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AN IMPROVED METHOD FOR THE SYNTHESES OF *p*-AMINOPHENYL 1-THIO-β-D-GLYCOSIDES

NOBUKO IINO AND KAZUO YOSHIDA

Daiichi College of Pharmaceutical Sciences, Tamagawacho, Takamiya, Minamiku, Fukuoka (Japan)

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ABSTRACT

The condensation of the appropriate acetylglycosyl bromides with *p*-aminobenzenethiol in the presence of sodium methoxide afforded *p*-aminophenyl 1-thio-β-D-glucopyranoside, 1-thio-β-D-galactopyranoside, 1-thio-β-D-xylopyranoside, and 2-acetamido-2-deoxy-1-thio-β-D-glucopyranoside. *p*-Aminophenyl 1-thio-β-D-glucopyranosiduronic acid was synthesized by condensation of methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide)uronate with *p*-aminobenzenethiol, followed by saponification with sodium hydroxide.

INTRODUCTION

The recent reports on the affinity chromatography of glycosidases, and the purification of 2-acetamido-2-deoxy-β-D-glucosidase[1] and β-D-galactosidase[2–6] by affinity chromatography using *p*-aminophenyl 2-acetamido-2-deoxy-1-thio-β-D-glucopyranoside and *p*-aminophenyl 1-thio-β-D-galactopyranoside, respectively, as ligand, have shown the usefulness of *p*-aminophenyl 1-thio-β-D-glycosides for affinity chromatography. *p*-Aminophenyl 1-thioglycosides were synthesized by Chipowsky and Lee[7], Bahl and associates[8–10], and Wagner and Lenk[11] by condensation of the appropriate acetylglycosyl halides with *p*-nitrobenzenethiol, followed by deacetylation, and catalytic hydrogenation of the nitro to the amino group. The overall yields of *p*-aminophenyl 1-thio-β-D-galactopyranoside, -β-D-glucopyranoside, and 2-acetamido-2-deoxy-β-D-glucopyranoside from each acetylglycosyl halide were rather poor, 23.8%, 22%, and 38.7%, respectively. The present investigation was undertaken to improve the yields in the syntheses of *p*-aminophenyl 1-thio-β-D-glycosides, by condensing *p*-aminobenzenethiol with the appropriate acetylglycosyl halide in dry methanol containing sodium methoxide to give the corresponding *p*-aminophenyl 1-thio-β-D-glycosides in one step. This method is simple and gave fairly good yields, except for *p*-aminophenyl 2-acetamido-2-deoxy-1-thio-β-D-glucopyranoside. *p*-Aminophenyl 1-thio-β-D-glucopyranoside, -β-D-galactopyranoside, -β-D-xylopyranoside and -2-acetamido-2-deoxy-β-D-glucopyranoside were obtained in 48%, 45%, 46%, and 20% yield, respectively. *p*-Aminophenyl 1-thio-β-D-glucopyranosiduronic acid

was prepared in 58% yield by condensation of *p*-aminobenzenethiol with methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide)uronate by the same method just described, followed by saponification with methanol–sodium hydroxide. To the best of our knowledge, this is the first synthesis of *p*-aminophenyl 1-thio-β-D-glucopyranosiduronic acid and 1-thio-β-D-xylopyranoside.

EXPERIMENTAL

General methods. — Melting points were determined with a Yanagimoto MP-S2 apparatus and are uncorrected. I.r. spectra were recorded with a Hitachi Model EPI-G3 spectrophotometer. Optical rotations were measured at room temperature in a 0.5-dm cell with a Yanagimoto Model OR-10 polarimeter. N.m.r. spectra were recorded at 60 MHz with a Hitachi R-20A spectrometer in chloroform-*d* with Me_4Si as the internal reference, unless otherwise stated. Mass spectra were recorded with a JMS-D100 spectrometer. Thin-layer chromatography was performed on plates precoated with Wakogel B-5 with the following solvents (v/v): I, 1:3:2 methanol–ethyl acetate–benzene; and II, 5:2:2 ethyl acetate–acetic acid–water. At first, the spots were detected by the Ehrlich reagent (*A*) for the amino group, and then by heating at 100–110° on a hot plate the same t.l.c. plates presprayed with 50% H_2SO_4 (*B*), for carbohydrate components. After hydrolysis of the acetamido group with M HCl, acetylated derivatives were detected by *A* as well as *B*. The absence of α anomer in the β-D-glycosides was ascertained by g.l.c.[12] with a Shimazu Gas-Chromatograph GC-5A, equipped with a hydrogen-flame, ionization detector and a stainless-steel column (2 m × 3 mm) packed with 1.5% silicone SE-30 on 60–80 mesh Chromosorb W (SE-30) or with 3% silicone SE-52 on 60–80 mesh Chromosorb W (SE-52), N_2 being the carrier gas at a flow rate of 60 ml/min. The trimethylsilylation was performed as described by Sweeley *et al.*[13] and the trifluoroacetylation as described by Vilkas *et al.*[14].

p-*Aminophenyl 1-thio-β-D-galactopyranoside.* — A solution of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (1 g) in abs. methanol (5 ml) was added, dropwise, to a solution of *p*-aminobenzenethiol (0.3 g) in chilled (−14°) 0.586M methanolic sodium methoxide (4.3 ml). The reaction mixture was stirred for 1 h at −14°, and then for 4 h at room temperature. The reaction mixture was evaporated under reduced pressure. Water (10 ml) and ethyl acetate (4 ml) were added to the residue, and the mixture was vigorously shaken. The water layer was washed with ethyl acetate several times to remove the unreacted bromide. The water layer was applied to a column of Amberlite IR-120 (H^+) ion-exchange resin (7.5 ml), and the column was washed with water and then with 1:9 (v/v) pyridine–water to elute a fraction that was evaporated under reduced pressure to give crude crystals (314 mg, 44.9%), m.p. 164–175°. Recrystallization from ethanol gave a compound having m.p. 175–177°, $[\alpha]_D^{23}$ −44.4° (*c* 1, methanol); t.l.c.: R_F: 0.25(I), 0.37(II); i.r.: ν_{max}^{KBr}: 3300 (broad, OH and NH), 1615 (NH_2 shoulder), 1594 and 1487 (aromatic), 1268 (C–N), and 818 cm^{-1} (*p*-subst. Ph); m.s.: *m/e* 287 (M^+); lit.[8]: m.p. 169–172°, $[\alpha]_D$ −46.7° (*c* 0.90, methanol);

lit.[7]: m.p. 173–173.5°, $[\alpha]_D$ −49.1° (water); lit.[15]: m.p. 173–174°, $[\alpha]_D^{20}$ −44.6° (*c* 1, methanol).

Anal. Calc. for $C_{12}H_{17}NO_5S$: C, 50.18; H, 5.92; N, 4.88. Found: C, 49.90; H, 6.01; N, 4.90.

On g.lc. on a column of SE-52, the per(trimethylsilyl) ether (16.5 min) and the trifluoroacetate (7.4 min) gave single peaks at 230° and 200°, respectively.

p-*Aminophenyl 1-thio-β-*D*-glucopyranoside.* — 2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl bromide was condensed with *p*-aminobenzenethiol as described for the galactoside to give crude *p*-aminophenyl 1-thio-β-D-glucopyranoside (48.3% yield), m.p. 144–145°. Recrystallization from ethanol afforded the analytical sample, m.p. 147–148°, $[\alpha]_D^{22}$ −35.4° (*c* 1.0, methanol); t.l.c.: R_F 0.33(I), and 0.45(II); i.r.: ν_{max}^{KBr} 3300 (OH and NH), 1620 (NH_2), 1600 and 1494 (aromatic), 1265 (C–N), and 820 cm^{-1} (*p*-subst. Ph); m.s.: *m/e* 287 ($M^{\ddagger}$); lit.[11]: m.p. 146°, $[\alpha]_D^{22}$ −63.5° (*c* 5.0, water); lit.[15]: m.p. 147°, $[\alpha]_D^{20}$ −35.9° (*c* 0.8, methanol).

Anal. Calc. for $C_{12}H_{17}NO_5S$: C, 50.18; H, 5.92; N, 4.88. Found: C, 50.34; H, 6.13; N, 5.11.

On g.l.c. on a column of SE-52, the per(trimethylsilyl)ether (14.1 min) and the trifluoroacetate (7.4 min) gave single peaks at 230° and 200°, respectively.

p-*Aminophenyl 2-acetamido-2-deoxy-1-thio-β-*D*-glucopyranoside.* — 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride was condensed with *p*-aminobenzenethiol as described for the galactoside to give crude (20% yield) *p*-aminophenyl 2-acetamido-2-deoxy-1-thio-β-D-glucopyranoside, m.p. 240–246°. Recrystallization from ethanol afforded fine crystals, m.p. 246–249°, $[\alpha]_D^{21}$ +6.0° (*c* 1.0, methanol); t.l.c.: R_F 0.046(I), and 0.48(II); i.r.: ν_{max}^{KBr} 3475, 3425, 3340 and 3240 (OH and NH), 1600 and 1495 (aromatic), 1640 (Amide I), 1555 (Amide II), and 1275 cm^{-1} (C–N); m.s.: *m/e* 328 ($M^{\ddagger}$); lit.[10]: m.p. 246–248°, $[\alpha]_D$ +3.9° (*c* 1.06, methanol).

Anal. Calc. for $C_{14}H_{20}N_2O_5S$: C, 51.22; H, 6.10; N, 8.54. Found: C, 51.04; H, 6.46; N, 8.28.

On g.l.c. on a column of SE-52, the per(trimethylsilyl) ether gave one sharp peak at 200° (2.1 min).

p-*Aminophenyl 1-thio-β-*D*-xylopyranoside.* — To a solution of *p*-aminothiophenol (0.9 g) in chilled (−14°) 0.586M methanolic sodium methoxide (13 ml) was added in portions with stirring 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide (2.8 g). The reaction mixture was treated as described for the galactoside to give crude (46.2% yield) *p*-aminophenyl 1-thio-β-D-xylopyranoside, m.p. 149°. Recrystallization from ethanol afforded the analytical sample, m.p. 150–151°, $[\alpha]_D^{22}$ −80.6° (*c* 1.0, methanol); t.l.c.: R_F 0.45(I) and 0.61(II); i.r.: ν_{max}^{KBr} 3300 (OH and NH), 1615 (NH_2), 1596 and 1490 (aromatic), 1255 (C–N), and 818 cm^{-1} (*p*-subst. Ph); m.s.: *m/e* 257 ($M^{\ddagger}$).

Anal. Calc. for $C_{11}H_{15}NO_4S$: C, 51.36; H, 5.84; N, 5.45. Found: C, 51.08; H, 5.94; N, 5.05.

On g.l.c. on a column of SE-52, the per(trimethylsilyl) ether (10.3 min) and the trifluoroacetate (7.0 min) gave sharp peaks at 230° and 200°, respectively.

p-*Aminophenyl 1-thio-β-*D*-glucopyranosiduronic acid.* — Methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide)uronate (4.0 g) was added to a solution of *p*-aminobenzenethiol (1.2 g) in 0.586M methanolic sodium methoxide (17 ml) precooled to −14°. The reaction mixture was stirred for 1 h at −14° and then for 4 h at room temperature. Methanol (100 ml) was added, and the solution was cooled to 0°. M NaOH (100 ml) was added, and the solution was warmed for 1 h at 40°. The methanol was evaporated under reduced pressure to give an insoluble material that was filtered off, and the filtrate (~100 ml) was applied to a column of Amberlite IR-120 (H^+) ion-exchange resin (200 ml). The column was washed with water until the eluate was no longer acidic, and then with 1:9 (v/v) pyridine–water. The pyridine–water solution was evaporated under reduced pressure to give crystals (1.87 g, 58.2%), m.p. 159–161°. Recrystallization from water (charcoal) gave pale-yellow needles, becoming opaque at 105–120° and melting clearly at 166.5–168°, $[\alpha]_D^{22}$ −68.8° (*c* 1.0, methanol); t.l.c.: R_F 0.02(I) and 0.37(II); i.r.: ν_{max}^{KBr} 3320 (OH and NH), 1635 and 1588 (CO_2^-), 1495 (aromatic), 1270 (C–N), and 820 cm^{-1} (*p*-subst. Ph); m.s.: *m/e* 301 (M^+).

Anal. Calc. for $C_{12}H_{15}NO_6S \cdot H_2O$: C, 45.14; H, 5.33; N, 4.39. Found: C, 45.47; H, 5.34; N, 4.66.

p-*Acetamidophenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-*D*-galactopyranoside.* — To a solution of *p*-aminophenyl 1-thio-β-D-galactopyranoside (100 mg) in cold, dry pyridine (2 ml) was added acetic anhydride (2 ml). After being kept overnight, the solution was evaporated after addition of toluene. Ice–water was added to the syrupy residue with stirring to give crystals (77.5 mg, 44.8%) that were recrystallized several times from ethanol–water to give the analytical sample, m.p. 134–135°, $[\alpha]_D^{19}$ −9.2° (*c* 0.25, chloroform); t.l.c.: R_F 0.58(I) and 0.89(II); i.r.: ν_{max}^{KBr} 3400 (NH), 1745 (C=O), 1225 (CO of Ac), and 828 cm^{-1} (*p*-subst. Ph); m.s.: *m/e* 497 (M^+); n.m.r.: δ 1.95 (s, 3 H, OAc), 2.02 (s, 3 H, OAc), 2.09 (s, 6 H, 2 OAc), 2.15 (s, 3 H, NAc) and 7.41 (s, 4 H, Ph).

Anal. Calc. for $C_{22}H_{27}NO_{10}S$: C, 53.11; H, 5.43; N, 2.81. Found: C, 52.62; H, 5.82; N, 2.76.

p-*Acetamidophenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-*D*-glucopyranoside.* — To a solution of *p*-aminophenyl 1-thio-β-D-glucopyranoside (200 mg) in dry pyridine (5 ml) precooled at 0° was added acetic anhydride (5 ml). After being kept overnight, the mixture was poured into ice–water (50 ml) with stirring, and the crystals formed were filtered off (300 mg, 86.7%), m.p. 154–156°. Recrystallization from ethanol gave the analytical sample, m.p. 155–156°, $[\alpha]_D^{24}$ −34.0° (*c* 1.0, chloroform); t.l.c.: R_F 0.63 (I) and 0.95 (II); i.r.: ν_{max}^{KBr} 3360 (NH), 1745 (C=O), 1220 (CO of Ac), and 825 cm^{-1} (*p*-subst. Ph); m.s.: *m/e* 497 (M^+); n.m.r. δ 1.98 (s, 3 H, OAc), 2.00 (s, 3 H, OAc), 2.07 (s, 6 H, 2 OAc), 2.16 (s, 3 H, NAc), and 7.04 (s, 4 H, Ph); lit.[11]: m.p. 153–155°, $[\alpha]_D^{21}$ −35.3° (*c* 5.0, chloroform).

Anal. Calc. for $C_{22}H_{27}NO_{10}S$: C, 53.11; H, 5.43; N, 2.81. Found: C, 53.07; H, 5.39; N, 2.91.

p-*Acetamidophenyl 2,3,4-tri*-O-*acetyl-1-thio-β*-D-*xylopyranoside.* — *p*-Aminophenyl 1-thio-β-D-xylopyranoside (100 mg) was treated as just described for the glucoside to afford white crystals (161 mg, 97.6%), m.p. 112–144°. Recrystallization from ethanol–water gave the analytical sample, m.p. 117–118.5°, $[\alpha]_D^{25}$ −69.2° (*c* 1.0, chloroform); t.l.c.: R_F 0.68 (I) and 0.92 (II); i.r.: ν_{max}^{KBr} 3300 (NH), 1740 (C=O), 1210–1250 (CO of Ac), and 825 cm^{-1}; m.s.: *m/e* 425 ($M^{\mp}$); n.m.r.: δ 2.03 (s, 6 H, 2 OAc), 2.09 (s, 3 H, OAc), 2.15 (s, 3 H, NAc), and 7.40 (s, 4 H, Ph).

Anal. Calc. for $C_{19}H_{23}NO_8S$: C, 53.65; H, 5.41; N, 3.29. Found: C, 53.93; H, 5.87; N, 3.08.

p-*Acetamidophenyl 2-acetamido-3,4,6-tri*-O-*acetyl-2-deoxy-1-thio-β*-D-*glucopyranoside.* — Treatment of *p*-aminophenyl 2-acetamido-2-deoxy-1-thio-β-D-glucopyranoside (200 mg) as just described for the glucoside gave 187 mg (61.9%), m.p. 237–238°. Recrystallization from ethanol gave the analytical sample, m.p. 248°, $[\alpha]_D^{28}$ −32.8° (*c* 0.25, chloroform); t.l.c.: R_F 0.40(I) and 0.90(II); i.r.: ν_{max}^{KBr} 3320 and 3250 (NH), 1660 (Amide I), 1585 and 1495 (aromatic), and 1522 cm^{-1} (Amide II); m.s.: *m/e* 496 ($M^{\mp}$); n.m.r. (dimethyl sulfoxide-d_6): δ 1.87, 1.95, 2.00, 2.02 and 2.10 (s, 15 H, 3 OAc and 2 NAc), and 7.44 (d, 4 H, Ph). On g.l.c. on a column of SE-30, the compound showed one sharp peak at 200° (3.5 min).

Anal. Calc. for $C_{22}H_{28}N_2O_9S$: C, 53.22; H, 5.64; N, 5.64. Found: C, 53.37; H, 5.23; N, 5.43.

Methyl (p-*acetamidophenyl 2,3,4-tri*-O-*acetyl-1-thio-β*-D-*glucopyranosid*)-*uronate.* — To an ethereal solution of diazomethane (prepared from 2 g of 1-methyl-1-nitrosourea) was added a solution of *p*-aminophenyl 1-thio-β-D-glucopyranosiduronic acid (200 mg) in abs. methanol (30 ml). After 3 h, the solution was evaporated under reduced pressure to afford a syrupy residue that was dissolved in dry pyridine (5 ml), and acetic anhydride (5 ml) was added. After being kept overnight, the mixture was diluted with ice–water with vigorous stirring to yield crude crystals (261 mg, 86%), m.p. 195–197°. Recrystallization from methanol (charcoal) gave m.p. 197–198°, $[\alpha]_D^{24}$ −35.0° (*c* 1.0, chloroform); t.l.c.: R_F 0.63(I) and 0.93(II); i.r.: ν_{max}^{KBr} 3360 (NH), 1750 (C=O), 1690 (CO_2^-), 1205–1240 (CO of Ac), and 825 cm^{-1}; m.s.: *m/e* 483 ($M^{\mp}$); n.m.r.: δ 1.97 (s, 6 H, 2 OAc), 2.06 (s, 3 H, OAc), 2.15 (s, 3 H, NAc), 3.73 (s, 3 H, OMe), and 7.45 (s, 4 H, Ph). On g.l.c. on a column of SE-30, the compound gave one sharp peak at 280° (6.1 min).

Anal. Calc. for $C_{21}H_{25}NO_{10}S$: C, 52.17; H, 5.18; N, 2.90. Found: C, 52.24; H, 5.24; N, 2.58.

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