SYNTHESIS OF 6-AMINO-6-^{14}C-HEXYL 1-THIO-α-D-MANNOPYRANOSIDE

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SUMMARY

The radiolabeled insulin-like carbohydrate derivative, 6-amino-6-^{14}C-hexyl 1-thio-α-D-mannopyranoside (6), has been synthesized for receptor binding and mechanism-of-action studies by a two-step process from 5-bromopentyl 1-thio-α-D-mannopyranoside (4). Nucleophilic displacement reaction on compound 4 with sodium cyanide-^{14}C and subsequent catalytic reduction of the intermediate nitrile 5 afforded after chromatography the radiochemically pure amine 6 with a specific activity of 47.3 mCi/mmol.

Key Words: Insulin-like agent, 6-aminohexyl 1-thio-α-D-mannopyranoside, carbon-14

INTRODUCTION

We have recently reported the *in vitro* insulin-like activity on rat adipocytes of 6-aminohexyl 1-thio-α-D-mannopyranoside and structural analogs (1,2). To assist in the elucidation of the mechanism of action in cellular and cell membrane systems, a radiolabeled form of this novel saccharide derivative was required. A synthesis of carbon-14 labeled material has been developed, and the route is depicted by the following sequence:

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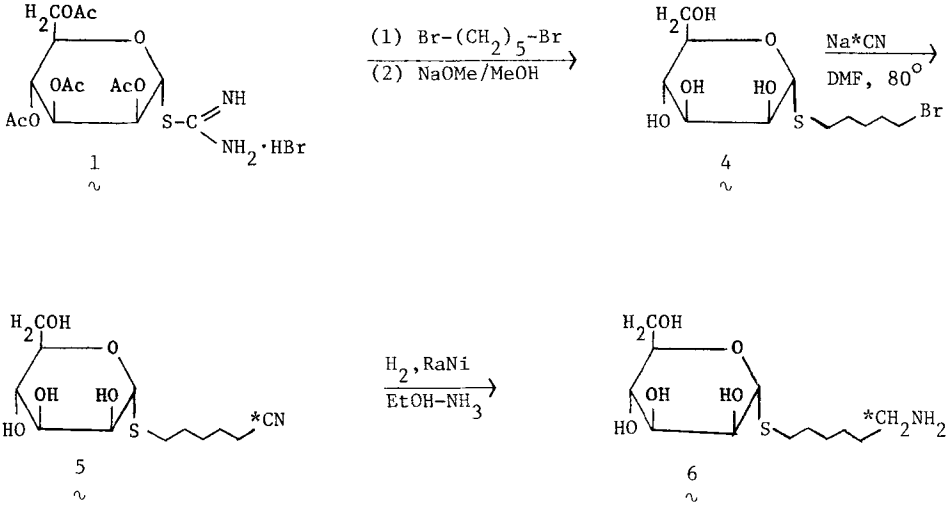

1 ~ 4 ~ 5 ~ 6 ~

Reaction of 2-S-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-2-thiopseudourea hydrobromide (1) with two equivalents of 1,5-dibromopentane in the presence of potassium carbonate and potassium metabisulfite gave after chromatography crystalline 5-bromopentyl 2,3,4,6-tetra-O-acetyl-1-thio-α-D-mannopyranoside (2). 1,5-Di-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosylthio)-pentane (3) was also obtained as a by-product of the reaction. Zemplén deacetylation of 2 afforded 5-bromopentyl 1-thio-α-D-mannopyranoside (4), which could not be induced to crystallize. Since bromide 4 as a syrup proved to be unstable, the subsequent step in the sequence was carried out without delay. Introduction of the carbon-14 label into the molecule was achieved via a nucleophilic displacement reaction on compound 4 with sodium cyanide-^{14}C. Chromatography of the crude product provided 45.2 mCi (77.3% radiochemical

yield) of the pure nitrile-^{14}C 5. Catalytic hydrogenation of this intermediate in saturated ethanolic ammonia in the presence of Raney nickel yielded the desired labeled amine 6. Purification to tracer quality material was achieved by gradient silica column chromatography. Those fractions which contained, on the basis of t.l.c., radiochemically pure product were combined to yield 20.3 mCi of 6 with a specific activity of 47.3 mCi/mmol. The 6-amino-6-^{14}C-hexyl 1-thio-α-D-mannopyranoside (6) has been employed in investigations on the mechanism of action of ω-aminoalkyl glycosides on fat cells (3) and in adipocyte surface membrane binding experiments (4).

EXPERIMENTAL

General methods. - Solutions were evaporated below 50°C under diminished pressure. Melting points were determined with a Thomas-Hoover "Unimelt" apparatus and are uncorrected. Optical rotations were measured with a Zeiss polarimeter. N.m.r. spectra were recorded at 60 and 300 MHz with Varian T-60 and SC-300 n.m.r. spectrometers, respectively. Column chromatography was conducted with either silica gel 60 No. 7734 (E. Merck; 70-230 mesh) or silica gel 60HR No. 7744 (E. Merck). T.l.c. was performed on plates (250 μm) of Silica Gel GF_{254} (Analtech), and visualization was effected with a ceric sulfate (1%) - sulfuric acid (10%) spray. Radioactive zones on t.l.c. were located with a Varian Aerograph/Berthold Model LB2722 scanner. Radioactivity was determined with a Packard Tri-Carb Model 3320 liquid scintillation spectrometer using 0.4% omnifluor in toluene-ethanol (7:3) as scintillator medium.

2-S-(2,3,4,6-Tetra-O-acetyl-α-D-mannopyranosyl)-2-thiopseudourea hydrobromide (1). - A solution of tetra-O-acetyl-α-D-mannopyranosyl bromide (5) (78.8 g, 19.2 mmol) and thiourea (15 g, 19.7 mmol) in dry acetone (90 ml) was heated at reflux for 2 h. The mixture was then concentrated, and the residue partitioned between water (100 ml) and chloroform (70 ml). The aqueous layer was extracted with chloroform (50 ml), and the product allowed to crystallize from the aqueous layer overnight at 5° to yield 52.6 g (54%). An analytical sample was obtained by recrystallization from water; m.p. 125-128°; $[\alpha]_D^{27}$ + 103 ± 0.5° (c 1, acetone) {lit. (6) m.p. 131-133°; $[\alpha]_D^{20}$ + 106.8° (c 1, methanol)}.

Anal. Calc. for $C_{15}H_{23}BrN_2O_9S \cdot H_2O$: C, 35.65; H, 4.99; N, 5.54; Br, 15.81; S, 6.34. Found: C, 35.54; H, 5.03; N, 5.61; Br, 15.90; S, 6.40.

5-Bromopentyl 2,3,4,6-tetra-O-acetyl-1-thio-α-D-mannopyranoside (2). - To a mixture of 1 (2.3 g, 4.6 mmol), potassium carbonate (0.63 g, 4.6 mmol), and potassium metabisulfite (1.1 g, 4.9 mmol) in water (10 ml) was added a solution of 1,5-dibromopentane (2.1 g, 9.1 mmol) in acetone (10 ml). The mixture was stirred for 2 h at room temperature, concentrated, and the residue partitioned between dichloromethane and water. The organic layer was dried (sodium sulfate) and evaporated to a syrup that was applied to a column of silica gel (E. Merck, #7734) that was developed with 19:1 chloroform-ethyl acetate. The title compound 2 was eluted first and was obtained as a solid that was recrystallized twice from ethanol; yield 1.5 g (64%); m.p. 91.5-93°; $[\alpha]_D^{27}$ + 85.8 ± 0.5° (c 1, chloroform).

Anal. Calc. for $C_{19}H_{29}BrO_9S$: C, 44.45; H, 5.69; Br, 15.56; S, 6.24. Found: C, 44.63; H, 5.64; Br, 15.88; S, 6.40.

Further development with the same solvent system afforded the slower-moving 1,5-di-(tetra-O-acetyl-α-D-mannopyranosylthio)-pentane (3). Recrystallization from ethanol gave pure 3 (0.32 g); m.p. 119-120°; $[\alpha]_D^{25}$ + 115 ± 0.5° (c 1, chloroform).

Anal. Calc. for $C_{33}H_{48}O_{18}S_2$: C, 49.74; H, 6.07; S, 8.05. Found: C, 49.84; H, 6.08; S, 8.16.

5-Bromopentyl 1-thio-α-D-mannopyranoside (4). - To a mixture of the tetraacetate 2 (600 mg, 1.2 mmol) in dry methanol (6 ml) was added a catalytic amount of sodium methoxide. The mixture was kept overnight at room temperature, made neutral with Bio-Rad AG50W-X4 (H^+) ion-exchange resin, the suspension filtered, and the filtrate evaporated. The residue was applied to a column of silica gel (E. Merck, #7734), and the desired product was obtained by development with 9:1 chloroform-methanol; yield 355 mg (88%). Since the unprotected bromide 4 as a syrup was unstable when exposed to the atmosphere at room temperature, it was not further characterized but used immediately for the nucleophilic displacement reaction described below.

5-Cyano-5-^{14}C-pentyl 1-thio-α-D-mannopyranoside (5). - Into a 100-ml round-bottom flask containing a magnetic bar for stirring was weighed 53.3 mg (1.04 mmol, 57.7 mCi/mmol, American Radiochemical Corporation Lot #376) of sodium cyanide-^{14}C. A solution of 5-bromopentyl 1-thio-α-D-mannopyranoside (4) (347 mg, 1.0 mmol) in dry DMF (10 ml) was then added and the mixture stirred under nitrogen for 4 h at 80°C. The cooled mixture was then evaporated *in vacuo* at ~35°C, and the residue applied to a column of silica gel (E. Merck, #7744). Elution with 19:1 chloroform-methanol afforded the chromatographically homogeneous nitrile 5 (300.3 mg, 45.2 mCi, 77.3% radiochemical yield), isographic on t.l.c. with authentic material (7).

6-Amino-6-^{14}C-hexyl 1-thio-α-D-mannopyranoside (6). - A solution of the ^{14}C-nitrile 5 (300.3 mg, 45.2 mCi) in saturated ethanolic ammonia (50 ml) was hydrogenated at 35 psi for 6 h at 25° in the presence of Raney nickel catalyst (100 mg). The catalyst was then removed by filtration through Celite under an atmosphere of nitrogen, the filter was washed with methanol, and the combined filtrates were evaporated under diminished pressure to a final constant weight of 262 mg. T.l.c. analysis of the reaction mixture before and after high-vacuum constant-weight drying indicated the presence of a volatile and ninhydrin-positive impurity running at the solvent front and accounting for 20-25% of the total radioactivity. The residue, only about 80-85% pure by t.l.c. radioscan, was applied to a column of silica gel (17 g, E. Merck, #7744) that was developed with at first 1:1 chloroform-methanol and subsequently with 50:50:2, 50:50:4, and finally 50:50:10 chloroform-methanol-28.5% ammonium hydroxide. The fractions containing radiochemically pure product by t.l.c. analysis were combined and evaporated to afford the ninhydrin-positive amine 6 (20.3 mCi, 127 mg, 47.3 mCi/mmol), isographic with authentic material (7). The radiochemical yield was 44.9% (34.7% overall from sodium cyanide-^{14}C). Analysis of this material by scintillation counting of sections scraped from a t.l.c. plate developed in either 4:1:1 n-butanol-acetic acid-water or 2:2:1 chloroform-methanol-28.5% ammonium hydroxide indicated a radiochemical purity of 99%. Its 300-MHz n.m.r. spectrum in D_2O was identical to that of an authentic sample (7,8).

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7. The synthesis of unlabeled nitrile and 6-aminohexyl 1-thio-α-D-manno-pyranoside will be reported elsewhere.

8. Unlabeled 6-aminohexyl 1-thio-α-D-mannopyranoside (7) exhibited the following physical characteristics: m.p. 93-95°, $[\alpha]_D^{25}$ + 188° (c 1, methanol), n.m.r. (300-MHz, D_2O): δ 1.34-1.72 [m, 8H, $-S-CH_2-(CH_2)_4-CH_2NH_2$], 2.62-2.78 [m, 4H, $-S-CH_2-(CH_2)_4-CH_2NH_2$], and 5.30 (s, 1H, H-1). Anal. Calc. for $C_{12}H_{25}NO_5S$: C, 48.79; H, 8.53; N, 4.74; S, 10.85. Found: C, 48.59; H, 8.38; N, 4.57; S, 10.79.