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Synthesis of [1-^{2}H]- and [3-^{2}H]-Guanosine

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Summary

Reliable methods are reported for the total synthesis of both [1-^{2}H]- and [3-^{2}H]-guanosine for use in studies of the stereochemistry and mechanism of two enzyme catalysed reactions

Key Words : deuteriated ribose, deuteriated guanosine, nucleoside, pentose.

Introduction

The biosynthetic pathway leading to the coenzymes, tetrahydrofolic acid and tetrahydrobiopterin is extremely important. The first enzyme on the pathway, GTP cyclohydrolase I (EC 3.5.4.16; **(i)** in Scheme 1), is common to the synthesis of both coenzymes. The second enzyme, dihydroneopterin aldolase (EC 4.1.2.25; **(ii)**) is unique to the tetrahydrofolate pathway and so is a target for anti-cancer and anti-bacterial therapy. GTP cyclohydrolase I catalyses conversion of guanosine triphosphate (GTP) (**1**) to dihydroneopterin triphosphate (**5**). The step (**3**) to (**4**) in the reaction is thought to involve an interesting Amadori rearrangement which would involve the chiral centre C-1' in (**1**) becoming a prochiral centre in (**4**). Labelling of H_A in GTP (**1**, $\mathbf{H_A=^2H}$) and incubation with GTP cyclohydrolase I would therefore result in dihydroneopterin triphosphate (**5**) labelled stereospecifically at C-7. Labelling at H_C in GTP (**1**, $\mathbf{H_C=^2H}$) and

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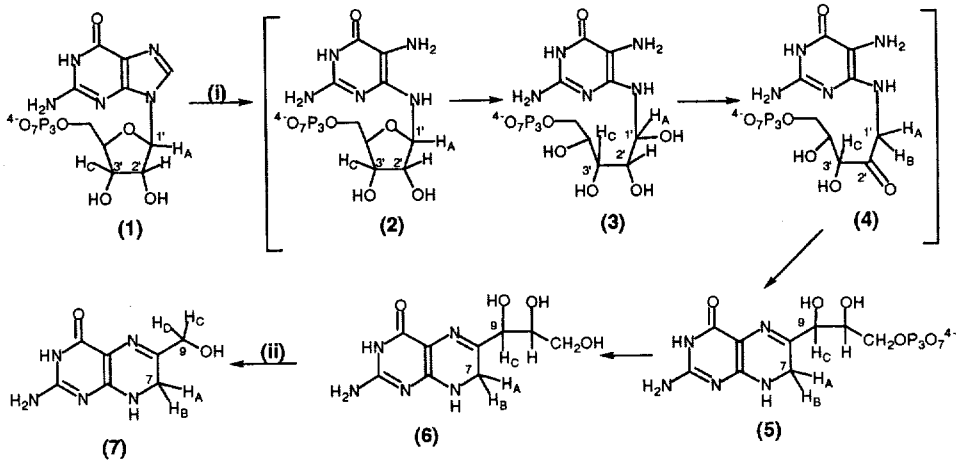
incubation with both of the above enzymes and a kinase would result in enantiotopic labelling in the product (**7**). Since we wished to assess the stereochemical consequences of both enzyme catalysed reactions, we required [1-^{2}H]-GTP and [3-^{2}H]-GTP, (**1**, $\mathbf{H_A}$=^{2}H) and (**1**, $\mathbf{H_C}$=^{2}H) respectively. We now wish to report the synthesis of the unphosphorylated precursors [1-^{2}H]-guanosine and [3-^{2}H]-guanosine, (**8**, $\mathbf{H_A}$=^{2}H) and (**8**, $\mathbf{H_C}$=^{2}H) respectively.



Scheme 1

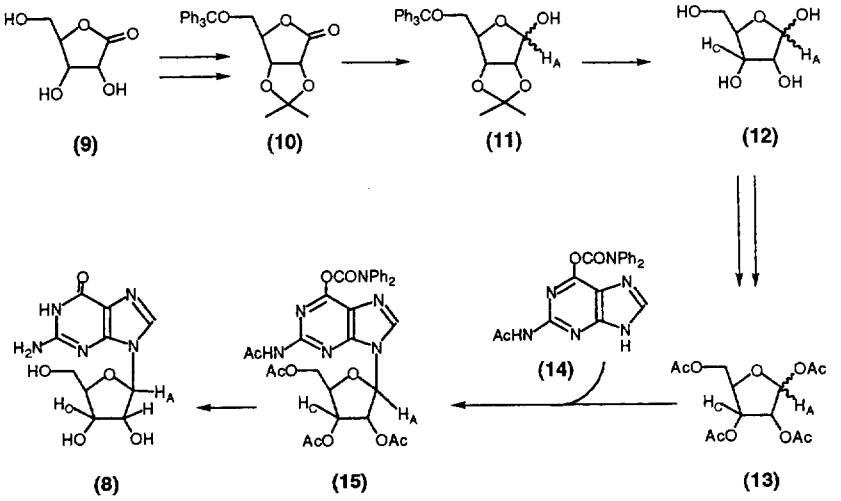
Results and Discussion

Synthesis of [1-^{2}H]-guanosine (8, $\mathbf{H_A}$=^{2}H)

The obvious substrate for the synthesis of [1-^{2}H]-guanosine (**8**, $\mathbf{H_A}$=^{2}H) was [1-^{2}H]-ribose (**12**, $\mathbf{H_A}$=^{2}H). A direct method for synthesis of [1-^{2}H]-ribose by reduction of ribonolactone (**9**) using sodium amalgam in ^2H_2O has been reported[1] but, when we attempted this reaction, it was obvious that hydrolysis of ribonolactone to the corresponding acid was the sole reaction in our hands. Other attempts at direct reduction were equally futile and so, to allow us to use organic solvents in the reduction, we decided to use a protected ribonolactone in our synthesis. Ribonolactone (**9**) was therefore converted to its 2,3-acetonide by a literature procedure[2] and this was reacted with triphenylmethyl chloride in pyridine to yield the 5-trityl derivative (**10**). Reduction with di-isobutylaluminium hydride (DIBAl-H) at –60 °C was unsuccessful but, when this

reduction was carried out at room temperature, the product 2,3-isopropylidene-5-O-trityl-D-ribose (**11**) was a 1 : 4 mixture of α and β-anomers. Initially a 0.5 M solution of DIBAl-^{2}H in toluene from Aldrich gave a product with only 54 % deuteriation. A fresh solution of DIBAl-^{2}H in hexane/diethyl ether prepared by the method of Kalvin and Woodard,[3] however, gave [1-^{2}H]-2,3-isopropylidene-5-O-trityl-D-ribose (**11, H_A=^{2}H**) as a mixture of anomers with 95 % deuteriation. There were also varying amounts of the over-reduced alcohol present. Deprotection using *para*-toluenesulfonic acid in methanol gave a crude sample of [1-^{2}H]-ribose (**12, H_A=^{2}H**) which was converted to the anomeric mixture of tetra-acetates (**13, H_A=^{2}H**) by a method which had been used to prepare the unlabelled compound by Guthrie and Smith.[4]

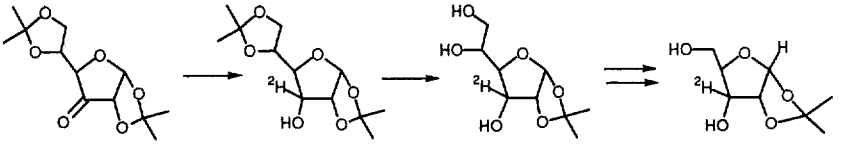
2-Acetylamino-6-O-(N,N-diphenylcarbamoyl)-guanine (**14**) was now prepared by the method of Zou and Robins[5] and reacted with N,O-bis(trimethylsilyl)-acetamide at 80 °C followed by trimethylsilyltrifluoromethanesulfonate and [1-^{2}H]-1,2,3,5-tetra-O-acetylribose (**13, H_A=^{2}H**). The product [1-^{2}H]-2,2',3',5'-tetraacetyl-6-O-(N,N-diphenylcarbamoyl)-guanosine (**15, H_A=^{2}H**) was obtained in 56 % yield as a single anomer due to assistance from the 2α-acetyl group during the condensation. Deprotection using 1N aqueous ammonium hydroxide in methanol gave the target compound, [1-^{2}H]-guanosine (**8, H_A=^{2}H**).



Scheme 2

Synthesis of [3-^{2}H]-guanosine (8, $H_C = {}^2H$)

Since we had succeeded in preparing [1-^{2}H]-guanosine (**8**, $H_A={}^2H$), using [1-^{2}H]-ribose (**12**, $H_A={}^2H$), we expected that use of [3-^{2}H]-ribose (**12**, $H_C={}^2H$) in the same procedure should yield [3-^{2}H]-guanosine (**8**, $H_C={}^2H$). Synthesis of [3-^{2}H]-ribose (**12**, $H_C={}^2H$) was achieved by a simple adaptation of the method which had been used to prepare the [3-^{3}H]-analogue.[6] Since we could now verify stereochemistry by NMR methods, the regio- and stereochemistry of labelling in this synthesis was placed on a firm footing. 1,2:5,6-di-isopropylidene-α-D-ribohexafuranose-3-ulose (**16**) was first reduced with NaB^2H_4 to afford [3-^{2}H]-1,2:5,6-di-isopropylidene-α-D-allofuranose (**17**).[7,8] This was now selectively hydrolysed using 3N H_2SO_4 at room temperature to yield the triol (**18**) and cleaved by sodium periodate to an aldehyde which was reduced *in situ* to the corresponding alcohol (**19**). Hydrolysis with refluxing 3N H_2SO_4 now gave [3-^{2}H]-ribose (**12**, $H_C={}^2H$) and this was converted to the target compound [3-^{2}H]-guanosine (**8**, $H_C={}^2H$) by the methods used in our synthesis of the [1-^{2}H]-isotopomer described above.



(16) (17) (18) (19)

Scheme 3

Experimental

Melting points were determined on a Kofler hot-stage apparatus and optical rotations (10^{-1} deg cm^2 g^{-1}) on a Perkin-Elmer PE241 polarimeter using a 1 dm path-length micro cell. I.R. spectra were recorded on a Perkin-Elmer 1710 Fourier transform instrument and UV spectra on a ATI Unicam UV2-100 Fourier transform scanning spectrophotometer. ^{1}H-NMR spectra were determined using Bruker WM360 (360 MHz) and DPX300 (300 MHz) Fourier transform instruments. J Values are given in Hz. ^{2}H-NMR spectra were recorded on a Bruker AC-P250 (38.4 MHz) Fourier transform instrument and ^{13}C-NMR spectra on Bruker DPX300 (75.48 MHz) and AC-P250 (62.88 MHz) Fourier transform instruments. INEPT and DEPT experiments were used to help assign ^{13}C resonances where necessary. Residual undeuteriated solvent peaks were used as internal references in NMR spectra. Low resolution mass spectra were recorded on Kratos MS80RF and Fisons/VG AutoSpec machines at Sussex, and accurate mass measurements were carried out by the EPSRC

National Mass Spectrometry Service Centre at the University of Wales, Swansea. Column chromatography was carried out using Merck Kieselgel 60 (230-400 Mesh) - Art. 9385, Sorbsil C60 40/60 A and Fluka Silica Gel 60 (220-440 Mesh)

2,3-O-Isopropylidene-5-O-trityl-D-ribonic-γ-lactone (10)

2,3-O-Isopropylidene-D-ribonic-γ-lactone[2] (3.0 g, 16 mmol) was dissolved in dry pyridine (19 ml) at room temperature under nitrogen. Triphenylmethyl chloride (4.44 g, 16 mmol) was added and the solution was stirred at room temperature for 1 h. The mixture was left under nitrogen for 2 days. The solution was filtered and the solvent was removed *in vacuo*. The residue was dissolved in dichloromethane (20 ml) and the precipitate was removed by filtration. The organic layer was washed with water and brine and dried ($MgSO_4$). The solvent was removed *in vacuo* to yield the product as a white foam (3.75 g, 55 %); $[\alpha]_D^{31}$ +2.2 (c 1.0, $CHCl_3$) (lit.[9] $[\alpha]_D^{20}$ +2.1 (c 1.0, $CHCl_3$); m/z [+ve FAB (3-NBA)] 430 ([M]$^+$) and 453 ([M+Na]$^+$); ν_{max} (KBr)/cm^{-1} 1786 (lactone); δ_H (360 MHz, C^2HCl_3) 1.28 (3H, s, C$\underline{H}_3$), 1.42 (3H, s, C$\underline{H}_3$), 3.01 (1H, dd, $J_{5B,5A}$ 10.7 $J_{5B,4}$ 1.34, $\underline{H}$-5*B*), 3.68 (1H, dd, $J_{5A,5B}$ 10.7, $J_{5A,4}$ 2.4, $\underline{H}$-5*A*), 4.37 (1H, d, $J_{3,2}$ 5.6, $\underline{H}$-3), 4.54 (1H, s, $\underline{H}$-4), 4.93 (1H, d, $J_{2,3}$ 5.6, $\underline{H}$-2) and 7.20-7.32 (15H, Ar$\underline{H}$); δ_C (62.88 MHz, C^2HCl_3) 25.57 (C$\underline{H}_3$), 26.74 (C$\underline{H}_3$), 62.76 ($\underline{C}$-5), 75.74 ($\underline{C}$-4), 78.54 ($\underline{C}$-3), 81.85 ($\underline{C}$-2), 87.81 ($\underline{C}Ph_3$), 113.18 ($\underline{C}(CH_3)_2$), 127.39-128.41 (Ar$\underline{C}$H), 142.85 (Ar$\underline{C}$-quat.) and 174.40 ($\underline{C}$-1).

2,3-O-Isopropylidene-5-O-trityl-D-ribose (11)

2,3-O-Isopropylidene-5-O-trityl-D-ribonic-γ-lactone **(10)** (100 mg, 0.23 mmol) was dissolved in dry toluene (1.7 ml) under nitrogen and heated to 40 °C. Di-isobutylaluminium hydride (0.46 ml, 0.46 mmol, 1 M solution in THF) was added over a period of 15 min. TLC indicated that starting material was still present after stirring for 3 h at 40 °C. Di-isobutylaluminium hydride (0.23 ml, 0.23 mmol) was added and the solution was stirred for a further 1 h 30 min at 40 °C. Methanol (0.3 ml) was added to yield a gel which was broken down by addition of saturated aqueous sodium potassium tartrate (0.6 ml). The product was extracted into diethyl ether, washed with brine and dried ($MgSO_4$). The solvent was removed *in vacuo* to yield a foam which was chromatographed on silica gel using EtOAc / petroleum ether (60/80) (1:4) as eluant. 2,3-O-Isopropylidene-5-O-trityl-D-ribose **(11)** was isolated as a glass (25 mg, 25 %); m/z [+ve FAB (3-NBA)] 432 ([M]$^+$); δ_H (360 MHz, C^2HCl_3) (mixture of anomers, 1:4 α:β): α-anomer, 1.38 (3H, s, C$\underline{H}_3$), 1.57 (3H, s, C$\underline{H}_3$), 3.02 (1H, dd, $J_{5B,5A}$ 10.2, $J_{5B,4}$ 2.7, $\underline{H}$-5*B*), 3.43 (1H, $\underline{H}$-5*A* (overlap with βH-5*A*)), 4.02 (1H, d, $J_{OH1,1}$ 11.2, O$\underline{H}$-1, exch. ^2H_2O), 4.21 (1H, br s, $\underline{H}$-4), 4.60 (1H, d, $J_{3,2}$ 6.1, $\underline{H}$-3), 4.76 (1H, dd, $J_{2,3}$ 6.1, $J_{2,1}$ 4.1, $\underline{H}$-2), 5.77 (1H, dd, $J_{1,OH1}$ 11.2, $J_{1,2}$ 4.1, $\underline{H}$-1 [d in ^2H_2O]), 7.24-7.43 (15H, Ar$\underline{H}$, overlap with β-anomer); β-anomer, 1.36 (3H, s, C$\underline{H}_3$), 1.50 (3H, s, C$\underline{H}_3$), 3.35 (1H, dd, $J_{5B,5A}$ 10.4, $J_{5B,4}$ 3.7, $\underline{H}$-5*B*), 3.43 (1H, dd, $J_{5A,5B}$ 10.4, $J_{5A,4}$ 3.4 $\underline{H}$-5*A*, overlap with αH-5*A*), 3.97 (1H, d, $J_{OH1,1}$ 9.2, O$\underline{H}$-1, exch. ^2H_2O), 4.37 (1H, t, $J_{4,5}$ 3.4, $\underline{H}$-4), 4.68 (1H, d, $J_{3,2}$ 5.9, $\underline{H}$-3), 4.80 (1H, d, $J_{2,3}$ 5.9, $\underline{H}$-2), 5.34 (1H, d, $J_{1,OH1}$ 9.2, $\underline{H}$-1 [s in ^2H_2O]), and 7.24-7.43 (15H, Ar$\underline{H}$, overlap with α-anomer); δ_C (62.88 MHz, C^2HCl_3) 24.73 and 25.08 (C$\underline{H}_3$), 26.14 and 26.50 (C$\underline{H}_3$), 65.05 and 65.41 ($\underline{C}$-5), 79.42 and 80.08 ($\underline{C}$-3), 81.91 and 82.17 ($\underline{C}$-4), 86.05 and 87.04 ($\underline{C}$-2), 87.50 and 88.18 ($\underline{C}Ph_3$), 97.99 and 103.51 ($\underline{C}$-1), 112.22 and 113.10 ($\underline{C}(CH_3)_2$), 127.19, 127.47, 127.96, 128.07, 128.56 and 128.65 (Ar$\underline{C}$H) and 142.79 and 143.46 (Ar$\underline{C}$-quat.).

[1-^{2}H]-2,3-O-Isopropylidene-5-O-trityl-D-ribose (11, H_A=^{2}H)

This was prepared as above using 2,3-O-isopropylidene-5-O-trityl-D-ribonic-γ-lactone **(10)** (2.27 g, 5.27 mmol) and freshly prepared[3] di-isobutylaluminium deuteride (21 ml, 21 mmol, 1M solution

in hexane / diethyl ether) to yield [1-^{2}H]-2,3-O-isopropylidene-5-O-trityl-D-ribose (**11, H_A=^{2}H**) as a white solid (380 mg, 17 %); mp 153-155 °C; m/z [+ve FAB (3-NBA)] 456 ([M+Na]$^+$); ν_{max} (KBr)/cm^{-1} 3462 (OH, br); δ_H (300 MHz, C^{2}HCl$_3$) (mixture of anomers - 3:7 α:β), α-anomer, 1.28 (3H, s, C$\underline{H}_3$), 1.47 (3H, s, C$\underline{H}_3$), 2.92 (1H, dd, $J_{5B,5A}$ 10.1, $J_{5B,4}$ 2.6, $\underline{H}$-5*B*), 3.37 (1H, dd, $J_{5A,5B}$ 10.1, $J_{5A,4}$ 2.9 $\underline{H}$-5*A*, overlap with βH-5*A*), 3.91 (1H, br s, O$\underline{H}$-1, exch. ^2H_2O, overlap with β-anomer), 4.11 (1H, br s, $\underline{H}$-4), 4.49 (1H, d, $J_{3,2}$ 6.2, $\underline{H}$-3), 4.66 (1H, d, $J_{2,3}$ 6.2, $\underline{H}$-2), 5.68 (0.03H, dd, $\underline{H}$-1, [d in ^2H_2O]) and 7.13-7.34 (15H, Ar$\underline{H}$, overlap with β-anomer); β-anomer, 1.26 (3H, s, C$\underline{H}_3$), 1.40 (3H, s, C$\underline{H}_3$), 3.25 (1H, dd, $J_{5B,5A}$ 10.3, $J_{5B,4}$ 3.7, $\underline{H}$-5*B*), 3.34 (1H, dd, $J_{5A,5B}$ 10.3, $J_{5A,4}$ 3.4, $\underline{H}$-5*A*, overlap with αH-5*A*), 3.91 (1H, br s, O$\underline{H}$-1, exch. ^2H_2O), 4.27 (1H, t, $J_{4,5}$ 3.4, $\underline{H}$-4), 4.57 (1H, d, $J_{3,2}$ 5.9, $\underline{H}$-3), 4.70 (1H, d, $J_{2,3}$ 5.9, $\underline{H}$-2), 5.24 (0.06H, d, $\underline{H}$-1, [s in ^2H_2O]), and 7.13-7.34 (15H, Ar$\underline{H}$, overlap with α-anomer); δ_D (38.4 MHz, CHCl$_3$) 5.38 and 5.76 (α and β ^{2}H-1); δ_C (75.48 MHz, C^{2}HCl$_3$) 25.18 and 25.52 (α (C$\underline{H}_3$), 26.60 and 26.95 (C$\underline{H}_3$), 65.50 and 65.90 ($\underline{C}$-5), 79.77 and 80.49 ($\underline{C}$-3), 82.34 and 82.63 ($\underline{C}$-4), 86.41 and 87.41 ($\underline{C}$-2), 87.95 and 88.63 (C$\underline{P}$h$_3$), 97 and 107 (2 x t, $\underline{C}$-1), 112.68 and 113.53 (C$\underline{(CH_3)_2}$), 127.65, 127.93, 128.36, 128.42, 128.53, 129.00 and 129.09 (Ar$\underline{C}$H) and 143.24 and 143.91 (Ar$\underline{C}$-quat.).

The over-reduced by-product [1,1-$^2H_2$]-2,3-O-isopropylidene-5-O-trityl-ribitol eluted as an oil (940 mg, 41 %); $[\alpha]_D^{26}$ +19.0 (c 1.0, CHCl$_3$); m/z [+ve FAB (3-NBA)] 459 ([M+Na]$^+$); ν_{max} (film)/cm^{-1} 3400 (OH, br); δ_H (300 MHz, C^{2}HCl$_3$) 1.22 and 1.23 (6H, 2 x s, C$\underline{H}_3$), 2.92 (2H, br s, O$\underline{H}$-1 and O$\underline{H}$-4, exch. ^2H_2O), 3.23 (1H, dd, $J_{5B,5A}$ 9.7, $J_{5B,4}$ 6.9, $\underline{H}$-5*B*), 3.40 (1H, dd, $J_{5A,5B}$ 9.7 $J_{5A,4}$ 2.8, $\underline{H}$-5*A*), 3.76 (1H, m, $\underline{H}$-4), 4.03 (1H, dd, $J_{3,4}$ 9.5, $J_{3,2}$ 5.8, $\underline{H}$-3), 4.25 (1H, d, $J_{2,3}$ 5.8 $\underline{H}$-2) and 7.18-7.40 (15H, m, Ar$\underline{H}$); δ_D (38.4 MHz, CHCl$_3$) 3.84 (s, 2$\underline{H}$-1); δ_C (75.48 MHz, C^{2}HCl$_3$) 25.74 (C$\underline{H}_3$), 28.24 (C$\underline{H}_3$), 65.48 ($\underline{C}$-5), 69.42 ($\underline{C}$-4), 77.25 ($\underline{C}$-3), 77.87 ($\underline{C}$-2), 87.51 (C$\underline{P}$h$_3$), 108.95 ($\underline{C}$(CH$_3$)$_2$), 127.64, 128.37 and 129.01 (Ar$\underline{C}$H) and 144.08 (Ar$\underline{C}$-quat.).

[3-^{2}H]-1,2:5,6-Di-O-isopropylidene-α-D-allofuranose (17)

1,2:5,6-Di-O-isopropylidene-α-D-ribohexafuranose-3-ulose (**16**) (1.40 g, 5.42 mmol) was dissolved in [$^2H_1$]-ethanol (19 ml) and ^2H_2O (8 ml) under nitrogen. The solution was cooled in an ice/water bath and NaO^{2}H (40 % wt. in ^2H_2O) was added until pD = 8. NaB$^2H_4$ (786 mg, 18.8 mmol) was added and the solution was cooled. The cloudy solution was stirred at 0 °C for 40 min, allowed to warm to room temperature and stirred for a further 1 h. Water (84 ml) was added, causing the solution to become clear and stirring was continued for 10 min. The product was extracted with EtOAc, washed with water and brine and dried ($MgSO_4$). The solvent was removed *in vacuo* to yield a white crystalline solid (951 mg, 67 %); mp 75-76 °C (lit.[10] mp 76-78 °C); m/z [+ve FAB, (3-NBA)] 262 ([M+H]$^+$); ν_{max} (KBr)/cm^{-1} 3347 (OH, br); δ_H (360 MHz, C^{2}HCl$_3$) 1.38 (3H, s, C$\underline{H}_3$), 1.39 (3H, s, C$\underline{H}_3$), 1.48 (3H, s, C$\underline{H}_3$), 1.59 (3H, s, C$\underline{H}_3$), 2.53 (1H, s, 3-O$\underline{H}$, exch. ^2H_2O), 3.82 (1H, d, $J_{4,5}$ 4.6, $\underline{H}$-4), 4.00-4.11 (2H, m, $\underline{H}$-6), 4.32 (1H, m, $\underline{H}$-5), 4.62 (1H, d, $J_{2,1}$ 3.8, $\underline{H}$-2) and 5.82 (1H, d, $J_{1,2}$ 3.8, $\underline{H}$-1); δ_D (38.4 MHz, CHCl$_3$) 4.05 (s, $^2\underline{H}$-3); δ_C (62.88 MHz, C^{2}HCl$_3$) 25.20, 26.23, 26.44 and 26.50 (4 x C$\underline{H}_3$), 65.75 ($\underline{C}$-6), 71.9 (t, $\underline{C}^2$H-3), 75.47 ($\underline{C}$-5), 78.85 ($\underline{C}$-4), 79.58 ($\underline{C}$-2), 103.86 ($\underline{C}$-1), 109.78 ($\underline{C}$(CH$_3$)$_2$) and 112.77 ($\underline{C}$(CH$_3$)$_2$).

[3-^{2}H]-1,2-O-isopropylidene-α-D-allofuranose (18)

[3-^{2}H]-1,2:5,6-Di-O-isopropylidene-α-D-allofuranose (**17**) (900 mg, 3.44 mmol) was dissolved in methanol (4.8 ml) and treated with 0.3N aqueous sulfuric acid (4.8 ml). The mixture was stirred at room temperature for 3.5 h. Solid $BaCO_3$ was added to neutralise the solution and the suspension was heated to reflux for 20 min and allowed to cool to room temperature. The suspension was filtered through Celite® and the solvent was removed *in vacuo* to yield a clear oil which crystallised under high vacuum (0.1 mm Hg) (720 mg, 95 %); mp 131-133 °C (lit.[8] 127-129 °C); $[\alpha]_D^{26}$ +43.7

(c 1.0, MeOH); m/z [+ve FAB (3-NBA)] 222 ([M+H]$^+$) and 244 ([M+Na]$^+$); ν_{max} (KBr)/cm^{-1} 3285 (OH, br); δ_H (360 MHz, $(C^2H_3)_2SO$) 1.24 (3H, s, C$\underline{H}_3$), 1.42 (3H, s, C$\underline{H}_3$), 3.31-3.46 (m, $\underline{H}$-6, obscured by H_2O, [in ^2H_2O 3.31-3.36 (1H, dd, $J_{6B,6A}$ 11.3, $J_{6B,5}$ 6.7, $\underline{H}$-6*B*) 3.41-3.46 (1H, dd, $J_{6A,6B}$ 11.3, $J_{6A,5}$ 5.0, $\underline{H}$-6*A*)]), 3.66 (1H, m, $\underline{H}$-5), 3.77 (1H, d, $J_{4,5}$ 2.1, $\underline{H}$-4), 4.42 (1H, d, $J_{2,1}$ 3.6, $\underline{H}$-2), 4.53 (1H, t, $J_{OH6,6}$ 5.8, O$\underline{H}$-6, exch. ^2H_2O), 4.73 (1H, d, $J_{OH5,5}$ 4.6, O$\underline{H}$-5, exch. ^2H_2O), 4.84 (1H, s, O$\underline{H}$-3, exch. ^2H_2O) and 5.61 (1H, d, $J_{1,2}$ 3.6, $\underline{H}$-1); δ_D (38.4 MHz, $(CH_3)_2SO$) 3.88 ($^2\underline{H}$-3); δ_C (62.88 MHz, $(C^2H_3)_2SO$) 27.20, 27.48 (2 x $\underline{C}H_3$), 64.43 ($\underline{C}$-6), 71.92, (t, $\underline{C}^2$H-3), 73.00 ($\underline{C}$-5), 81.64 ($\underline{C}$-4), 82.04 ($\underline{C}$-2), 105.81 ($\underline{C}$-1) and 114.22 ($\underline{C}(CH_3)_2$).

[3-^{2}H]-1,2-O-Isopropylidene-α-D-ribofuranose (19)

[3-^{2}H]-1,2-O-Isopropylidene-α-D-allofuranose (**18**) (665 mg, 3.0 mmol) was dissolved in water (6.5 ml) and treated with 0.5 M aqueous sodium periodate (6.5 ml). The mixture was allowed to stand for 8 h at room temperature. Methanol (54 ml) was added, forming a suspension which was left overnight at -15 °C. The solution was filtered and the solvent was removed *in vacuo*. The residue was dissolved in methanol and filtered. The solvent was removed *in vacuo* to yield [3-^{2}H]-1,2-O-isopropylidene-α-D-ribopentodialdo-1,4-furanose as a foam. This was dissolved in 70 % (v/v) aqueous ethanol (13.5 ml) and $NaBH_4$ (271 mg, 7.17 mmol) was added. The reaction was stirred for 1 h. Dowex 50W-X8 (H^+) (20-50 mesh) was added until the solution was neutral and stirring was continued for a further 30 min. The mixture was filtered and the solvent was removed *in vacuo*. The residue was azeotroped with methanol and the residue was left under high vacuum to yield a clear yellow oil which crystallised to yield the product, [3-^{2}H]-1,2-O-isopropylidene-α-D-ribofuranose (**19**), as off-white crystals (561 mg, 98 %); mp 85-87 °C (lit.[8] mp 86-87 °C) $[\alpha]_D^{27}$ +41.6 (c 0.7, H_2O); m/z [+ve FAB (3-NBA)] 214 ([M+Na]$^+$); ν_{max} (KBr)/cm^{-1} 3388 (OH, br); δ_H (360 MHz, $(C^2H_3)_2SO$) 1.24 (3H, s, C$\underline{H}_3$), 1.42 (3H, s, C$\underline{H}_3$), 3.33-3.39 (dd, H-5*B*, obscured by H_2O, [simplifies with ^2H_2O to 3.32-3.37 (1H, dd, $J_{5B,5A}$ 12.2, $J_{5B,4}$ 5.0, $\underline{H}$-5*B*)]), 3.59-3.63 (1H, dd, $J_{5A,5B}$ 12.2, $J_{5A,4}$ 3.5, $\underline{H}$-5*A*), 3.68 (1H, d, J 4.6, $\underline{H}$-4), 4.41 (1H, d, $J_{2,1}$ 3.6, $\underline{H}$-2), 4.68 (1H, t, J 5.5, O$\underline{H}$-5, exch. ^2H_2O), 5.02 (1H, s, O$\underline{H}$-3, exch. ^2H_2O), and 5.63 (1H, d, $J_{1,2}$ 3.6, $\underline{H}$-1); δ_D (38.4 MHz, $(CH_3)_2SO$) 3.83 ($^2\underline{H}$-3); δ_C (62.88 MHz, $(C^2H_3)_2SO$) 25.67, 25.91 (2 x $\underline{C}H_3$), 60.72 ($\underline{C}$-5), 70.75 (t, $\underline{C}^2$H-3), 79.73 ($\underline{C}$-4), 80.58 ($\underline{C}$-2), 104.26 ($\underline{C}$-1) and 112.62 ($\underline{C}(CH_3)_2$).

[1-^{2}H]-D-Ribose (12, H_A=^{2}H)

[1-^{2}H]-2,3-O-Isopropylidene-5-O-trityl-D-ribose (**11, H_A=^{2}H**) (135 mg, 0.31 mmol) was dissolved in methanol (4.5 ml). *para*-Toluenesulfonic acid monohydrate (59 mg, 0.31 mmol) was added and the reaction was stirred at room temperature. TLC indicated complete consumption of starting material after 2 h. The solvent was removed *in vacuo* to yield the crude product as a crystalline solid which was used directly in the next stage.

[3-^{2}H]-D-Ribose (12, H_C=^{2}H)

[3-^{2}H]-1,2-O-Isopropylidene-α-D-ribofuranose (**19**) (510 mg, 2.67 mmol) was dissolved in methanol (15 ml). 0.3N Aqueous sulfuric acid (15 ml) was added and the solution was heated at reflux for 2 h and cooled to room temperature. Solid $BaCO_3$ was added to neutralise the solution, which was filtered through Celite®. The solvent was removed *in vacuo* to yield a yellow oil (399 mg, 99 %); m/z [+ve FAB (glycerol)] 174 ([M+Na]$^+$); ν_{max} (film)/cm^{-1} 3367 (OH, br); δ_H (360 MHz, $(C^2H_3)_2SO$) 3.1-3.8 (m) and 4.4-4.9 (m); δ_C (62.88 MHz, $(C^2H_3)_2SO$) 54.47 ($\underline{C}$H), 54.77 ($\underline{C}$H), 61.87 ($\underline{C}H_2$), 62.92 ($\underline{C}H_2$), 63.18 ($\underline{C}H_2$), 63.32 ($\underline{C}H_2$), 63.40 ($\underline{C}H_2$), 68.54 ($\underline{C}$H), 70.73 (t, $\underline{C}^2$H-3), 71.35 ($\underline{C}$H), 71.97 ($\underline{C}$H), 74.32 ($\underline{C}$H), 75.62 ($\underline{C}$H), 82.93 ($\underline{C}$H), 83.61 ($\underline{C}$H), 85.12 ($\underline{C}$H), 93.81 ($\underline{C}$H), 94.61 ($\underline{C}$H), 101.58 ($\underline{C}$H), 102.99 ($\underline{C}$H) and 108.22 ($\underline{C}$H).

[3-^{2}H]-1,2,3,5-Tetra-O-acetyl-D-ribofuranose (13, H_C=^{2}H)

[3-^{2}H]-D-Ribose **(12, H_C=^{2}H)** (118 mg, 0.78 mmol) was dissolved in methanol (1.85 ml) under nitrogen and cooled in an ice/water bath. Conc. sulfuric acid (9 µl) was added and the mixture was allowed to stand at 4 °C overnight and neutralised using dry pyridine (370 µl). The solvents were removed *in vacuo* to yield the methylfuranoside as an oil which was dissolved in dry pyridine (0.92 ml) under nitrogen and cooled in an ice/water bath. Acetic anhydride (370 µl) was added and the solution was allowed to warm to room temperature and stirred for 2 days. EtOAc was added and the solution was washed with water and brine. The organic layer was dried ($MgSO_4$) and the solvent was removed *in vacuo* to give 1-methyl-2,3,5-tri-O-acetyl-D-ribofuranose as a yellow oil. This was dissolved in acetic acid (1.11 ml) under nitrogen and cooled in an ice/water bath. The solution was treated with acetic anhydride (260 µl) and conc. sulfuric acid (60 µl), allowed to warm to room temperature and stirred overnight. Ice (1.5 g) was added to the resulting dark yellow solution, which was stirred for 10 min. The product was extracted into chloroform, washed with water and saturated aqueous sodium carbonate and dried ($MgSO_4$). The solvent was removed *in vacuo* to yield a pale yellow oil (170 mg, 69 %); m/z 320 ([M+H]$^+$) and 342 ([M+Na]$^+$); ν_{max} (film)/cm^{-1} 1744 (ester); δ_H (360 MHz, C^2HCl_3) (mixture of α and β anomers in a ratio of 3:7) 2.08, 2.09, 2.10 and 2.11 (12H, 4 x s, C<u>H</u>$_3$), 4.31-4.45 (2H, m, <u>H</u>-4), 4.18-4.23 (0.3H, dd, $J_{5A,5B}$ 12.2, $J_{5A,4}$ 3.9, α<u>H</u>-5*A*), 5.22 (0.27H, d, $J_{2,1}$ 4.6, α<u>H</u>-2), 6.43 (0.27H, d, $J_{1,2}$ 4.6, α<u>H</u>-1), 4.12-4.17 (0.7H, dd, $J_{5A,5B}$ 11.8, $J_{5A,4}$ 5.2, β<u>H</u>-5*A*), 5.34 (0.73H, s, β<u>H</u>-2) and 6.16 (0.73H, s, β<u>H</u>-1); δ_D (38.4 MHz, C^2HCl_3) 5.37 (s, ^{2}<u>H</u>-3); δ_C (75.48 MHz, C^2HCl_3) 19.49, 19.63, 19.72 and 20.02 (4 x <u>C</u>H$_3$), 62.26 (α<u>C</u>-5), 62.59 (β<u>C</u>-5), 68.88 (α<u>C</u>-4), 73.02 (β<u>C</u>-4), 78.17 (β<u>C</u>-2), 80.54 (α<u>C</u>-2), 93.02 (α<u>C</u>-1), 97.14 (β<u>C</u>-1), 168.00, 168.42, 168.70 and 169.46 (4 x <u>C</u>=O,). C-3 was not visible above the baseline.

[1-^{2}H]-1,2,3,5-Tetra-O-acetyl-D-ribofuranose (13, H_A=^{2}H)

This was prepared as above using crude [1-^{2}H]-D-Ribose **(12, H_A=^{2}H)** to yield [1-^{2}H]-1,2,3,5-tetra-O-acetyl-D-ribofuranose **(13, H_A=^{2}H)**, as an oil (mixture of anomers; 40 mg, 40% overall from [1-^{2}H]-2,3-O-isopropylidene-5-O-trityl-D-ribose **(11, H_A=^{2}H))**; $[\alpha]_D^{26}$ -8.7 (c 1.0, $CHCl_3$) (lit.[11] β-anomer $[\alpha]_D^{26}$ -11.4 (c 10, $CHCl_3$) (lit.[12] α-anomer $[\alpha]_D^{22}$ +78.7 (c 3.73, MeOH))); m/z [+ve FAB (3-NBA)] 342 ([M+Na]$^+$); ν_{max} (film)/cm^{-1} 1749 (ester); δ_H (300 MHz, C^2HCl_3) 2.01, 2.03, 2.04 and 2.06 (12H, 4 x s, C<u>H</u>$_3$), 4.14 (1H, dd, $J_{5B,5A}$ 11.7, $J_{5B,4}$ 5.2, <u>H</u>-5*B*), 4.31-4.40 (2H, m, <u>H</u>-5*A* and <u>H</u>-4), 5.34 (2H, s, <u>H</u>-3 and <u>H</u>-2) and 6.17 (0.05H, s, <u>H</u>-1); δ_D (38.4 MHz, $CHCl_3$) 6.18 (s, ^{2}<u>H</u>-1); δ_C (75.48 MHz, C^2HCl_3) 22.71, 22.74, 22.98 and 23.27 (4 x OCO<u>C</u>H$_3$), 65.88 (<u>C</u>-5), 72.73 (<u>C</u>-3), 76.28 (<u>C</u>-4), 81.49 (<u>C</u>-2), 100.1 (t, <u>C</u>^{2}H-1), 171.3, 171.69, 171.95 and 172.73 (O<u>C</u>OCH$_3$).

[3'-^{2}H]-2-Acetylamino-2',3',5'-tri-O-acetyl-6-O-(N,N-diphenylcarbamoyl)-guanosine (15, H_C=^{2}H)

2-Acetylamino-6-O-diphenylcarbamoylguanine **(14)**[5] (150 mg, 0.38 mmol) was suspended in 1,2-dichloroethane (3.2 ml) under nitrogen and treated with N,O-bis-(trimethylsilyl)-acetamide (0.16 ml). The mixture was heated to 80 °C and stirred for 15 min when some of the suspension dissolved. The solvents were removed *in vacuo*. The residue was suspended in dry toluene (1.6 ml) under nitrogen and fresh trimethylsilyltrifluoromethanesulfonate (80 µl) was added. [3-^{2}H]-1,2,3,5-Tetra-O-acetyl-D-ribofuranose **(13, H_C=^{2}H)** (123 mg, 0.38 mmol) dissolved dry toluene (1.6 ml) was added to the mixture which was heated to 80 °C for 1 h. The solution was cooled, diluted with EtOAc, washed with water and brine and dried ($MgSO_4$). The solvent was removed *in*

vacuo to yield a yellow/brown foam, which was absorbed onto silica gel and purified by column chromatography on silica gel using diethyl ether / acetone (4:1) as eluant. The product was obtained as a glass which was azeotroped with diethyl ether to yield a foam (112 mg, 45 %); $[\alpha]_D^{26}$ -4.2 (c 0.5, $CHCl_3$); λ_{max} ($CHCl_3$)/nm 246 and 280 (ε 23,495 and 14,338); m/z [+ve FAB (3-NBA)] 648 ([M+H]$^+$) and 670 ([M+Na]$^+$); Found 648.216509, [$C_{31}H_{29}{}^2HN_6O_{10}$+H] requires 648.216443; ν_{max} (film)/cm^{-1} 3325 (NH) and 1745 (C=O); δ_H (360 MHz, $(C^2H_3)_2SO$) 1.97, 2.04, 2.10 (9H, 3 x s, 3 x CH_3COO), 2.15 (3H, s, CH_3CON), 4.28-4.44 (3H, m, H-4', H-5'), 5.91 (1H, d, $J_{2',1'}$ 4.7, H-2'), 6.25 (1H, d, $J_{1',2'}$ 4.7, H-1'), 7.30-7.46 (10H, ArH) and 8.61 (1H, s, H-8); δ_D (38.4 MHz, C^2HCl_3) 5.73 (^{2}H-3'); δ_C (75.48 MHz, C^2HCl_3) 20.83, 20.96, 21.20 (3 x CH_3COO), 25.55 (CH_3CON), 63.59 (C-5'), 73.55 (C-4'), 80.65 (C-2'), 87.27 (C-1'), 121.61 (urethane), 127-129 (ArCH), 142.04 (C-8), 142.65 (CON), 150.62, 152.71, 154.71 and 156.75 (C-5, C-2, C-6, C-4), 169.82, 169.99 and 170.85 (3 x COO). C-3 was not visible above the baseline.

[1'-^{2}H]-2-Acetylamino-2',3',5'-tri-O-acetyl-6-O-(N,N-diphenylcarbamoyl)-guanine (15, H_A=^{2}H)

This was prepared from [1-^{2}H]-1,2,3,5-tetra-O-acetyl-D-ribofuranose (**13**, H_A=^{2}H) (42 mg, 0.13 mmol) using the method above. The product was obtained as a glass (50 mg, 60 %); $[\alpha]_D^{27}$ -5.8 (c 1.0, $CHCl_3$); λ_{max} ($CHCl_3$)/nm 244 and 281 (ε 18,005 and 11,035); m/z [+ve FAB (3-NBA)] 648 ([M+H]$^+$) and 670 ([M+Na]$^+$); Found 648.218894, [$C_{31}H_{29}{}^2HN_6O_{10}$+H] requires 648.216443; ν_{max} (film)/cm^{-1} 3351 (NH) and 1746 (C=O); δ_H (300 MHz, C^2HCl_3) 2.02 (6H, s, 2 x $COCH_3$), 2.07 (3H, s, $COCH_3$), 2.10 (3H, s, $COCH_3$), 4.30-4.41 (3H, m, H-4' and H-5'), 5.66 (1H, t, J 4.9, H-3'), 5.81 (1H, d, $J_{2',3'}$ 5.5, H-2'), 7.19-7.34 (10H, ArH), 8.04 (1H, s, H-8) and 8.19 (1H, s, 2-NH, exch. with ^2H_2O); δ_D (38.4 MHz, $CHCl_3$) 6.12 (s, ^{2}H-1); δ_C (75.48 MHz, C^2HCl_3) 20.83, 20.97 and 21.21 (3 x $COCH_3$), 25.56 ($COCH_3$), 63.60 (C-5'), 70.96 (C-3'), 73.54 (C-4'), 80.73 (C-2'), 88 (weak t, C^2H-1'), 121.4 (urethane), 126-129 (ArCH), 142.0 (C-8), 142.61 (CON), 150.59, 152.74, 154.63 and 156.70 (C-5, C-2, C-6, C-4), 169.82, 169.99 and 170.85 (3 x C=O).

[3'-^{2}H]-Guanosine (8, H_C=^{2}H)

[3'-^{2}H]-2-Acetylamino-2',3',5'-tri-O-acetyl-6-O-(N,N-diphenylcarbamoyl)-guanine (**15**, H_C=^{2}H) (64 mg, 0.1 mmol) was dissolved in methanol (1 ml). 1N Aqueous ammonium hydroxide was added until a precipitate formed (1 ml). Methanol was added (3 ml) to dissolve the precipitate, followed by 1N aqueous NH_4OH (2 ml). The solution was stirred at room temperature for 4 h but λ_{max} 280 nm indicated incomplete reaction. Further 1N NH_4OH (0.5 ml) was added and after stirring for 2 days at room temperature, λ_{max} 280 nm was absent. The solvents were removed *in vacuo* and the residue was recrystallised from water as an off-white solid (12 mg, 42 %); mp 231-233 °C (lit.[13] mp 248-251 °C; lit.[14] mp 230-235 °C); $[\alpha]_D^{27}$-74.5 (c 0.11, 0.1N NaOH) (lit.[15] $[\alpha]_D^{25}$ -71.9 (c 1.078, 0.1N NaOH); lit.[16] $[\alpha]_D$ -56 (c 0.48, 0.1N NaOH)); λ_{max} (MeOH)/nm 255 and 275 (sh) (ε 11,482 and 7,389); λ_{max} (OH^-)/nm 259; m/z [+ve FAB (3-NBA)] 285 ([M+H]$^+$); ν_{max} (KBr)/cm^{-1} 3412 (OH, NH, br) and 1693 (amide); δ_H (360 MHz, $(C^2H_3)_2SO$) 3.47-3.62 (2H, m, H-5'), 3.84 (1H, t, $J_{4',5'}$ 3.7, H-4'), 4.37 (1H, t, J 6.0, H-2', [d, $J_{2',1'}$ 6.0 in ^2H_2O]), 5.04 (1H, t, $J_{OH5',5'}$ 5.4, OH-5', exch. ^2H_2O), 5.11 (1H, s, OH-3', exch. ^2H_2O), 5.40 (1H, d, $J_{OH2',2'}$ 6.0, OH-2', exch. ^2H_2O), 5.67 (1H, d, $J_{1',2'}$ 6.0, H-1'), 6.46 (2H, s, 2-NH, exch. ^2H_2O), 7.93 (1H, s, H-8) and 10.62 (1H, s, NH-3, exch. ^2H_2O).

[1'-^{2}H]-Guanosine (8, H_A=^{2}H)

This was prepared as above, using [1'-^{2}H]-2-acetylamino-2',3',5'-tri-O-acetyl-6-O-(N,N-diphenylcarbamoyl)-guanine (**15**, H_A=^{2}H) (17 mg, 26 μmol) to yield [1'-^{2}H]-guanosine (**8**,

H_A=^{2}H) as a white solid (3.0 mg, 40 %); mp 233-237 °C (lit.[13] mp 248-251 °C; lit.[14] mp 230-235 °C); $[\alpha]_D^{27}$-55 (c 0.1, 0.1N NaOH) (lit.[15] $[\alpha]_D^{25}$ -71.9 (c 1.078, 0.1N NaOH); lit.[16] $[\alpha]_D$ -56 (c 0.48, 0.1N NaOH)); λ_{max} (MeOH)/nm 257 and 273 (sh) (ε 16,739 and 13,954), λ_{max} (OH^-) /nm 268; m/z [+ve FAB (glycerol/water)] no parent ion was detected; ν_{max} (KBr)/cm^{-1} 3392 (OH, NH, br) and 1695 (amide); δ_H (300 MHz, $(C^2H_3)_2SO$) 3.65-3.85 (~2H, m, H-5' overlapping with H_2O), 4.08 (1H, d, J 3.6, H-4'), 4.30 (1H, br s, H-3'), 4.61 (1H, t, J 5.4, H-2' [d $J_{2',3'}$ 4.9 in ^2H_2O]), 5.28 (1H, t, $J_{OH5',5'}$ 5.4, OH-5', exch. ^2H_2O), 5.37 (1H, d, $J_{OH3',3'}$ 4.6, OH-3', exch. ^2H_2O), 5.63 (1H, d, $J_{OH2',2'}$ 6.0, OH-2', exch. ^2H_2O), 6.69 (2H, s, 2-NH, exch. ^2H_2O), 8.16 (1H, s, H-8) and 10.85 (1H, s, NH-3, exch. ^2H_2O).

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