Use of a ^{13}C Atom To Differentiate Two ^{15}N-Labeled Nucleosides. Syntheses of [$^{15}NH_2$]-Adenosine, [1,NH_2-$^{15}N_2$]- and [2-^{13}C-1,NH_2-$^{15}N_2$]-Guanosine, and [1,7,NH_2-$^{15}N_3$]- and [2-^{13}C-1,7,NH_2-$^{15}N_3$]-2′-Deoxyguanosine

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We report the first examples of the specifically ^{15}N and ^{13}C multilabeled nucleosides: [1,NH_2-$^{15}N_2$]- and [2-^{13}C-1,NH_2-$^{15}N_2$-]-guanosine; [1,7,NH_2-$^{15}N_3$]- and [2-^{13}C-1,7,NH_2-$^{15}N_3$]-2′-deoxyguanosine. In each set, the [^{13}C] atom functions as a "tag" that allows the N1 and N2 ^{15}N atoms of two ^{15}N-labeled guanines to be unambiguously differentiated in RNA and DNA fragments. The syntheses employ high-yield reactions in which protecting groups are not required and use relatively low cost sources of isotopes: [^{15}N]-ammonium chloride and [^{15}N]- or [^{13}C,^{15}N]-potassium cyanide.

Specific ^{15}N-labeling of DNA fragments with single [^{15}N] labels has demonstrated the value of these labels for NMR studies of nucleic acid structure and interactions.[1−11] To maximize the information available from a single NMR experiment and a single synthesis, it would be most useful to have in the molecule or complex as many ^{15}N labels as can be unambiguously distinguished. The synthetic routes reported to date for ^{15}N labeling of bases[12−14] or nucleosides,[2,15−27] however, generally give singly labeled products. We have begun development of routes designed to give multilabeled nucleosides, and the syntheses of [7,NH_2-$^{15}N_2$]-adenosine and 2′-deoxyadenosine have been reported recently.[28] Unfortunately, introduction of more than one such multilabeled monomer into a particular DNA or RNA fragment raises uncertainty about the identity of the ^{15}N labels. The solution we have devised is to add a ^{13}C atom as a "tag" to one of a pair of ^{15}N-multilabeled nucleosides. Modern NMR spectrometers are well suited to the use of molecules with both ^{13}C and ^{15}N labels. This ^{13}C "tag" then offers a means for differentiation of two otherwise identical ^{15}N-labeled residues. Because the most straightforward way to use such a "tag" would be to have the ^{13}C atom directly connected to at least some of the ^{15}N atoms involved, we chose [1,NH_2-$^{15}N_2$]- (**8a**) and [2-^{13}C-1,NH_2-$^{15}N_2$]-guanosine (**8b**) and [1,7,NH_2-$^{15}N_3$]- (**8c**) and [2-^{13}C-1,7,NH_2-$^{15}N_3$]-2′-deoxyguanosine (**8d**) as the first examples of this type of multilabeled nucleoside pair.

The syntheses of **8a** and **8b** are carried out by the same route, which is shown in Scheme 1. In the first transformation, inosine (**1**) is converted to the 6-chloro nucleoside (**2**) in 80% yield by the trifluoroacetic anhydride/thionyl chloride/DMF procedure developed by Robins for 2′-deoxyinosine.[29] Here, the trifluoroacetyl groups are used for transient hydroxyl protection, rather than for stabilization of the glycosidic bond. They are easily removed by methanolysis after the chlorination. Ammonolysis of **2** using [^{15}N]-ammonium chloride and potassium bicarbonate, as we reported recently,[28] gives [$^{15}NH_2$]-adenosine (**3a**) in 90% yield. The subsequent steps constitute an adenosine to guanosine transformation, in which the adenosine amino group becomes the guanosine N1, and the guanosine C2 and amino come from potassium cyanide. It is based on a route we reported previously for singly [^{15}N]-labeled 2′-deoxyguanosine,[22]

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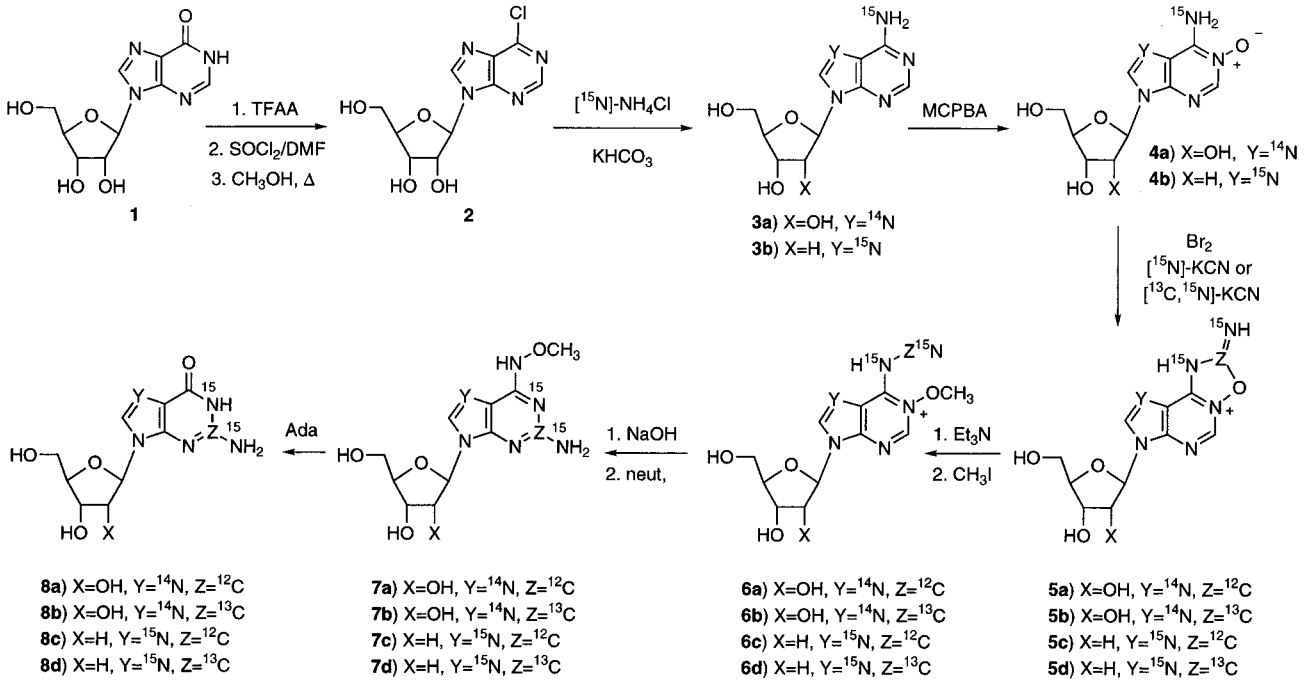
Scheme 1



which had been derived from a set of transformations first reported by Ueda.[30] Oxidation of **3a** to the *N*-oxide **4a** is followed by reaction with cyanogen bromide generated in situ from bromine and labeled KCN. The synthesis of **8a** uses [^{15}N]-KCN, while synthesis of **8b** uses [^{13}C,^{15}N]-KCN. The conversion of **4a** to the 6-*N*-methoxy derivatives **7a** and **7b** is carried out as a one-flask set of reactions without purification of intermediates **5a**, **5b**, **6a**, or **6b**. Treatment of **5a** or **5b** with triethylamine opens the oxazolidine ring so that the *N*-oxide can be methylated with methyl iodide to give **6a** or **6b**. Aqueous sodium hydroxide then opens the pyrimidine ring of **6a** or **6b**. Neutralization of the mixture followed by heating at 60 °C results in deformylation and ring closure to give **7a** or **7b**.[22,30] After purification of **7a** or **7b** by preparative reversed-phase HPLC, enzymatic deamination converts **7a** or **7b** to **8a** or **8b** to complete the synthesis. Preparation of **8c** and **8d**, starting from [7,NH_2-$^{15}N_2$]-deoxyadenosine, **3b**,[28] uses the same sequence of reactions. During the oxidation to form **4b**, depurination of the deoxyadenosine by the *m*-chlorobenzoic acid produced is minimal as long as the reaction time is kept to 3 h.

The key steps in the adenosine to guanosine transformation now have been optimized to a substantial extent. Specifically, we have (1) improved the procedure for preparation of the *N*-oxide to a 92% yield by using *m*-chloroperoxybenzoic acid for the oxidation and preparative reversed-phase HPLC for purification; (2) found that it is important to avoid the presence of excess bromine (orange color), in the reaction of the *N*-oxide with the labeled cyanogen bromide; and (3) found that an 8:1 excess of methyl iodide in the methylation reaction is preferable to the smaller excess we had used previously. We have prepared 2−3 g quantities of **8a**−**d** by these procedures.

The use of these tagged nucleosides in NMR studies of DNA and RNA fragments relies on coupling between

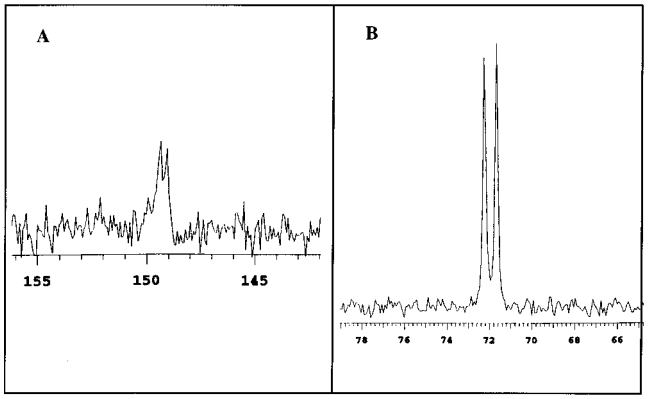


Figure 1. The ^{1}H-decoupled ^{15}N NMR resonances of (A) ^{15}N1, and (B) $^{15}NH_2$, of **8b** at 40.543 MHz, in 10 mM sodium phosphate, pH 7, δ (ppm) 149.18 (d, J_{N-C} = 12.2 Hz), 71.95 (d, J_{N-C} = 24.4 Hz). Chemical shifts are reported relative to NH_3, using external 1 M [^{15}N]HNO_3 in 90% D_2O at 25 °C at 375.8 ppm as a reference.

the ^{13}C2 and the ^{15}N1 and $^{15}NH_2$ atoms in **8b** and **8d**. As the ^{1}H decoupled ^{15}N NMR spectrum of **8b** (Figure 1) shows, these one-bond coupling constants are 12.2 Hz for ^{15}N1−^{13}C2 and 24.4 Hz for $^{15}NH_2$−^{13}C2 in water and about the same in DMSO. The analogous coupling constants for the deoxy compound **8d** have the same values, and they all are assumed to be negative. These observed couplings are more than adequate to allow differentiation of the ^{15}N resonances of **8a** and **8b** in a synthetic RNA fragment and all but the N7 ^{15}N resonance of **8c** and **8d** in a DNA fragment.

When the Fermi contact term is the dominant spin−spin coupling mechanism, most $^1J_{C-N}$ values are in the range of −10 to −15 Hz and appear often to reflect the percentage of s character (*S*) of the carbon and nitrogen atoms ($80J_{CN} = S_C S_N$).[31−34] The 12.2 Hz coupling for the ^{15}N1 is in this range and is consistent with significant

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sp^2 character. However, the 24 Hz coupling for the $^{15}NH_2$ is significantly larger. In addition, the $^{15}NH_2-^{13}C6$ coupling constant in $^{15}NH_2$-adenosine reported here is nearly as large, 20.6 Hz in DMSO. Although these purine amino groups have unusually large $^{15}N-^{13}C$ coupling constants, their $^{15}N-^{1}H$ coupling constants are more typical (about 90 Hz). As we gain more information about these $^{15}N-^{13}C$ couplings within oligomers, we may find that they provide additional structural information. At this time, both **8a** and **8b** have been incorporated into RNA fragments and were used successfully to help determine the complete structure of an aptamer−AMP complex[35−37] and to characterize an intrahelical GU wobble pair.[38] In addition, **8c** and **8d** have been incorporated into several different DNA molecules for NMR studies which are under way.

Experimental Section

General Methods. Melting points (mp) were determined in soft glass capillary tubes and are uncorrected. The ^{15}N chemical shifts are reported relative to NH_3, using external 1 M [^{15}N]HNO_3 in 90% D_2O at 25 °C at 375.8 ppm as a reference. Analytical HPLC was carried out with Waters C-18 Nova-Pak cartridges (8 × 100 mm) using a gradient of 2−40% acetonitrile/0.1 M triethylammonium acetate (TEAA) at a flow rate of 2 mL/min. Preparative HPLC was carried out with three Waters Delta-Pak PrepPak cartridges (40 × 100 mm, C_{18} 300 Å, 15 μm) in series at a flow rate of 40 mL/min.

The MCPBA from Aldrich (50−60% pure, along with 3-chlorobenzoic acid) was purified before use by dissolving in ether and washing with three portions of 0.1 M aqueous potassium phosphate (pH 7.5). Care should be taken while using this peroxy acid. The [^{15}N]NH_4Cl, [^{15}N]KCN, and [^{13}C,^{15}N]KCN were obtained from Isotec Inc. Adenosine deaminase (A-5773) was obtained from Sigma Chemical Co. General reagents were obtained from Aldrich Chemical Co.

6-Chloro-9-(β-D-ribofuranosyl)purine (2). A mixture of inosine (**1**) (9.24 g, 34.4 mmol) and trifluoroacetic anhydride (55 mL) in CH_2Cl_2 (180 mL) was stirred at rt for 16 h and then concentrated to a white foam (high vacuum), dissolved in CH_2Cl_2 (920 mL), and cooled to 0 °C. To this solution was added over 10 min a premixed solution of DMF (7 mL) and thionyl chloride (14 mL) in CH_2Cl_2 (370 mL), and the resulting cloudy suspension was refluxed for 16 h. This solution was concentrated to ca. 300 mL and washed with saturated $NaHCO_3$ (200 mL). The aqueous phase was back extracted with CH_2Cl_2 (3 × 30 mL), and the combined organic phases were dried with Na_2SO_4 and concentrated. The resulting yellow oil was dissolved in MeOH (30 mL), and the mixture was refluxed for 16 h. Ether (60 mL) was added to the cooled (0 °C) suspension, and the resulting precipitate was filtered and dried under vacuum (7.91 g, 80%): mp 160−163 °C (lit.[39] 182−183.5 °C); ^{1}H NMR (DMSO-d_6) δ 8.97 (s, 1 H), 8.84 (s, 1 H), 6.06 (d, 1 H, J = 5.1 Hz), 5.59 (d, 1 H, J = 5.9 Hz), 5.28 (d, 1 H, J = 5.1 Hz), 5.12 (t, 1 H, J = 5.5 Hz), 4.58 (m, 1 H), 4.21 (m, 1 H), 4.10 (m, 1 H), 3.5−3.8 (m, 2H); ^{13}C NMR (DMSO-d_6) δ 151.8, 151.6, 149.4, 145.8, 131.4, 88.3, 85.8, 74.1, 70.1, 61.0; MS (CI) 289 (M^+ [^{37}Cl] + H)$^+$, 287 (M^+ [^{35}Cl] + H)$^+$. Anal. Calcd for $C_{10}H_{11}O_4N_4Cl$: C, 41.90; H, 3.87; N, 19.56. Found: C, 42.00; H, 3.77; N, 19.59.

[$^{15}NH_2$]-Adenosine (3a). A mixture of **2** (5.35 g, 18.7 mmol), [^{15}N]-NH_4Cl (1.99 g, 37.2 mmol), and $KHCO_3$ (5.62 g, 56.1 mmol) in DMSO (28 mL) was sealed in a 100 mL bottle which was kept in an oven at 80 °C for 2 days. The cooled (0 °C) bottle was opened carefully (CO_2 pressure), the contents were filtered and rinsed with DMSO, and the filtrate was concentrated to ca. 10 mL. This solution was diluted with water (20 mL) and the product 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 4.27 g (84%) of **3a**·0.25H_2O: mp 232−234 °C (lit.[40] 233−234 °C); ^{1}H NMR (DMSO-d_6) δ 8.34 (s, 1 H), 8.12 (s, 1 H), 7.56 (d, 2 H, J = 90.1 Hz), 5.86 (d, 1 H, J = 6.2 Hz), 5.4 (m, 2 H), 5.17 (d, 1 H, J = 4.4 Hz), 4.60 (m, 1 H), 4.14 (m, 1 H), 3.95 (m, 1 H), 3.8−3.4 (m, 2 H); ^{13}C NMR (DMSO-d_6) δ 156.4 (d, $J_{C-^{15}N}$ = 20.6), 152.7, 149.3, 140.2, 119.7, 88.2, 86.2, 73.7, 70.9, 62.0; MS (EI) m/z (relative intensity) 268 (M^+, 3), 136 ([b + H]$^+$, 100). Anal. Calcd for $C_{10}H_{13}O_4N_4{}^{15}N_1$·0.25$H_2O$: C, 44.04; H, 4.80; N, 25.68. Found: C, 43.78; H, 4.82; N, 25.69.

[$^{15}NH_2$]-Adenosine N^1-Oxide (4a). To 2.55 g (9.19 mmol) of [$^{15}NH_2$]-adenosine hydrate **3a** dissolved in 300 mL of 30% aqueous dioxane was added 3.17 g (18.4 mmol) of 3-chloroperoxybenzoic acid (MCPBA). The mixture was allowed to stir in the dark at room temperature for 3 h and was then concentrated to about 50 mL. This solution was then washed with 3 × 100 mL of ethyl ether, and the aqueous layer was concentrated to a small volume and purified by preparative reversed phase HPLC with a gradient of 0 to 12% CH_3CN in H_2O in 30 min at a flow rate of 40 mL/min. Evaporation of appropriate fractions gave pure **4a** (2.31 g, 8.44 mmol, 92%): mp 217 °C dec; UV (H_2O) λ_{max} 235, 263 nm; UV (H_2O) λ_{min} 254 nm; ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm) 8.62 (s, 1), 8.53 (s, 1), 8.30 (br, 2), 5.87 (d, 1, J = 5.6 Hz), 5.54 (d, 1, J = 6.0 Hz), 5.22 (d, 1, J = 5.0 Hz), 5.05 (t, 1, J = 5.8 Hz), 4.52 (m, 1), 4.13 (m, 1), 3.93 (m, 1), 3.60 (m, 2); ^{13}C NMR (^{1}H decoupled, 50.3 MHz, DMSO-d_6) δ (ppm) 148.6 (d, J = 21.8 Hz), 143.6, 142.7, 142.2, 119.1, 87.7, 86.8, 74.1, 70.5, 61.5.

[1,NH_2-$^{15}N_2$]-2-Amino-6-(methoxyamino)-9-(β-D-ribofuranosyl)purine (7a). To 1.00 g (15 mmol) of [^{15}N]KCN dissolved in 250 mL of anhydrous methanol and cooled to 0 °C was added bromine (0.773 mL, 15 mmol). After 3 h of stirring, 2.84 g (10 mmol) of **4a** was added. After an additional 3 h, the reaction mixture was concentrated to dryness. The residue was dissolved in a mixture of anhydrous DMF (45 mL) and triethylamine (4 mL, 28.8 mmol) under N_2. The mixture was stirred at room temperature for 40 min, after which 5 mL of CH_3I (80.6 mmol) was added. Stirring was continued for a further 4 h in darkness, whereupon the reaction mixture was concentrated to dryness and the residue dissolved in 170 mL of 0.25 N NaOH. After 1 h, the pH was adjusted to 7.4 with 1 N HCl. 95% Ethanol (180 mL) was then added, and the mixture was heated at 60 °C for 4 h. The mixture was then concentrated to a small volume and purified by preparative reversed phase HPLC with a gradient of 2 to 14% acetonitrile in 0.1 M ammonium bicarbonate in 40 min at a flow rate of 40 mL/min. Evaporation of appropriate fractions gave pure **7a** (2.25 g, 7.15 mmol, 72%): UV (H_2O) λ_{max} 214, 281 nm; UV (H_2O) λ_{min} 241 nm; ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm) 7.75 (s, 1), 6.45 (d, 2, J = 89.7 Hz), 5.62 (d, 1, J = 5.8 Hz), 4.37 (m, 1), 4.05 (m, 1), 3.84 (m, 1), 3.72 (s, 3), 3.54 (m, 2).

[1,NH_2-$^{15}N_2$]-Guanosine (8a). To 2.25 g (7.15 mmol) of **7a** dissolved in 150 mL of 0.1 M phosphate buffer (pH 7.4) was added adenosine deaminase (430 units). The mixture was shaken in an oven at 37 °C for 2 days, during which time the product crystallized. The mixture was then cooled to 0 °C and filtered, and the crude product was purified by recrystallization from water to give 1.78 g of **8a** (5.01 mmol, 70%): mp 239 °C dec; ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm) 10.61 (d, 1, J = 88.5 Hz), 7.92 (s, 1), 6.44 (d, 2, J = 89.5 Hz), 5.68 (d, 1, J = 5.8 Hz), 5.37 (d, 1, J = 5.9 Hz), 5.09 (d, 1, J = 4.3 Hz), 5.02 (t,

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1, $J = 5.0$ Hz), 4.36 (m, 1), 4.07 (m, 1), 3.85 (m, 1), 3.56 (m, 2); ^{13}C NMR (^1H decoupled, 50.3 MHz, DMSO-d_6) δ (ppm) 156.8 (d, $J = 10.7$ Hz), 153.7 (d of d, $J = 23$ Hz, $J = 13$ Hz), 151.4, 135.6, 116.3 (d, $J = 7.9$ Hz), 86.3, 85.2, 73.7, 70.4, 61.4; ^{15}N NMR (40.543 MHz, DMSO-d_6) δ (ppm) 149.98 (d, $J = 70.2$ Hz), 75.87 (t, $J = 88.5$ Hz); EI MS *m/z* 285, 153, 135, 109.

[2-^{13}C-1,NH$_2$-^{15}N$_2$]-Guanosine (8b). To 2.03 g (30 mmol) of [^{15}N,^{13}C]-KCN dissolved in 450 mL of anhydrous methanol and cooled to 0 °C was added bromine (1.55 mL, 30 mmol). After 3 h of stirring, 5.67 g (20.0 mmol) **4a** was added. After an additional 3 h, the reaction mixture was concentrated to dryness. The residue was dissolved in a mixture of anhydrous DMF (80 mL) and triethylamine (8 mL, 57.6 mmol) under N_2. The mixture was stirred at room temperature for 40 min, after which 8 mL of CH_3I (129 mmol) was added. Stirring was continued for a further 4 h in darkness, whereupon the reaction mixture was concentrated to dryness and the residue dissolved in 340 mL of 0.25 N NaOH. After 1 h, the pH was adjusted to 7.4 with 1 N HCl. 95% Ethanol (400 mL) was then added, and the mixture was heated at 60 °C for 4 h. The mixture was then concentrated to a small volume and purified by preparative reversed phase HPLC with a gradient of 2 to 14% acetonitrile in 0.1 M ammonium bicarbonate in 35 min at a flow rate of 40 mL/min. Evaporation of appropriate fractions gave 4.04 g (12.8 mmol, 64%) of pure [2-^{13}C-1,NH$_2$-^{15}N$_2$]-2-amino-6-(methoxyamino)-9-(β-D-ribofuranosyl)purine which was dissolved in 270 mL of 0.1 M phosphate buffer (pH 7.4). Adenosine deaminase (770 units) was then added. The mixture was shaken in an oven at 37 °C for 2 days, during which time the product crystallized. The mixture was then cooled to 0 °C and filtered. The crude product was purified by recrystallization from water to give 2.63 g of **8b** (9.13 mmol, 71%): mp 239 °C dec; ^1H NMR (200 MHz, DMSO-d_6) δ (ppm) 10.61 (d, 1, $J = 89.0$ Hz), 7.92 (s, 1), 6.44 (d, 2, $J = 89.5$ Hz), 5.68 (d, 1, $J = 5.9$ Hz), 5.38 (d, 1, $J = 6.2$ Hz), 5.11 (d, 1, $J = 4.7$ Hz), 5.02 (t, 1, $J = 5.0$ Hz), 4.38 (m, 1), 4.07 (m, 1), 3.84 (m, 1), 3.56 (m, 2); ^{13}C NMR (^1H decoupled, 50.3 MHz, DMSO-d_6) δ (ppm) 156.8 (d, $J = 10.7$ Hz), 153.6 (d of d, $J = 22.6$ Hz, $J = 13.1$ Hz), 151.4, 135.6, 116.3 (d, $J = 7.9$ Hz), 86.3, 85.2, 73.7, 70.4, 61.4; ^{15}N NMR (40.543 MHz, DMSO-d_6) δ (ppm) 150.01 (d of d, $J_{N-H} = 88.5$ Hz, $J_{N-C} = 12.2$ Hz), 75.79 (t of d, $J_{N-H} = 88.5$ Hz, $J_{N-C} = 24.4$ Hz); EI MS *m/z* 286, 154, 136, 110.

[7,NH$_2$-^{15}N$_2$]-2′-Deoxyadenosine *N*^{1}-Oxide (4b). To 2.72 g (10.0 mmol) of [7,NH$_2$-^{15}N$_2$]-2′-deoxyadenosine hydrate, **3b**,[28] dissolved in 400 mL of 50% aqueous methanol was added 6.67 g (38.6 mmol) of 3-chloroperoxybenzoic acid (MCPBA). The mixture was stirred in the dark at room temperature for 3 h and was then concentrated to give about 150 mL of a cloudy mixture. It was washed with 2 × 200 mL of ethyl ether, and the aqueous layer was immediately neutralized to pH 7 with 0.25 M NaOH. The solution was then concentrated to a small volume and purified by preparative reversed phase HPLC with a gradient of 0 to 12% acetonitrile in water in 30 min at a flow rate of 40 mL/min. Evaporation of appropriate fractions gave pure **4b** (2.35 g, 8.74 mmol, 87%): mp 212 °C dec; UV (H_2O) λ_{max} 232, 262 nm; λ_{min} 251 nm; ^1H NMR (200 MHz, DMSO-d_6) δ (ppm) 8.62 (s, 1), 8.50 (d, 1, $J_{N-H} = 12.1$ Hz), 8.20 (br, 2), 6.31 (t, 1, $J = 6.6$ Hz), 5.34 (d, 1, $J = 4.0$ Hz), 4.95 (t, 1, $J = 5.2$ Hz), 4.39 (m, 1), 3.85 (m, 1), 3.55 (m, 2), 2.69 and 2.32 (m and m, 2); ^{13}C NMR (^1H decoupled, 50.3 MHz, DMSO-d_6) δ (ppm) 148.3 (d of d, $J = 21.9$ Hz, $J = 6.0$ Hz), 143.3, 142.3, 141.6, 118.8, 88.0, 83.6, 70.6, 61.6.

[1,7,NH$_2$-^{15}N$_3$]-2-Amino-6-(methoxyamino)-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine (7c). The procedure for **7c** was analogous to that for **7a**, using 0.73 g (11.0 mmol) of [^{15}N]KCN, dissolved in 250 mL anhydrous methanol, 0.55 mL, (10.6 mmol) of bromine, 2.11 g (7.85 mmol) of **4b**, 40 mL of DMF, 4 mL of triethylamine, and 4 mL of CH_3I (64.5 mmol), to give **7c** (1.77 g, 5.91 mmol, 75%): ^1H NMR (200 MHz, DMSO-d_6) δ (ppm) 7.74 (d, 1, $J_{N-H} = 12.0$ Hz), 6.45 (d, 2, $J_{N-H} = 89.2$ Hz), 6.05 (t, 1, $J = 6.6$ Hz), 5.20 (m, 2); 4.30 (m, 1), 3.77 (m, 1), 3.72 (s, 3), 3.50 (m, 2); 2.50 and 2.16 (m and m, 2).

[1,7,NH$_2$-^{15}N$_3$]-2′-Deoxyguanosine (8c). To 1.77 g (5.91 mmol) of **7c** dissolved in 120 mL of 0.1 M TEAA (pH 7.4) was added adenosine deaminase (355 units). The mixture was shaken in an oven at 37 °C for 5 days and then directly purified by preparative reversed phase HPLC with a gradient of 0 to 12% acetonitrile in 0.1 M ammonium bicarbonate in 40 min at a flow rate of 40 mL/min. Evaporation of appropriate fractions gave 1.51 g of **8c** (5.59 mmol, 95%): mp 245 °C dec; ^1H NMR (200 MHz, DMSO-d_6) δ (ppm) 10.59 (d, 1, $J_{N-H} = 88.3$ Hz), 7.90 (d, 1, $J_{N-H} = 12.0$ Hz), 6.43 (d, 2, $J_{N-H} = 89.5$ Hz), 6.10 (d, 1, $J = 6.2$ Hz), 5.24 (d, 1, $J = 4.0$ Hz) 4.93 (t, 1, $J = 5.5$ Hz), 4.32 (m, 1), 3.79 (m, 1), 3.85 (m, 1), 3.50 (m, 2), 2.49 and 2.20 (m and m, 2); ^{13}C NMR (^1H decoupled, 50.3 MHz, DMSO-d_6) δ (ppm) 156.9 (d of d, $J = 10.8$ Hz, $J = 6.3$ Hz), 153.7 (d of d, $J = 22.8$ Hz, $J = 13.3$ Hz), 150.9, 135.3, 116.7 (d, $J = 7.7$ Hz), 87.6, 82.6, 70.8, 61.8; ^{15}N NMR (40.543 MHz, DMSO-d_6) δ (ppm) 250. 81 (d, $J_{N-H} = 12.2$ Hz), 149.71 (s), 75.42 (t, $J = 91.6$ Hz); EI MS *m/z* 270, 154, 136, 110.

[2-^{13}C-1,7,NH$_2$-^{15}N$_3$]-2-Amino-6-(methoxyamino)-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine (7d). The procedure for **7d** was analogous to that for **7a**, using 0.73 g (11.0 mmol) of [^{13}C,^{15}N]-KCN, dissolved in 250 mL of anhydrous methanol, 0.55 mL (10.6 mmol) of bromine, 2.11 g (7.85 mmol) of **4b**, 40 mL of DMF, 4 mL of triethylamine, and 4 mL of CH_3I (64.5 mmol), to give **7d** (1.75 g, 5.83 mmol, 74%): ^1H NMR (200 MHz, DMSO-d_6) δ (ppm) 7.71 (d, 1, $J_{N-H} = 12.1$ Hz), 6.49 (d, 2, $J_{N-H} = 89.3$ Hz), 6.04 (t, 1, $J = 6.2$ Hz), 5.22 (m, 1), 4.97 (m, 1), 4.30 (m, 1), 3.76 (m, 1), 3.72 (s, 3), 3.50 (m, 2), 2.50 and 2.16 (m and m, 2).

[2-^{13}C-1,7,NH$_2$-^{15}N$_3$]-2′-Deoxyguanosine (8d). The procedure for **8d** was analogous to that for **8c** using 1.75 g (5.83 mmol) of **7d** dissolved in 120 mL of 0.1 M TEAA (pH 7.4), and 350 units of adenosine deaminase to give 1.58 g of **8d** (5.82 mmol, 99%): mp 245 °C dec; ^1H NMR (200 MHz, DMSO-d_6) δ (ppm) 10.59 (d, 1, $J_{N-H} = 88.9$ Hz), 7.90 (d, 1, $J_{N-H} = 12.0$ Hz), 6.43 (d, 2, $J_{N-H} = 89.7$ Hz), 6.10 (d, 1, $J = 6.2$ Hz), 5.24 (d, 1, $J = 3.8$ Hz), 4.93 (t, 1, $J = 5.5$ Hz), 4.31 (m, 1), 3.79 (m, 1), 3.85 (m, 1), 3.51 (m, 2); 2.49 and 2.20 (m and m, 2); ^{13}C NMR (^1H decoupled, 50.3 MHz, DMSO-d_6) δ (ppm) 156.8 (d of d, $J = 10.6$ Hz and $J = 6.4$ Hz), 153.6 (d of d, $J = 22.8$ Hz and $J = 13.3$ Hz), 150.9, 135.3, 116.7 (d, $J = 5.5$ Hz), 87.6, 82.6, 70.8, 61.8; ^{15}N NMR (40.543 MHz, DMSO-d_6) δ (ppm) 250.81 (d, $J_{N-H} = 12.2$ Hz), 149.64 (d, $J_{N-C} = 12.2$ Hz), 75.42 (t of d, $J_{N-H} = 88.5$ Hz, $J_{N-C} = 24.4$ Hz); EI MS *m/z* 271, 155, 137, 111.

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