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SYNTHESIS OF 2-DEOXY-D-GLUCOSE [2,6,6'-^{3}H] WITH HIGH SPECIFIC ACTIVITY

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Summary: A new tritiation method was used to obtain 2-deoxy-D-glucose [2,6,6'-^{3}H] starting from a mixture of protected bromo deoxy glucose derivatives. Intermediates were characterized by ^{1}H- and ^{3}H-NMR.

Key words: 2-deoxy-D-glucose, borotritide reduction, tritium, ^{3}H-NMR.

INTRODUCTION

2-Deoxy-D-glucose (dG) is taken up into cells by the same transport system as D-glucose (1,2) and phosphorylated to 2-deoxy-D-glucose-6-phosphate (dG-6-P) by hexokinase (1,3,4). Hexokinase is not inhibited by dG-6-P (3). Thus, at this level, there is no control of dG influx into the hexose-6-phosphate pool of cells. Since dG-6-P is not further metabolized (1,4) and cell membrane permeability is low for dG-6-P, this compound accumulates in cells. Therefore, the rate of its intracellular accumulation may be used as an index of glucose utilization in tissues (12).

This property of dG-6-P has been used for example in studies of hexose transport across cell membranes (5,6), brain metabolism (7,8), variations of cell permeability (9) and alteration of function of the cerebral capillaries during ischemia (10,11).

The first studies used 2-deoxy-D-glucose labelled with ^{14}C (dG-^{14}C) (7). The use of dG-^{14}C for the resolution of autoradiograms allows the exploration of larger brain areas but is not suitable for the determination of distinct cerebral activities of glia, of neurone cell bodies and neurophil ramifications.

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SHARP (13) used tritiated deoxyglucose for the first time. The resolution of autoradiograms is higher due to the low penetration of radiation emitted by tritium. Thus, an autoradiographic resolution on the cellular level can be achieved with dG-^{3}H of specific activity 30 Ci/mmole (1480 GBq/mmole) (14).

In order to obtain a higher detection sensitivity of dG-^{3}H by autoradiography, we synthesized dG-^{3}H with a high specific activity (50 Ci/mmole; 1.85 TBq/mmole).

METHODS

Tritiation of 1 and 2

Tritiolysis (15) of a 40/60 mixture of methyl 1-O-(1-methylethyl)-3,4-di-O-acetyl-2-deoxy-2-bromo-β-D-glucopyranoside 2 and methyl 1-O(1-methylethyl)-3,4-di-O-acetyl-2-deoxy-2-bromo-α-D-mannopyranoside 1 gives a 4/2/1 mixture of three compounds 3, 4 and 5:

3: methyl 1-O-(1-methylethyl)-3,4-di-O-acetyl-2-deoxy α-D-glucopyranosiduronate [2-^{3}H].

4: methyl 1-O-(1-methylethyl)-3,4-di-O-acetyl-2-deoxy β-D-glucopyranosiduronate [2-^{3}H].

5: methyl 1-O-(1-methylethyl)-4-O-acetyl-2,3-dideoxy α-(ou β) D-glucopyranosiduronate [2,3-^{3}H].

Compound 5 certainly results from a $C_2 = C_3$ double bond formation after tritium reduction in the described experimental conditions.

Compounds 3, 4, and 5 were determined by ^{1}H-NMR and mass spectrometry. However, $C_{1\alpha}$ and $C_{1\beta}$ stereoisemers of 3 and 4 could not be separated by these methods.

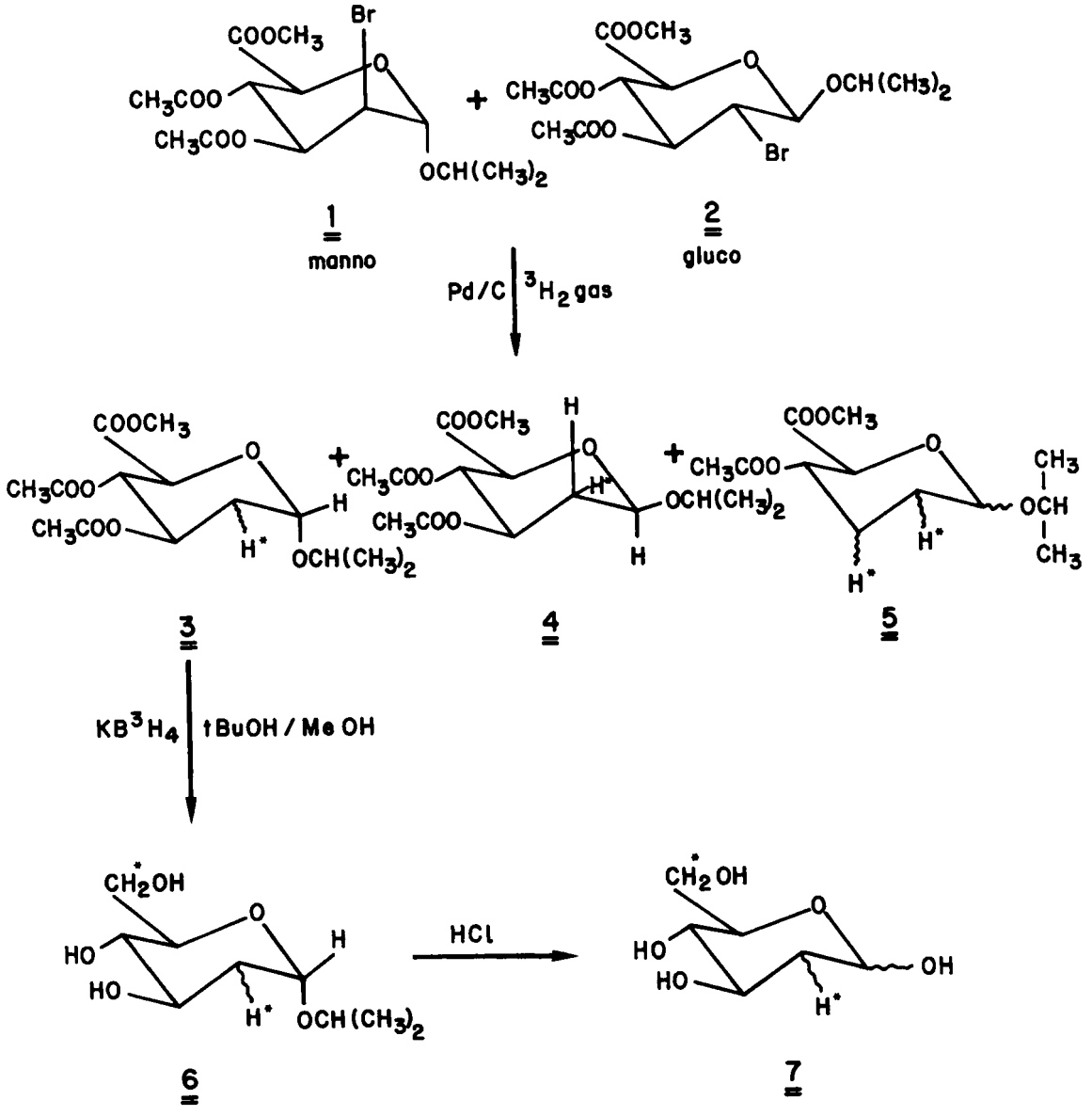
Determination of formulas 3 and 4

1) Compound 4 shows only one signal in ^{3}H-NMR (δ = 2.06 ppm in DMSO) with corresponds to an equatorial tritium atom in position 2, T_{2eq} (16).

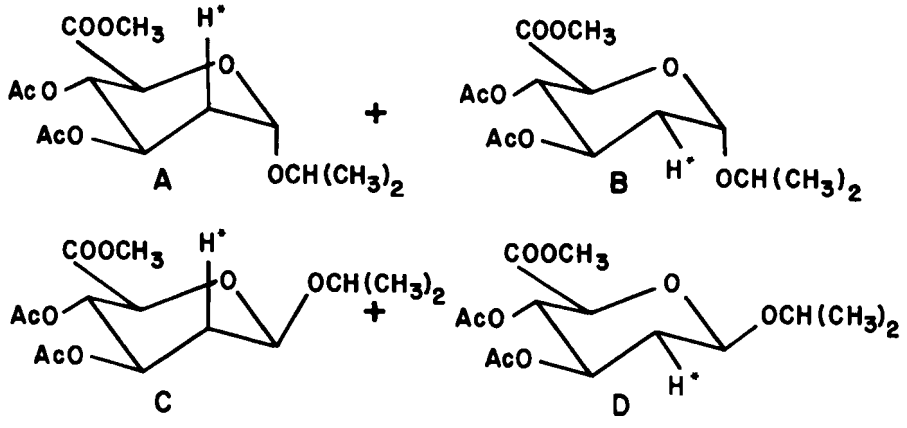
^{1}H-NMR of 4 with homodecoupling of residual H_{2eq} protons leads to the coupling constants H_1-T_{eq} (10 Hz) and H_1-H_{2a} (2 Hz), which correspond respectively (17) to a H_{1a}-H_{2a} and H_{1a}-H_{2eq} coupling. Thus, compound 4 has an axial proton in position 1 (scheme 1).

Scheme 1

Synthesis of 2-deoxy-D-glucose [2, 6, 6'-^3H]



2) Compound 3 shows two uncoupled signals in ^{3}H-NMR, which correspond to two possible couples of axial and equatorial isotopomers in position 2:(A+B) or (C+D).



Isotopomer D corresponds to compound 4. As compounds 3 and 4 are separated by chromatography and have different ^{1}H- and ^{3}H-NMR spectra, compound 3 corresponds to couple (A+B) having a H_1 equatorial proton (18).

Reduction and deprotection of 3 or 4

Potassium borotritide reduction of 3 or 4 gives 1-O-(1-methylethyl)-2-deoxy-α-D-glucopyranoside [2,6,6'-^{3}H] 6 which deprotection with HCl (19) leads to 2-deoxy-D-glucose [2,6,6'^{3}H] 7.

EXPERIMENTAL SECTION

Radioactive compounds were characterized by HPLC, ^{1}H- and ^{3}H-NMR and mass spectrometry. The NMR spectra were obtained using a Bruker AC 300 spectrometer (200 MHz for ^{1}H, 318 MHz for ^{3}H). The mass spectra were obtained with a Finnigan CH7A spectrometer equipped with an INCOS 2000 calculator.

1. Methyl 1-O-(1-methylethyl)-3,4-di-O-acetyl-2-deoxy-α-D-glucopyranosiduronate [2-^{3}H] 3, methyl 1-O-(1-methylethyl)-3,4-di-O-acetyl-2-deoxy-β-D-glucopyranosiduronate [2-^{3}H] 4 and methyl 1-O-(1-methylethyl)-4-O-acetyl-2,3-dideoxy-α(ou β)-D-glucopyranosiduronate [2,3-^{3}H] 5.

80 mg of a 40/60 mixture of 2 and 1 in 2 ml methanol and 20 µl diethylamine were reduced with 25 Ci tritium gas using 80 mg of 10% Pd/C for 15 minutes. After filtration of the catalyst, elimination of labile tritium atoms and solvent removal in vacuo, the residue was dissolved in 60 ml diethyl ether.

This ethereal solution was washed successively with 3 x 5 ml water. The aqueous extracts contained about 370 mCi. The ether phase contained 3.2 Ci which were purified by high performance liquid chromatography (reversed phase C_{18} column: ϕ 21 mm; h = 25 cm; solvent: methanol/water: 50/50).

Three main products were obtained and analysed by TLC (see table 1).

TABLE 1

HPLC and TLC of 3, 4 and 5

	HPLC α^*	TLC** Rf	Radioactivity (Ci)	Radiochemical purity
3	5.24	0.58	1.44	> 99%
4	3.56	0.46	0.42	> 99%
5	4.12	0.40	0.77	> 99%

*α = Selectivity of the separation.

** = Silica gel - Chloroform/methanol: 99/1.

Compound 4 (α = 3.56)

^{1}H-NMR (solvent: DMSO)

δ = 1.03 ppm - t - J = 7 Hz - 6H [-CH(CH_3)$_2$]; δ = 1.48 ppm - m - 1H - H_{2ax}; δ = 1.91 ppm - s - 6-H (-OCOCH_3); δ = 2.05 ppm - m - 1H - H_{2eq}; δ = 3.83 ppm - m - 1H - CH(CH_3)$_2$; δ = 4.16 ppm - d - J = 11 Hz - 1H - H_5; δ = 4.73 ppm - t - J = 9.5 Hz 1H - H_4; δ = 4.87 ppm - d - J = 11 Hz - 1H - H_1; δ = 5.07 ppm - m - 1H - H_3.

^{3}H-NMR (solvent: DMSO) proton decoupled

δ = 2.05 ppm - s - ^{3}H - 2eq

^{3}H-NMR (solvent: DMSO) proton coupled

δ = 2.05 ppm - d.d.d: J = 2 Hz; J = 5.2 Hz; J = 13 Hz.

Compound 5 (α = 4.12)

^{1}H-NMR solvent: DMSO)

δ = 1.00 ppm - d - 2H - 7 Hz; δ = 1.03 ppm - d - 2H - J = 7 Hz;
δ = 1.92 ppm - s - 3H; δ = 3.57 ppm - s - 3H;
δ = 3.82 ppm - m - 1H; δ = 4.06 ppm - d - J = 10 Hz - 1H;
δ = 4.7 ppm - d - J = 10 Hz - 1H.

^{3}H-NMR (solvent: DMSO)

δ = 1.6 ppm - d - J = 4 Hz; δ = 1.72 ppm - d - J = 4 Hz.

Mass spectrometry (C.I. - NH_3)

$[MNH_4]^+$· 282, 280, 278.

Compound 3 (α = 5.24)

^{1}H-NMR (solvent: DMSO)

δ = 1.04 ppm - d - J = 6.5 Hz - 3H-$CH(CH_3)_2$; δ = 1.08 ppm - d - J = 6.5 Hz - 3H-$CH(CH_3)_2$; δ = 1.73 ppm - m - H_{2ax};
δ = 1.91 ppm - s - 3H - $COCH_3$; δ = 1.93 ppm - s - 3H - $COCH_3$;
δ = 1.96 ppm - m - H_{2eq}; δ = 3.58 ppm - s - 3H-OCH_3;
δ = 3.78 ppm - m - 1H - CHiPr; δ = 4.19 ppm - d - J = 9.5 Hz - 1H-H-5;
δ = 4 86 ppm - t - J = 9.5 Hz - 1H-H_4;
δ = 5.03 ppm - m - 1H - H-3; δ = 5.09 ppm - m - 1H-H_1.

^{3}H-NMR (solvent: DMSO)

δ = 1.73 ppm - s - H_{2ax}; δ = 1.96 ppm - s - H_{2eq}.

^{3}H-NMR (solvent: DMSO) proton coupled

δ = 1.73 ppm - d.d.d -- J = 3.6 Hz J = 11 Hz - J = 13.9 Hz;
δ = 1.96 ppm - d.d.d -- J = 2.7 Hz J = 5.1 Hz - J = 14 Hz.

Mass spectrometry (C.I - NH_3)

$[MNH_4]^+$· 336, 338

2. 1-O-(1-Methylethyl)-2-deoxy-α-D-glucopyranoside [2,6,6'^{3}H] 6

In a 20 ml three-necked flask (vacuum or N_2, tert-butanol, methanol) were placed 35 Ci (1.3 TBq) of potassium borotritide (S.A. = 60 Ci/mmole; 2.22 TBq/mmole). Compound 3 (3 Ci; 0.11 TBq) in diethyl ether (5 ml) was

introduced. The mixture was evaporated under vacuo. To the residue were added 8 ml of tert-butanol and the solution was heated to gentle reflux. Methanol (1.6 ml; 1 ml/hour) was introduced and the solution was refluxed for 40 minutes. After cooling to room temperature and transfer of the reaction mixture in a 250 ml flask, water (5 ml), acetic acid (0.5 ml) and methanol (30 ml) were added. The solution was stirred one hour then dried under vacuo. The residue was dissolved in methanol yielding a total radioactivity of 4.48 Ci (166 GBq). After purification by HPLC (reversed phase C_{18} column: ϕ = 20 mm; h = 25 cm; solvent: methanol/water: 1/1) 3.26 Ci (120 GBq) were obtained and chromatographed on a silica gel column (ϕ=1 cm; h = 7 cm; solvent: chloroform/methanol: 96/4).

Compound 6 (1.52 Ci; 56 GBq) had a radiochemical purity of 98% by thin-layer chromatography (R_f = 0.2; solvent: chloroform/methanol: 9/1).

^{3}H-NMR (solvent: deuterated DMSO)

δ = 1.38 ppm - s - H_{2a}; δ = 1.75 ppm - s - H_{2e};

δ = 3.37 ppm - d - $J_{T-6,T-6'}$ = 18 Hz ditritiated form in position 6;

δ = 3.38 ppm - s - monotritiated form in position 6;

δ = 3.56 ppm - d - $J_{T6-T6'}$ = 18 Hz - ditritiated form in position 6';

δ = 3.57 ppm - s - monotritiated form in position 6'.

3. D-Glucose-[2,6,6'^{3}H] 7

Compound 6 (1.52 Ci; 56 GBq) in 18 ml N hydrochloric acid is heated at 60 °C for 3 hours under nitrogen. After cooling, the solution was neutralized on Dowex 1 x 8 (carbonate form), filtered and dried. The residue was dissolved in methanol (0.5 ml) and purified by preparative paper chromatography (washed Whatman SS 2316; solvent: n-butanol/acetic acid/water: 2/1/1). Compound 7 (1.12 Ci; 41 GBq) was obtained with a 99% radiochemical purity checked by TLC on cellulose (R_f = 0.49; n-butanol/acetic acid/water: 2/1/1) and by HPLC (Aminex HPX87, solvent: water).

Specific activity of 7 was determined by colorimetry with 2,4-dinitrophenyldrazine and by liquid scintillation counting (50 Ci/mmole; 1.85 TBq/mmole).

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