# Synthesis of [$^{15}$N]Guanosines and Deoxy[$^{15}$N]guanosines from 5-Amino-1-(β-D-ribofuranosyl)imidazole-4-carboxamide ('AICA-riboside')

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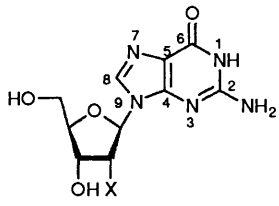
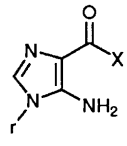
Methods are described for the syntheses of $^{15}$N-labelled guanosines and deoxyguanosines, suitable for incorporation into oligonucleotides. The $^{15}$N is located either at N-1 (*e.g.* [$^{15}$N$^1$]guanosine **1a** and deoxy[$^{15}$N$^1$]guanosine **1b**) or at the $NH_2$ (*e.g.* [$^{15}NH_2$]guanosine **1c** and deoxy[$^{15}NH_2$]guanosine **1d**). One of the synthetic methods starts from 5-amino-1-(β-D-ribofuranosyl)imidazole-4-carboxamide (AICA-riboside, **2a**) and leads *via* a cyclonucleoside to mixtures of compounds **1a** and **1c**, in which either compound predominates depending on the source of $^{15}$N (ammonia or benzoyl isothiocyanate). The other method utilises the same starting material, but a different mode of formation of the pyrimidine ring and yields either guanosine **1a** or **1c** (as its 2-*N*-benzoyl derivative **8b**), exclusively.

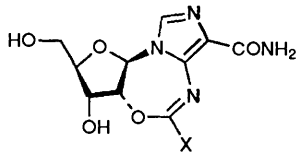
We are studying the reactions of genotoxic chemicals with nucleosides and oligonucleotides,[1-3] and wished to explore the potential application in such studies of specific $^{15}$N-labelling of guanine residues. This is because guanine residues are frequently the site of chemical attack on DNA, *e.g.* by *N*-methyl-*N*-nitrosourea.[4,5] $^{15}$N-labelling of a particular guanine in a synthetic oligonucleotide is expected to aid the mass spectral and NMR analysis of complex mixtures of products from reactions of the oligonucleotide with such chemicals. We have already shown the value of $^{15}$N-labelling of guanosine in a study of its reaction with formaldehyde[2] and glycidaldehyde.[1,6] In this paper, we describe efficient syntheses of guanosine and deoxyguanosine containing $^{15}$N either at N-1 ([$^{15}$N$^1$]-guanosine **1a** and deoxy[$^{15}$N$^1$]guanosine **1b**) or at the $NH_2$ ([$^{15}NH_2$]guanosine **1c** and deoxy[$^{15}NH_2$]guanosine **1d**). Other syntheses of $^{15}$N-labelled nucleosides or deoxynucleosides have been reported: adenosine ($^{15}NH_2$,[7-10] $^{15}$N$^1$,[11] $^{15}$N$^3$[12]), 2-aminopurine deoxynucleoside ($^{15}$N$^1$ and $^{15}NH_2$),[13] cytidine ($^{15}NH_2$,[7,9,14,15] $^{15}$N$^{1,3}$,[14] and $^{15}$N$^{1,3,4}$[14]), guanosine ($^{15}NH_2$,[6,16] $^{15}$N$^1$,[6,16] and N$^7$[17]) and uridine ($^{15}$N$^3$).[18] Several of these have been incorporated into oligonucleotides[8,9,15,17,19] and a 'RNA[20] for spectroscopic studies. For example, Jones and co-workers[11,16] have described efficient routes to deoxy-$^{15}$N-adenosines and deoxy-$^{15}$N-guanosines, their incorporation into oligonucleotides, and the characterisation of the oligonucleotides by $^{15}$N NMR spectroscopy. Baker and Dervan[21] have used DNA specifically labelled with $^{15}$N at one adenine to show sequence-specific cleavage by *N*-bromoacetyldistamycin.



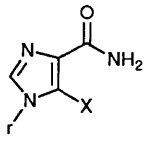
**1a**; $^{15}$N at N-1, X = OH
**b**; $^{15}$N at N-1, X = H
**c**; $^{15}$N at $NH_2$, X = OH
**d**; $^{15}$N at $NH_2$, X = H



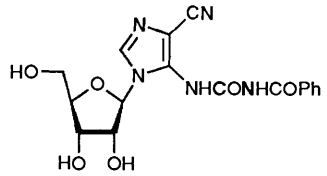
**2a**; X = $NH_2$, r = ribose
**b**; X = $O^-Na^+$, r = ribose
**c**; X = OH, r = triacetylribose
**d**; X = O-*N*-succinimidyl, r = triacetylribose
**e**; X = $^{15}NH_2$, r = triacetylribose



**3a**; X = NHCOPh
**b**; X = $^{15}$NHCOPh



**4a**; X = PhCONHCSNH
**b**; X = PhCO$^{15}$NHCSNH



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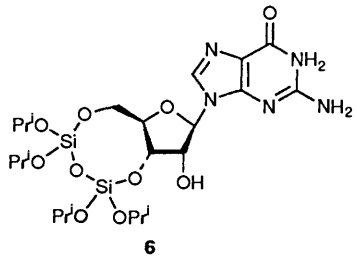

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## Results and Discussion

We have focussed on the production of guanosines and deoxyguanosines labelled with $^{15}$N at their critical reactive sites. Syntheses have been developed in which guanosine or deoxyguanosine is labelled with $^{15}$N either at N-1 (**1a** and **1b**) or at the $NH_2$ (**1c** and **1d**), in each case starting with the readily available 5-amino-1-(β-D-ribofuranosyl)imidazole-4-carboxamide ('AICA-riboside', **2a**). A preliminary communication has been published on part of this work[6] (see also ref. 2), and this formed the basis of the present studies. During the investigation, we found a method to obtain exclusively compound **1c**, *i.e.* free of compound **1a**. The methodology to

pure product **1c** was adapted to the preparation of the 2-*N*-benzoyl derivative of **1a**, *via* conversion of the carboxamide group of compound **2a** into a carboxylic acid and re-formation of the carboxamide from [$^{15}$N]ammonia. We have also converted a $^{15}$N-labelled guanosine into the corresponding deoxyguanosine, essentially as described for unlabelled materials.[22] Recently, Jones has reported the synthesis of $^{15}$N-labelled deoxyguanosines by different routes from those described here.[16,17]

*Synthesis of* [$^{15}$N]*Guanosines* (*Mixtures of* **1a** *and* **1c**).—For the syntheses of [$^{15}$N]guanosines we initially exploited the published conversion of the commercially available 'AICA-riboside' **2a** into guanosine.[23] The conversion of substrate **2a** into guanosine proceeds *via* the cyclonucleoside 2′,1″-anhydro-5-[(1″-benzamido-1″-hydroxymethylene)]amino-1-(β-D-ribofuranosyl)imidazole-4-carboxamide **3a**. Thus, AICA-riboside **2a** was treated with benzoyl isothiocyanate to yield the 5-[(*N*′-benzoylthiocarbamoyl)amino]imidazole-4-carboxamide derivative **4a** as a syrup after removal of dimethylformamide (DMF) solvent. Treatment of compound **4a** with iodomethane in 0.1 mol dm$^{-3}$ aq. sodium hydroxide gave, on neutralisation, the cyclonucleoside **3a** as a precipitate in 68% yield. This compound possessed the reported spectroscopic properties.[23] The alternative structure **5** has been suggested for this compound.[24] However, its IR spectrum lacks a cyanide stretching absorption and its $^{13}$C NMR spectrum is not consistent with this proposal. Cyano groupings resonate in the region $\delta_C$ 115–125, whilst a carboxamide signal is found at $\delta \sim 160$.[25] Compound **3a** shows no resonances between $\delta_C$ 115 and 125, but does possess a resonance at $\delta_C$ 163 ($CONH_2$). Furthermore, the alternative structure would not be expected to convert into a guanosine species under the reaction conditions described below.

Okutsu and Yamazaki[23] produced guanosine in 72% yield by heating compound **3a** with an excess of ammonia in an autoclave at 100 °C for 3 h. This procedure was clearly not suitable for the preparation of $^{15}$N-labelled guanosine from $^{15}NH_3$. However, we found (*cf.* ref. 6) that when compound **3a** was treated with 1 mol equivalent of ammonia (generated *in situ* from ammonium chloride and lithium hydroxide) in wet dimethyl sulfoxide (DMSO) in a sealed tube at 100 °C for 8–10 days, guanosine was obtained in 51% yield by column chromatography of the crude reaction product on silica and recrystallisation from water.

Extrapolating from the proposed mechanism[23] for the conversion of compound **3a** into compound **1** suggested that treatment of compound **3a** with [$^{15}$N]ammonia would lead to [$^{15}NH_2$]guanosine **1c**, whereas employment of the cyclonucleoside **3b** derived from [$^{15}$N]benzoyl isothiocyanate would give [$^{15}N^1$]guanosine **1a**. However, analysis of the NMR spectra of the $^{15}$N-labelled guanosines obtained showed a very different labelling pattern from that expected. Thus, from compound **3a** and [$^{15}$N]ammonia we obtained ~75% **1a** and 15–20% **1c** (*N.B*: both NMR and mass spectra showed this substance to contain ~5% unlabelled guanosine, reflecting the isotopic content of the [$^{15}$N]ammonium chloride used as the precursor of [$^{15}$N]ammonia).* This can be accounted for by the mechanism given below and suggested that starting from [$^{15}$N]benzoyl isothiocyanate would lead to [$^{15}$N]guanosine containing 75% $^{15}$N at the $NH_2$ and 15–20% at N-1.

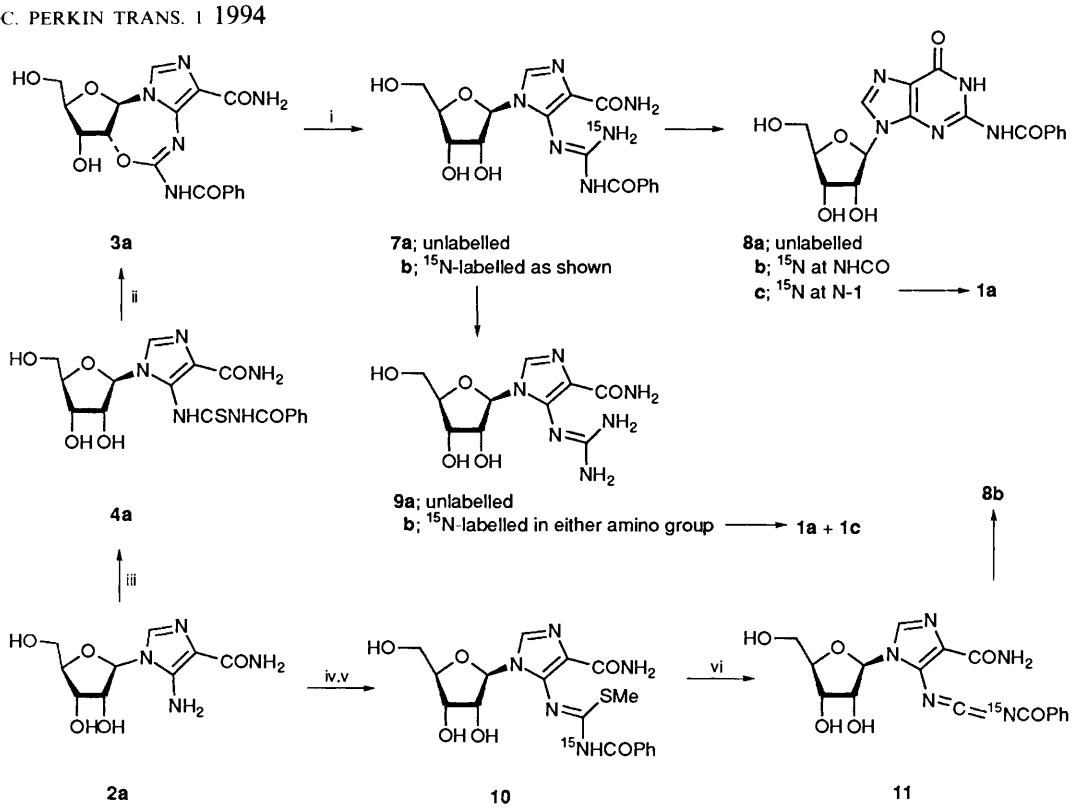
Following a procedure for the preparation of unlabelled benzoyl isothiocyanate,[26] K$^{15}$CN (containing 95% $^{15}$N in initial experiments, and 98% $^{15}$N in later experiments) was treated with one mole equivalent of sulfur in refluxing ethanol to give K$^{15}$NCS in 80% yield. Reaction of the $^{15}$N-labelled potassium thiocyanate with one mole equivalent of benzoyl chloride in acetone gave, after filtration of NaCl, evaporation of acetone under reduced pressure, and distillation, benzoyl [$^{15}$N]isothiocyanate, quantitatively. The IR absorbance spectrum of the isothiocyanate possessed a band at 1950 cm$^{-1}$, corresponding to the NCS asymmetric stretching vibration (*cf.* benzoyl isothiocyanate, 2095 cm$^{-1}$). The absence of a band at 1760 cm$^{-1}$ and GLC analysis indicated that the compound was free of benzoyl chloride. The benzoyl [$^{15}$N]isothiocyanate was used to prepare cyclonucleoside **3b**, essentially as described.[23] Finally, compound **3b** was converted into labelled guanosine by reaction with ammonia in DMSO. This guanosine was found to contain the predicted labelling pattern (75% **1c** and 15–20% **1a**).

The proposed route for guanosine formation is *via* ammonia-induced ring-opening of compound **3a** to give the *N*-benzoylguanidine derivative **7a** (see Scheme 1). This compound is formed quantitatively (by HPLC analysis) on treatment of compound **3a** with ammonia (1.2 mol equiv.) in DMSO at 100 °C for 1 h. Cyclisation of compound **7a** to 2-*N*-benzoylguanosine **8a**, and hence to guanosine after ammonolysis, may occur *via* intramolecular nucleophilic attack of the amino group on the carbamoyl group with release of ammonia. In support of this hypothesis, the intermediacy of 2-*N*-benzoylguanosine and the formation of benzamide were observed during monitoring of the reaction by HPLC. Partial removal of the benzoyl group from compound **7a** by the action of ammonia may occur, resulting in the formation of the guanidine **9a**, which can cyclise directly to guanosine. Reaction of compound **3a** with $^{15}NH_3$ gives compound **7b**, cyclisation of which affords, *via* compound **8c**, labelled guanosine **1a** and unlabelled ammonia. The latter converts unchanged intermediate **7b** into the guanidine **9b**, cyclisation of which yields compounds **1a**/**1c**. Assuming that both amino groups of the guanidine **9** can participate equally in intramolecular attack on the carbamoyl group and that the formation of intermediates **7** is much faster than its conversion into debenzoylated product **9**, then the observed pattern of $^{15}$N-labelling can be explained by postulating that the extent of conversion of compounds **7** into compounds **9** is 30–40%. Thus, unlabelled compound **3a** with $^{15}NH_3$ gives 75% **1a** and 15–20% **1c** as products, whilst reaction of unlabelled ammonia with compound **3b** affords 75% **1c** and 15–20% **1a** as products.

*Synthesis of Deoxy*[$^{15}$N]*guanosine* (**1b**/**1d** *Mixture*).—Deoxyguanosine was prepared from guanosine essentially as described by Robins *et al.*[22] Thus, treatment of guanosine with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane and pyridine in DMF gave 3′,5′-*O*-(tetraisopropyldisiloxan-1,3-diyl)guanosine **6** in high yield. The crude product was esterified at the 2′-position in 79% yield with phenoxythiocarbonyl chloride and 4-(dimethylamino)pyridine (DMAP) in acetonitrile. For the reduction of the ester with tributyltin hydride in toluene at 75 °C (complete after 3 h[22]) we found that increasing the reaction time to 16 h gave good yields of deoxyguanosine after removal of the 3′,5′-protecting group with tetrabutylammonium fluoride (TBAF). Attempts to purify the product with XAD-4 resin in ethanol, and silica gel (230–400 mesh, medium pressure) in (4:1) ethanol–conc. ammonia were unsuccessful. However, the use of Dowex 1-X2-200 resin in 0.25 mol dm$^{-3}$ triethylammonium hydrogen carbonate (TEAB) buffer at pH 9.0 led to the isolation of deoxyguanosine.

*Synthesis of* [$^{15}NH_2$]*Guanosine* **1c**.—The application of the $^{15}$N-labelled guanosines described above to mechanistic studies

* Similarly, all other $^{15}$N-labelled compounds described in this paper contained, according to NMR and mass spectra, >90% [$^{15}N_1$]-species, with no [$^{15}N_2$]-species.




**Scheme 1** Reaction pathways from 5-amino-1-(β-D-furanosyl)imidazole-4-carboxamide **2a** to $^{15}$N-labelled guanosines and deoxyguanosines. *Reagents:* i, $NH_3$ (or $^{15}NH_3$); ii, MeI, NaOH; iii, PhCONCS; iv, PhCO$^{15}$NCS; v, MeI; vi, EtONa

of nucleosides and oligonucleotides is complicated by the dual labelling, which leads, for example, to complex NMR spectra.[2] To enable studies to be undertaken that are not compromised by such complexities, we sought a synthesis of isotopically pure [$^{15}NH_2$]guanosine **1c**. We have found a simple synthesis of this compound by modifying the methodology of Okutsu and Yamazaki.[23] These authors reported that cyclonucleoside **3a** could be converted into 2-*N*-benzoylguanosine **8a** by treatment with sodium ethoxide. The cyclonucleoside was prepared by treatment of AICA-riboside **2a** with benzoyl isothiocyanate to give compound **4a**, which was converted into tricycle **3a** by treatment with iodomethane and aq. sodium hydroxide. We have discovered that reaction of crude compound **4a** with iodomethane, followed by addition of sodium ethoxide, leads directly to compound **8a** in excellent overall yield (71%). Moreover, when compound **4b** (specifically labelled with $^{15}$N as shown) is used, the $^{15}$N-benzoylguanosine **8b** obtained is exclusively labelled in its amino group (see spectroscopic analysis below). Hence, the mechanism of formation of compound **8b** must involve nucleophilic attack by the carbamido group (probably deprotonated) on a carbon atom (that eventually becomes C-2 of guanosine) in an intermediate derived from the sulfide **10** (*e.g.* compound **11**; see Scheme 1).

The positive-ion fast-atom bombardment (FAB) mass spectrum of compound **8b** exhibited an $(M + H)^+$ ion at *m/z* 389, indicating the incorporation of only one $^{15}$N atom, and a peak at *m/z* 257, corresponding to the loss of the ribofuranosyl group. A single resonance at $\delta$ −247.8 in the $^{15}$N NMR spectrum (relative to external $CD_3NO_2$ set at $\delta$ 0) indicated that the $^{15}$N nucleus is positioned in only one chemical environment with its chemical shift within the typical range for secondary amides.[27] Conclusive evidence for the location of the $^{15}$N label was obtained from the proton-decoupled $^{13}$C NMR spectrum. This exhibited the expected signals, four of which were split into doublets, as a consequence of $^{15}$N–$^{13}$C coupling. These were assigned to C-2, C=O, *ipso*-Ar and C-4. The resonance for C-2 appears at $\delta_C$ 148.3 (*J* 22.4 Hz) and C=O at $\delta_C$ 168.9 (*J* 13.2 Hz), both possessing characteristic, large 'one-bond' $^{15}$N–$^{13}$C coupling constants.[27] The resonance due to *ipso*-Ar is found at $\delta_C$ 132.4 (*J* 8.8 Hz), its splitting being due to a 'two-bond' $^{15}$N–$^{13}$C coupling. Usually, $^{15}$N–$^{13}$C coupling constants of this type are significantly smaller than those across one bond, but in this case the coupling is across a carbonyl carbon, which increases the coupling magnitude. The resonance for C-4 appears at $\delta_C$ 149.0 (*J* 4.1 Hz) and has the smallest *J*-value since it is split by a 'three-bond' coupling to $^{15}$N-2. The C-6 resonance is a singlet because of the absence of $^{15}$N at the N-1 position.

Reese and Saffhill[28] have reported that ethanolic methylamine cleaved the benzoyl group from $N^2$-benzoylguanosine at a faster rate than did either ammonia or dimethylamine. Similarly, we found that compound **8b** was efficiently debenzoylated to [$^{15}NH_2$]guanosine **1c** by methylamine in methanol. The positive-ion FAB mass spectrum of compound **1c** possessed an $(M + H)^+$ ion at *m/z* 285 and a peak at *m/z* 153 corresponding to the loss of a ribofuranosyl fragment. The $^1$H NMR spectrum showed a doublet centred at $\delta$ 6.49 with a $^{15}$N–$^1$H coupling constant of 89.4 Hz, assigned to the amino protons attached to the $^{15}$N atom. A small central peak was due to the presence of ~1% [$^{14}N^2$]guanosine. The 100 MHz proton-decoupled $^{13}$C NMR spectrum showed a single resonance at $\delta$ 154.0 which is split by $^{15}$N–$^{13}$C coupling (*J* 22.7 Hz).

*Synthesis of 2-N-Benzoyl*[$^{15}N^1$]*guanosine* **8c**.—Having established the route from AICA-riboside to [$^{15}NH_2$]guanosine **1c** (see above), it was obvious that prior hydrolysis of the carboxamide of AICA-riboside and re-formation of the amide using [$^{15}$N]ammonia would afford a synthesis of [$^{15}N^1$]-guanosine. The viability of this approach has been demonstrated, without optimisation of yields, by utilising a literature method[29] to convert AICA-riboside, *via* sodium 5-amino-1-(β-D-ribofuranosyl)imidazole-4-carboxylate **2b** into 5-amino-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxylic acid **2c**. This was converted into *N*-succinimidyl 5-amino-1-

(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxylate **2d**, which was allowed to react with [$^{15}$N]ammonia to give 5-amino-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-[$^{15}$N]carboxamide **2e**. The latter was treated with benzoyl isothiocyanate and the product was treated with iodomethane followed by sodium ethoxide in ethanol, which resulted in removal of acetyl groups and cyclisation to compound **8c**.

We have used the mixture of deoxy[$^{15}$N$^1$]guanosine and deoxy[$^{15}$NH$_2$]guanosine to prepare, by phosphoramidite methodology, 5′-d(GT*GCAC)-3′ labelled with $^{15}$N at the central G (designated as *G).[2,20]

## Experimental

*General.*—Solvents were dried as follows: pyridine was distilled from KOH, acetonitrile was dried with 3 Å molecular sieves or used as supplied (HPLC grade) and chloroform was filtered through basic alumina. FAB spectra were measured in the positive-ion mode using either glycerol or *m*-nitrobenzyl alcohol as matrix. NMR spectra were obtained with Bruker instruments operating at the frequency given. Mass spectra were determined with a Kratos MS80 instrument.

*Preparation of 2′,1″-Anhydro-5-[(1″-benzamido-1″-hydroxymethylene)amino]-1-(β-D-ribofuranosyl)imidazole-4-carboxamide* **3a**.—To a solution of AICA-riboside **2a** (1.986 g, 7.69 mmol) in dry DMF (40 cm$^3$) under nitrogen was added benzoyl isothiocyanate (1.05 cm$^3$, 7.8 mmol). The reaction mixture was stirred for 4 h at room temperature, after which the solvent was removed under reduced pressure. The resultant red gum was taken up in aq. 0.5 mol dm$^{-3}$ sodium hydroxide (30 cm$^3$) and filtered to remove a small amount of insoluble material. Iodomethane (0.72 cm$^3$, 11.5 mmol) was added in portions over *ca.* 30 min to the vigorously stirred mixture. The mixture was stirred overnight. The resultant heterogeneous reaction mixture was neutralised with 0.5 mol dm$^{-3}$ hydrochloric acid, and the precipitate was collected by filtration and washed with water to yield the title compound as a powder (2.01 g, 68%), m.p. > 260 °C; $\lambda_{max}$(pH 1) 242 (ε 20 200 dm$^3$ mol$^{-1}$ cm$^{-1}$) and 295 nm (14 400); $\lambda_{max}$(pH 13) 241 (18 700) and 338 nm (12 500); $\delta_H$[200 MHz; $(CD_3)_2SO$] 3.52 (2 H, m, 5′-H), 4.27 (1 H, br t, $J_{4,5}$ 4.3, 4′-H), 4.37 (1 H, t, $J_{3,OH}$ 4.8, 3′-H), 4.90 (1 H, dd, $J_{2,3}$ 4.8, $J_{1,2}$ 7.4, 2′-H), 5.09 (1 H, t, $J_{5,OH}$ 5.4, 5′-OH), 5.87 (1 H, d, *J* 4.8, 3′-OH), 5.91 (1 H, d, $J_{1,2}$ 7.4, 1′-H), 7.08 (1 H, s, $CONH_2$), 7.46–7.7 (4 H, m, *m*- and *p*-ArH and 8-H), 7.88–7.92 (2 H, m, *o*-ArH), 8.58 (1 H, s, $CONH_2$) and 11.14 (1 H, s, CONH); *m*/*z* (FAB) 388 ($MH^+$, 33%) and 105 (100).

*Mixture of* [$^{15}$N$^1$]*Guanosine* (**1a**, 75%) *and* [$^{15}$NH$_2$]*Guanosine* (**1c**, 15–20%).—A solution of compound **3a** (3.072 g, 7.93 mmol), [$^{15}$N]ammonium chloride (0.519 g, 9.52 mmol, nominally 97 atom-% $^{15}$N), and lithium hydroxide monohydrate (0.399 g, 9.52 mmol) in a mixture of DMSO (35 cm$^3$) and water (3.3 cm$^3$) was heated and stirred in a sealed vessel at 100 °C for 9 days. The solvent was removed under reduced pressure to leave a syrup, which was purified by medium-pressure chromatography (Kieselgel 60; 230–400 mesh; 150 g) with ethanol–conc. ammonia (4:1) as eluent. Recrystallisation of the product from water, using charcoal for decolourisation, yielded [$^{15}$N$^1$]/[$^{15}$NH$_2$]guanosine as the monohydrate (1.21 g, 51%); $\delta_H$[300 MHz; $(CD_3)_2SO$] 3.58 (2 H, m, 5′-$H_2$), 3.88 (1 H, m, 4′-H), 4.09 (1 H, m, 3′-H), 4.40 (1 H, m, 2′-H), 5.05 (1 H, t, *J* 5, 5′-OH), 5.15 (1 H, d, *J* 4.5, 3′-OH), 5.42 (1 H, d, *J* 6, 2′-OH), 5.72 (1 H, d, *J* 6, 1′-H), 6.45 (1.6 H, s, $^{14}NH_2$), 6.45 (0.4 H, d, *J* 90, $^{15}NH_2$), 7.93 (1 H, s, 8-H), 10.63 (0.3 H, s, $^{14}$NH) and 10.63 (0.7 H, d, *J* 88, $^{15}$NH); $\delta_C$[75 MHz; $(CD_3)_2SO$] 61.3 (s, C-5′), 70.3 (s, C-3′), 73.6 (s, C-2′), 85.1 (s, C-4′), 86.3 (s, C-1′), 117.0 (s and d, *J* 9, C-5), 133.8 (s, C-8), 151.2 (s, C-4), 153.9 [s, d and d, *J* (C, $^{15}$N$^1$) 22, *J* (C, $^{15}NH_2$) 13, C-2] and 157.0 (t, *J* 11, C-6); $\delta_N$[30.42 MHz; $(CD_3)_2SO$] −302.6 (0.25 $^{15}$N, t, *J* 91, [$^{15}NH_2$]guanosine) and −228 (0.75 $^{15}$N, d, *J* 87, [$^{15}$N$^1$]guanosine) [resonances upfield from those of the external standard $NH_4NO_3$ set at δ 0]; *m*/*z* (FAB) 285 ($MH^+$, 14%) and 75 (100).

*Preparation of 3′,5′-O-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)*[$^{15}$N$^1$]/[$^{15}NH_2$]*guanosine* **6**.—To a suspension of dry [$^{15}$N$^1$]/[$^{15}NH_2$]guanosine (1.05 g, 3.69 mmol) in dry DMF (55 cm$^3$) and pyridine (3.7 cm$^3$) under nitrogen was added 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (1.19 cm$^3$, 3.80 mmol). The mixture was stirred overnight to give a homogeneous solution, which was poured into ice–water (1 dm$^3$). The resultant precipitate was collected by filtration, washed with water, and dried to yield the title compound as a powder (1.95 g, 100%); $\delta_H$[300 MHz; $(CD_3)_2SO$] 1.05–1.15 (28 H, m, Pr$^i$), 4.00–4.21 (3 H, m, 4′-H and 5′-$H_2$), 4.35 (1 H, v br s, 2′-H), 4.44 (1 H, dd, $J_{2,3}$ 5.0, $J_{3,4}$ 8.0, 3′-H), 5.74 (1 H, d, $J_{OH,2}$ 5.0, 2′-OH), 5.77 (1 H, d, $J_{1,2}$ 1.5, 1′-H), 6.62 (1.52 H, s, $^{14}NH_2$), 6.62 (0.48 H, d, *J* 88, $^{15}NH_2$), 7.86 (1 H, s, 8-H), 10.77 (0.76 H, d, *J* 89, $^{15}$NH) and 10.77 (0.24 H, s, $^{14}$NH).

*Preparation of 2′-O-Phenoxythiocarbonyl-3′,5′-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)*[$^{15}$N$^1$]/[$^{15}NH_2$]*guanosine.*—To vacuum-dried 3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)[$^{15}$N$^1$]/[$^{15}NH_2$]guanosine (1.804 g, 3.43 mmol) were added DMAP (0.858 g, 7.03 mmol), anhydrous acetonitrile (51 cm$^3$) and *O*-phenyl chlorothiomethanoate (0.69 cm$^3$, 4.99 mmol). The mixture was stirred at room temperature under nitrogen overnight. The resultant precipitate was collected by filtration, washed with water and acetonitrile, and dried under vacuum (1.49 g). From the filtrate and washings a further crop of precipitate (0.31 g) was collected, to give the title compound (total yield 1.80 g, 79%); $\delta_H$[300 MHz; $(CD_3)_2SO$] 1.10–1.22 (28 H, m, Pr$^i$), 4.07–4.21 (3 H, m, 4′-H and 5′-$H_2$), 4.97 (1 H, m, 3′-H), 6.18 (1 H, d, $J_{1,2}$ 2.6, 1′-H), 6.32 (1 H, dd, $J_{1,2}$ 2.6, $J_{2,3}$ 5.8, 2′-H), 6.50 (1.48 H, s, $^{14}NH_2$), 6.50 (0.52 H, d, $^{15}NH_2$), 7.22–7.61 (5 H, m, ArH), 8.04 (1 H, s, 8-H), 10.87 (0.74 H, d, *J* 89, $^{15}$NH) and 10.87 (0.26 H, s, $^{15}NH_2$).

*Mixture of Deoxy*[$^{15}$N$^1$]*guanosine* (**1b**, 75%) *and Deoxy*-[$^{15}NH_2$]*guanosine* (**1d**, 15–20%).—To a suspension of 2′-*O*-phenoxythiocarbonyl-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)[$^{15}$N$^1$]/[$^{15}NH_2$]guanosine (1.691 g, 2.55 mmol) in toluene (51 cm$^3$) were added azoisobutyronitrile (82 mg, 0.51 mmol) and tributyltin hydride (1.02 cm$^3$, 3.83 mmol). The solution was degassed with oxygen-free nitrogen for 20 min and was then heated at 75 °C overnight. TBAF (2 mol equiv. of a 1 mol dm$^{-3}$ solution in THF) was added and heating was continued for a further 1 h. The solvents were removed and the residue was partitioned between water and diethyl ether. The aqueous fraction was concentrated and applied to a column (10 × 2 cm) of Dowex 1-X2 (200 mesh, $OH^-$ form) and eluted with 0.25 mol dm$^{-3}$ TEAB buffer (pH 9.0). The eluent was removed under reduced pressure and then water was repeatedly evaporated from the residue to remove excess of buffer. Finally the residue was resuspended in cold water and the product was collected by filtration to yield the title mixture (0.61 g, 84%) (containing guanosine that could be removed by further chromatography), $\delta_H$[200 MHz; $(CD_3)_2SO$] 2.30 (1 H, ddd, 2′-H), 2.60 (1 H, m, 2′-H), 3.63 (2 H, m, 5′-$H_2$), 3.90 (1 H, m, 4′-H), 4.46 (1 H, m, 3′-H), 5.06 (1 H, t, 5′-OH), 5.37 (1 H, d, 3′-OH), 6.21 (1 H, dd, 1′-H), 6.57 (0.76 H, s, $^{14}NH_2$), 6.57 (0.24 H, d, *J* 89, $^{15}NH_2$), 8.02 (1 H, s, 8-H), 10.72 (0.76 H, br d, *J ca.* 80, $^{15}$NH) and 10.72 (0.24 H, s, $^{14}$NH).

*Potassium* [$^{15}$N]*Thiocyanate.*—A mixture of potassium [$^{15}$N]cyanide (133 mg, 2 mmol, nominally 98 atom-% $^{15}$N)

and sulfur (71 mg, 2.2 mmol) in absolute ethanol (2 $cm^3$) was refluxed for 3 h. The potassium [$^{15}$N]thiocyanate which separated on cooling was filtered and collected. Light petroleum (distillation range 40–60 °C) was added to the filtrate and the precipitate was filtered off. The combined crude products were recrystallised from ethanol to give the title compound (158 mg, 80%), m.p. 174–176 °C; (lit.,[31] 173–176 °C).

*Benzoyl* [$^{15}$N]*Isothiocyanate.*—To a stirred solution of potassium [$^{15}$N]thiocyanate (158 mg, 1.6 mmol, prepared as described above; *N.B.* in initial experiments material containing nominally 95 atom-% $^{15}$N was used*) in acetone (2 $cm^3$) at room temperature was added benzoyl chloride (200 $mm^3$, 1.6 mmol) over a period of 30 min to give a precipitate of sodium chloride. The mixture was stirred under nitrogen at 20 °C for 30 min. Filtration through Celite and removal of the solvent gave an orange oil, which was distilled *in vacuo* to give the title compound as an oil (264 mg, 100%), b.p. 45 °C at 3 mmHg; $\nu_{max}$(film)/cm$^{-1}$ 1950s (NCS) and 1690s (CO).

2′,1″-*Anhydro*-5-[(1″-[$^{15}$N]*benzamido*-1″-*hydroxymethylene*)*amino*]-1-(β-D-*ribofuranosyl*)*imidazole*-4-*carboxamide* **3b**.—To a solution of AICA-riboside (907 mg, 3.52 mmol) in DMF (15 $cm^3$) was added a solution of benzoyl [$^{15}$N]isothiocyanate (633 mg, 3.86 mmol) in DMF (2 $cm^3$). The reaction mixture was stirred under nitrogen at 20 °C for 2 h. Removal of the DMF under reduced pressure gave an orange/red gum, which was dissolved in 0.5 mol $dm^{-3}$ aq. sodium hydroxide (13.7 $cm^3$, 6.85 mmol). Iodomethane (324 $mm^3$, 5.2 mmol) was added dropwise to the rapidly stirred solution over a period of 45 min and the reaction mixture was stirred at 20 °C for 1 h. Filtration of the reaction mixture, followed by neutralisation with 0.5 mol $dm^{-3}$ hydrochloric acid, caused the precipitation of a solid. The solid was collected, dissolved in DMSO, and precipitated by addition of water to give the title compound as a solid (474 mg, 35%), m.p. 236–237 °C (decomp.); (lit.,[23] 236 °C); $\lambda_{max}$(EtOH)/nm 234 (log ε 4.07) and 313 (3.86); $\delta_C$[125 MHz; $(CD_3)_2SO$] 61.7 (s, C-5′), 70.2 (s, C-3′), 80.4 (s, C-2′), 85.1 (s, C-4′), 90.9 (s, C-1′), 126.6 (s, C-4), 128.8 and 128.9 (2 × s, *m*- and *o*-Ar), 130.3 (s, C-5), 132.3 (s, *p*-Ar), 133.1 (s, C-8), 134.2 (d, $^2J$ 9, *ipso*-Ar), 142.4 (d, $^1J$ 25, C-1″), 163.7 (s, $CONH_2$) and 166.3 (d, $^1J$ 12, $CO^{15}NH$); *m/z* (FAB) 389 ($MH^+$, 65%) and 105 ($PhCO^+$, 100).

*Mixture of* [$^{15}$N$^1$]*Guanosine* (**1a**, 15–20%) *and* [$^{15}NH_2$]-*Guanosine* (**1c**, 75%).—Cyclonucleoside **3b** was treated with ammonium chloride–lithium hydroxide in DMSO in the manner described above for the preparation of the mixture of [$^{15}$N$^1$]guanosine (**1a**, 75%) and [$^{15}NH_2$]guanosine (**1c**, 15–20%) to yield [$^{15}$N$^1$]/[$^{15}NH_2$]guanosine as a powder (43%); spectroscopic data as for the mixture of [$^{15}$N$^1$]guanosine (**1a**, 75%) and [$^{15}NH_2$]guanosine (**1c**, 15–20%), but with the expected reversals of peak intensities.

2-N-*Benzoyl*[$^{15}NH_2$]*guanosine* **8b** *from Compound* **3b**.—A solution of compound **3b** (496 mg, 1.28 mmol) in ethanol containing 2.75 mol $dm^{-3}$ sodium ethoxide (12 $cm^3$, 33 mmol NaOEt) was refluxed under nitrogen. A precipitate formed after 5 min and the reaction mixture was refluxed for a further 15 min. Neutralisation of the reaction mixture with 6 mol $dm^{-3}$ hydrochloric acid and evaporation under reduced pressure gave an off-white solid, which was recrystallised twice from water to give the title compound (301 mg, 61%), m.p. 236–237 °C; (lit.,[23] 232 °C); $\delta_H$[400 MHz; $(CD_3)_2SO$] 3.59 (2 H, m, 5′-$H_2$), 3.90 (1 H, q, *J* 3.9, 4′-H), 4.16 (1 H, m, 3′-H), 4.49 (1 H, m, 2′-H), 5.12 (1 H, m, 5′-OH), 5.28 (1 H, m, 3′-OH), 5.57 (1 H, m, 2′-OH), 5.89 (1 H, d, *J* 6.1, 1′-H), 7.53–7.68 (3 H, m, ArH), 8.04–8.06 (2 H, m, ArH), 8.32 (1 H, s, 8-H), 12.01 (1 H, br s, $^{15}NHCOPh$) and 12.37 (1 H, s, NH); $\delta_C$[100.62 MHz; $(CD_3)_2SO$] 61.3 (s, C-5′), 70.4 (s, C-3′), 74.0 (s, C-2′), 85.5 (s, C-4′), 86.5 (s, C-1′), 120.6 (s, C-5), 128.5 (s, *o*- and *m*-Ar), 132.4 (d, *J* 8.2, *ipso*-Ar), 133.1 (s, C-8), 138.0 (s, *p*-Ar), 148.3 (d, *J* 22.4, C-2), 149.0 (d, *J* 4.1, C-4), 155.2 (s, C-6) and 168.9 (d, *J* 13.2, $^{15}NCOPh$); $\delta_N$[40.56 MHz; $(CD_3)_2SO$] −247.8 (br s, $^{15}NCOPh$) [resonance upfield from the external standard $CD_3NO_2$ set at $\delta$ 0].

2-N-*Benzoyl*[$^{15}NH_2$]*guanosine* **8b** *directly from AICA-riboside.*—Benzoyl [$^{15}$N]isothiocyanate (263 mg, 1.61 mmol) was added to a solution of AICA-riboside (415 mg, 1.61 mmol) in dry DMF (10 $cm^3$). The reaction mixture was stirred under nitrogen at 20 °C for 2 h. The DMF was removed under reduced pressure to leave an orange gum, which was dissolved in dry ethanol (10 $cm^3$). To the resulting solution was added iodomethane (110 $mm^3$, 1.76 mmol) and the mixture was stirred under nitrogen at 20 °C for 1 h. 2.2 Mol $dm^{-3}$ ethanolic sodium ethoxide (20 $cm^3$, 44 mmol) was added and initially caused a solid to be precipitated, but this redissolved on addition of the remaining sodium ethoxide. The reaction mixture was refluxed for 1 h, and then cooled to room temperature, and water (10 $cm^3$) was added to dissolve the precipitate that had formed. This solution was neutralised with 5 mol $dm^{-3}$ hydrochloric acid and concentrated under reduced pressure to give a solid. This was recrystallised twice from water to afford the title compound (441 mg, 71%), m.p. 253–254 °C; $\lambda_{max}$(EtOH)/nm 238 (log ε 4.01) and 300 (3.96); $\delta_H$[200 MHz; $(CD_3)_2SO$] 3.60 (2 H, m, 5′-$H_2$), 3.93 (1 H, m, 4′-H), 4.16 (1 H, m, 3′-H), 4.49 (1 H, m, 2′-H), 5.2–5.6 (3 H, m, 5′-, 3′- and 2′-OH), 5.90 (1 H, d *J* 6.0, 1′-H), 7.54–7.68 (3 H, m, ArH), 8.04–8.08 (2 H, m, ArH), 8.34 (1 H, s, 8-H) and 12.2 (2 H, br, $N^2H$, $HN^1$); $\delta_C$[50.3 MHz; $(CD_3)_2SO$] 61.6 (s, C-5′), 70.7 (s, C-3′), 74.3 (s, C-2′), 85.8 (s, C-4′), 86.9 (s, C-1′), 120.8 (s, C-5), 128.8 (s, *o*- and *m*-Ar), 132.9 (d, *J* 8.7, *ipso*-Ar), 133.3 (s, C-8), 138.3 (s, *p*-Ar), 148.9 (d, *J* 22, C-2), 149.3 (s, C-4), 155.5 (s, C-6) and 169.4 (d, *J* 12.3, NH*C*OPh); *m/z* (FAB) 411 (M + Na, 31%), 389 ($MH^+$, 49) and 290 (100) (peaks > 210).

[$^{15}NH_2$]*Guanosine* **1c**.—A solution of 2-*N*-benzoyl[$^{15}NH_2$]-guanosine (279 mg, 0.72 mmol) in 33% ethanolic methylamine (10 $cm^3$) was stirred at 20 °C for 5 h, after which a significant amount of the product had precipitated out. After evaporation under reduced pressure the white solid was suspended in dichloromethane and collected by filtration. The resulting solid was recrystallised from water to give the title compound (176 mg, 86%), m.p. 250–252 °C (decomp.); $\delta_H$[200 MHz; $(CD_3)_2SO$] 3.57 (2 H, m, 5′-$H_2$), 3.87 (1 H, m, 4′-H), 4.09 (1 H, m, 3′-H), 4.40 (1 H, m, 2′-H), 5.06 (1 H, t, *J* 5, 5′-OH), 5.15 (1 H, d, *J* 4, 3′-OH), 5.41 (1 H, d, *J* 5.7, 2′-OH), 5.70 (1 H, d, *J* 5.9, 1′-H), 6.49 (2 H, d, *J* 89, $^{15}NH_2$), 6.49 (0.04 H, s, $^{14}NH_2$), 7.95 (1 H, s, 8-H) and 10.64 (1 H, s, $HN^1$); $\delta_C$[50.3 MHz; $(CD_3)_2SO$] 61.7 (s, C-5′), 70.7 (s, C-3′), 74.0 (s, C-2′), 85.5 (s, C-4′), 86.6 (s, C-1′), 117.0 (s, C-5), 135.9 (s, C-8), 151.7 (s, C-4), 154.0 (d, *J* 22.7, C-2) and 157.1 (s, C-6); *m/z* (FAB) 285 ($MH^+$, 14%), 153 (M − ribose, 23) and 75 (100).

*Sodium* 5-*Amino*-1-(β-D-*ribofuranosyl*)*imidazole*-4-*carboxylate* **2b**.—A solution of AICA-riboside (1.212 g, 4.7 mmol) in 6 mol $dm^{-3}$ aq. sodium hydroxide (4.84 $cm^3$, 29 mmol) was refluxed under nitrogen for 4 h, and then was cooled in ice. Ethanol (2 $cm^3$) was added and the mixture was rapidly stirred. The supernatant was pipetted off to leave a syrup, which was triturated with ethanol (3 × 1 $cm^3$, then 3 × 0.5 $cm^3$), the supernatant being pipetted off each time. The thick syrup was dried *in vacuo* over silica gel (24 h). The residue was triturated with methanol (2 $cm^3$) and the solid that formed was filtered off.

* We thank Dr. I. M. Lockhart (BOC, Morden) for this gift.

This was dried *in vacuo* to give tht title compound as an off-white solid (721 mg, 55%), m.p. 240–242 °C [lit.,[29] 244–245 °C (decomp.)]; $\lambda_{max}$(water/nm) 250 (log ε 4.00); $\lambda_{max}$(pH 11) 251 (log ε 4.04); $\nu_{max}$/cm$^{-1}$ 1589 and 1415 ($CO_2^-$); $\delta_H$(200 MHz; $D_2O$) 3.75 (1 H, m, 4′-H), 4.11 (2 H, m, 5′-$H_2$), 4.49 (1 H, t, *J* 5.5, 3′-H), 4.81 (2′-H, HOD), 5.47 (1 H, d, *J* 6.1, 1′-H) and 7.37 (1 H, s, 2-H); *m*/*z* (FAB) 282 ($MH^+$, 2%) and 115 (100).

5-*Amino*-1-(2,3,5-*tri*-O-*acetyl*-β-D-*ribofuranosyl*)*imidazole*-4-*carboxylic Acid* **2c**.—Acetic anhydride (19 cm$^3$, 200 mmol) was added over a period of 1 h to a suspension of the sodium salt **2b** (2.00 g, 7.1 mmol) in pyridine (37 cm$^3$). The mixture was stirred under nitrogen at 20 °C for 4 h, and then was kept at 4 °C for 63 h. The mixture was filtered and the clear filtrate was evaporated under reduced pressure (≤ 20 °C). Water (10 cm$^3$) was added to the residue and was then removed under reduced pressure. This process was repeated until, on the addition of water to the residue, a fine yellow precipitate was formed. This was filtered off, washed successively with cold water and cold acetone, and dried *in vacuo* to give the title compound as a fine powder (1.01 g, 37%), m.p. 144–145 °C (decomp.) (lit.,[29] 145–146 °C); $\delta_C$[50.3 MHz; $(CD_3)_2SO$] 20.4, 20.6 and 20.7 (s, 3 × CO*Me*), 63.2 (s, C-5′), 69.8 (s, C-3′), 72.0 (s, C-2′), 79.5 (s, C-4′), 84.1 (s, C-1′), 110.1 (s, C-5), 128.7 (s, C-4), 146.2 (s, C-2), 165.5 (s, $CO_2H$) and 169.5, 169.7 and 170.3 (s, 3 × *C*OMe); *m*/*z* (EI) 341.1230 (M − $CO_2^+$; Calc. for $C_{14}H_{19}N_3O_7$; *m*/*z*, 341.1223), 259 (M − base, 54%) and 43 (100).

N-*Succinimidyl* 5-*Amino*-1-(2,3,5-*tri*-O-*acetyl*-β-D-*ribofuranosyl*)*imidazole*-4-*carboxylate* **2d**.—Dicyclohexylcarbodiimide (595 mg, 2.89 mmol) was added to a mixture of *N*-hydroxysuccinimide (302 mg, 2.63 mmol) and triacetate **2c** (1.01 g, 2.63 mmol) in DMF (10 cm$^3$). The mixture was stirred under nitrogen at 20 °C for 48 h. Acetic acid (90 mm$^3$) was added and the mixture was stirred for 1 h. The precipitate of dicyclohexylurea was filtered off, and washed with ethyl acetate (30 cm$^3$), and the filtrate was evaporated under reduced pressure. The residue was dissolved in ethyl acetate and washed with water (2 × 15 cm$^3$). The organic layer was dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure. The residue was recrystallised from benzene to give the title compound as a crystalline solid (764 mg, 60%), m.p. 105–110 °C (lit.,[29] 110 °C); $\delta_H$[200 MHz; $(CD_3)_2SO$] 2.07 (6 H, s, 2 × Ac), 2.12 (3 H, s, Ac), 2.84 (4 H, s, $COCH_2CH_2CO$), 4.31 (3 H, m, 4′-H and 5′-$H_2$), 5.35 (1 H, m, 3′-H), 5.60 (1 H, t, *J* 6.3, 2′-H), 6.01 (1 H, d, *J* 6.4, 1′-H), 6.80 (2 H, s, $NH_2$) and 7.63 (1 H, s, 2-H).

5-*Amino*-1-(2,3,5-*tri*-O-*acetyl*-β-D-*ribofuranosyl*)*imidazole*-4-[$^{15}$N]*carboxamide* **2e**.—[$^{15}$N]Ammonium chloride (6.7 mg, 1.24 × 10$^{-4}$ mol) was added to a solution of ester **2d** (54.3 mg, 1.13 × 10$^{-4}$ mol) in 1 : 1 acetonitrile–triethylamine (2 cm$^3$). The mixture was vigorously stirred under nitrogen at 20 °C for 19 h, quenched by the addition of ethanol (1 cm$^3$), and evaporated under reduced pressure. The residue was dissolved in ethanol, evaporated onto silica, and chromatographed on silica [elution with dichloromethane–methanol (95:5)] to give the title compound as a foam (21 mg, 48%); $\delta_H$[200 MHz; $(CD_3)_2SO$] 2.07 (6 H, s, 2 × Ac), 2.12 (3 H, s, COMe), 4.30 (3 H, m, 4′-H and 5′-$H_2$), 5.35 (1 H, m, 3′-H), 5.59 (1 H, t, *J* 6.3, 2′-H), 5.89 (1 H, d, *J* 6.4, 1′-H), 5.97 (2 H, s, $NH_2$), 6.62 (1 H, d, *J* 27.3, $^{15}$NH′), 7.06 (1 H, d, *J* 24.5, $^{15}$NH″) and 7.81 (1 H, s, 2-H); $\delta_C$[50.3 MHz; $(CD_3)_2SO$] 20.5, 20.7 and 20.9 (s, 3 × CO*Me*), 63.39 (s, C-5′), 70.0 (s, C-3′), 72.1 (s, C-2′), 79.6 (s, C-4′), 84.3 (s, C-1′), 114.0 (br s, C-4), 127.9 (s, C-2), 143.2 (s, C-5), 166.8 (d, *J* 16.1, $CONH_2$), 169.6, 169.8 and 170.4 (s, 3 × *C*OMe); *m*/*z* (FAB) 386 ($MH^+$, 35%), 259 (M − base, 73) and 196 (100).

2-N-*Benzoyl*[$^{15}$N$^1$]*guanosine* **8c**.—Benzoyl isothiocyanate (66 mm$^3$, 0.49 mmol) was added to a solution of amide **2e** (143 mg, 0.37 mmol) in dry DMF (2 cm$^3$). The reaction mixture was stirred under nitrogen at 20 °C for 2 h and then concentrated under reduced pressure to give an orange gum, which was dissolved in dry ethanol (5 cm$^3$). Iodomethane (25.5 mm$^3$) was added and the mixture was stirred under nitrogen at 20 °C for 1 h. Ethanolic sodium ethoxide (0.96 mol dm$^{-3}$; 10 cm$^3$, 9.65 mmol NaOEt) was added and the mixture was refluxed under nitrogen for 1 h. Water (5 cm$^3$) was added and the resulting solution was carefully neutralised with 5 mol dm$^{-3}$ hydrochloric acid. The resulting solution was evaporated under reduced pressure to give a solid, to which methanol was added. The mixture was filtered and the solid was washed with methanol (5 × 10 cm$^3$). The combined filtrates were evaporated onto silica and chromatographed on silica [elution with methanol–dichloromethane (15:85 to 55:50)] to give a solid. This was recrystallised from water to give the title compound (50 mg, 35%), m.p. 254–255 °C (lit.,[23] 232 °C); $\lambda_{max}$(EtOH)/nm 237 (log ε 4.01) and 309 (3.95); $\delta_H$[200 MHz; $(CD_3)_2SO$] 3.59 (2 H, m, 5′-$H_2$), 3.90 (1 H, m, 4′-H), 4.13 (1 H, m, 3′-H), 4.47 (1 H, ddd, *J* 6, 2′-H), 5.06 (1 H, t, *J* 5.3, 5′-OH), 5.20 (1 H, d, *J* 5.3, 3′-OH), 5.50 (1 H, d, *J* 5.8, 2′-OH), 5.89 (1 H, d, *J* 6.0, 1′-H), 7.51–7.71 (3 H, m, ArH), 8.02–8.06 (2 H, m, ArH), 8.30 (1 H, s, 8-H), 11.98 (1 H, s, N*H*COPh) and 12.34 (1 H, d, *J* 89, $^{15}$NH$^1$); $\delta_C$[50.3 MHz; $(CD_3)_2SO$] 61.7 (s, C-5′), 70.8 (s, C-3′), 74.4 (s, C-2′), 85.9 (s, C-4′), 86.8 (s, C-1′), 121.0 (d, *J* 7.8, C-5), 128.9 (s, *m*- and *o*-Ar), 132.7 (s, *ipso*-Ar), 133.6 (s, C-8), 138.3 (s, *p*-Ar), 148.7 (d, *J* 13.7, C-2), 149.4 (s, C-4), 155.5 (d, *J* 9.9, C-6) and 169.5 (s, *C*OPh).

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