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GLYCOSIDASES. LIGANDS FOR AFFINITY CHROMATOGRAPHY: I. SYNTHESES OF 1,2-*trans* *p*-AMINOPHENYL 1-THIO-D-GLYCOPYRANOSIDES

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ABSTRACT

1,2-*trans* *p*-Aminophenyl 1-thioglycosides derived from β-D-galactose, β-D-fucose, and α-D-mannose were synthesized as potential ligands for the purification of β-D-galactosidase and α-D-mannosidase by affinity chromatography. The appropriate acetylglycosyl bromides were condensed with *p*-nitrothiophenol in the presence of potassium hydroxide. Deacylation of the 1,2-*trans* *p*-nitrophenyl *O*-acetyl-1-thio-D-glycopyranosides thus obtained, followed by reduction with hydrogen over palladium on barium sulfate, afforded the desired *p*-aminophenyl 1-thioglycopyranosides.

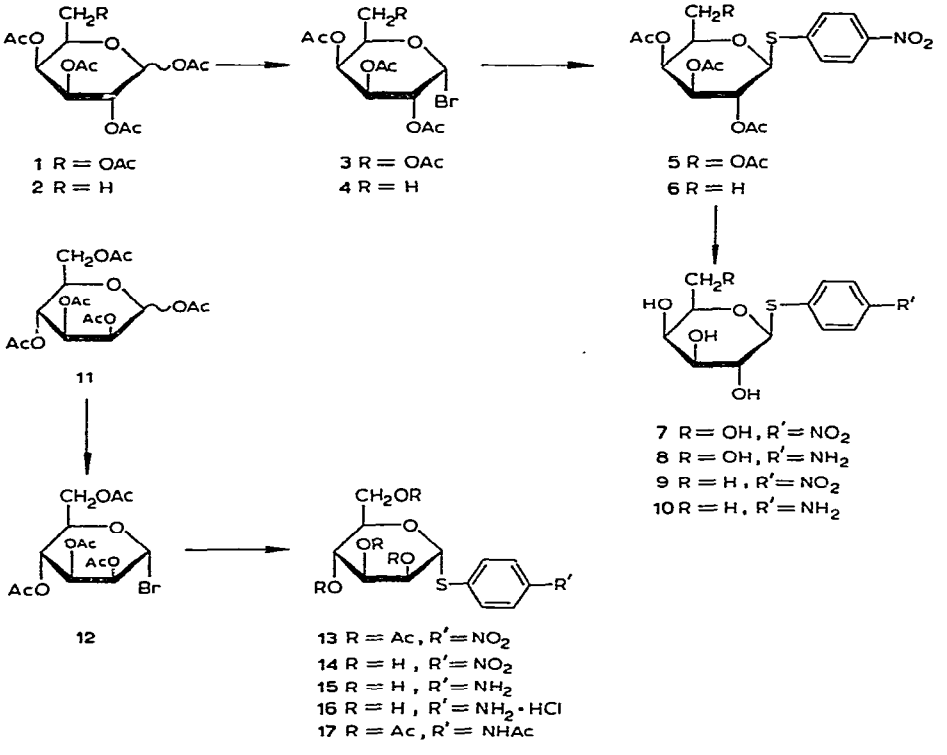
INTRODUCTION

The utility of exoglycosidases for determining the monosaccharide sequence of complex carbohydrate units in glycoproteins and glycolipids is quite evident from the number of recent reports[1–4]. One of the obstacles encountered in this approach is the inaccessibility of highly purified enzymes that are free of cross-contamination and proteases. The conventional procedures of enzyme purification are laborious, as all of the sources of the enzymes employed thus far for their isolation contain numerous other glycosidases closely related in their physico-chemical and specificity properties. Affinity chromatography[5] offers a convenient method for obtaining preparations of enzymes suitable for the monosaccharide-sequence studies. Availability of appropriate inhibitory ligands is an essential prerequisite for affinity chromatography. Hence the syntheses of such ligands were undertaken. The *p*-aminophenyl 1-thioglycosides were selected as a first choice because of some evidence in the literature regarding their inhibitory properties[6].

This communication describes the syntheses of the 1,2-*trans* *p*-aminophenyl 1-thio-D-glycopyranosides (**8**, **10**, **15**) derived from β-D-galactose, β-D-fucose, and α-D-mannose, respectively. To the best of our knowledge, no details regarding either their syntheses or their physical properties are available in the literature.

Of the several methods[7–11] available for glycosidation, the procedure of Purves[7,8] appeared best suited for our objectives. The acetylated α-D-glycopyranosyl

bromides (**3**, **4**, and **12**) of galactose, fucose, and mannose were condensed with *p*-nitrothiophenol in the presence of potassium hydroxide. The resulting *p*-nitrophenyl 1-thioglycosides **5**, **6**, and **13** were deacetylated and then reduced with hydrogen over palladium on barium sulfate to afford the 1,2-*trans* *p*-aminophenyl 1-thio-D-glycopyranosides **8**, **10**, and **15**. These ligands are well suited for covalent attachment to a solid matrix such as Sepharose or polyacrylamide.



RESULTS AND DISCUSSION

The reaction of the acetylglycosyl bromides **3**, **4**, and **12** with *p*-nitrothiophenol formed the acetylated *p*-nitrophenyl 1-thio-β-D-galactopyranoside (**5**), -β-D-fucopyranoside (**6**), and -α-D-mannopyranoside (**13**). It has been reported[8] that some deacetylation of the product might occur under the conditions of the coupling reaction. Hence the crude product from each reaction was subjected to acetylation with pyridine and acetic anhydride. Subsequently, the crystalline thioglycosides **5**, **6**, and **13** were isolated in 29%, 46%, and 29% yields from their respective pyranose acetates **1**, **2**, and **11**.

The 1,2-*trans* relationship in thioglycosides **5**, **6**, and **13** is predicted from the *trans*-rule of Tipson[12]. This assignment is further corroborated by Hudson's isorotation rules[13], in view of the negative specific rotations of the β-D-galactosides

(**5**, **7**, and **8**) and -fucosides (**6**, **9**, and **10**), and the large positive rotations of the α-D-mannosides (**13**, **14**, **16**, **17**). Unequivocal support in favor of the β-D-configuration in **5** and **6** was deduced from their 100 MHz n.m.r. spectra. The assignment of signals (Table I) in the *p*-nitrophenyl 1-thioglycosides **5** and **6** was made by analogy with the n.m.r. spectrum of 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl cyanide[14]. The diaxial orientation of H-1 and H-2 in **5** and **6** was indicated by a large $J_{1,2}$ coupling (10 Hz). For methyl D-galactosides, the observed $J_{1,2}$ values for the α-pyranoside, β-pyranoside, α-furanoside, and β-furanoside are 3.5, 8.0, 4.5, and 2.0 Hz, respectively[15]. The larger magnitude of the H-1–H-2 splitting (10 Hz) in **5** and **6**, as compared to 8.0 Hz for methyl β-D-galactopyranoside[15], may be attributed to replacement of oxygen by the less-electronegative sulfur at C-1. This accords with the observation that increased electronegativity of the first substituent atom on the carbon atom bearing one of the coupling hydrogen atoms tends to decrease the spin-splitting between vicinal protons[14,16]. This enhanced coupling may also arise from a specific orientation of the phenylthio group[17]. For the thiogalactoside **5**, all of the proton resonances, except for H-5 and the H-6 resonances, were well separated whereas the thiofucoside **6** displayed identifiable signals for H-1, H-3, H-5, and the H-6 protons, and a multiplet between τ 4.58–4.78 from which the H-2 triplet and H-4 doublet could easily be discerned. The $J_{4,5}$ value of less than 1 Hz in both **5** and **6** is consistent with the small H-4–H-5 coupling observed in 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl cyanide ($J_{4,5}$ 0.7 Hz)[14] and in diethylsulfonylgalactopyranosylmethane ($J_{4,5}$<0.5 Hz)[18].

TABLE I

CHEMICAL SHIFTS (τ VALUES) AND COUPLING CONSTANTS (Hz) FROM 100-MHz N.M.R. SPECTRA[a]

Compound	*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*	*Acetoxyl*
5	5.09 (d)	4.65 (t)	4.86 (q)	4.49 (d)	5.66———6.10[b]		7.84 (C-4), 7.92, 7.94 (sh), 8.01
	$J_{1,2}$ 10.0	$J_{2,3}$ 10.0	$J_{3,4}$ 3.0	$J_{4,5}$ <1			
6	5.10 (d)	4.67 (t)	4.85 (q)	4.65 (d)	6.01 (q)	8.72 (d)	7.82 (C-4), 7.93, 8.02
	$J_{1,2}$ 10.0	$J_{2,3}$ 10.0	$J_{3,4}$ 3.0	$J_{4,5}$ <1	$J_{5,6}$ 7.0		
13	4.25 (d)	4.48 (b)	4.56———4.81[b]		5.37———6.01[b]		7.80 (C-2), 7.92, 7.96[c]
	$J_{1,2}$ *ca.* 1						

[a]Abbreviations: b = broad, d = doublet, q = quartet, t = triplet, sh = shoulder. [b]Unresolved multiplet. [c]Two acetoxyl groups.

The n.m.r. spectrum of the thiomannoside **13** was only partially resolved. It showed two one-proton signals at τ 4.25 and 4.48 (H-1, H-2), a two-proton multiplet at τ 4.56–4.81 (H-3, H-4), and a three-proton multiplet at τ 5.37–6.01 (H-5, two H-6).

In the absence of double-resonance studies the signals at τ 4.25 (*J ca.* 1) and 4.48 were tentatively assigned to H-1 and H-2, respectively.

It has been reported that signals due to axial acetoxyl protons appear downfield from those due to equatorial acetoxyl protons[19–23] which, in turn, have been suggested to appear downfield from primary equatorial acetoxyl proton resonances[22]. On this basis, the low-field resonances were assigned to axial acetoxyl protons at C-4 in **5** (τ 7.84) and **6** (τ 7.82), and at C-2 in **13** (τ 7.80). In the case of the galactoside **5**, the signal due to the C-6 acetoxyl group appeared not to be at the highest field (τ 8.01), as the fucoside **6**, in which the C-6-acetoxyl group is absent, also displayed a peak at τ 8.02.

The acetylated thioglycosides **5**, **6**, and **13** were transesterified with sodium methoxide in methanol[24]. The crystalline, deacylated *p*-nitrophenyl 1-thioglycosides **7**, **9**, and **14** were obtained in yields of over 95%.

Catalytic hydrogenation of **14** over palladium on barium sulfate for 24 h at atmospheric pressure was unsuccessful, only unchanged **14** being recovered. Hence reduction of **7**, **9**, and **14** was accomplished over the same catalyst with hydrogen under pressure. The crystalline *p*-aminophenyl 1-thioglycosides **8** and **10** were obtained in 84% and 63% yields, respectively. The *p*-aminophenyl 1-thio-α-D-mannopyranoside (**15**) was obtained as a syrup and could not be crystallized. Examination of the syrupy product on t.l.c. revealed, in addition to **15**, traces of other components. However, reduction of **14** in the presence of a small excess of hydrochloric acid afforded the crystalline hydrochloride (**16**) of **15** in 77% yield. On t.l.c., **16** migrated essentially as a homogeneous spot. The hydrochloride **16** was characterized further by acetylation to the crystalline *p*-acetamidophenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-mannopyranoside (**17**). The thioglycosides **8**, **10**, **15**, and **16** appeared to be susceptible to light and air. Generally, the spots on thin-layer plates became visible when the plates were kept for a few days. Although the *p*-aminophenyl 1-thioglycosides **8**, **10**, and **16** contained traces of other components, they were analytically pure. An attempt to recrystallize **8** from propyl alcohol resulted in extensive degradation. Consequently, no effort was made to free the thioglycosides **8**, **10**, and **16** of trace contaminants.

The elemental analyses and the i.r. spectra of all compounds described here were in total accord with their proposed structures.

EXPERIMENTAL

General. — Melting points were determined on a Fisher–Johns apparatus and are uncorrected. I.r. spectra were recorded with a Perkin–Elmer Model 257 spectrophotometer. Optical rotations were measured at ambient temperature in a 1-dm cell with a Perkin–Elmer Model 141 spectropolarimeter. N.m.r. spectra were recorded at 100 MHz in chloroform-*d* with tetramethylsilane as an internal reference. The chemical shifts represent the midpoints of multiplets and are approximate. These data are summarized in Table I. The R_F values given are those on thin-layer plates coated

with Silica Gel G or cellulose powder. The spots were visualized either by exposure to iodine vapor or to short-wave u.v. light. The solvents for t.l.c. were: *A*, 5:1 benzene–ethyl acetate; *B*, 40:11:19 butyl alcohol–ethyl alcohol–10% ammonium hydroxide; and *C*, 3:1:1 ethyl acetate–acetic acid–water. The acetylated derivatives were examined on silica gel in solvent *A*. For deacetylated derivatives, solvent *B* was used for cellulose plates and solvent *C* was used for silica gel plates. The silica gel used for column chromatography was Davison, grade 923, 100–200 mesh. Fractions from columns were monitored on microscope slides coated with silica gel G and eluted with solvent *A*. All evaporations were performed under diminished pressure on a rotary evaporator. Celite refers to Celite analytical filter-aid (Johns–Manville Co., New York, N.Y.). *p*-Nitrothiophenol was of technical grade of 80+% purity (Aldrich Chemical Co., Inc., Cedar Knolls, N.J.). The elementary analyses were performed by Galbraith Laboratories, Knoxville, Tennessee.

p-*Nitrophenyl 2,3,4,6-tetra*-O-*acetyl-1-thio-β*-D-*galactopyranoside* (**5**). — A solution of the crude syrupy bromide[25] **3**, prepared from 1,2,3,4,6-penta-*O*-acetyl-D-galactopyranose (**1**, 16.3 g), in dry ($CaCl_2$) chloroform (80 ml) was added during 15 min to a solution of *p*-nitrothiophenol (5.23 g) in 0.5M methanolic potassium hydroxide (75 ml). After 22 h at room temperature under nitrogen, the insoluble solid was filtered off and washed with 1:1 chloroform–methanol. The filtrates were evaporated to dryness and the residue was triturated with methanol (200 ml). The insoluble solid was removed by filtration and washed with methanol. The combined filtrates were evaporated to dryness. Last traces of methanol were removed from the residue by evaporation of pyridine from it.

The residue was dissolved in pyridine (100 ml) and to the solution was added acetic anhydride (100 ml). After being kept at room temperature for 18 h, the solvent was coevaporated off with toluene at 50°. The pale-yellow solid residue was dissolved in benzene (250 ml), washed successively with cold water (2 × 50 ml), cold saturated aqueous sodium hydrogen carbonate solution (2 × 50 ml), and cold water (2 × 50 ml), dried with $CaCl_2$, and evaporated to dryness. The solid residue was recrystallized from methanol to give pale-yellow crystals of **5**; yield, 5.94 g (29.3%); m.p. 157–158°. An analytical sample, m.p. 158–159°, was obtained from a previous run by an additional recrystallization from methanol; it had $[\alpha]_D$ −7.0° (*c* 0.96, chloroform); R_F 0.42; ν_{max}^{KBr} 1747 and 1735 (C=O), 1590, 1577, and 1478 (aromatic), 1513 and 1340 (NO_2), 1257 and 1228 (acetate C–O–C), 853 (C–N), 842 (*p*-disubstituted phenyl), and 743 cm^{-1} (C–N–O); n.m.r.: see Table I.

Anal. Calc. for $C_{20}H_{23}NO_{11}S$: C, 49.48; H, 4.78; N, 2.89. Found: C, 49.42; H, 4.84; N, 2.74.

p-*Nitrophenyl 2,3,4-tri*-O-*acetyl-1-thio-β*-D-*fucopyranoside* (**6**). — 1,2,3,4-Tetra-*O*-acetyl-D-fucopyranose[25] (**2**, 12.3 g) was dissolved in 30–32% HBr in acetic acid (48 ml) by mixing on a shaker for *ca.* 10 min[25]. The solution was kept for 2 h at room temperature and then toluene was evaporated several times from it to remove hydrogen bromide and acetic acid; a $CaCl_2$ tube was placed in the vacuum line for precaution against moisture backup from the water aspirator. The crude syrupy

bromide **4** was dissolved in dry ($CaCl_2$) chloroform (72 ml) and added during 15 min to a solution of *p*-nitrothiophenol (4.71 g) in 0.5M methanolic potassium hydroxide (67 ml) under nitrogen. The subsequent steps were as described for **5**. The crude syrup containing the thiofucoside **6** was dissolved in benzene (20 ml) and placed on the top of a column (45 cm × 4.3 cm) of silica gel (375 g) packed in benzene. The column was washed with benzene (*ca.* 1700 ml) until all by-products arising from *p*-nitrothiophenol had been removed. Elution was then continued with 4:1 benzene–ethyl acetate to recover **6**. The residual syrup obtained by pooling of appropriate fractions was crystallized from methanol to afford pale-yellow crystals of **6**; yield 7.43 g (46.6%); m.p. 138–140°. An additional recrystallization from methanol furnished the analytical sample in a similar run; m.p. 139–140°, $[\alpha]_D$ −4.3° (*c* 0.82, chloroform); R_F: 0.58; ν_{max}^{KBr} 1750 (C=O), 1595, 1578, and 1480 (aromatic), 1510 and 1335 (NO_2), 1248 and 1226 (acetate C–O–C), 856 (C–N), 842 (*p*-disubstituted phenyl), and 741 cm^{-1} (C–N–O); n.m.r.: see Table I.

Anal. Calc. for $C_{18}H_{21}NO_9S$: C, 50.58; H, 4.95; N, 3.28. Found: C, 50.56; H, 5.06; N, 3.21.

p-*Nitrophenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-*D*-mannopyranoside* (**13**). — 1,2,3,4,6-Penta-*O*-acetyl-D-mannopyranose[25] (**11**, 19.4 g) was treated[25] with 30–32% HBr in acetic acid (60 ml) to obtain crude syrupy bromide **12**, which was dissolved in dry ($CaCl_2$) chloroform (95 ml) and condensed with *p*-nitrothiophenol (6.23 g) dissolved in 0.5M methanolic potassium hydroxide (90 ml) as described for **5**. The crude syrup containing **13** was chromatographed on silica gel as for the fucoside **6**. The solvent was evaporated from appropriate fractions and the residue was crystallized from methanol to afford pale-yellow crystals of **13**; yield 7.09 g (29.4%) m.p. 137–138°. The analytical sample, m.p. 135–136°, was prepared in another run by recrystallization from methanol; $[\alpha]_D$ +142.6° (*c* 0.93, chloroform); R_F 0.44; ν_{max}^{KBr} 1752 and 1742 (C=O), 1592, 1572, and 1475 (aromatic), 1508 and 1340 (NO_2), 1243, 1225, and 1210 (shoulder) (acetate C–O–C), 852, 750, and 745 cm^{-1}; n.m.r.: see Table I.

Anal. Calc. for $C_{20}H_{23}NO_{11}S$: C, 49.48; H, 4.78; N, 2.89. Found: C, 49.55; H, 4.87; N, 2.65.

p-*Nitrophenyl 1-thio-β-*D*-galactopyranoside* (**7**). — To a suspension of 1-thiogalactoside **5** (5.88 g) in dry methanol (22 ml) was added 0.5M methanolic sodium methoxide[24] (2.4 ml). After 2.5 h at room temperature the yellow solution was deionized by passage through a small column of methanol-washed Amberlite IR-120 (H^+) resin. The crystalline residue remaining after removal of solvent from the eluate was triturated with anhydrous ether. Pale-yellow crystals of **7** were filtered off and washed with additional ether; yield 3.74 g (97.4%); m.p. 165–170°. T.l.c. indicated a single component. This product was suitable for further use.

Two recrystallizations from methanol–ether afforded pale-yellow crystals of analytical **7** from an earlier run; m.p. 173–174°, $[\alpha]_D$ −103.4° (*c* 0.80, methanol); R_F 0.81 (cellulose), 0.71 (silica gel); ν_{max}^{KBr} 3500–3250 (OH), 1592, 1575, and 1479 (aromatic), 1508 and 1340 (NO_2), 860, 852, 845, 840, 808, and 745 cm^{-1}.

Anal. Calc. for $C_{12}H_{15}NO_7S$: C, 45.42; H, 4.76; N, 4.41. Found: C, 45.54; H, 4.81; N, 4.52.

p-*Nitrophenyl 1-thio-β-*D-*fucopyranoside* (**9**). — *p*-Nitrophenyl 1-thiofucoside **6** (2.30 g) was deacetylated[24] with sodium methoxide in methanol as described for **7** to afford 1.52 g (93.8%) of nearly colorless crystals of **9**, m.p. 176–178°. This product was homogeneous on t.l.c. and was suitable for further use. Two recrystallizations from methanol–ether provided the analytical sample; m.p. 177–178°, $[\alpha]_D$ −113.2° (*c* 0.80, methanol); R_F 0.91 (cellulose), 0.81 (silica gel); ν_{max}^{KBr} 3470, 3380, and 3300 (OH), 1592, 1578, and 1480 (aromatic), 1510 and 1340 (NO_2), 857 (C–N), 840 (*p*-disubstituted phenyl), and 741 cm^{-1} (C–N–O).

Anal. Calc. for $C_{12}H_{15}NO_6S$: C, 47.84; H, 5.02; N, 4.65. Found: C, 47.99; H, 5.09; N, 4.60.

p-*Nitrophenyl 1-thio-α-*D-*mannopyranoside* (**14**). — Deacylation[24] of **13** (7.02 g) as described for **7** afforded 4.46 g (97.2%) of pale-yellow crystals of **14**, m.p. 184–186°. An additional recrystallization of **14** from another run from methanol–ether furnished the analytical sample; m.p. 186–188°, $[\alpha]_D$ +317.6° (*c* 0.89, methanol); R_F 0.85 (cellulose), 0.74 (silica gel); ν_{max}^{KBr} 3500–3300 (OH), 1580, 1573, and 1472 (aromatic), 1505 and 1338 (NO_2), 850 (shoulder), 845, 792, 760, and 741 cm^{-1}.

Anal. Calc. for $C_{12}H_{15}NO_7S$: C, 45.42; H, 4.76; N, 4.41. Found: C, 45.36; H, 4.67; N, 4.31.

p-*Aminophenyl 1-thio-β-*D-*galactopyranoside* (**8**). — The *p*-nitrophenyl thiogalactoside **7** (0.159 g) and 5% palladium on barium sulfate (0.100 g) in methanol (50 ml) were shaken at an initial pressure of 50 $lb.in^{-2}$ for 17 h. The catalyst was filtered off through Celite and the filtrate was concentrated to a light-yellow fluffed glass. Crystallization from propyl alcohol yielded the *p*-aminophenyl 1-thio-β-D-galactoside (**8**) as pale-yellow crystals; yield 0.12 g (83.6%); m.p. 169–172°, $[\alpha]_D$ −46.7° (*c* 0.90, methanol); R_F 0.60 (major), 0.67 (trace) (cellulose); 0.46 (major), 0.64 and 0.71 (traces), (silica gel). The mobility of **8** was identical to that of authentic *p*-aminophenyl 1-thio-β-D-galactopyranoside (Calbiochem, La Jolla, Calif.); ν_{max}^{KBr} 3300 (broad, OH and NH), 1620 (NH_2), 1598 and 1492 (aromatic), 1272 (C–N), 867 (NH_2), 818 cm^{-1} (*p*-disubstituted phenyl).

Anal. Calc. for $C_{12}H_{17}NO_5S$: C, 50.16; H, 5.96; N, 4.88. Found: C, 50.02; H, 6.07; N, 4.56.

p-*Aminophenyl 1-thio-β-*D-*fucopyranoside* (**10**). — The *p*-nitrophenyl 1-thiofucoside **9** (0.150 g) was hydrogenated with 5% palladium on barium sulfate (0.100 g) in methanol (50 ml) as described for **8**. Crystallization from propyl alcohol gave light-yellow crystals of *p*-aminophenyl 1-thio-β-D-fucoside **10**; yield 0.085 g (62.7%); m.p. 75–78°, $[\alpha]_D$ −76.6° (*c* 0.75, methanol); R_F 0.73 (cellulose), 0.61 (silica gel); ν_{max}^{KBr} 3440, 3360, and 3210 (shoulder) (OH, NH), 1620 (NH_2), 1595 and 1494 (aromatic), 1272 (C–N), 860 (NH_2), and 828 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, 53.09; H, 6.37; N, 4.89.

p-*Aminophenyl 1-thio-α-*D-*mannopyranoside hydrochloride* (**16**). — *p*-Nitrophenyl

1-thiomannoside **14** (0.635 g) and 5% palladium on barium sulfate (0.400 g) in methanol (200 ml) containing conc. HCl (0.8 ml) were shaken 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, leaving a nearly colorless crystalline residue. Recrystallization from methanol afforded the hydrochloride **16** as pale-yellow crystals; yield 0.497 g (76.9%); m.p. 175–185° dec., $[\alpha]_D$ +220.2° (*c* 1.14, water); R_F 0.69 (major), 0.58 (trace) (cellulose); 0.57 (major), 0.19 and 0.74 (traces) (silica gel); ν_{max}^{KBr} 3300 (broad, OH), 2870, 2590, and 2300 (NH_3^+), 1605 (broad, NH_3^+ and aromatic), 1510 (NH_3^+), 1490 (aromatic), 850, 820, 800, and 772 cm^{-1}.

Anal. Calc. for $C_{12}H_{17}NO_5S \cdot HCl$: C, 44.51; H, 5.60; N, 4.33. Found: C, 44.39; H, 5.71; N, 3.86.

When **14** was hydrogenated in the absence of HCl, **15** was obtained as a light-yellow syrup that could not be induced to crystallize. On t.l.c. the syrup showed a major spot [R_F 0.71 (cellulose), 0.56 (silica gel)] corresponding to **15** and traces of other components [R_F 0.57 and 0.77 (cellulose), 0.40, 0.48, 0.63, and 0.76 (silica gel)]. Acetylation of syrupy **15** afforded the crystalline acetate **17** (following experiment) in *ca.* 55% yield.

p-*Acetamidophenyl 2,3,4,6-tetra*-O-*acetyl-1-thio*-α-D-*mannopyranoside* (**17**). — To a solution of **16** (0.201 g) in pyridine (4 ml), cooled in an ice-bath, was added acetic anhydride (2 ml). After 18 h at room temperature, the solvents were removed by coevaporation with toluene. The nearly colorless, crystalline residue was suspended in water (4 ml), filtered off, and washed with additional water; yield 0.282 g (90.7%); m.p. 139–141°. One recrystallization from methanol–water with the aid of charcoal afforded analytical **17**; m.p. 139–141°, $[\alpha]_D$ +97.8° (*c* 0.81, chloroform); R_F 0.33; ν_{max}^{KBr} 3355 (NH), 1740 (ester C=O), 1680 (amide, type I band), 1587 and 1490 (aromatic), 1510 (amide, type II band), 1242 and 1220 (acetate C–O–C), 840, 828, and 748 cm^{-1}.

Anal. Calc. for $C_{22}H_{27}NO_{10}S$: C, 53.11; H, 5.47; N, 2.82. Found: C, 53.16; H, 5.39; N, 2.89.

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