Syntheses of [6,7-^{15}N]-Adenosine, [6,7-^{15}N]-2′-Deoxyadenosine, and [7-^{15}N]-Hypoxanthine

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Abstract: We have developed a high-yield route for the synthesis of [7-^{15}N]-hypoxanthine in four steps in an overall yield of 81%. This procedure uses [^{15}N]-sodium nitrite as the ^{15}N source and an inexpensive pyrimidine to provide an economical route to this useful ^{15}N-labeled intermediate. Conversion to [7-^{15}N]-6-chloropurine followed by enzymatic transglycosylation gives the corresponding ribo- and 2′-deoxyribonucleosides. Ammonolysis of the 6-chloro moiety to give the [6,7-^{15}N]-labeled nucleosides is effected simply and in high yield using 2 equiv of [^{15}N]-ammonium chloride and 3 equiv of potassium bicarbonate.

NMR studies using oligonucleotides specifically labeled with ^{15}N have provided unique information about nucleic acid−ligand interactions.[1−12] These initial studies were designed primarily to demonstrate the value of specific ^{15}N-labeling. For this purpose singly labeled molecules were appropriate, and the monomer synthetic routes reported to date therefore have been directed largely to singly labeled bases[13−15] or nucleosides.[2,16−28] In general, however, the way to maximize the information available from the NMR experiment, while preserving the advantages of specific labeling, would be to have as many labels as can be unambiguously distinguished. To this end, we have begun development of a new series of routes designed to produce multilabeled nucleosides, both ribonucleosides and 2′-deoxynucleosides, efficiently and economically. We now report syntheses of [6,7-^{15}N]-adenosine and [6,7-^{15}N]-2′-deoxyadenosine, based on a highly efficient synthesis of [7-^{15}N]-hypoxanthine.

The combination of chemical and enzymatic steps used in these syntheses are shown in Scheme 1. Our previous route to [7-^{15}N]-adenine and 2,6-diaminopurine[20] used expensive di- or triaminopyrimidines and did not lend itself to easy incorporation of a second label at the N6. The present approach uses an inexpensive pyrimidine, 6-amino-2-thioxo-1,2-dihydro-4(3*H*)-pyrimidinone (**1**),[29] and takes advantage of the facile conversion of hypoxanthine to 6-chloropurine for introduction of the second ^{15}N atom. In addition, the lipophilic 6-chloro moiety in the nucleosides **7a** and **7b** proved to be useful in the isolation of these compounds.

In our previous approach to [7-^{15}N]-labeling,[20] we used an azo coupling reaction for introduction of the ^{15}N, while in this case we found that direct nitrosation of **1** gave the best yields. Thus, reaction of **1** with 1.1 equiv of [^{15}N]-$NaNO_2$[30] gives quantitative conversion of **1** to **2** (or 91% based on [^{15}N]-$NaNO_2$). The product (**2**) precipitates from the reaction mixture and is isolated by filtration. Reduction of the nitroso group and removal of the thiol can be done concomitantly with Raney nickel,[31] but this procedure required inconveniently large amounts of Raney nickel and gave only moderate yields. Instead, we adopted the two-step procedure shown in which **2** was first reduced with sodium hydrosulfite at 0 °C to give **3** in

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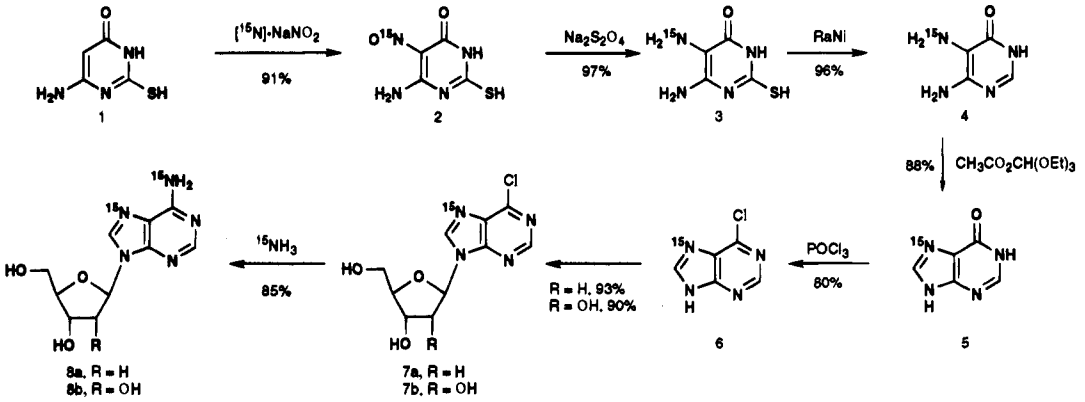
Scheme 1



97% yield, and the thiol then was cleaved using Raney nickel to afford **4** in 96% yield.[32,33]

There are many procedures and reagents available for ring closure of 4,5-diaminopyrimidines to the purine ring system,[34] and we have previously used diethoxymethyl acetate in DMF with good results.[20,22] In optimizing the ring closure of **4** to **5**, however, we found that a two-step procedure using a combination of formic acid and diethoxymethyl acetate in DMF gave the best overall yield. Thus **4** was heated for 1 h in formic acid to give a mixture of formylated products along with a small amount of **5**. This mixture was concentrated to a solid to which DMF, 1 equiv of formic acid, and 2 equiv of diethoxymethyl acetate were added, and it was heated for 1 h at 130 °C. Removal of solvents and trituration of the residue in acetonitrile gives **5** as a nearly pure off white solid in 88% yield.

The overall yield to [7-^{15}N]-hypoxanthine (**5**) is 81% from **1** or 75% from [^{15}N]-$NaNO_2$. We have prepared more than 100 g of **5** by this route, in batches of up to 20 g. Hypoxanthine is a versatile intermediate for nucleoside synthesis, and **5** is readily converted to [7-^{15}N]-6-chloropurine (**6**) using $POCl_3$.[35,36] By optimization of the reaction and isolation conditions, we obtain pure, colorless **6** reproducibly in 80% yield. The minor colored impurities produced in the reaction are removed by a simple procedure using a preparative reversed-phase column under conditions where **6** is not retained, but the impurities are, so that large (12 g) amounts of **6** can be purified by this method. At this point, it is possible to displace the chloro group of **6** using ammonia, as we have done previously,[22] although it requires forcing conditions. Conversely, similar reaction of **7a/b** proceeds under much milder, more convenient conditions. We have found, moreover, that the chloro nucleosides **7a/b** are sufficiently more lipophilic than **8a/b**, that **7a/b** are significantly easier to isolate after the enzymatic coupling reactions than are **8a/b**.

The enzymatic transglycosylation to produce **7a** from **6** uses thymidine, thymidine phosphorylase, and phosphate buffer to generate the 2-deoxyribose-α-1-phosphate, and purine nucleoside phosphorylase to effect the coupling with **6**.[20,37-39] The reaction mixture is kept at 45 °C for 2 days, and **7a** is then isolated by continuous extraction into methylene chloride. Continuous extraction is more convenient, and more amenable to large-scale work, than the ion-exchange chromatography we had employed previously for isolation of [7-^{15}N]-labeled 2′-deoxyadenosine[20] and may be a general procedure for moderately lipophilic derivatives. The methylene chloride extract also contains 8% of **6** and small amounts of thymine and thymidine. Pure **7a** is obtained by preparative reversed-phase chromatography using a water/acetonitrile gradient. The yield of **7a** after chromatography is 86%, based on the amount of **6** used initially, but since the 8% of unreacted **6** is recovered by the chromatography, the yield based on the amount of **6** consumed is 93%.

The enzymatic conversion of **6** to the ribonucleoside **7b** uses 7-methylguanosine, rather than uridine, as the ribose source. In this case the purine nucleoside phosphorylase both generates the ribose-α-1-phosphate and couples it to **6**.[40] The 7-methylguanine produced in the reaction has very low water solubility, so that the reverse reaction does not occur, and the bulk of the 7-methylguanine is easily removed by filtration. The 7-methylguanosine is obtained from inexpensive guanosine in a straightforward procedure.[41] Preparative reversed-phase chromatography using a water/acetonitrile gradient gives pure **7b** in 90% yield.[42]

Ammination of **7a/b** to **8a/b** occurs readily under a variety of conditions, but most employ ammonia in a large excess, which is not practical for [^{15}N]-ammonia.[43] The least expensive source of ^{15}N is [^{15}N]-ammonia gas, but [^{15}N]-NH_4Cl is similar to [^{15}N]-ammonia gas in cost and is more convenient to use in controlled amounts. After investigating numerous combinations of [^{15}N]-ammonium salts, bases, and solvents, we found that use of 2 equiv of [^{15}N]-NH_4Cl in DMSO with 3 equiv of $KHCO_3$ at 80 °C in a sealed container for 2-3 days gives efficient conversion to the [6,7-^{15}N]-nucleosides **8a/b**. This is a simple procedure that is amenable to large scales and does not require any complicated apparatus or difficult manipulations. Both **8a** and **8b** are obtained in 85 to 90% yield after reversed-phase chromatography using a water/acetonitrile gradient.

The route described above is the first synthesis of these doubly labeled adenine nucleosides. More importantly, the steps are generally high-yield, avoid protection/deprotection steps, and

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are amenable to multigram scales, so that these compounds can be obtained efficiently and economically. The overall viability of this synthetic route is particularly important in that **8a/b** will serve as intermediates for synthesis of a large family of [7-^{15}N]-labeled purine nucleosides. We have in hand routes capable of converting **8a/b** to the corresponding [1,7-^{15}N]- and [1,6,7-^{15}N]-adenine nucleosides[18] as well as [1,2,7-^{15}N]-guanine nucleosides.[23] Moreover, improvements to these routes to make more efficient use of **8a/b** are under development and will be reported shortly.

Experimental Section

General Methods. Melting points (mp) were determined in Kimax soft glass capillary tubes with a Thomas-Hoover apparatus and are uncorrected. The ^{1}H (200 MHz) and ^{13}C (50.3 MHz) NMR spectra were recorded on a Varian XL-200 and referenced to DMSO-d_6 (99.9 atom % D, 2.49 δ ^{1}H NMR; 39.5 δ ^{13}C NMR) or 3-(trimethylsilyl)-propanoic-*2,2,3,3-d*$_4$ acid, sodium salt (0.0 δ) when the NMR solvent was D_2O. The ^{15}N (40.5 MHz) NMR spectra were recorded on a Varian XL-400 and were referenced to H^{15}NO$_3$ (375.8 δ). Mass spectral (MS) data by electron impact (EI) were measured at 70 eV on a Kratos MS-9/50 double-focusing high-resolution mass spectrometer. Combustion analyses were performed by Galbraith Laboratories, Knoxville, TN or Quantitative Technologies Inc. Whitehouse, NJ. Enzyme reactions were performed in a Boekel Industries Inc. Model 136400 Incubator-Shaker II. Analytical HPLC was carried out with Waters C-18 Nova-Pak cartridges (8 × 100 mm) using a gradient of 2−20% CH_3CN/0.1 M TEAA, 2 mL/min. Preparative reversed-phase HPLC was performed on a system consisting of a Waters 590 EEF pump and Model 660 gradient controller with an Autochrome OPG to allow a single pump gradient and a Linear UVIS 200 detector. Three Waters Delta-Pak PrepPak cartridges (40 × 100 mm, C_{18} 300 Å, 15 μm) were used in series contained in a Waters prepPak holder assembly (gradient was monitored at 280 nm, 45 min at 40 mL/min). The [^{15}N]-$NaNO_2$ and [^{15}N]-NH_4Cl were purchased from Isotec Inc., thymidine phosphorylase was purchased from Sigma Chemical Co., and purine nucleoside phosphorylase was a gift form Burroughs Wellcome Co. Clear threaded sample vials with rubber lined closures (03-339-22G) and bottles (06-414-1A) were obtained from Fisher. Other general reagents were purchased from Aldrich Chemical Co.

[5-^{15}N]-6-Amino-5-nitroso-2-thioxo-1,2-dihydro-4(3*H*)-pyrimidinone (2). To a chilled (0 °C) suspension of 6-amino-2-thioxo-1,2-dihydro-4(3*H*)-pyrimidinone (**1**) (24.8 g, 0.154 mol) in aqueous HCl (1 N, 650 mL) was added [^{15}N]-sodium nitrite (12.0 g, 0.169 mmol) in water (60 mL) during 20 min. The resulting red suspension was stirred at 0 °C for 7 h, and the product was isolated by suction filtration followed by washing (60 mL) with cold water, ethanol, and acetone. The brick red filter cake was dried in a vacuum desiccator over P_2O_5 for 24 h to give 26.6 g of **2** (91% yield based on [^{15}N]-$NaNO_2$, 99% yield based on **1**): mp 270−285 dec °C (lit.[32,44,45] no mp given); ^{13}C NMR (D_2O/NaOD) δ 198.7 (C2), 174.5 (C4), 154.3 (C6), 144.7 (d, J = 3.9 Hz, C5) (The spectrum must be obtained immediately because the product decomposes quickly in this solvent mixture); MS (EI) *m/z* (rel intensity) 173 (M^+, 100), 157 ($M^+ - NH_2$, 50).

[5-^{15}N]-5,6-Diamino-2-thioxo-1,2-dihydro-4(3*H*)-pyrimidinone (3). The procedure of Taylor[33] and Albert[32] was altered by suspending **2** (26.6 g, 0.154 mol) in saturated aqueous $NaHCO_3$ (600 mL) instead of dissolving in 1 N aqueous NaOH. Sodium hydrosulfite ($Na_2S_2O_4$, 64.5 g, 0.369 mol) was added in portions over 10 min. The yellow mixture was stirred at 0 °C for 7 h, then acetic acid (36 mL) was added, and the cold suspension was filtered by suction, washed (20 mL) with cold water and ethanol, and finally dried in a vacuum desiccator over P_2O_5 for 24 h to give 23.8 g (97%) of **3** as a fine yellow powder: mp > 300 °C (lit.[32,33,45] no mp given); ^{1}H NMR (DMSO-d_6) δ 12−10 (br s, 1 H, NH), 8−5 (br s, 5 H, NH_2's and SH); ^{13}C NMR (DMSO-d_6) δ 167.5 (C2), 157.9 (C4), 140.6 (d, J = 3.3 Hz, C6), 102.5 (d, J = 11.7 Hz, C5); ^{15}N NMR (DMSO-d_6) δ 29.9; MS (EI) *m/z* (rel intensity) 159 (M^+, 100); HRMS *m/z* 159.0228 (calcd for $C_4H_6N_3{}^{15}NOS$ 159.0233).

[5-^{15}N]-5,6-Diamino-4(3*H*)-pyrimidinone (4). To a solution of **3** (24.5 g, 0.154 mol) in NH_4OH (664 mL, 5% NH_3) was added Raney nickel (68.6 g, of a 50% water suspension) over 5 min, and this mixture was refluxed for 1.5 h. The hot reaction mixture was filtered through a bed of Celite, and the Raney nickel filter cake was washed with boiling water. The filtrate was concentrated and then dried in a vacuum desiccator over P_2O_5 for 24 h to give 20.5 g (96%) of yellow solid: mp 226−227 °C (lit.[32,45] mp 239 °C); ^{1}H NMR (DMSO-d_6) δ 7.50 (s, 1 H, CH), 7−4 (br s, 5 H, NH and NH_2's); ^{13}C NMR (DMSO-d_6) δ 156.5 (C4), 147.4 (C6), 138.3 (C2), 110.5 (d, J = 11.3 Hz, C5); ^{15}N NMR (DMSO-d_6) δ 37.1; MS (EI) *m/z* (rel intensity) 127 (M^+, 100); HRMS *m/z* 127.0518 (calcd for $C_4H_6N_3{}^{15}NO$ 127.0512).

[7-^{15}N]-Hypoxanthine (5). A suspension of **4** (17.6 g, 0.139 mol) in 100 mL of formic acid was refluxed for 1 h and then concentrated to a yellow solid. Diethoxymethyl acetate (45 mL, 0.28 mol), formic acid (6.5 mL, 0.17 mol), and DMF (440 mL) were added, and the resulting suspension was heated (130 °C) for 2.5 h. The suspension was concentrated to a grayish solid, resuspended in 200 mL of refluxing acetonitrile for 10 min, chilled (0 °C), filtered, and then dried in a vacuum desiccator over P_2O_5 for 24 h to give 16.7 g of **5** (88%, off white solid): ^{1}H NMR (D_2O/NaOD) δ 8.06 (s, 1 H, H2), 7.86 (d, J = 12.3 Hz, 1H, H8); ^{13}C NMR (D_2O/NaOD) δ 170.2 (d, J = 3.9 Hz, C5), 162.9 (C6), 153.9 (C2, C8), 153.5 (C2, C8), 127.1 (C4); ^{15}N NMR (D_2O/NaOD) δ 224.8 (d, J = 12.2 Hz); MS (EI) *m/z* (rel intensity) 137 (M^+, 100); HRMS *m/z* 137.0359 (calcd for $C_5H_4N_3{}^{15}NO$ 137.0356). An analytical sample was prepared by reversed phase preparative chromatography using a gradient of 0−5% CH_3CN in water: mp > 300 °C (lit.[33,34,46] no mp given). Anal. Calcd for $C_5H_4N_3{}^{15}NO\cdot 0.1H_2O$: C, 43.23; H, 3.05; N, 40.33. Found: C, 43.33; H, 3.01; N, 40.33.

[7-^{15}N]-6-Chloropurine (6). A mixture of **5** (13.4 g, 98.4 mmol), $POCl_3$ (390 mL), and *N,N*-dimethylaniline (33 mL, 0.258 mol) was refluxed until a homogeneous black solution was obtained (not longer than 30 min). The solution was allowed to cool and then concentrated to a black gum (it is important to remove all of the $POCl_3$, oil pump vacuum is required). The black gum was dissolved in 100 mL of NH_4OH (30%) with cooling, filtered through a bed of Celite, and extracted with ethyl acetate (100 mL) and then ether (2 × 50 mL). During the extraction it is important that the pH is kept above 10. This solution was acidified to pH 2 with concentrated HCl (with cooling in an ice bath) and then continuously extracted with ether for 24 h. To the ether layer was added 20 mL of NH_4OH (30%) (to neutralize any residual HCl), and this mixture was concentrated until the ether had just been removed, affording the ammonium salt of **6** in *ca.* 20 mL of water which was then applied to a reversed phase preparative column eluted with water only. The product fractions were concentrated to a white solid and dried in a vacuum desiccator over P_2O_5 for 24 h to give 12.2 g (80%) of **6**: mp > 300 °C (lit.[34,35] 175−177 °C); ^{1}H NMR (DMSO-d_6) δ 8.70 (s, 1 H, H2), 8.67 (d, 1 H, J = 12.5 Hz, H8); ^{13}C NMR (DMSO-d_6) δ 154.3 (C6), 151.4 (C2, C8), 147.7 (d, J = 5.5 Hz, C5), 146.4 (C2, C8), 129.3 (C4); ^{15}N NMR (DMSO-d_6) δ 230.4; MS (EI) *m/z* (rel intensity) 157 (M^+ [^{37}Cl], 32), 155 (M^+ [^{35}Cl], 100), 120 (M^+ − Cl, 50); HRMS *m/z* 155.0013 (calcd for $C_5H_3N_3{}^{15}N^{35}Cl$ 155.0016). Anal. Calcd for $C_5H_3N_3{}^{15}NCl$: C, 38.61; H, 1.94; N, 36.01. Found: C, 38.54; H, 1.94; N, 35.91.

[7-^{15}N]-6-Chloro-9-(2-deoxy-β-D-erythro-pentofuranosyl)purine (7a). A pH 7 suspension of **6** (1.86 g, 12.0 mmol), thymidine (8.77 g, 36.2 mmol),[47] purine nucleoside phosphorylase (400 units), and thymidine phosphorylase (400 units) in aqueous K_2HPO_4 (50 mL, 0.02 M) was incubated with gentle agitation at 45 °C for 2 days (*ca.* 95% complete; HPLC analysis). Sodium chloride (9 g) was added, and the reaction mixture was continuously extracted with CH_2Cl_2 for 24 h after which HPLC analysis indicated that all the **7a** and **6** had been extracted as well as small amounts of thymidine and thymine. The organic phase (white suspension) was concentrated and purified by reversed phase preparative chromatography (0−15% CH_3CN/water) (the order of elution from the column was thymine, thymidine, **6**, and

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(47) Recent test reactions indicate that use of as little as 1.5 equiv of thymidine gives the same equilibrium concentration of **6** and **7a**.

7a). The fractions containing **6** (0.149 g, 8% recovered yield) and **7a** (2.77 g, 86%) were collected, concentrated, and then dried in a vacuum desiccator over P_2O_5 for 24 h (**7a**): mp 147−148 °C (lit.[48] 139−143 °C); ^{1}H NMR (DMSO-d_6) δ 8.87 (d, 1 H, J = 12.2 Hz, H8), 8.76 (s, 1 H, H2), 6.45 (t_{app}, 1 H, J = 6.6 Hz, H1′), 5.37 (d, 1 H, J = 4.2 Hz, 3′-OH), 4.98 (t, 1 H, J = 5.4 Hz, 5′-OH), 4.43 (m, 1 H, H3′), 3.89 (m, 1 H, H4′), 3.9−3.4 (m, 2 H, H5′, H5″), 2.8−2.7 (m, 1 H, H2′), 2.4−2.3 (m, 1H, H2″); ^{13}C NMR (D_2O) δ 154.2 (C2), 153.1 (C6), 152.6 (d, J = 5.3 Hz, C5), 148.5 (C8), 133.5 (C4), 90.4 (C4′), 87.9 (C1′), 73.8 (C3′), 64.3 (C5′), 42.1 (C2′); ^{15}N NMR (DMSO-d_6) δ 231.6 (d, J = 11.1 Hz); MS (EI) m/z (rel intensity) 273 (M^+ [^{37}Cl], 0.2), 271 (M^+ [^{35}Cl], 0.6), 117 (100); HRMS m/z 271.0506 (calcd for $C_{10}H_{11}O_3N_3{}^{15}N^{35}Cl$ 271.0491). Anal. Calcd for $C_{10}H_{11}O_3N_3{}^{15}NCl$: C, 44.21; H, 4.08; N, 20.62. Found: C, 44.17; H, 4.09; N, 20.56.

[6,7-^{15}N]-2′-Deoxyadenosine (8a). A mixture of **7a** (3.89 g, 14.4 mmol), [^{15}N]-NH_4Cl (1.6 g, 29.9 mmol), and $KHCO_3$ (4.35, 43.4 mmol) in DMSO (22 mL) was sealed in a 100 mL bottle and placed in an oven at 80 °C for 2 days. The cooled (0 °C) reaction vial was opened carefully (CO_2 pressure buildup) and diluted with 22 mL of water, the pH was adjusted to 6.5−6.8 with glacial acetic acid, and the mixture was purified by reversed phase preparative chromatography (0−20% CH_3CN/water). The combined fractions of **8a** were concentrated to dryness, and the solid was dried in a vacuum desiccator over P_2O_5 for 24 h to afford 3.52 g (90%) of pure **8a** monohydrate: mp 184.5−186 °C (lit.[49] 191−192 °C); ^{1}H NMR (DMSO-d_6) δ 8.31 (d, 1 H, J = 12.0 Hz, H8), 8.12 (s, 1 H, H2), 7.29 (d, 2 H, J = 90.1 Hz, NH_2), 6.33 (t_{app}, 1 H, J = 6.3 Hz, H1′), 5.29 (d, 1 H, J = 4.0 Hz, 3-OH′), 5.22 (t, 1 H, J = 6.5 Hz, 5′-OH), 4.40 (m, 1 H, H3′), 3.87 (m, 1 H, H4′), 3.7−3.4 (m, 2 H, H5′, H5″), 2.8−2.6 (m, 1 H, H2′), 2.3−2.1 (m, 1H, H2″); ^{13}C NMR (D_2O, data acquired at 65 °C) δ 158.1 (d, J = 17.1 Hz, C5), 155.3 (C2), 151.1 (C6), 142.9 (C8), 121.7 (C4), 90.3 (C4′), 87.4 (C1′), 74.1 (C3′), 64.7 (C5′), 42.1 (C2′); ^{15}N NMR (DMSO-d_6) δ 244.15 (d, J = 11.9 Hz, N7), 84.96 (t, J = 90.2 Hz, N6); MS (EI) m/z (rel intensity) 253 (M^+, 2), 137 (100, [b + 2H]$^+$); HRMS m/z 253.0946 (calcd for $C_{10}H_{13}O_3N_3{}^{15}N_2$ 253.0959). Anal. Calcd for $C_{10}H_{13}O_3N_3{}^{15}N_2{\cdot}H_2O$: C, 44.28; H, 5.57; N, 25.82. Found: C, 44.22; H, 5.60; N, 25.58.

7-Methylguanosine. A suspension of guanosine (10.1 g, 36.0 mmol) and dimethyl sulfate (7.0 mL, 74 mmol) in *N,N*-dimethylacetamide (100 mL) was stirred at room temperature for 6 h. The pH of the homogeneous solution was adjusted to 10 with NH_4OH (30%), the solution was poured into 300 mL of chilled acetone (0 °C), and the resulting white precipitate was filtered by suction. The filter cake was suspended in absolute ethanol (300 mL), refiltered, suspended in dry ether (300 mL), and filtered again. The white filter cake was allowed to air dry for a few minutes and then dried in a vacuum desiccator over P_2O_5 for 24 h to give 9.21 g (86%) of 7-methylguanosine, which was used without further purification: mp 150−158 °C (lit.[41]mp 159−160 °C).

[7-^{15}N]-6-Chloro-9-(β-D-erythro-pentofuranosyl)purine (7b). To a suspension of **6** (0.714 g, 4.59 mmol) and 7-methylguanosine (2.75 g, 9.23 mmol) in aqueous K_2HPO_4 (15 mL, 0.02 M) was added 6 N sodium hydroxide until the pH was 7.4. To this mixture was added purine nucleoside phosphorylase. The mixture was kept at 30 °C with gentle agitation for 2 days. The reaction mixture then was suspended in 35 mL of boiling water, allowed to cool slightly, and filtered to remove most of the 7-methylguanine. The filtrate (cloudy suspension) was concentrated and suspended in 20 mL of refluxing 50% aqueous MeOH, and this warm suspension was syringe filtered directly onto the reversed phase preparative column and eluted with 0−15% acetonitrile in water [the order of elution from the column was 7-methylguanosine, 7-methylguanine (elutes as a wide band), **7b**, followed by traces of the side product [7-^{15}N]-6-*N,N*′-dimethyladenosine. The fractions containing **7b** were concentrated to a white solid and then dried in a vacuum desiccator over P_2O_5 for 24 h (1.19 g, 90%): mp 166−167 °C (lit.[13] 170−171 °C); ^{1}H NMR (DMSO-d_6) δ 8.94 (d, 1 H, J = 12.3 Hz, H8), 8.81 (s, 1 H, H2), 6.01 (d, 1 H, J = 5.3 Hz, H1′), 5.57 (d, 1 H, J = 5.7 Hz, 2′-OH), 5.25 (d, 1 H, J = 5.2 Hz, 3′-OH), 5.09 (t, 1 H, J = 5.5 Hz, 5′-OH), 4.58 (m, 1 H, H2′), 4.18 (m, 1 H, H3′), 3.98 (m, 1 H, H4′), 3.5−3.8 (m, 2H, H5′); ^{13}C NMR (DMSO-d_6) δ 151.8 (C2), 151.6 (C6), 149.4 (d, J = 6.0 Hz, C5), 145.8 (C8), 131.5 (C4), 88.4 (C1′), 85.8 (C4′), 74.2 (C2′), 70.2 (C3′), 61.1 (C5′); ^{15}N NMR (DMSO-d_6) δ 244.65 (d, J = 12.2 Hz); MS (EI) m/z (rel intensity) 287 (M^+ [^{35}Cl], 3), 186 (M^+ [^{37}Cl] − $C_4H_7O_3$, 32), 184 (M^+ [^{35}Cl] − $C_4H_7O_3$, 100); HRMS m/z 287.0431 (calcd for $C_{10}H_{11}O_4N_3{}^{15}N^{35}Cl$ 287.0439). Anal. Calcd for $C_{10}H_{11}O_4N_3{}^{15}NCl$: C, 41.75; H, 3.85; N, 19.48. Found: C, 41.59; H, 3.70; N, 19.44. The fractions containing side product [7-^{15}N]-6-*N,N*′-dimethyladenosine were also concentrated to a white solid and then dried in a vacuum desiccator over P_2O_5 for 24 h (0.011 g, 1%): mp 183−184 °C (lit.[50] 183−184 °C); ^{1}H NMR (DMSO-d_6) δ 8.36 (d, 1 H, J = 12.2 Hz, H8), 8.2 (s, 1 H, H2), 5.89 (d, 1 H, J = 5.9 Hz, H1′), 5.43 (d, 1 H, J = 6.2 Hz, 2′-OH), 5.36 (m, 1 H, 5′-OH), 5.17 (d, 1 H, J = 4.8 Hz, 3′-OH), 4.57 (m, 1H, H2′), 4.13 (m, 1 H, H3′), 3.94 (m, 1 H, H4′), 3.3−3.8 (m, 8 H, H5′, NMe_2); ^{13}C NMR (DMSO-d_6) δ 154.3 (d, J = 3.3 Hz, C6), 151.7 (C2), 149.9 (C4), 138.6 (C8), 119.8 (C5), 87.9 (C1′), 85.8-(C4′), 73.5 (C2′), 70.5 (C3′), 61.6 (C2′); ^{15}N NMR (DMSO-d_6) δ 248.0 (d, J = 11.9 Hz); MS (EI) m/z (rel intensity) 296 (M^+, 20), 135 ([(b + H)$^+$ − NMe_2]$^+$, 100).

[6,7-^{15}N]-Adenosine (8b). A mixture of **7b** (1.19 g, 4.15 mmol), [^{15}N]-NH_4Cl (0.453 g, 8.46 mmol), and $KHCO_3$ (1.25 g, 12.5 mmol) in DMSO (6 mL) was sealed (Teflon tape) in a 23 mL standard sample vial which was kept in an oven at 80 °C for 3 days. The cooled (0 °C) reaction vial was opened carefully (CO_2 pressure build up), the contents were diluted with 11 mL of water, the pH was adjusted to 7 with glacial acetic acid, and the product was purified by reversed phase preparative chromatography (0−20% CH_3CN/water). The combined product fractions were concentrated to dryness, and the solid was dried in a vacuum desiccator over P_2O_5 for 24 h to afford 0.977 g (85%) of **9** as the hemihydrate: mp 233−235 °C (lit.[49] 233−234 °C); ^{1}H NMR (DMSO-d_6) δ 8.33 (d, 1 H, J = 12.1 Hz, H8), 8.13 (s, 1 H, H2), 7.26 (d, 2 H, J = 90.1 Hz, NH_2), 5.86 (d, 1 H, J = 6.2 Hz, H1′), 5.4 (m, 2 H, 2′-OH and 5′-OH), 5.15 (m, 1 H, 3′-OH), 4.60 (m, 1 H, H2′), 4.14 (m, 1 H, H3′), 3.95 (m, 1 H, H4′), 3.8−3.4 (m, 2 H, H5′); ^{13}C NMR (DMSO-d_6) δ 156.3 (dd, $J_{C.15N6}$ = 20.4, $J_{C.15N7}$ = 4.5 Hz, C6), 152.6 (C2), 149.2(C4), 140.1 (C8), 119.5 (C5), 88.1 (C1′), 86.1 (C4′), 73.7 (C2′), 70.8 (C3′), 61.8 (C5′); ^{15}N NMR (DMSO-d_6) δ 244.15 (d, J = 11.9 Hz, N7), 85.4 (t, J = 90.1 Hz, N6); MS (EI) m/z (rel intensity) 269 (M^+, 10), 137 ([b + H]$^+$, 100); HRMS m/z 269.0910 (calcd for $C_{10}H_{13}O_4N_3{}^{15}N_2$ 269.0908). Anal. Calcd for $C_{10}H_{13}O_4N_3{}^{15}N_2{\cdot}0.5H_2O$: C, 43.17; H, 5.07; N, 25.17. Found: C, 42.99; H, 5.00; N, 25.16.

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Supporting Information Available: ^{1}H, ^{13}C, ^{15}N NMR and mass spectra for **5** to **8a/b** (24 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for the ordering information and Internet access instructions.

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