First synthesis of β-2′-deoxy[9-^{15}N]adenosine

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1 PERKIN COMMUNICATION

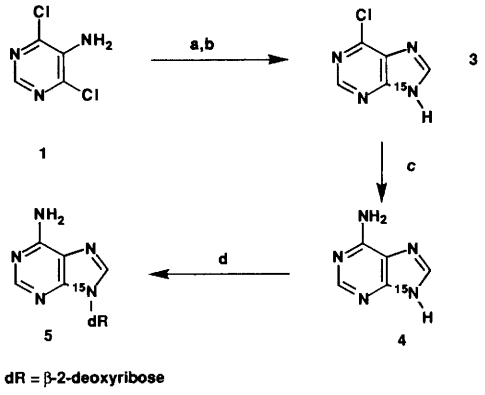
We report the first synthesis of β-2′-deoxy[9-^{15}N] adenosine: the last of the singly labelled ^{15}N β-2′-deoxyadenosines to be synthesized. Using commercially available 5-amino-4,6-dichloropyrimidine and 2–10 equivalents of $^{15}NH_3$, β-2′-deoxy[9-^{15}N]adenosine has been constructed in four steps in good overall yield.

We have been interested in the synthesis of ^{13}C, ^{15}N and ^{77}Se labelled nucleic acids because the spectroscopic information obtained from studying labelled oligonucleotides can provide significant structural and functional insight into biological assemblies that contain nucleic acids.[1] The construction and characterization of site specific ^{15}N-labelled 2′-deoxynucleic acids which have been incorporated into oligonucleotides have been the focus of a great deal of synthetic effort.[2] Most notably, Jones and co-workers have developed efficient synthetic routes to many 2′-deoxynucleic acids containing a ^{15}N label at a single position.[3] Moreover, Jones has incorporated these stable isotope labelled monomeric units into biologically relevant oligonucleotide sequences. These probes have provided a basis for elucidating the structure and function, *via* NMR spectroscopy, of these important biomolecular complexes.[4]

Our most recent effort in this area has given rise to an improved synthesis of β-2′-[^{15}N-amino]deoxyadenosine.[5] We now report that β-2′-deoxy[9-^{15}N]adenosine can be constructed in four steps from commercially available chemicals. This labelled compound is significant because its NMR, IR and Raman spectra are likely to provide detailed information about the local conformations of oligonucleotides. For example, Thomas and co-workers have exploited Raman spectroscopy in the study of DNA because of the sensitive nature of its vibrational states.[6] Difference Raman spectroscopy also provides useful information on the nature of ligand binding to nucleic acids. Label placement at the location of ligand binding serves to shift the absorption bands associated with the motion of the base. Therefore, placement of the label at the 9-position of purine (Scheme 1) is critical to the execution of these types of investigations. Ultimately, this labelled position has the potential to provide for the identification of base-specific and sequence-specific Raman (and IR) tags. These could ultimately be applied to structural studies of a great variety of biomolecular complexes containing nucleic acids.

Results and discussion

The synthesis of the title compound required the conversion of 5-amino-4,6-dichloropyrimidine into 5,6-[6-^{15}N]diamino-4-chloropyrimidine (Scheme 1). Treatment of **1** with 2–3 equivalents of $^{15}NH_3$ in ethanol and *N*,*N*′-diisopropylethylamine at 200 °C and a pressure of ≈100 psi over a period of 12 h gave the monosubstituted product **2** (95%). Examination of the ^{13}C NMR of **2** revealed four resonances, and the C-6 resonance, as expected, was split into a doublet ($^1J_{C,N}$ 20). The



Scheme 1 *Reagents:* (*a*) 26% aq. $^{15}NH_3$, 120 °C (97%); (*b*) diethoxymethyl acetate (DEMA) (86–90%); (*c*) aq. NH_3 (95%); (*d*) thymidine, thymidine phosphorylase, nucleoside phosphorylase (55–75%)

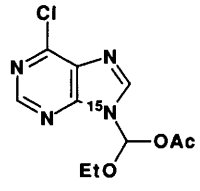


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Fig. 1

extent of isotopic enrichment of **2** was obtained by integration of the resolved carbon signals from the C-6 singlet (arising from the remaining ^{14}N isotopomer) and from the C-6 ^{13}C–^{15}N doublet. These analyses indicated a ^{15}N enrichment of **2** greater than 98%, identical to that of the starting ammonia (98.2%). Based on these results, we concluded that dilution of the label at C-6 by a Dimroth ring opening/closing process did not occur.[7] Since the excess of labelled ammonia was difficult to recover from the reaction medium, we investigated the possibility that by using 4–10 mol equivalents of $^{15}NH_3$ the reaction would proceed cleanly. We were pleased that the reaction proceeded rapidly and in excellent yield (97%). Recovery of the excess of $^{15}NH_3$ was accomplished by simply trapping it with HCl (1 mol dm^{-3}).

Ring closure to the 6-chloropurine was effected using diethoxymethylacetate (DEMA) in *N*,*N*-dimethylformamide (DMF) at room temperature for 8 h. The DMF and unreacted DEMA were then removed and the residue was taken up in HCl (0.1 mol dm^{-3}) in methanol. The reaction mixture was then stirred for 2 h and evaporated under reduced pressure, giving the crude chloropurine **3**. Purification by flash chromatography using 15% methanol in methylene dichloride afforded the labelled 6-chloropurine in 90% yield. Alternatively, the annulation reaction could be performed in neat DEMA at 100 °C for 3.5 h. Purification gave an 82% yield of the desired product.[8] In addition, we have isolated in 4% yield the adduct **6** (Fig. 1) which presumably arises from addition of DEMA

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to **3**. Reaction of **6** with methanolic HCl readily converts **6** into **3**. Reaction of **3** with an excess of natural abundance NH_3 at 150 °C gives rise to **4**. Purification by crystallization of the hydrochloride of **4** from water proceeds by slow diffusion of ethanol (95% yield). Enzymatic ribosylation, effected with thymidine phosphorylase, nucleoside phosphorylase and thymidine over a period of four days, followed by purification, yields the β-2′-deoxy[9-^{15}N]adenosine (55–75%).[5]

This four-step process constitutes an efficient synthesis of the previously unreported β-2′-deoxy[9-^{15}N]adenosine. The process uses an economical source of ^{15}N, does not require any protection or deprotection steps, and the excess isotope used in the reaction can be recovered as its HCl salt.[9] Moreover, this route provides synthetic flexibility for multiply labelling the purine ring. We are currently exploring the feasibility of applying this route to the synthesis of β-2′-deoxy[6,9-$^{15}N_2$]- and [8-^{13}C,6,9-$^{15}N_2$]-adenosines and these results will be reported in due course.

Experimental

^{1}H, ^{13}C and ^{15}N NMR spectra were recorded on either a Bruker WM-300 or AMX-500 spectrometer. δ_H Values are expressed relative to tetramethylsilane; δ_C values are referenced with respect to internal $(CD_3)_2SO$ (δ_C 39.5). Positive chemical shifts denote resonances deshielded with respect to the reference. δ_N Values were referenced with respect to concentrated solutions of sodium nitrate (δ_N 0). Measurements were made at, or near, ambient probe temperature in 5 mm NMR tubes using the solvent as an internal lock. Accurate mass spectra were measured on a VG 70 SQ GC/MS or HP 5989 spectrometer. Microanalyses were performed on a Perkin-Elmer Series II CHNS/O Analyzer #2400 or by Atlantic Microlab. Thin-layer chromatography was carried out on glass plates (silica gel 60 Å 250 μm thickness). Liquid chromatography separations were carried out on silica gel. The columns were hand packed with silica gel 60 (230–400 mesh, Merck). Pressures used were usually between 5 and 8 psi. Fractions were monitored by thin-layer chromatography (TLC).

5-Amino-4,6-dichloropyrimidine and DEMA were obtained from Aldrich Chemical Co. and used without purification. Thymidine phosphorylase, nucleoside phosphorylase and thymidine were obtained from Sigma Chemical Co.

5,6-[6-^{15}N]Diamino-4-chloropyrimidine 2

In a 175 cm^3 stainless steel Parr pressure reactor fitted with an internal thermocouple and pressure gauge was placed 5-amino-4,6-dichloropyrimidine (2.00 g, 12.2 mmol), aq. $^{15}NH_3$ [26.2%; 8.50 g, 124 mmol (10 mol equiv.)] and ethanol (24 cm^3). The mixture was heated to 120–130 °C for 7 h and allowed to cool overnight. The reaction mixture was vented into ice-cooled aq. HCl (1 mol dm^{-3}) contained in two flasks in tandem. The reaction vessel was opened and the crystals of **2** which formed (1.24 g) were filtered off under suction and washed with cold methanol. The combined filtrate and methanol wash were concentrated under reduced pressure to give a semisolid to which water was added and the mixture stirred for 1 h, then filtered. The solids were purified by flash column chromatography on silica gel. Elution of the column [methylene dichloride–methanol–aq. NH_3 (80:18:2) v/v], followed by evaporation of the fractions under reduced pressure, afforded an additional 0.480 g of **2** (combined yield 1.72 g, 97%). The compound was recrystallized from methanol as yellow needles. From the combined HCl solutions, after removal of water and crystallization from ethanol–water, $^{15}NH_4Cl$ (4.6 g, 77%) was isolated, mp 240–242 °C; ν_{max}/cm^{-1} 3419, 3367, 3324, 3284, 3227, 3123, 1670, 1635, 1573, 1550, 1507, 1429, 1344 and 1306; $\delta_H[(CD_3)_2SO]$ 4.88 (2 H, br s, NH_2), 6.67 (2 H, d, $J_{N,H}$ 90, $^{15}NH_2$) and 7.59 (1 H, s); $\delta_C[(CD_3)_2SO]$ 123.2, 137.6, 145.9 and 153.5 (C-6, $J_{C,N}$ 20); $\delta_N[(CD_3)_2SO]$ −290.1 (Found: C, 33.2; H, 3.5; N, 38.7. Calc. for $C_4H_5Cl^{14}N_3{}^{15}N$: C, 33.01; H, 3.46; N, 38.50%).

6-Chloro[9-^{15}N]purine 3

To DEMA (10 cm^3) in a reaction flask was added **2** (1.40 g, 9.62 mmol) and the mixture was maintained at 100 °C for 3.5 h. Unreacted DEMA was evaporated off under reduced pressure, and the crude reaction mixture was purified by flash column chromatography. Elution with ethyl acetate gave two bands (R_f2 0.81 and R_f1 0.15): R_f1 afforded **3** (1.23 g, 82.2%) as a white solid with a yellow tinge; R_f2 gave an oily residue (0.160 g) which was further separated by flash column chromatography (diethyl ether) to give **6** (0.130 g, R_f 0.73) and the tautomer of **3**[8] as a solid (0.002 g, R_f 0.10). Compound **6** was dissolved in methanolic HCl (0.06 mol dm^{-3}) and stored in the refrigerator overnight. Aqueous sodium hydrogen carbonate (5%) was added until evolution of CO_2 ceased. The mixture was then concentrated. Methanol was added and the mixture was heated and filtered while hot. The filtrate was concentrated to give a residue which was purified by flash column chromatography (methanol–methylene dichloride, 1:10 v/v) to give **3** (0.060 g) for a combined yield of 1.29 g (86%). Compound **3** was recrystallized from methanol as a fine, white powder, mp > 300 °C (decomp.);[10] ν_{max}/cm^{-1} 3048, 2929, 2755, 2656, 2529, 1570, 1387, 1332, 1230 and 949; $\delta_H[(CD_3)_2SO]$ 8.67 (1 H, d, J 7.4), 8.73 (1 H, s) and 13.9 (1 H, d, J 87.3, 9-H); δ_H(D_2O–DCl) 4.82 (s, H_2O/N-H), 8.63 (1 H, s) and 8.70 (d, $J_{N,H}$ 8.6); δ_C(D_2O–DCl) 128.1 (d, $J_{N,C}$ 4.0, C-5), 147.2 (d, $J_{N,C}$ 8.6, C-8), 150.8 (s, C-6), 154.7 (s, C-2) and 155.6 (d, $J_{C,N}$ 16.6, C-4); m/z 155 (Found: M$^+$, 156.0103. $C_5H_3Cl^{14}N_4{}^{15}N$ requires M, 156.0095).

[9-^{15}N]Adenine. HCl 4[11]

In a 175 cm^3 stainless steel Parr pressure reactor was placed **3** (0.380 g, 2.46 mmol) and aq. NH_3 (28–29%; 4.70 g, 80.2 mmol). The reactor was heated at 150 °C for 7 h. Purification by flash column chromatography (methanol–methylene dichloride, 3:7 v/v) gave **4** (0.430 g, may contain silica gel from the column). This material, as the hydrochloride salt, was crystallized from water by slow diffusion of ethanol to give **4** (0.409 g, 95%); mp 270 °C; ν_{max}/cm^{-1} 3310, 3129, 2975, 2788, 2672, 1674, 1606, 1417, 1309, 1252, 1211, 1154, 934, 908, 796 and 638; δ_H(D_2O–NaOD) 7.33 (1 H, d, J 13), 7.45 (1 H, s); δ_C(D_2O–NaOH) 121.2 (C-5), 150.9 (d, $J_{C,N}$ 32), 153.9 (d, $J_{C,N}$ 25), 155.3 (s) and 160.6 (d, $J_{C,N}$ 5.3); δ_N −149.3 (N-9, d, J 13) (Found: M$^+$, 137.0593. $C_5H_6{}^{14}N_4{}^{15}N$ requires M, 137.0597).

β-2′-Deoxy[9-^{15}N]adenosine 5

In a 100 cm^3 round-bottomed flask was placed **4** (0.030 g, 0.174 mmol), thymidine (0.270 g, 1.11 mmol) and KH_2PO_4 (10 mmol dm^{-3}; 3.5 cm^3) (pH = 7.2). The resulting mixture was stirred for 5 min and the pH was then adjusted to 7.4 with KOH. Nucleoside phosphorylase (6 units) and thymidine phosphorylase (9 units) were added and the mixture was stirred at 40–44 °C for 4 days. The crude reaction mixture was evaporated to dryness and the solid residue was triturated several times with methanol. The methanol solution was concentrated and purified by dry column chromatography (ethyl acetate–methanol, 4:1 v/v) to give **5** (27 mg, 62%). The reaction was multiply repeated giving yields in the range 55–75%. The product was crystallized from methanol by slow diffusion of diethyl ether; mp 189–190 °C (lit.,[11] 184.4–186 or 191–192 °C); ν_{max}/cm^{-1} 3297, 3109, 2920, 1635, 1599, 1575, 1204, 1150, 1094 and 503; $\delta_H(CD_3OD)$ 2.40 (1 H, m), 2.79 (1 H, m), 3.78 (1 H, dd, J 3.4, 12), 3.84 (1 H, dd, J 2.9, 12), 4.07 (1 H, q, J 2.9), 4.58 (1 H, m), 6.42 [1 H, ddd, J 1.5 (^{1}H–^{15}N), 6, 7], 8.16 (1 H, s) and 8.30 (1 H, d, J 7.8); δ_C 41.5, 63.6, 73.0, 87.1 (d, $J_{C,N}$ 11, C-1′), 89.9, 120.8 (d, $J_{C,N}$ 8, C-5), 141.4 (d, $J_{C,N}$ 5, C-8), 149.8 (d, $J_{C,N}$ 19, C-4), 152.4 and 156.1; δ_N −202.0 (N-9, s) (Found: M$^+$, 253.1076. $C_{10}H_{14}{}^{14}N_4{}^{15}NO_3$ requires M, 253.1067).

9-[Acetoxy(ethoxy)methyl]-6-chloro[9-^{15}N]purine 6
δ_H($CDCl_3$) 1.33 (3 H, t, J 7, CH_3), 2.19 (3 H, s, CH_3CO), 3.85–3.91 (1 H, m, CH_2), 3.96–4.01 (1 H, m, CH_2), 7.72 (1 H, d, $J_{N,H}$ 2.3, CH), 8.46 (1 H, d, $J_{N,H}$ 7.8, 8-H) and 8.79 (1 H, s, 2-H); δ_C 14.6 (CH_3), 20.7 (CH_3COO), 65.5 (CH_3CH_2O), 94.5 (d, $J_{N,C}$ 15.8, *C*HOEtOAc), 131.4 (d, $^2J_{N,C}$ 8.7, C-5), 142.1 (d, $J_{N,C}$ 10.4, C-4), 150.5 (d, $J_{N,C}$ 20.1, C-8), 151.2 (C-2), 152.5 (d, $^3J_{N,C}$ 2.4, C-6), 168.6 (s, C=O); δ_N −175.6 (N-9, s); m/z 271 (unlabelled compound $C_{10}H_{11}{}^{14}N_4O_3Cl$) (For labelled compound, found: M^+, 272.0585. $C_{10}H_{12}{}^{14}N_3{}^{15}NO_3Cl$ requires M, 272.0568).

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