (water aspirator). It was then cooled to room temperature and partitioned between ice–water (25 ml) and chloroform (25 ml). The aqueous layer was further extracted with chloroform (25 ml). The combined chloroform extracts were washed successively with cold, saturated sodium hydrogen carbonate solution and water, dried ($CaCl_2$), and evaporated to dryness. T.l.c. revealed the presence of the α-anomer (**2a**, R_F 0.66), β-anomer (**2b**, R_F 0.58), and the byproducts of the reaction.

The syrupy mixture (1.71 g) was dissolved in benzene (5 ml) and applied to a

column (60 × 1 cm) of silica gel (28 g) packed in benzene. Elution with benzene (300 ml) removed most of the byproducts. The elution was then continued with solvent *A* to give a mixture of **2a**, **2b**, and traces of the byproducts. This mixture was then separated on 4 preparative thin-layer plates (20 × 40 cm) with solvent *A*. The bands containing **2a** and **2b** were scraped off separately, and eluted with ethyl acetate. The solvent was evaporated and the residual syrups were crystallized from methanol.

α-Anomer (**2a**): yield, 0.45 g (35%); m.p. 133–134°. Recrystallization from methanol afforded the analytical sample without any change in m.p., $[\alpha]_D$ −273.5° (*c* 1.0, chloroform); R_F 0.66; ν_{max}^{KBr} 1748 (C=O), 1590, 1580, and 1477 (aromatic), 1515 and 1333 (NO_2), 1380 (C–CH_3), 1230–1260 (acetate C–O–C), 850 (C–N), 830 (*p*-disubstituted phenyl), 740 (C–N–O), 880, 827 (shoulder), and 760 cm^{-1}; n.m.r. data: τ 1.92 (2 H, doublet, *J* 9.0 Hz; $H_{o\text{-}nitro}$) 2.42 (2 H, doublet) ($H_{m\text{-}nitro}$); 3.80 (1 H, doublet, $J_{1,2}$ 4.2 Hz, H-1), 4.40–4.80 (3 H, multiplet, H-2,3,4), 5.47 (1 H, quartet, $J_{5,6}$ 6.5 Hz, H-5), 8.87 (3 H, doublet, H-6), 7.81 (3 H, 4-OAc), 7.90 and 7.98 (6 H, 2,3-OAc).

Anal. Calc. for $C_{18}H_{21}NO_9S$: C, 50.58; H, 4.95; N, 3.28. Found: C, 50.30; H, 4.89; N, 3.23.

β-Anomer (**2b**): yield, 0.45 g (35%); m.p. 141–141.5°. The analytical sample of **2b** was obtained by recrystallization from methanol; m.p. 141–141.5° (reported[1] for *β*-D-isomer, 139–140°), $[\alpha]_D$ +4.7° (*c* 1.0, chloroform) (lit.[1] −4.3° for the D-enantiomer); R_F 0.58 (identical with that of *β*-D-isomer). The i.r. and n.m.r. spectra were indistinguishable from those of the D-enantiomer.

The proportions of anomers **2a** and **2b**, and their overall yields, were essentially the same when the reaction was scaled up 15-fold. In another experiment **2a** and **2b** were obtained in a yield of 97%.

p-*Nitrophenyl 2,3,4-tri-O-acetyl-1-thio-β-L-fucopyranoside.* — B. *By using zinc chloride.* Reaction of 1,2,3,4-tetra-*O*-acetyl-α-L-fucopyranose (**1**, 1.0 g) with *p*-nitrothiophenol (2.0 g) at atmospheric pressure at 130° in 4:1 acetic acid–acetic anhydride (2.5 ml) gave the thio-*β*-fucopyranoside (**2b**) (0.90 g, 70%) exclusively when anhydrous zinc chloride (0.5 g) was employed as the catalyst. Its m.p., specific rotation, i.r. spectrum, and R_F value were identical with those of **2b** already described.

p-*Nitrophenyl 1-thio-α-L-fucopyranoside* (**3a**). — To a suspension of **2a** (2.03 g) in methanol (12.2 ml) was added 0.5M methanolic sodium methoxide (1.42 ml). The mixture was stirred for 1.5 h at room temperature. The product (**3a**) partially crystallized from the solution and was dissolved in methanol. The solution was then passed through a column (0.5 × 30 cm) of methanol-washed Amberlite IR-120 (H^+) resin (5 g). The resin was eluted with methanol, and the eluates were evaporated to dryness. The crystalline residue, on trituration with ether, gave colorless crystals of **3a**; yield, 1.34 g (97%); m.p. 224–226°. Recrystallization from methanol afforded the analytical sample without altering the m.p.; $[\alpha]_D$ −382° (*c* 1.0, methanol); R_F 0.88; ν_{max}^{KBr} 3330 (OH), 1600, 1585, and 1480 (aromatic), 1515 and 1350 (NO_2), 855 (C–N), 840 (shoulder, *p*-disubstituted phenyl), 745 (C–N–O), 1382 (shoulder), 880, 770, and 685 cm^{-1}.

Anal. Calc. for $C_{12}H_{15}NO_6S$: C, 47.84; H, 5.02; N, 4.65. Found: C, 47.91; H, 5.08; N, 4.68.

p-*Nitrophenyl 1-thio-β-*L-*fucopyranoside* (**3b**). — Deacetylation of **2b** (1.0 g) as described for **3a** gave 0.68 g (96.5%) of β-L-thiofucopyranoside **3b**; m.p. 177–178° (same as reported[1] for the D-enantiomer), $[\alpha]_D$ +116.0° (*c* 1.0, methanol), −113.2° for the D-isomer (Shah and Bahl[1]). The R_F value and the i.r. spectrum of **3b** were identical to those of the β-D-enantiomer[1].

p-*Aminophenyl 1-thio-α-*L-*fucopyranoside* (**4a**). — The α-L-thiofucopyranoside **3a** (0.15 g) was hydrogenated overnight with 5% palladium on barium sulfate (0.10 g) in methanol (50 ml) at an initial pressure of 50 lb.in^{-2}. The catalyst was removed by filtration through Celite. The filtrate was evaporated and the syrupy residue crystallized from propyl alcohol to yield **4a** as almost colorless crystals yield, 0.11 g (78%); m.p. 138–139°, $[\alpha]_D$ −357° (*c* 1.0, methanol); R_F 0.66; ν_{max}^{KBr} 3300 (OH and NH), 1615 (shoulder, NH_2), 1590 and 1575 (aromatic), and 830 cm^{-1} (*p*-disubstituted phenyl).

Anal. Calc. for $C_{12}H_{17}NO_4S$: C, 53.12; H, 6.32; N, 5.16. Found: C, 53.03; H, 6.35; N, 5.08.

p-*Aminophenyl 1-thio-β-*L-*fucopyranoside* (**4b**). — Hydrogenation of **3b** (0.40 g) as described for **4a** afforded 0.32 g (88%) of pale-yellow crystals of **4b**; m.p. 65–78°, $[\alpha]_D$ +66.0°; for β-D-enantiomer m.p. 75–78° and $[\alpha]_D$ −76.6° (Shah and Bahl[1]). The i.r. spectrum and the mobility on a thin-layer plate of **4b** were indistinguishable from those of the β-D-isomer[1].

*Assay for α-*L-*fucosidase.* — The enzyme assay was carried out essentially according to the procedure described earlier[11]. A mixture of 50 µl of 2mM aqueous *p*-nitrophenyl α-L-fucopyranoside and 50 µl of 0.2M sodium acetate buffer (pH 5.0) was preincubated for 5 min at 37°. After the addition of 50 µl of the enzyme (0.023 units), the reaction mixture was incubated for another 15 min. The enzymic reaction was stopped by the addition of 1 ml of 0.2M sodium carbonate solution and absorbance was read at 400 nm against a suitable blank.

One unit of the enzyme is defined as the amount that liberates 1 µmole of *p*-nitrophenol per min at 37°. Protein was determined by the method of Lowry, *et al*[12].

REFERENCES

1 R. Shah and O. P. Bahl, *Carbohyd. Res.*, 32 (1974) 15.
2 B. Helferich and E. Schmitz-Hillebrecht, *Ber.*, 66 (1933) 378.
3 E. M. Montgomery, N. K. Richtmyer, and C. S. Hudson, *J. Amer. Chem. Soc.*, 64 (1942) 690.
4 G. N. Bollenback, J. W. Long, D. G. Benjamin, and J. A. Lindquist, *J. Amer. Chem. Soc.*, 77 (1955) 3310.
5 W. E. Trevelyan, *Carbohyd. Res.*, 2 (1966) 418.
6 T. R. Ingle and J. L. Bose, *Carbohyd. Res.*, 12 (1970) 459.
7 A. B. Landge, T. R. Ingle, and J. L. Bose, *Indian J. Chem.*, 7 (1969) 1200.
8 T. D. Audichya, T. R. Ingle, and J. C. Bose, *Indian J. Chem.* 9 (1971) 315.
9 D. H. Leaback, E. C. Heath, and S. Roseman, *Biochemistry*, 8 (1969) 1351.
10 C. S. Hudson, *J. Amer. Chem. Soc.*, 31 (1909) 66.
11 G. A. Levvy and A. McAllan, *Biochem. J.*, 80 (1961) 435.
12 O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, 193 (1951) 265.