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A Direct Synthesis of 2-Deoxy-2-fluoro-α-D-[6-^{3}H]glucopyranosyl Uridine-5′-diphosphate

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The synthesis of 2-deoxy-2-fluoro-α-D-[6-^{3}H]glucopyranosyl uridine-5′-diphosphate, with the late introduction of the radiolabel, has been achieved from 3,4-di-*O*-benzyl-2-deoxy-2-fluoro-α-D-glucosyl diphenyl phosphate, by an oxidation–reduction sequence, followed by protecting group removal and morpholidate coupling to uridine-5′-monophosphate.

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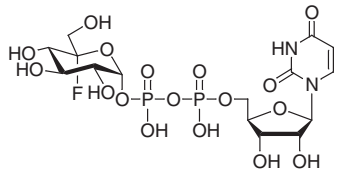
5-Fluoro and 2-deoxy-2-fluoro sugars are excellent mechanism-based inhibitors of many retaining glycoside hydrolases, so much so that they may often be used to label the catalytic nucleophile of the enzyme.[1,2] Naturally, some efforts have been made to investigate the use of related molecules, e.g. (1) and (2), for the inhibition of those glycosyl transferases that utilize a nucleoside-diphosphate activated sugar for the construction of the glycosidic linkage.[3–5]

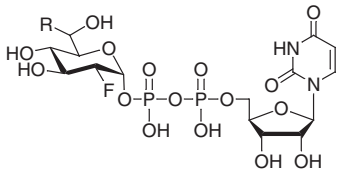
We have recently reported on our synthetic endeavours towards (2).[6] In early inhibition experiments with (2) and the self-glucosylating protein glycogenin, it soon became apparent that we needed a radiolabelled version of (2), and chose tritium. We initially prepared tritium-labelled (3) starting from D-[6-^{3}H]glucose—the whole procedure was extremely unsatisfactory, generating amounts of low-level radioactive waste at every step and yielding a product with a very poor specific activity. We decided to rectify the situation.

It seemed logical to develop a synthesis of (3) that introduced the label (tritium) late in the sequence. Thus, D-glucal was converted into the silyl ether (4); the attempted introduction of fluorine (at C2) using Selectfluor™ was accompanied by some loss of the silicon protecting group, and so the silyl ether (4) was transformed into the acetate (5). Now, treatment with Selectfluor™ gave a mixture of 2-deoxy-2-fluoro sugars, from which the α-D-anomer (6) fortuitously crystallized!

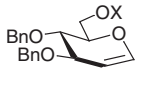
Treatment of the hemiacetal (6) with diphenyl chlorophosphate gave the phosphate (7), but the attempted removal of the acetyl group with sodium methoxide in methanol was accompanied by some loss of the anomeric



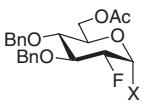
(1)



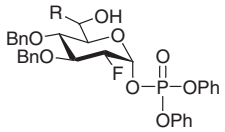
(2) R = H
(3) R = ^{3}H



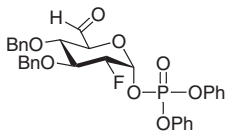
(4) X = $SiPh_2Bu^t$
(5) X = Ac



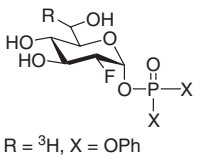
(6) X = OH
(7) X = $OP(O)(OPh)_2$



(8) R = H
(10) R = ^{3}H



(9)



(11) R = ^{3}H, X = OPh
(12) R = ^{3}H, X = $O^-(Bu_3NH)^+$
(13) R = H, X = $O^-(Bu_3NH)^+$

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phosphate. However, treatment of (7) with guanidine/guanidinium nitrate in methanol gave a good yield of the alcohol (8).[7]

Oxidation of (8) with pyridinium chlorochromate in the presence of molecular sieves[8] smoothly gave the aldehyde (9), and a subsequent reduction with an overly generous amount of sodium borotritide (100 mCi), followed by sodium borohydride, gave the alcohol (10). Treatment of (10) with hydrogen, first in the presence of palladium-on-carbon [to give (11)], and then platinum(IV) oxide, gave the phosphate, isolated as the bis(tributylammonium) salt (12).

The salt (12) was converted into the labelled target (3) according to published procedures.[6,9] The 2-deoxy-2-fluoro sugar (3), labelled at C6 with tritium, now had an extremely high, and useful, specific activity.

Experimental

General experimental procedures have been given previously.[10] Sodium boro[^{3}H]hydride was purchased from Amersham. Selectfluor™ was donated by Air Products and Chemicals, Inc.

6-O-*Acetyl-3,4-di*-O-*benzyl*-D-*glucal (5)*

Acetic anhydride (5 mL) was added to 3,4-di-*O*-benzyl-D-glucal[11] (6.7 g) in dry pyridine (30 mL), and the solution left to stand at room temperature (1 h). The solution was then concentrated under vacuum and subjected to a usual workup (EtOAc). Flash chromatography (15% EtOAc/petrol) then gave the acetate (5) as colourless needles (7.0 g, 92%), m.p. 67–69°C (lit.[12] 71°C), $[\alpha]_D$ +5° (lit.[12] +6.5°). The ^{1}H NMR spectrum was consistent with that reported.[12]

6-O-*Acetyl-3,4-di*-O-*benzyl-2-deoxy-2-fluoro*-α-D-*glucose (6)*

Selectfluor™ (6 g) was added to the D-glucal (5) (4.3 g) in dimethylformamide (10 mL) and water (20 mL), and the mixture left to stir at room temperature (3 h). The resultant solution was concentrated under vacuum and subjected to a usual workup (EtOAc). Flash chromatography (25% EtOAc/petrol) then gave the *hemiacetal* (6) as fine needles (2.0 g, 41%), m.p. 119–123°C (EtOH), $[\alpha]_D$ +82° (*c*, 1.0 in $CHCl_3$) (Found: C, 65.2; H, 6.2. $C_{22}H_{25}FO_6$ requires C, 65.3; H, 6.2%). ^{1}H NMR (500 MHz) δ 2.03, s, CH_3; 3.52, dd, $J_{3,4}$ 9.2, $J_{4,5}$ 9.9 Hz, H4; 4.12, ddd, $J_{5,6}$ 2.2, 4.3 Hz, H5; 4.15, dt, $J_{2,3}$ 9.1, $J_{3,F}$ 12.3 Hz, H3; 4.23, dd, $J_{6,6}$ 12.1 Hz, H6; 4.31, dd, H6; 4.51, ddd, $J_{1,2}$ 3.8, $J_{2,F}$ 49.6 Hz, H2; 4.58, 4.89, ABq, *J* 10.9 Hz, C**H**$_2$Ph; 4.77, 4.93, ABq, *J* 11.2 Hz, C**H**$_2$Ph; 5.39, d, H1; 7.25–7.39, m, 10H, Ph. ^{13}C NMR (125.8 MHz) δ 20.82, CH_3; 62.80, C6; 68.64, C5; 75.11, 75.16, 2C, **C**H$_2$Ph; 76.33, d, $J_{4,F}$ 8.7 Hz, C4; 80.11, d, $J_{3,F}$ 15.8 Hz, C3; 90.45, d, $J_{1,F}$ 21.8 Hz, C1; 91.43, d, $J_{2,F}$ 190 Hz, C2; 127.86–138.05, Ph; 170.78, C=O. High-resolution mass spectrum (fast-atom bombardment, FAB) *m*/*z* 405.1729 $[C_{22}H_{26}FO_6\ (M + H)^{+\bullet}$ requires 405.1713].

Diphenyl 6-O-*Acetyl-3,4-di*-O-*benzyl-2-deoxy-2-fluoro*-α-D-*glucosyl Phosphate (7)*

Diphenyl chlorophosphate (0.66 mL, 3.20 mmol) was added to the hemiacetal (6) (1.25 g, 3.11 mmol) and 4-dimethylaminopyridine (400 mg, 3.20 mmol) in dry CH_2Cl_2 (10 mL) at –10°C and the solution was left to stir at this temperature (1 h). The solution was allowed to warm to room temperature, subjected to a usual workup (CH_2Cl_2) and the residue purified using flash chromatography (20% EtOAc/petrol) to give the *phosphate* (7) as a colourless oil (1.8 g, 89%), $[\alpha]_D$ +84° (*c*, 1.0 in $CHCl_3$) (Found: C, 64.1; H, 5.4. $C_{34}H_{34}FO_9P$ requires C, 64.2; H, 5.4%). ^{1}H NMR (500 MHz) δ 1.95, s, CH_3; 3.57, dd, $J_{3,4}$ 9.3, $J_{4,5}$ 9.7 Hz, H4; 3.78–3.94, m, H5; 4.03, dd, $J_{5,6}$ 2.0, $J_{6,6}$ 12.6 Hz, H6; 4.06, dt, $J_{2,3}$ 9.2, $J_{3,F}$ 11.8 Hz, H3; 4.17, dd, $J_{5,6}$ 3.8, H6; 4.57, 4.88, ABq, *J* 10.9 Hz, C**H**$_2$Ph; 4.60, dddd, $J_{1,2} \approx J_{2,P}$ 3.6, $J_{2,F}$ 48.8 Hz, H2; 4.74, 4.86, ABq, *J* 11.0 Hz, C**H**$_2$Ph; 6.12, dd, $J_{1,P}$ 6.4 Hz, H1; 7.22–7.40, m, 20H, Ph. ^{13}C NMR (125.8 MHz) δ 20.55, CH_3; 61.76, C6; 70.73, C5; 75.07, 75.17, 2C, **C**H$_2$Ph; 75.08, d, $J_{4,F}$ 8.8 Hz, C4; 79.65, d, $J_{3,F}$ 16.0 Hz, C3; 90.43, dd, $J_{2,P}$ 8.0, $J_{2,F}$ 189 Hz, C2; 95.25, dd, $J_{1,P}$ 5.7, $J_{1,F}$ 23.2 Hz, C1; 119.93–150.19, Ph; 170.38, C=O. High-resolution mass spectrum (FAB) *m*/*z* 637.1982 $[C_{34}H_{35}FO_9P\ (M + H)^{+\bullet}$ requires 637.2002].

Diphenyl 3,4-Di-O-*benzyl-2-deoxy-2-fluoro*-α-D-*glucosyl Phosphate (8)*

The phosphate (7) (305 mg, 0.48 mmol) was added to a guanidine/guanidinium nitrate solution[7] (50 mL) and the mixture left to stir at 0°C (40 min). The solution was neutralized (Amberlite IR 120, H^+ form), concentrated under vacuum and the residue purified using flash chromatography (30% EtOAc/petrol) to give the *alcohol* (8) as a colourless oil (220 mg, 79%), $[\alpha]_D$ +64° (*c*, 1.1 in $CHCl_3$) (Found: C, 64.5; H, 5.3. $C_{32}H_{32}FO_8P$ requires C, 64.5; H, 5.4%). ^{1}H NMR (500 MHz) δ 2.00, br s, OH; 3.51, dd, $J_{5,6}$ 2.3, $J_{6,6}$ 12.5 Hz, H6; 3.58, dd, $J_{5,6}$ 3.1, H6; 3.63, dd, $J_{3,4}$ 9.1, $J_{4,5}$ 9.2 Hz, H4; 3.68, ddd, H5; 3.98, dt, $J_{2,3}$ 9.1, $J_{3,F}$ 17.8 Hz, H3; 4.50, dddd, $J_{1,2} \approx J_{2,P}$ 3.4, $J_{2,F}$ 48.3 Hz, H2; 4.59, 4.82, ABq, *J* 10.9 Hz, C**H**$_2$Ph; 4.68, 4.83, ABq, *J* 11.0 Hz, C**H**$_2$Ph; 6.04, dd, $J_{1,P}$ 6.4 Hz, H1; 7.18–7.45, m, 20H, Ph. ^{13}C NMR (125.8 MHz) δ 58.90, C6; 73.96, C5; 75.57, 76.17, 2C, **C**H$_2$Ph; 75.79, d, $J_{4,F}$ 8.9 Hz, C4; 79.87, d, $J_{3,F}$ 18.2 Hz, C3; 90.73, dd, $J_{2,P}$ 8.2, $J_{2,F}$ 185 Hz, C2; 96.74, dd, $J_{1,P}$ 5.8, $J_{1,F}$ 24.0 Hz, C1; 118.25–150.27, Ph. High-resolution mass spectrum (FAB) *m*/*z* 595.1913 $[C_{32}H_{33}FO_8P\ (M + H)^{+\bullet}$ requires 595.1897].

Diphenyl 3,4-Di-O-*benzyl-2-deoxy-2-fluoro*-α-D-gluco-*hexodialdo-1,5-pyranosyl Phosphate (9)*

Pyridinium chlorochromate (235 mg, 1.10 mmol) was added to a vigorously stirred mixture of the alcohol (8) (130 mg, 0.22 mmol) and 3 Å molecular sieves (300 mg) in dry CH_2Cl_2 (10 mL), and stirring was continued (1 h). The suspension was then filtered through Celite and concentrated under vacuum. Rapid silica filtration (25% EtOAc/petrol) of the residue then gave the aldehyde (9) as a colourless oil (115 mg, 89%) that was used without further purification. ^{1}H NMR (500 MHz) δ 3.62, dd, $J_{3,4}$ 9.9, $J_{4,5}$ 10.4 Hz, H4; 4.08, dt, $J_{2,3}$ 9.1, $J_{3,F}$ 11.7 Hz, H3; 4.09, dd, $J_{5,6}$ 0.6 Hz, H5; 4.57, dddd, $J_{1,2} \approx J_{2,P}$ 3.3, $J_{2,F}$ 49.4 Hz, H2; 4.63, 4.81, ABq, *J* 10.9 Hz, C**H**$_2$Ph; 4.72, 4.86, ABq, *J* 11.0 Hz, C**H**$_2$Ph; 6.13, dd, $J_{1,P}$ 6.6 Hz, H1; 7.24–7.34, m, 20H, Ph; 9.39, d, H6. ^{13}C NMR (125.8 MHz) δ 75.13, 75.44, 2C, **C**H$_2$Ph; 75.32, C5; 75.80, d, $J_{4,F}$ 7.0 Hz, C4; 79.45, d, $J_{3,F}$ 16.1 Hz, C3; 89.51, dd, $J_{2,P}$ 6.1, $J_{2,F}$ 190 Hz, C2; 94.87, dd, $J_{1,P}$ 5.9, $J_{1,F}$ 25.0 Hz, C1; 119.98–150.17, Ph; 195.11, C6.

Diphenyl 3,4-Di-O-*benzyl-2-deoxy-2-fluoro*-α-D-*[6-^{3}H]glucosyl Phosphate (10)*

A suspension of NaB[^{3}H]$_4$ (100 mCi) in MeOH (0.5 mL) was added to a stirred solution of the aldehyde (9) (620 mg, 1.05 mmol) in MeOH (5 mL), and stirring was continued (30 min). $NaBH_4$ (19 mg, 0.50 mmol) was then added to the mixture and stirring was continued (30 min). AcOH (0.3 mL) was added with stirring, and then the mixture was concentrated under vacuum and subjected to a usual workup (EtOAc). Flash chromatography (25% EtOAc/petrol) then gave the alcohol (10) as a colourless oil (597 mg, 96%). The spectroscopic data (^{1}H and ^{13}C NMR) were consistent with those observed above for (8).

Bis(tributylammonium) 2-Deoxy-2-fluoro-α-D-*[6-^{3}H]glucopyranosyl Phosphate (12)*

Pd/C (100 mg, 10% w/w) was added to the alcohol (10) (560 mg) in MeOH (10 mL), and the mixture was stirred vigorously under a hydrogen atmosphere at room temperature (2 h). The mixture was filtered through Celite, and PtO_2 (40 mg) then added to the filtrate. The mixture was again stirred vigorously under an atmosphere of hydrogen at room temperature (overnight). The mixture was filtered through Celite, concentrated under vacuum, and the residue taken up in water (5 mL) and passed through a column of Dowex 50W-X8 (H^+) resin into a vigorously stirred mixture of water and excess tributylamine. Concentration under vacuum and subsequent lyophilization gave the α-D-phosphate (12) as a colourless solid (542 mg, 91%). The spectroscopic data (^{1}H and ^{13}C NMR) were consistent with those reported for (13).[6]

2-Deoxy-2-fluoro-α-D-[6-^{3}H]glucopyranosyl Uridine-5′-diphosphate (3)

The phosphate (12) (410 mg, 650 μmol) was treated with 4-morpholine-*N*,*N*′-dicyclohexylcarboxamidinium uridine 5′-monophosphomorpholidate (660 mg, 960 μmol) according to the literature procedure[6] to give (3) as a colourless powder (268 mg, 68%). The ^{1}H NMR spectrum was consistent with that reported for (2).[6]

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