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Note

Synthesis of 4-deoxy-4-fluoro-D-[6-^{3}H]glucose

JOHN SAMUEL AND NORMAN FLETCHER TAYLOR*

Biochemistry Laboratory, Department of Chemistry, University of Windsor, Windsor, Ontario N9B 3P4 (Canada)

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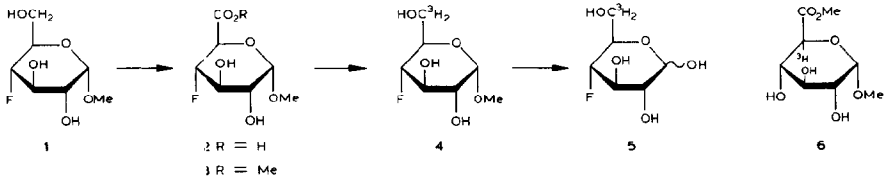
Our biochemical studies with deoxyfluoro carbohydrates frequently necessitate the introduction of a specific radiolabel. For example, 4-deoxy-4-fluoro-D-[3-^{3}H]glucose has been used for metabolic[1] and transport studies[2,3]. Recently we reported the reaction of 4-deoxy-4-fluoro-D-glucose with a membrane protein in *Pseudomonas putida*[4]. To investigate the biochemistry of this reaction further necessitated the synthesis of 4-deoxy-4-fluoro-D-[6-^{3}H]glucose. The methods for the incorporation of tritium into sugars have been extensively reviewed[5–7]. Random-labeling methods, such as catalytic labeling[8] and the Wilzbach method[9], are unsuitable for the tritiation of carbohydrates. The most convenient method for the stereospecific introduction of tritium into sugars involves the reduction of a sugar aldehyde, ketone, lactone, or ester by a tritiated hydride reagent[10]. We report, herein an application of this method to the synthesis of 4-deoxy-4-fluoro-D-[6-^{3}H]glucose (**5**).



Sodium borohydride reduction of glycosiduronic esters is known to yield the parent glycosides[11]. These esters may be obtained by platinum-catalyzed, selective oxidation of glycosides[12,13], followed by reaction with diazomethane[14]. Since methyl 4-deoxy-4-fluoro-α-D-glucopyranoside (**1**) is readily available[15], the following route was used for the introduction of tritium at C-6 of 4-deoxy-4-fluoro-D-glucose. Methyl (methyl 4-deoxy-4-fluoro-α-D-glucopyranosid)uronate (**3**), was

*To whom correspondence should be addressed.

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obtained, by platinum-black oxidation of **1** and subsequent treatment of acid **2** with diazomethane, as a chromatographically pure syrup. The presence of an ester carbonyl group was confirmed by a strong absorption band at 1760 cm^{-1} and by a signal in the ^1H-n.m.r. at δ 3.76 assigned to the methoxycarbonyl group. The ^{19}F-n.m.r. chemical shift (δ 119.5) was comparable with that reported for other deoxyfluoromonosaccharides[16]. The geminal ^{19}F–^1H coupling constant ($J_{F,H-4}$ 54.5 Hz) was also consistent with the presence of a sp^3-hybridized carbon atom in a six-membered ring[17]. The observed vicinal-coupling (J 14.7 Hz) was assigned to $J_{F,H-3}$ and is comparable to that reported for 4-deoxy-4-fluoro-D-glucose[17]. Owing to limited resolution, coupling constants of lower magnitudes could not be determined. Reduction of the ester with sodium borotritide at room temperature resulted in the formation of two products. The major component corresponded chromatographically to methyl 4-deoxy-fluoro-α-D-[6-^3H]glucopyranoside (**4**), and a minor component to the uronic acid **2** considered to arise from alkaline hydrolysis of **3**. The reduction was repeated at 0–4°, therefore, with lower concentrations of sodium borotritide. This resulted in a slower rate of reduction but still trace amounts of the side product **2**. The pure glycoside **4** was eventually obtained in crystalline form in 54% yield by preparative t.l.c., and was identical in all physical and chemical respects with authentic material[15]. Acid hydrolysis of **4** with 2M sulfuric acid gave 4-deoxy-4-fluoro-D-[6-^3H]glucose (**5**) in 59% yield.

As it was intended to use **5** for mechanistic studies, the position of radiolabel at C-6 had to be established unequivocally. The method of radiochemical synthesis does not necessarily preclude the incorporation of label in an entirely unexpected position[18]. Furthermore, Prihar *et al.*[19] have reported an 85% loss of tritium label during sodium borohydride reduction of methyl (methyl α-D-[5-^3H]glucopyranosid)uronate (**6**). This unexpected loss of tritium at C-5 during reduction at C-6 was explained by the base-catalyzed enolization of **6**. If such tautomeric transformations are associated with sodium borotritide reduction of **3**, then extensive incorporation of tritium at C-5 may occur. In order to ascertain this possibility, **4** was reoxidized to **2**. After purification of the resulting uronic acid by preparative t.l.c., the radioactivity was found to be only 3.7% of the specific activity of the glycoside **4**. From this result, it may be assumed that 96.3% of the tritium label is located at C-6 in **5**.

EXPERIMENTAL

Material and methods. — Methyl 4-deoxy-4-fluoro-α-D-glucopyranoside was obtained as reported[15]. Sodium borotritide (8.45 GBq/mmol) was from Amersham Corp., (Arlington Heights, IL 60005). Reagent-grade chemicals were used unless otherwise stated and obtained from Fisher Scientific Co. (Pittsburgh, PA 15219). Melting points are uncorrected. Optical rotations were measured with a manual polarimeter (Rudolph) for solutions in a 0.5-dm tube. I.r. spectra were recorded over the range of 4000–400 cm^{-1} with a Beckman IR-12 spectrophotometer. ^1H-

N.m.r. spectra were recorded with a Jeolco-C60HL spectrometer; chemical shifts (δ) are expressed downfield from the signal of tetramethylsilane as internal standard for solutions in chloroform-*d*. ^{19}F-N.m.r. spectra were recorded with a Bruker n.m.r. spectrometer CSD.100; ^{19}F-chemical shifts are expressed relative to the signal of trifluoroacetic acid. Radioactive counting was done with a Beckman LS7500 ligand-scintillation system using Ready-Solv ™MP as a "cocktail"; sugars were dissolved in water before addition of the "cocktail". Thin-layer chromatography was carried out on 20 × 20-cm plastic plates, precoated with a 0.2-mm layer of Silica gel 60 F_{254} (BDH Chemicals Canada Ltd., Toronto, ON M8Z 1K5). The plates were developed in the solvent specified. Carbohydrates were detected by spraying with a 50% (v/v) of concentrated sulfuric acid in ethanol, followed by heating at 110° for 5–10 min. Reducing sugars were detected with the aniline hydrogenphthalate reagent[20]. Preparative t.l.c. was performed on 20 × 20-cm glass plates, precoated with a 2.0-mm layer of silica gel (Fisher). The developed plates were air dried and the band corresponding to the R_F values of the compound was removed and eluted with the appropriate solvent. Column chromatography was performed with Silica gel H (Fisher). The following solvent systems (all v/v) were used: (*A*) 3:3:1 ethyl acetate–acetic acid–water; (*B*) 7:2:1 ethyl acetate–acetic acid–methanol; and (*C*) 3:1 ethyl acetate–petroleum ether (b.p. 30–65°). Elemental microanalyses were performed by Guelph Chemical Laboratories Ltd.

*Methyl (methyl 4-deoxy-4-fluoro-α-*D*-glucopyranosid)uronate* (**3**). — Methyl 4-deoxy-4-fluoro-α-D-glucopyranoside (2.0 g; R_F 0.74, solvent *A*), Pt black (0.4 g), and $NaHCO_3$ (0.21 g) in water (100 mL) were mechanically stirred while oxygen was bubbled through the solution. The temperature was maintained at 40–50° and the pH maintained at 7.5–8.0 by the addition of small quantities of $NaHCO_3$. The progress of the oxidation was monitored by t.l.c. (R_F of **3** 0.6, solvent *A*) and appeared to be complete after 4 h. The suspension was filtered, and the filtrate cooled and made acid by stirring with Amberlite IR-120(H^+) cation-exchange resin. After filtration, the filtrate was evaporated to dryness *in vacuo*. The residual syrup was dissolved in anhydrous methanol (100 mL), and a solution of diazomethane (4 g) in ether (60 mL) added dropwise at room temperature. The solution was boiled with activated charcoal (100 mg), the suspension filtered, and the filtrate evaporated *in vacuo*. The resulting syrup was submitted to column chromatography over Silica gel H (mesh 60-200, 300 g) and eluted with solvent *C*. Evaporation of the eluate *in vacuo* gave a chromatographically pure product as a syrup (1.5 g), R_F 0.84 (solvent *A*) which was dried in the presence of P_2O_5 in a vacuum desiccator; $[\alpha]_D^{23}$ +93.8° (*c* 0.91, water); $\nu_{max}^{CHCl_3}$ 3600–3380(w) (OH stretch) and 1760(s) cm^{-1} (CO-stretch); ^{1}H-n.m.r. ($CDCl_3$): δ 3.7 (br, CO_2CH_3), 3.35 (br, OCH_3); ^{19}F-n.m.r. (CH_3OH): δ 119.2 (dd, $J_{F,H-4}$ 54.5, $J_{F,H-3}$ 14.7 Hz).

Anal. Calc. for $C_8H_{13}FO_6$: C, 42.86; H, 5.80; F, 8.48. Found: C, 42.85; H, 5.69; F, 6.80.

*Methyl 4-deoxy-4-fluoro-α-*D*-[6-^{3}H]glucopyranoside* (**4**). — A solution of methyl (methyl 4-deoxy-4-fluoro-α-D-glucopyranoside)uronate (1.0 g) in water (10

mL) was added dropwise to a solution of sodium borotritide (13 mg, 8.45 GBq/mmol) in water (1 mL) with stirring at 4°. After 10 min, an additional quantity of sodium borohydride (440 mg) was added and stirring continued for 1 h. Excess borohydride was eliminated by stirring with Amberlite R-120 (H^+; 20 mL) cation-exchange resin, and methyl borate removed by repeated addition and evaporation of methanol *in vacuo*. After filtration, the solution was evaporated to dryness *in vacuo*, and the resulting syrup purified by preparative t.l.c. (solvent *B*). Elution of the major band with ethyl acetate and removal of the solvent gave the pure title compound (yield, 440 mg; 0.39 GBq/mmol) which was crystallized from 1:1 ethyl acetate–acetone, m.p. 127–129°, $[\alpha]_D^{23}$ +129.2° (*c* 0.24, water); R_F 0.74 (solvent *A*); identical with an authentic sample of methyl 4-deoxy-4-fluoro-α-D-glucopyranoside[15].

4-Deoxy-4-fluoro-D-[*6-*^{3}*H*]*glucose* (**5**). — A solution of methyl 4-deoxy-4-fluoro-α-D-[6-^{3}H]glucopyranoside (200 mg) in 2M sulfuric acid (20 mL) was boiled gently under reflux for 4 h. After neutralization with solid barium carbonate, the mixture was filtered, and the filtrate evaporated to dryness *in vacuo*. The residue was extracted with absolute ethanol (15 mL), filtered off, and the solvent removed *in vacuo* to leave a syrup that crystallized from ethanol (100 mg) and had a specific activity of 0.40 GBq/mmol, m.p. 188–190° alone, or admixed with authentic 4-deoxy-4-fluoro-D-glucose. A single spot (R_F 0.66, solvent *A*) was observed in t.l.c. The total radiochemical yield from **3** to **5** was 12.5%.

Oxidation of methyl 4-deoxy-4-fluoro-α-D-[*6-*^{3}*H*]*glucopyranoside* (**4**). — A mixture of **4** (14 mg) and methyl 4-deoxy-4-fluoro-α-D-glucopyranoside (96 mg) was dissolved in water (15 mL) to give a specific activity of 51 mBq/mmol. To this solution were added sodium hydrogencarbonate (10 mg) and Pt black (40 mg). The mixture was stirred (magnetic) at 40–50° while O_2 was bubbled through the solution which was maintained at pH 7.5–8.0. After 4 h, the suspension was cooled and filtered, and the filtrate evaporated *in vacuo*. Water was added to the residue and the process repeated until no more tritium could be detected in the distillate. The syrupy oxidation product (**2**) was purified by preparative t.l.c. (solvent *B*) and had a specific radioactivity of 1.88 mBq/mmol (R_F 0.6, solvent *A*).

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