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Note

Synthesis of 7-bromo-3-(α-D-mannopyranosyloxy)-2-naphth-o-anisidide, a D-mannosyl derivative of naphthol AS-BI*

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Use of a chromogenic substrate is one of the standard procedures for the assay of glycosidases[1]. Glycosides composed of 7-bromo-3-hydroxy-2-naphth-*o*-anisidide (naphthol AS-BI) (**1**) as the chromogenic aglycon and 2-acetamido-2-deoxy-D-glucose or D-glucuronic acid are known[2], and the advantage of such derivatives of naphthol AS as substrates for histochemical detection has also been shown[3]. Generally, glycosides containing naphthol AS-BI have been synthesized by the condensation of an acetylated glycosyl halide with naphthol AS-BI in alkaline aqueous acetone[4], as developed originally by Michael[5]. Because glycosides of D-mannose with naphthol AS-BI are not available commercially, it was of interest to develop a convenient preparation of these derivatives. Courtin-Duchateau and Veyrières[6] reported the preparation of 4-methylumbelliferyl α- and β-D-mannopyranoside. We now describe a convenient synthesis of 7-bromo-3-(α-D-mannopyranosyloxy)-2-naphth-*o*-anisidide (**4**) by the nitromethane–mercuric cyanide procedure[7], which is known to be a useful method for the synthesis of nucleosides. Compound **4** has been successfully used for the histochemical detection of α-D-mannosidases in tissues[8].

Initial attempts to obtain **4** under the usual, alkaline conditions previously described[4] did not give satisfactory yields, whereas application of the nitromethane–mercuric cyanide procedure[7] afforded high yields. Thus, reaction under reflux of a mixture of naphthol AS-BI (**1**) and 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl chloride[6] (**2**) (2 equiv.) in the presence of an excess of mercuric cyanide gave 7-bromo-3-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyloxy)-2-naphth-*o*-anisidide (**3**)

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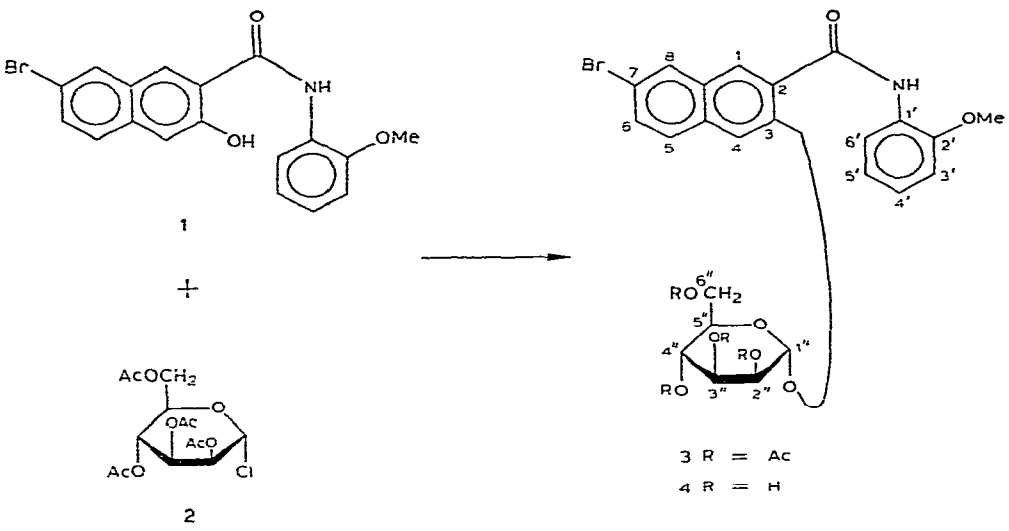
in 72% yield. *O*-Deacetylation was readily achieved by treatment of **3** with sodium methoxide in methanol, to give **4** in 88% yield.

As Onodera *et al.*[9] reported that 9-α-D-mannopyranosyltheophylline exists in the $^1C_4$(D) conformation, it was of interest to consider the n.m.r. data for such compounds as **3** and **4**, having a bulky aglycon. The 270-MHz, n.m.r. spectrum of **3** showed that the chemical shifts and the coupling constants of protons of the tetra-*O*-acetyl-D-mannosyl group are consistent with those of 4-methylumbelliferyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside, except for low-field shifts of H-1′ and H-2′, which were both strongly deshielded by the aglycon group. The coupling constants suggested that **3** might exist in the $^4C_1$ conformation expected. The n.m.r. spectrum of **4** in dimethyl sulfoxide-d_6 solution showed the H-1′ signal as a singlet at δ 5.76. Four signals (three doublets and one triplet) appeared in the region of δ 4.5–5.3, and these were attributed to the protons of hydroxyl groups of the D-mannosyl group: this was confirmed by the effect of addition of deuterium oxide to the dimethyl sulfoxide solution. The magnetic resonance properties of protons of hydroxyl groups of sugars have been discussed by Perlin[10]. The signals of the other protons of the D-mannosyl group appeared as indistinguishable peaks in the region of δ 4.2–3.3.

EXPERIMENTAL

General methods. — Melting points were determined with a Mettler T-P2 hot-stage apparatus equipped with a microscope. Optical rotations were measured in 1-dm, semimicro tubes with a Perkin–Elmer No. 141 polarimeter. Preparative t.l.c. was performed on precoated, p.l.c. plates of Silica Gel F254 (Merck). All solvent mixtures are v/v. The microanalyses were performed by Dr. W. Manser, Zurich, Switzerland. I.r. spectra were recorded with a Perkin–Elmer spectrophotometer, model 237, and n.m.r. spectra at 270 MHz with a Bruker HFX-270 instrument.

*7-Bromo-3-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyloxy)-2-naphth-o-anisidide* (**3**). — A mixture of 7-bromo-3-hydroxy-2-naphth-*o*-anisidide (**1**; 185 mg, 0.5 mmol; Sigma Chemical Co., St. Louis, MO 63178) and mercury(II) cyanide (200 mg) in dry nitromethane (20 mL) was dried by azeotropic distillation. 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl chloride (200 mg, 0.6 mmol) was added to the suspension, and the mixture was boiled under reflux and anhydrous conditions. After 2 h, additional **2** (80 mg, 0.4 mmol) was added, and the reaction was continued for 12 h under the same conditions. The mixture was evaporated to a syrup *in vacuo*, the residue was extracted with chloroform (50 mL), and the extract was washed successively with aqueous 10% potassium iodide (2 × 50 mL) and water (50 mL), dried (Na_2SO_4), and evaporated. The residue was applied to two preparative-t.l.c. plates (2-mm thick, 20 × 20 cm), which were developed in 5:1 ether–hexane. The band having R_F 0.45, corresponding to **3**, was extracted with 9:1 dichloromethane–methanol. Evaporation gave a syrup that crystallized from 95% ethanol. Recrystallization from absolute ethanol afforded **3** (253 mg, 72% based on **1**), m.p. 150–152°, $[\alpha]_D^{20}$ +288° (*c* 1.0, chloroform); λ_{max}^{MeOH} 237 nm (ε_{mM} 13.10), with shoulders at 283 and 302 nm;



ν_{max}^{KBr} 3455 (NH), 1760 and 1750 (OAc), 1673 (NHCO), 1605 (Ph), 1530 (NHCO), 1230 (OAc), 1070 (C-O-C), and 750 cm^{-1} (Ph); ^{1}H-n.m.r. data (chloroform-*d*): δ 9.69 (s, 1 H, NH), 8.67 (q, 1 H, $J_{5,6}$ 1.3 and 7.8 Hz, H-5), 8.53 (s, 1 H, H-1), 8.07 (s, 1 H, H-4), 7.62 (m, 3 H, H-6,8,6'), 7.11 (dq, 1 H, $J_{4',6'}$ 1.5, $J_{3',4'} = J_{4',5'} = 7.6$ Hz, H-4'), 7.06 (dq, 1 H, $J_{3',5'}$ 1.5, $J_{4',5'} = J_{5',6'} = 7.6$ Hz, H-5'), 6.93 (q, 1 H, $J_{3',5'}$ 1.5, $J_{3',4'}$ 7.6 Hz, H-3'), 5.84 (d, 1 H, $J_{1'',2''}$ 1.6 Hz, H-1''), 5.68 (q, 1 H, $J_{1'',2''}$ 1.6, $J_{2'',3''}$ 3.5 Hz, H-2''), 5.53 (q, 1 H, *J* 3.5, $J_{2'',3''}$ 9.8 Hz, H-3''), 5.35 (t, 1 H, $J_{3'',4''} = J_{4'',5''}$ 9.8 Hz, H-4''), 4.30 (q, 1 H, $J_{5'',6''a}$ 5.4, $J_{6''a,6''b}$ 12.2 Hz, H-6''a), 4.12 (q, 1 H, $J_{5'',6''a} = 5.4$, $J_{4'',5''}$ 9.8 Hz, H-5'') 4.02 (q, 1 H, $J_{5'',6''b}$ 2.3, $J_{6''a,6''b}$ 12.2 Hz, H-6''b), 3.90 (s, 3 H, OMe), 2.24 (s, 3 H, Ac), 2.00 (s, 3 H, Ac), 1.97 (s, 3 H, Ac), and 1.90 (s, 3 H, Ac).

Anal. Calc. for $C_{32}H_{32}BrNO_{12}$: C, 54.71; H, 4.59; N, 1.99. Found: C, 54.75; H, 4.55; N, 2.01.

*7-Bromo-3-(α-*D*-mannopyranosyloxy)-2-naphth-*O*-anisidide* (**4**). — A solution of **3** (165 mg, 234 μmol) in dry methanol (5 mL) was treated with sodium methoxide in methanol (3 mL, prepared from 100 mg of sodium in 10 mL of methanol), and the mixture was kept for 1 h at 4°. The crystals that formed during the reaction were filtered off, washed with a small volume of methanol, and recrystallized from methanol, to give fine needles of **4** (74 mg). Additional crystals (37 mg) were obtained from the combined mother-liquors, for a total of 111 mg (88%), m.p. 201–203°, $[\alpha]_D^{20}$ +43° (*c* 0.2, *N,N*-dimethylformamide); λ_{max}^{MeOH} 238 nm (ε_{mM} 12.90); ν_{max}^{KBr} 3390 (OH), 1660 (NHCO), 1600 (Ph), 1530 (NHCO), 1070 (C-O-C), and 750 cm^{-1} (Ph); ^{1}H-n.m.r. data (dimethyl sulfoxide-d_6): δ 9.78 (s, 1 H, NH), 8.53 (s, 1 H, H-1), 8.40 (d, 1 H, $J_{5,6}$ 7.8 Hz, H-5), 8.33 (s, 1 H, H-4), 7.90 (bs, 1 H, H-8), 7.87 (d, 1 H, $J_{5',6'}$ 7.8 Hz, H-6'), 7.69 (q, 1 H, $J_{6,8}$ 1.9, $J_{5,6}$ 7.8 Hz, H-6), 7.13 (m, 2 H, H-3',4'), 5.76 (s, 1 H, H-1''), 5.26 (d, *J* 4 Hz, OH), 4.93 (d, *J* 5.3 Hz, OH), 4.85 (d, *J* 5.3 Hz,

OH), 4.54 (t, *J* 5.3 Hz, OH), 4.11 (bs, 1 H), 3.93 (s, 3 H, OMe), and 3.7–3.2 (m, 5 H, H-1″,2″,3″,4″,5″).

Anal. Calc. for $C_{24}H_{24}BrNO_8$: C, 53.93; H, 4.53; N, 2.62. Found: C, 54.06; H, 4.43: N, 2.55.

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