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A CONVENIENT METHOD FOR THE SYNTHESIS OF RADIOACTIVELY LABELLED ARYL GLYCOSIDES

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Abstract: Fluoride-directed methylation of the aryl hydroxyl of hydroxyphenyl glycosides with radioactive methyl iodide allows the incorporation of label under mild conditions that preserve the glycosidic link. The effectiveness of this approach has been demonstrated by the synthesis of two ^{14}C-labelled aryl α-glycosides.

Key words: [^{14}C]-methyl iodide, fluoride-directed methylation, *p*-methoxyphenyl-α-D-glucopyranoside, 2-deoxy-(*p*-methoxyphenyl)-α-D-glucopyranoside

Introduction: Aryl glycosides are widely used as substrates and inhibitors in the study of glycosidases and glycoside transport across biological membranes[1,2,3]. Radiolabelling of analogues facilitates easy measurement of transport, and is required for the detection of photoaffinity labels. To date, introduction of radioactive labels into aryl glycosides has either involved use of ^{3}H- or ^{14}C-labelled free sugar as starting material [2,3] or the peroxidase-catalysed iodination ([^{125}I]) of the aryl group[4]. The former of these approaches suffers from cumulative losses at each step and, because most glycosylations produce a mixture of anomers and tautomers, only one of which is usually required, efficiency is very low. On the other hand, isotopes of iodine are not always the radioactive labels of choice, due to their highly energetic emissions, short half-lives and a tendency to accumulate in the human body. As an alternative to using ^{14}C-labelled glucose as starting material, we have developed a method for the synthesis of aryl glucopyranosides that incorporates this isotope into the aglycone moiety by [^{14}C]-methylation of an aromatic hydroxyl, subsequent to the formation of the glycosidic link and separation of the anomers.

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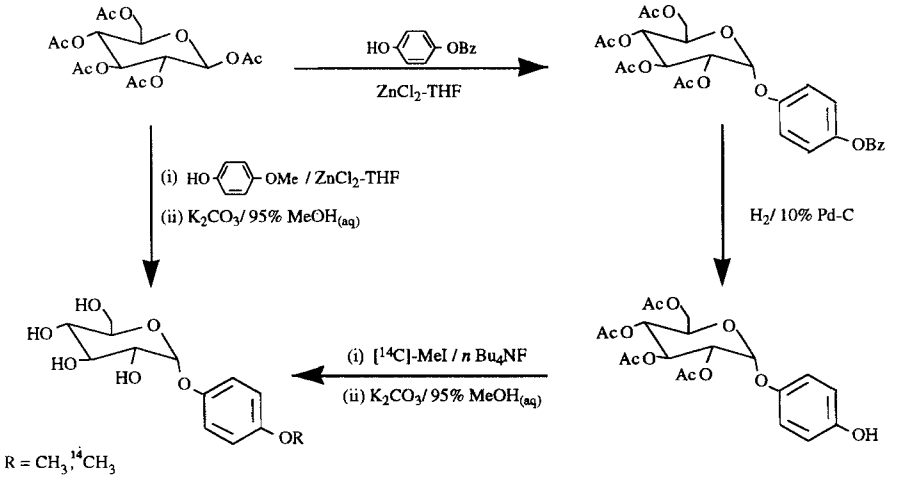
We describe the preparation of [^{14}C-*methyl*]-(*p*-methoxyphenyl)-α-D-glucopyranoside and of [^{14}C-*methyl*]-2-deoxy-(*p*-methoxyphenyl)-α-D-glucopyranoside by [^{14}C]-methylation subsequent to debenzylation of the corresponding acetylated (*p*-benzyloxyphenyl)-α-D-glycopyranoside.



Strategy for the synthesis of labelled and unlabelled *p*-methoxyphenyl-α-D-glucopyranoside

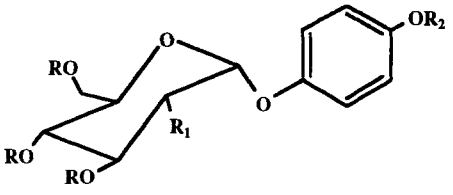
Fluoride directed methylation [5] by [^{14}C]-methyl iodide, at room temperature, not only gives high yields (70% with these substrates), but eliminates the need for strong base such as KOH [6] and K_2CO_3 [7]. The latter reagents are likely to lead to premature de-acetylation, risk cleavage of the glycosidic bond, and sometimes require elevated temperatures [7] which might be unsafe in the case of a volatile radioactive reagent.

Since methyl iodide is readily available isotopically labelled with either ^{3}H or ^{14}C, the following method therefore represents a useful general strategy for the synthesis of both ^{3}H- and ^{14}C-labelled aryl glycosides.

Experimental

General methods. Melting points are uncorrected. I.r. spectra were recorded using a Perkin Elmer 1760X IR FT spectrometer, and liquid scintillation counting was by a Beckman LS 1701 in Scintillator 299™ (Packard Instrument Co., Inc.). FAB+ mass spectra were obtained on a VG Analytical ZAB1F spectrometer, and CI(NH_3) and DCI(NH_3) mass spectra on a VG Biotech 20-250 spectrometer. T.l.c. was carried out on Silica Gel-60 F_{254} (Merck 5554) with detection by spraying

with 5% v:v sulphuric acid in ethanol, developed by charring. [^{14}C]-Methyl iodide was purchased from Amersham International PLC (Bucks., U.K.), other reagents were from Aldrich Chemical Company, and solvents were obtained from Merck Ltd. (Poole, U.K.) and dried according to Perrin and Armarego [8].



R	R_1	R_2	Compound
H	OH	CH_3	I
H	H	CH_3	II
Ac	OAc	CH_3	Ia
Ac	OAc	OBz	Ib
Ac	OAc	H	Ic
Ac	H	CH_3	IIa
Ac	H	OBz	IIb
Ac	H	H	IIc

4-Methoxyphenyl-α-D-glucopyranoside **(I)**[13]. An anomerically enriched sample of **(Ia)** and the β-anomer were prepared from β-D-glucose pentaactetate and 4-methoxyphenol by a modified method of Helferich and Reischel [9], using anhydrous zinc chloride in dry THF. Following deacetylation [10], traces of the β-anomer contaminant were removed by treatment with β-glucosidase (EC 3.2.1.21) (7 U.mL^{-1} in sodium acetate-acetic acid buffer 50 mM, pH 5.0). The resulting **(I)** was purified by flash chromatography on silica, eluting with (i) diethyl ether, and (ii) CH_3CN:H_2O, 9:1, v:v (33% yield).

4-Benzyloxyphenyl-α-D-glucopyranoside **(Ib)**[14] was prepared in a similar manner to **(Ia)**, from β-D-glucose pentaactetate and 4-benzyloxyphenol. The α-anomer was purified by fractional crystallisation [9] from CCl_4-ethanol (28% yield).

2-deoxy-1-O-(4-methoxyphenyl)-α-D-glucopyranoside **(IIa)**[16] was synthesised by a modified method of Ravi *et al.*[11]. To a solution of 4-methoxyphenol (5 g, 0.040 moles) and Tri-O-acetyl-2-deoxy-1-(2'-thiopyridyl)-β-D-glucopyranoside[12] (10.25 g, 0.027 moles), in dry dichloromethane (50 mL) containing 5% v/v methyl iodide, was added about 1 g of 4 Å molecular sieves. The mixture was refluxed for 36 hours after which the solvent was removed *in vacuo* and the residue triturated with toluene (100 mL). The toluene extract was filtered and washed with 5 x 1 vol.

2% w/v $NaOH_{(aq)}$ and 1 vol. distilled water, and dried over anhydrous $CaSO_4$. The solvent was removed *in vacuo* and the residue taken up in ethanol whereupon **(IIa)** was deposited as colourless needle-like crystals (38% yield). **(IIa)** was deacetylated [10] to give **(II)**[15] in 95% yield.

2-deoxy-1-O-(4-benzyloxyphenyl)-α-D-glucopyranoside **(IIb)**[17] was prepared as for **(IIa)**, from 4-benzyloxyphenol and Tri-O-acetyl-2-deoxy-1-(2'-thiopyridyl)-β-D-glucopyranoside. Purified by flash chromatography on silica, eluting with hexane:ethyl acetate (3:2 v:v) (30% yield).

(Ic) and **(IIc)** were prepared from **(Ib)** and **(IIb)** by hydrogenation (4 atm), in ethanol (Ib), or 1:1 v:v ethanol:ethyl acetate (IIb), over 10% palladium-charcoal until judged complete by t.l.c. (CCl_4: MeOH 95:5 v:v) (95% yield, homogenous by t.l.c.).

[^{14}C]-methylation

[^{14}C-*methyl*]-(*p*-methoxyphenyl)-α-D-glucopyranoside and [^{14}C-*methyl*]-2-deoxy-(*p*-methoxyphenyl)-α-D-glucopyranoside were prepared using a fluoride directed alkylation [5] of **(Ic)** and **(IIc)**. A solution of **(Ic)** or **(IIc)** (70 μmol) in dry THF (0.5 mL), followed by tetrabutyl ammonium fluoride hydrate (c. 1.5 eq.) in dry THF (0.5 mL), was added to [^{14}C]-methyl iodide (1 mCi, 50-60 $mCi.mmol^{-1}$) and agitated for 1 hour at room temperature. An excess of unlabelled methyl iodide (20 mL, 5 eq.) in dry THF (0.5 mL) was then introduced into the reaction mixture and the whole agitated overnight. The mixture was then dried under a stream of nitrogen, taking care to pass the vapours through liquid nitrogen and activated charcoal traps. After drying, the residue was taken up in ethyl acetate (c.1.5 mL) and applied to a small chromatography column of silica gel 60 equilibrated with *n*-Hexane, eluting with (i) Hexane, (ii) Hexane: Ethyl acetate 75:25 v:v. Fractions (c. 2 mL) could be assayed for radioactivity by scintillation counting. Fractions containing the major peak of radioactivity, which was eluted by (ii), were pooled and the solvent removed *in vacuo*. Following deacetylation [9] and removal of the solvent *in vacuo*, the residue was taken up in ethanol, filtered through silica gel, and purified by ascending paper chromatography on Whatman 3MM Chr paper with *n*-Butanol: Ethanol: Water (100:15:30 v:v:v). After 20 hours chromatography, a highly radioactive band was excised from the region of corresponding R_f to unlabelled markers (developed by spraying with (i) 5% conc. H_2SO_4 in ethanol (v/v), (ii) 1:1 0.1M $FeCl_{3(aq)}$: 0.1M $K_3FeCN_{6(aq)}$). The labelled compound was eluted with 80% $Ethanol_{(aq)}$ (v/v), dried briefly *in*

vacuo, and taken up in a known volume of distilled water containing 3% ethanol (v/v). The total activity of the [^{14}C-*methyl*]-analogues prepared in this way, was measured by scintillation counting (350-400 mCi, 35-40% overall incorporation). Radioactive products were shown homogenous by t.l.c. (CH_3CN:H_2O, 9:1, v:v) and of corresponding R_f to standard samples of **(I)** and **(II)**. The aqueous solutions were stored at -20°C.

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13 **I:** Recrystallised from ethanol as colourless prisms. m.p. 155-158°C, (Found C, 54.6% and H, 5.87%; $C_{13}H_{18}O_6$ calculated C, 54.5% and H, 6.33%). $[\alpha]_D^{20}$ +182.5 (*c* 1.0, MeOH). N.m.r. ^{1}H (500 MHz, D_2O) δ 3.45 (t, 1H, *J* 9.5 Hz, H-6), 3.64 - 3.77 (m, 4H, H-2,3,4,5), 3.72 (s, 3H, 4'-OCH_3), 3.84 (t, 1H, *J* 8.7 Hz, H-6'), 5.42 (d, 1H, *J* 4.7 Hz, H-1), 6.08, 7.05 (2d, 4H, *J* 8.8 Hz, H-2',3',5',6'). ^{13}C (50.2 MHz, D_2O) δ 56.42 (4'-OCH_3), 60.97 (C-6),

70.07, 71.89, 73.15, 73.75 (C-2,3,4,5), 98.97 (C-1), 115.86, 119.73 (C-2',3',5',6' aryl), 151.30, 158.58 (C-1',4'); *m/z* (NH_3 D.C.I.) 304 ($M+NH_4^+$, 31%), 286 (M^+, 10), 180 (44), 162 (12), 145 (14), 125 (17), 124 (base peak), 123 (10). ν_{max} (KBr disc) 3370, 2836, 1517, 1253, 1223, 1120, 1033, 917, 838 and 784 cm^{-1}

14 **Ib:** Recrystallised from ethanol. m.p. 100.5-102°C, (Found C, 61.35% and H, 5.82%; $C_{27}H_{30}O_{11}$ calculated C, 61.1% and H, 5.7%). $[\alpha]_D^{20}$ +141 (*c* 1.0 $CHCl_3$). N.m.r. ^1H (250 MHz, $CDCl_3$), δ 2.03, 2.04, 2.05, 2.06 (4s, 12H, 3 OAc), 4.06 (dd, 1H, $J_{6,6'}$ 12, $J_{5,6'}$ 2.0 Hz, H-6'), 4.12-4.19 (m, 1H, H-5), 4.25 (dd, 1H, $J_{5,6}$ 4.5 Hz, H-6), 5.02 (dd, 1H, $J_{2,3}$ 9.8 Hz, H-2), 5.02 (s, 3H, benzyl CH_2), 5.15 (t, 1H, $J_{4,5}$ 9.8 Hz, H-4), 5.63 (d, 1H, $J_{1,2}$ 3.7 Hz, H-1), 5.69 (t, 1H, $J_{3,4}$ 9.8 Hz, H-3), 6.90, 7.01 (2d, 4H, 9.1 Hz, H-2',3',5',6'), 7.29-7.44 (benzyl ring). ^{13}C (62.9 MHz, $CDCl_3$), δ 20.5, 20.6 (4 $OCOCH_3$), 61.6 (C-6), 67.8, 68.3, 70.0, 70.4 (C-2,3,4,5), 70.4 (benzyl CH_2), 94.9 (C-1), 115.7, 117.8 (C-2', 3', 5', 6'), 127.3, 127.9, 128.5 (C-2'',3'',4'',5'',6'' benzyl), 136.9 (C-1''), 150.2, 154.5 (C-1', 4'), 169.5, 170.1, 170.4 (4 $OCOCH_3$). *m/z* (NH_3 D.C.I.) 549 (31%), 548 ($M+NH_4^+$, base peak), 458 (11), 331 (35), 271 (<10%), 213 (12), 186 (<10%), 108 (20), 91 (12). ν_{max} (KBr disc) 1741, 1510, 1228, 1213, 1042, 982, 826, and 784 cm^{-1}.

15 **II:** Recrystallised from water or ethanol as colourless prisms. m.p. 174-176°C, (Found C, 57.75% and H, 6.53%; $C_{13}H_{18}O_6$ calculated C, 57.77% and H, 6.71%). $[\alpha]_D^{20}$ +166.5 (*c* 1.0, MeOH). N.m.r. ^1H (500 MHz, D_2O) δ 1.74 (ddd, 1H, H-2a), 2.24 (ddd, 1H, $J_{2a,2e}$ 12, $J_{2e,3}$ 5 Hz, H-2e), 3.28 - 3.39 (m, 2H, H-4, 6), 3.58 - 3.71 (m, 2H, H-5, 6'), 3.72 (s, 3H, 4'-OCH_3), 3.95-4.05 (m, 1H, H-3), 5.50 (d, 1H, *J* 2.4 Hz, H-1), 6.80, 7.01 (2d, 4H, *J* 6.9 Hz, H-2',3',5',6'). ^{13}C (50.2 MHz, D_2O) δ 39.0 (C-2), 56.1 (4'-OCH_3), 62.6 (C-6), 69.8, 73.0, 74.6 (C-3,4,5), 98.5 (C-1), 115.5, 119.2 (C-2',3',5',6' aryl), 152.3, 156.38 (C-1',4'). *m/z* (NH_3 C.I.) 288 ($M+NH_4^+$, base peak), 270 (M^+, <10%), 165 (17), 146 (28), 129 (60), 125 (21), 124 (28), 111 (22). ν_{max} (KBr disc) 3392, 2833, 1514, 1229, 1192, 1184, 982, 922, 825 and 787 cm^{-1}.

16 **IIa:** Recrystallised from ethanol-hexane as colourless slender needles. m.p. 103°C, (Found C, 57.78% and H, 5.93%; $C_{19}H_{24}O_9$ calculated C, 57.57% and H, 6.10%). $[\alpha]_D^{20}$ +138.5 (*c* 1.0 $CHCl_3$). N.m.r. ^1H (500 MHz, $CDCl_3$), δ 1.92-2.02 (m, 1H, H-2a), 2.04, 2.05 (2s, 9H, 3 OAc), 2.46 (dd, 1H, $J_{2a,2e}$ 12, $J_{2e,3}$ 5.4 Hz, H-2e), 3.77 (s, 3H, 4'-OCH_3), 4.01 (dd, 1H, $J_{6,6'}$ 12 Hz, H-6), 4.10 (ddd, 1H, $J_{4,5}$ 10, $J_{5,6}$ 4.6, $J_{5,6'}$ 2.1 Hz, H-5), 4.30 (dd, 1H, H-6'), 5.08 (t, 1H, 9.8Hz, H-4), 5.47-5.53 (m, 1H, H-3), 5.57 (d, 1H, $J_{1,2a}$ 2.2 Hz, H-1), 6.82, 7.02 (2d, 4H, 9.2 Hz, H-2',3',5',6'). ^{13}C (62.9 MHz, $CDCl_3$), δ 20.6, 20.9 (3 $OCOCH_3$), 35.0 (C-2), 55.5 (4'-OCH_3), 62.0 (C-6), 68.3, 68.8, 69.1 (C-3,4,5), 95.9 (C-1), 114.5, 117.5 (C-2', 3', 5', 6'), 150.1, 154.9 (C-1', 4'), 169.8, 170.1, 170.5 (3 $OCOCH_3$). *m/z* (FAB$^+$) 396 (M^+, 12%), 336 (54), 273 (75), 213 (71), 153 (50), 124 (75), 111 (base peak). ν_{max} (KBr disc) 2837, 1747, 1509, 1236, 1216, 972, 917, 831, and 788 cm^{-1}.

17 **IIb:** Recrystallised from ethanol as colourless slender needles. m.p. 130-131°C, (Found C, 63.32% and H, 5.88%; $C_{25}H_{28}O_9$ calculated C, 63.55% and H, 5.97%). $[\alpha]_D^{20}$ +124.0 (*c* 1.0, $CHCl_3$). N.m.r. ^1H (500 MHz, $CDCl_3$), δ 1.91-2.01 (m, 1H, H-2a), 2.02, 2.05 (2s, 9H, 3 OAc), 2.45 (dd, 1H, $J_{2a,2e}$ 12, $J_{2e,3}$ 5.5 Hz, H-2e), 4.00 (dd, 1H, $J_{6,6'}$ 12.1 Hz, H-6), 4.09 (ddd, 1H, $J_{4,5}$ 10.1, $J_{5,6}$ 4.6, $J_{5,6'}$ 2.1 Hz, H-5), 4.29 (dd, 1H, H-6'), 5.01 (s, 3H, benzyl CH_2), 5.08 (t, 1H, 9.8 Hz, H-4), 5.46-5.53 (m, 1H, H-3), 5.56 (d, 1H, $J_{1,2a}$ 2.7 Hz, H-1), 6.87, 7.00 (2d, 4H, 9.2 Hz, H-2',3',5',6'), 7.29-7.44 (benzyl ring). ^{13}C (62.9 MHz, $CDCl_3$), δ 20.6, 20.9 (3 $OCOCH_3$), 35.0 (C-2), 62.1 (C-6), 68.4, 68.8, 69.1 (C-3,4,5), 70.4 (benzyl CH_2), 95.8 (C-1), 115.6, 117.5 (C-2', 3', 5', 6'), 127.3, 127.8, 128.5 (C-2'',3'',4'',5'',6'' benzyl), 137.0 (C-1''), 150.3, 154.1 (C-1', 4'), 169.8, 170.1, 170.5 (3 $OCOCH_3$). *m/z* (NH_3 C.I.) 490 ($M+NH_4^+$, 29%), 273 (19), 213 (base peak), 200 (59), 153 (32), 111 (25), 108 (32), 91 (60). ν_{max} (KBr disc) 1751, 1505, 1230, 1212, 1009, 983, 827 and 766 cm^{-1}.