Syntheses of [1,7-$^{15}N_2$]- and [1,7,NH_2-$^{15}N_3$]Adenosine and 2′-Deoxyadenosine via an N^1-Alkoxy-Mediated Dimroth Rearrangement

Alex R. Pagano, Hong Zhao, Anthony Shallop, and Roger A. Jones*

Department of Chemistry, Rutgers, The State University of New Jersey, Piscataway, New Jersey 08855

Received October 1, 1997

We have found that the N^1-methoxy derivatives of adenosine and 2′-deoxyadenosine undergo a Dimroth rearrangement in which the intermediate *N*,*N*-dimethylamino adducts are stable compounds. This mild and high-yield rearrangement allows efficient conversion of [7,NH_2-$^{15}N_2$]-2′-deoxyadenosine and [7,NH_2-$^{15}N_2$]adenosine into the triply labeled [1,7,NH_2-$^{15}N_3$] derivatives. The [7,NH_2-$^{15}N_2$]nucleoside is first oxidized to the N^1-oxide with 3-chloroperoxybenzoic acid (MCPBA). Methylation of the N^1-oxide with methyl iodide or dimethyl sulfate is followed by treatment with dimethylamine to afford the 6-amino-N^1-methoxy-2-(*N*,*N*-dimethylamino) derivative. Dimroth rearrangement of these surprisingly stable intermediates is accomplished by refluxing in the presence of a dimethylammonium hydrohalide salt to give the [1,7-$^{15}N_2$]-6-*N*-methoxy nucleosides in high yield. Removal of the N^6-methoxy function to afford both [1,7-$^{15}N_2$]deoxyadenosine and [1,7-$^{15}N_2$]adenosine was accomplished readily with Raney nickel. No hydroxyl protection is required for these transformations. Introduction of the third label at the N^6 was accomplished by conversion into the 6-(1,2,4-triazol-4-yl)purine nucleosides, followed by nucleophilic displacement of the 6-triazole with [^{15}N]ammonia to afford the triply labeled title compounds.

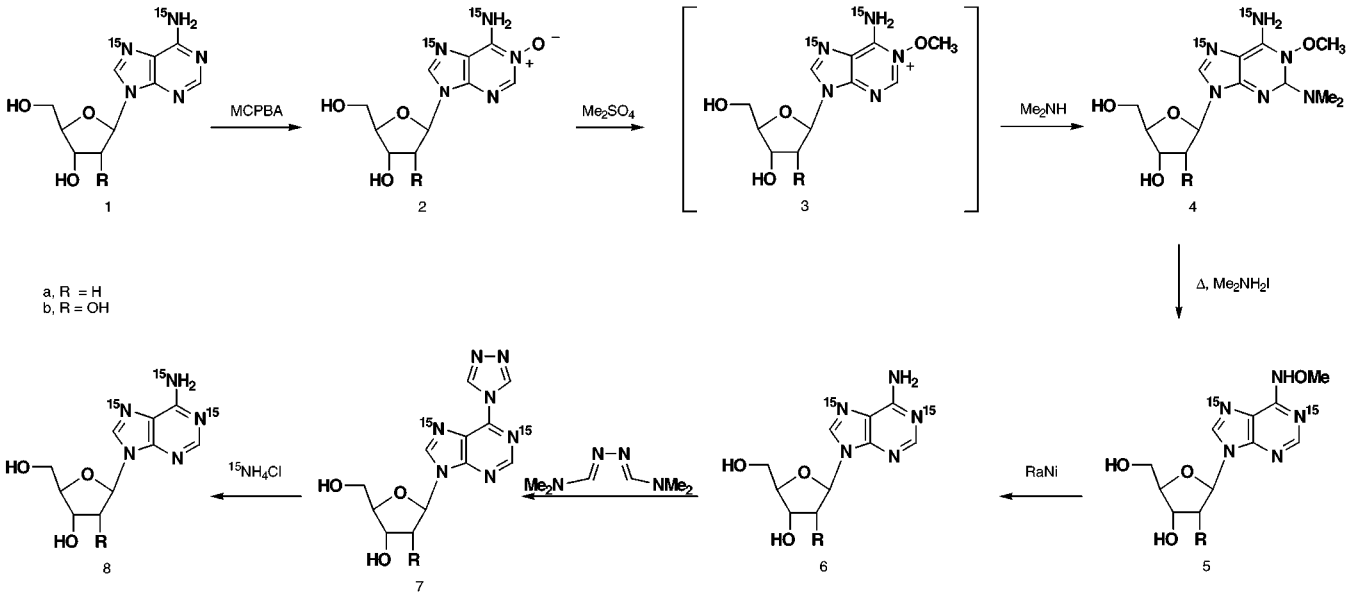
NMR studies of nucleic acid structure and interactions using ^{15}N-labeled DNA and RNA fragments have demonstrated the value of these labels.[1−11] Accordingly, there are now many synthetic routes available for ^{15}N labeling of bases[12−14] or nucleosides.[2,15−27] These routes, however, generally give singly labeled products. It would be more useful for NMR experiments to have in the molecule or complex as many ^{15}N labels as can be unambiguously distinguished. We have therefore begun development of routes designed to give multilabeled nucleosides. The syntheses of [7,NH_2-$^{15}N_2$]adenosine and 2′-deoxyadenosine,[28] and [1,NH_2-$^{15}N_2$]-, [1,NH_2-$^{15}N_2$,2-^{13}C]-, [1,7,NH_2-$^{15}N_3$]-, and [1,7,NH_2-$^{15}N_3$,2-^{13}C]guanosine and 2′-deoxyguanosine have been completed recently.[29] We now report a novel Dimroth rearrangement of the adenine N^6 to the N^1 in high yield by a procedure equally appropriate for both adenosine and 2′-deoxyadenosine, and application of Robins' 6-triazole derivative[30] to effect efficient introduction of ^{15}N to the 6-amino.

We had previously used a Dimroth rearrangement of an N^1-benzylated 2′-deoxyadenosine derivative for preparation of [1-^{15}N]deoxyadenosine, but competing depurination kept the yields moderate at best, and protection of the sugar hydroxyls was required.[17] Because of these modest overall yields, this procedure was not viable for preparation of multilabeled nucleosides. In attempting to find a new route that would avoid these problems, we chose to explore transformation of the nucleoside N^1-oxides. These are stable compounds that are obtained

(1) Gao, X.; Jones, R. A. *J. Am. Chem. Soc.* **1987**, *109*, 3169−3171.
(2) Kupferschmitt, G.; Schmidt, J.; Schmidt, T.; Fera, B.; Buck, R.; Rüterjans, H. *Nucleic Acids Res.* **1987**, *15*, 6225−6241.
(3) Massefski, W., Jr.; Redfield, A.; Sarma, U. D.; Bannerji, A.; Roy, S. *J. Am. Chem. Soc.* **1990**, *112*, 5350−5351.
(4) Wang, C.; Gao, X.; Jones, R. A. *J. Am. Chem. Soc.* **1991**, *113*, 1448−1450.
(5) Wang, C.; Gao, H.; Gaffney, B. L.; Jones, R. A. *J. Am. Chem. Soc.* **1991**, *113*, 5486-5488.
(6) Gaffney, B. L.; Wang, C.; Jones, R. A. *J. Am. Chem. Soc.* **1992**, *114*, 4047−4050.
(7) Goswami, B.; Gaffney, B. L.; Jones, R. A. *J. Am. Chem. Soc.* **1993**, *115*, 3832−3833.
(8) Rhee, Y.; Wang, C.; Gaffney, B. L.; Jones, R. A. *J. Am. Chem. Soc.* **1993**, *115*, 8742−8746.
(9) Macmillan: A. M.; Lee, R. J.; Verdine, G. L. *J. Am. Chem. Soc.* **1993**, *115*, 4921−4922.
(10) Gaffney, B. L.; Goswami, B.; Jones, R. A. *J. Am. Chem. Soc.* **1993**, *115*, 12607−12608.
(11) Gaffney, B. L.; Kung, P.-P.; Wang, C.; Jones, R. A. *J. Am. Chem. Soc.* **1995**, *117*, 12281−12283.
(12) Barrio, M. D. C. G.; Scopes, D. I. C.; Holtwick, J. B.; Leonard, N. J. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 3986−3988.
(13) Sethi, S. K.; Gupta, S. P.; Jenkins, E. E.; Whitehead, C. W.; Townsend, L. B.; McCloskey, J. A. *J. Am. Chem. Soc.* **1982**, *104*, 3349−3353.
(14) Griffey, R.; Poulter, C. D. *Nucleic Acids Res.* **1983**, *11*, 6497−6504.
(15) Wilson, M. H.; McCloskey, J. A. *J. Org. Chem.* **1973**, *38*, 2247−2249.
(16) Poulter, C. D.; Livingston, C. L. *Tetrahedron Lett.* **1979**, *9*, 755−758.
(17) Gao, X.; Jones, R. A. *J. Am. Chem. Soc.* **1987**, *109*, 1275−1278.
(18) Sarfati, S. R.; Kansal, V. K. *Tetrahedron* **1988**, *44*, 6367−6372.
(19) Gaffney, B. L.; Kung, P.-P.; Jones, R. A. *J. Am. Chem. Soc.* **1990**, *112*, 6748−6749.
(20) Adler, J.; Powell, W.; Wolfenden, R. *J. Am. Chem. Soc.* **1990**, *112*, 1247−1248.
(21) Rhee, Y. S.; Jones, R. A. *J. Am. Chem. Soc.* **1990**, *112*, 8174−8175.
(22) Goswami, B.; Jones, R. A. *J. Am. Chem. Soc.* **1991**, *113*, 644−647.
(23) Acedo, M.; Fabrega, C.; Aviño, A.; Goodman, M.; Fagan, P.; Wemmer, D.; Eritja, R. *Nucleic Acids Res.* **1994**, *22*, 2982−2989.
(24) Sako, M.; Ishikura, H.; Hirota, K.; Maki, Y. *Nucleosides Nucleotides* **1994**, *13*, 1239−1246.
(25) Bleasdale, C.; Ellwood, S. B.; Golding, B. T.; Slaich, P. K.; Taylor, O. J.; Watson, W. P. *J. Chem. Soc., Perkin Trans. 1* **1994**, 2859−2865.
(26) Ariza, X.; Bou, V.; Vilarrasa, J. *J. Am. Chem. Soc.* **1995**, *117*, 3665−3673.
(27) Kamaike, K.; Takahashi, M.; Utsugi, K.; Tomizuka, K.; Ishido, Y. *Tetrahedron Lett.* **1995**, *36*, 91−94.
(28) Pagano, A. R.; Lajewski, W. M.; Jones, R. A. *J. Am. Chem. Soc.* **1995**, *117*, 11669−11672.
(29) Zhao, H.; Pagano, A. R.; Wang, W.; Shallop, A.; Gaffney, B. L.; Jones, R. A. *J. Org. Chem.* **1997**, *62*, 7832−7835.

S0022-3263(97)01815-X CCC: $15.00
Published on Web 04/23/1998

Scheme 1



in high yield for both adenosine and 2′-deoxyadenosine by oxidation with 3-chloroperoxybenzoic acid (MCPBA).[31] By rough analogy to the procedure that has worked well for conversion of adenine to guanine nucleosides,[29] we planned to alkylate the N^1-oxides and then explore use of a Dimroth rearrangement, with the idea that the N^1-methoxy intermediates might prove more stable than the N^1-benzyl intermediates. In fact, we have observed no depurination during the transformation, and the adduct **4** is stable and has been isolated and characterized.

As shown in Scheme 1, the N^1-oxide **2** is first methylated by reaction with dimethyl sulfate, followed by addition of dimethylamine without isolation of the N^1-methoxy derivative **3**. In the absence of heating, the resulting adduct (**4**) does not undergo rearrangement. The rearrangement can be carried out without isolation of **4** simply by refluxing the methanolic reaction mixture. If **4** is isolated, however, the addition of an equivalent of amine hydrohalide is necessary for the rearrangement. This is analogous to the dimethylamine hydroiodide present in the reaction mixture which apparently serves as a weak acid promoter of the rearrangement. In either case, rearrangement proceeds cleanly with no evidence of depurination. Moreover, the N^1-methoxy group in **5** is amenable to facile removal with Raney nickel, again without the need for sugar hydroxyl protection. The conversion of **2** to **6** is best performed in a continuous sequence, without isolation and purification of intermediates to give **6a** and **6b** in 73% and 81% yields, respectively, from **2a** and **2b**. This set of reactions to **6** proceeds in significantly higher overall yield than our previous route and is applicable to preparation of multilabeled compounds. This route is not accompanied by depurination even for the deoxynucleoside, and no protection/deprotection steps are involved.

Incorporation of the third ^{15}N label is based on the conversion of the 6-amino function to the 6-(1,2,4-triazol-4-yl)purine derivative, recently reported by Robins.[30,32] We had previously used a nonaqueous diazotization reaction to convert 2′-deoxyadenosine to the 6-chloro derivative to introduce the [6-^{15}N] amino group, but the yield for the diazotization reaction was only about 50%.[17] In contrast, Robins' procedure for conversion into the 6-triazole derivative **7** proceeds in high yield. The conditions for optimal displacement of the 6-triazole moiety with [^{15}N]ammonia differed significantly between **7a** and **7b**. The former proceeds cleanly using 2 equiv of [^{15}N]ammonium chloride and potassium *tert*-butoxide in DMSO at 80 °C in a yield of 82%, while the latter requires much different conditions. In the case of **7b**, 10 equiv of [^{15}N]ammonium chloride is needed to obtain comparable yields, along with potassium carbonate as the base. The excess [^{15}N]ammonia required in this case was recovered by purging the reaction mixture with helium and bubbling the exit stream through 10% hydrochloric acid.

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.

[7,*NH*$_2$-$^{15}N_2$]-2′-Deoxyadenosine N^1-Oxide (2a). To a solution of **1a** hydrate (3.01 g, 11.1 mmol) in 80 mL of 50% aqueous methanol was added 2.8 g of 3-chloroperoxybenzoic acid (MCPBA). The reaction mixture was stirred at room temperature for 16 h, diluted with 100 mL of water, and

(30) Samano, V.; Miles, R. W.; Robins, M. J. *J. Am. Chem. Soc.* **1994**, *116*, 9331−9332.

(31) MacCoss, M.; Ryu, E. K.; White, R. S.; Last, R. L. *J. Org. Chem.* **1980**, *45*, 788−794.

(32) Miles, R. W.; Samano, V.; Robins, M. J. *J. Am. Chem. Soc.* **1995**, *117*, 5951−5957.

extracted with ether (2 × 50 mL). The aqueous phase was concentrated to 20 mL and the mixture purified by reversed phase preparative chromatography (0−20% CH_3CN/water). The combined fractions of **2a** were concentrated to dryness and the solid dried in a vacuum desiccator over P_2O_5 for 24 h to afford 2.73 g (91%) of pure **2a**: mp 216−218 °C (dec); ^{1}H NMR (D_2O) δ 8.61 (s, 1 H), 8.43 (d, 1 H, J = 11.4 Hz), 6.33 (t_{app}, 1 H, J = 6.3 Hz), 4.65 (m, 1 H), 3.85 (m, 1 H), 3.9−3.7 (m, 2 H), 2.92−2.81 (m, 1 H), 2.67−2.61 (m, 1H); ^{13}C NMR (50.3 MHz, D_2O) δ 151.14 (dd, $J_{C-^{15}N6}$= 23.3, $J_{C-^{15}N7}$ = 5.8 Hz, C6), 146.9, 145.8, 121.8, 100.3, 90.3, 87.5, 73.8, 64.4, 41.9; ^{15}N NMR (D_2O) δ 231.59 (d, J = 10.7 Hz, N7), 74.59 (s, NH_2); MS (EI) m/z (relative intensity) 269 (M^+, 5.4), 137 (100, $[b + 2H]^+$); HRMS m/z 269.0905 (calcd for $C_{10}H_{13}O_4N_3{}^{15}N_2$ 269.0908). Anal. Calcd for $C_{10}H_{13}O_4N_3{}^{15}N_2$: C, 44.61; H, 4.87; N, 26.01. Found: C, 44.58; H, 4.89; N, 26.18.

[7,*NH*$_2$-^{15}N$_2$]-6-Amino-2-(*N*,*N*-dimethylamino)-*N*^{1}-methoxy-9-(2-deoxy-β-*erythro*-pentofuranosyl)purine (4a). A. Unlabeled. A mixture of 2′-deoxyadenosine N^1-oxide (prepared as above, 1.07 g, 4.00 mmol) and dimethyl sulfate (0.615 g, 4.88 mmol) in *N*,*N*-dimethylacetamide (10 mL) was stirred at room temperature for 24 h. The reaction mixture was chilled (0 °C) and 10 mL of 1:1 dimethylamine:methanol was added slowly. The mixture was allowed to warm to room temperature and was stirred for 2 h, concentrated to a small volume at room temperature (oil pump vacuum is required), and applied to a preparative reversed phase HPLC system. Concentration of the product fractions gave 1.17 g (90%): mp 188−190 °C; ^{1}H NMR (DMSO-d_6) δ 8.60 (s, 1 H), 7.60 (s, 1 H), 6.03 (t_{app}, 1 H, J = 6.3 Hz), 5.5 (s, 2H), 5.20 (d, 1 H, J = 4.2 Hz), 4.85 (t, 1 H, J = 5.4 Hz), 4.25 (m, 1 H), 3.75 (m, 1 H), 3.65 (s, 3H), 3.5−3.4 (m, 2 H), 3.0−2.8 (2s, 6H), 2.4−2.05 (m, 2H); ^{13}C NMR (50.3 MHz, D_2O) δ 162.1, 153.7, 143.8, 134.3, 118.96, 89.3, 85.6, 73.7, 64.3, 63.4, 42.9, 41.7, 36.7; MS (EI) m/z (relative intensity) 326 (M^+, 52), 135 (100); HRMS m/z 326.1675 (calcd for $C_{13}H_{22}0_4N_6$ 326.1704). Anal. Calcd for $C_{13}H_{22}0_4N_6$: C, 47.85; H, 6.79; N, 25.75. Found: C, 47.45; H, 6.71; N, 25.68.

B. Labeled. Using a similar procedure **4a** was prepared: mp 188−190 °C; ^{1}H NMR (DMSO-d_6) δ 8.60 (s, 1 H), 7.60 (d, 1 H, J = 11.4 Hz, H8), 6.03 (t_{app}, 1 H, J = 6.3 Hz), 5.5 (s, 2H), 5.20 (d, 1 H, J = 4.2 Hz), 4.85 (t, 1 H, J = 5.4 Hz), 4.25 (m, 1 H,), 3.75 (m, 1 H), 3.65 (s, 3H), 3.5−3.4 (m, 2 H), 3.0−2.8 (2s, 6H), 2.4−2.05 (m, 2H).

[1,7-^{15}N$_2$]-*N*^{6}-Methoxy-2′-deoxyadenosine (5a). A mixture of **2a** (1.73 g, 6.43 mmol) and methyl iodide (2.20 g, 15.5 mmol) in *N*,*N*-dimethylacetamide (20 mL) was stirred at room temperature for 24 h. The reaction mixture was chilled (0 °C), 20 mL of 1:1 dimethylamine:methanol was added slowly, and the mixture was allowed to warm to room temperature and stir for 1 h. The *N*,*N*-dimethylacetamide was removed with gentle heating (45 °C, oil pump vacuum is required), and the residue was dissolved in methanol (30 mL) and refluxed for 36 h to complete the Dimroth rearrangement. This mixture was concentrated and the crude solid recrystallized from water (25 mL) to afford 1.46 g of **5a** as the hemihydrate. A further 0.100 g of **5a** was isolated by reversed phase preparative HPLC of the mother liquor (0−20% CH_3CN/H_2O), affording a total yield of 1.56 g (83%): mp 131−140 °C (dec); ^{1}H NMR (DMSO-d_6) δ 11.6−10.9 (m, 1H), 8.5−7.5 (m, 2 H), 6.5−6.1 (m, 1 H), 5.32 (br s, 1 H), 5.2−4.9 (m, 1 H), 4.40 (br s, 1 H), 3.85 (m, 1 H), 3.77 (s, 3H), 3.7−3.4 (m, 2 H), 2.9−2.2 (m, 1 H); ^{13}C NMR (50.3 MHz, DMSO-d_6) δ 151.9 (br s), 144.3 (m), 140.6 (m), 136.9, 118.5, 87.9, 83.7 (m), 77.7, 63.5−60.94 (m), 39.9; ^{15}N NMR (DMSO-d_6) δ 248.5 (d, J = 11.1 Hz, N7), 244.6−244.4 (br s), 230−236 (br s), 140.5 (d, J = 93.9 Hz, N1) (Additional resonances in the NMR spectra presumably reflect the presence of a significant amount of an imine tautomer with *E* and *Z* isomers); MS (EI) m/z (relative intensity) 283 (M^+, 10.2), 137 (100); HRMS m/z 283.1044 (calcd for $C_{11}H_{15}O_4N_3{}^{15}N_2$ 283.1065). Anal. Calcd for $C_{11}H_{15}O_4N_3{}^{15}N_2{\cdot}0.5H_2O$ C, 45.21; H, 5.57 N, 23.96. Found: C, 45.22; H, 5.59; N, 23.86.

[1,7-^{15}N$_2$]-2′-Deoxyadenosine (6a). To a solution of **5a** (1.15 g, 3.93 mmol) in NH_4OH (30%, 30 mL) was added Raney nickel (50% water suspension, 3.93 g) and the suspension was heated (90 °C) for 2 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 the residue purified by reversed phase preparative HPLC (0−20% CH_3CN/H_2O). The combined fractions of **6a** were concentrated to dryness and the solid dried in a vacuum desiccator over P_2O_5 for 24 h to afford (82%) of pure **6a** monohydrate.

[1,7-^{15}N$_2$]-2′-Deoxyadenosine (6a). One-Flask Procedure from 2a. A mixture of **2a** (2.36 g, 8.77 mmol) and methyl iodide (2.85 g, 20.1 mmol) in *N*,*N*-dimethylacetamide (30 mL) was stirred at room temperature for 24 h. The reaction mixture was chilled (0 °C), 20 mL of 1:1 dimethylamine:methanol was added slowly, and the mixture was allowed to warm to room temperature and stir for 1 h. The volatiles were removed with gentle heating (45 °C, oil pump vacuum is required), and the residue was dissolved in methanol (20 mL) and refluxed for 48 h. This mixture was concentrated, the crude **5a** was dissolved in NH_4OH (30%, 55 mL), Raney nickel (50% water suspension, 10.7 g) was added, and the suspension was heated (90 °C) for 2 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 the residue purified by reversed phase preparative HPLC (0−20% CH_3CN/H_2O). The combined fractions of **6a** were concentrated to dryness and the solid dried in a vacuum desiccator over P_2O_5 for 24 h to afford 1.74 g (73% yield from **2a**) of pure **6a** monohydrate: mp 189.5−191 °C (lit.[33] mp 189−190 °C); ^{1}H NMR (DMSO-d_6) δ 8.31 (d, 1 H, J = 12.1 Hz), 8.11 (d, 1 H, J = 15.6 Hz), 7.29 (br s, 2 H), 6.33 (t_{app}, 1 H, J = 6.3 Hz), 5.29 (d, 1 H, J = 4.0 Hz), 5.23 (t, 1 H, J = 6.5 Hz), 4.40 (m, 1 H), 3.87 (m, 1 H), 3.7−3.4 (m, 2 H), 2.8−2.6 (m, 1 H), 2.3−2.1 (m, 1 H); ^{13}C NMR (50.3 MHz, D_2O) δ 157.9, 155.1, 150.8, 142.8, 121.5, 90.2, 87.4, 74.1, 64.7, 42.1; ^{15}N NMR (DMSO-d_6) δ 244.07 (d, J = 10.7 Hz, N7), 239.20 (d, J = 17.0 Hz, N1); MS (EI) m/z (relative intensity) 253 (M^+, 4), 137 (100, $[b + 2H]^+$); HRMS m/z 253.0937 (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, 25.85.

[1,7-^{15}N$_2$]-9-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-6-(1,2,4-triazol-4-yl)purine (7a). The procedure of Robins was used.[30] A mixture of **6a** (1.74 g, 6.41 mmol) was concentrated three times from pyridine (20 mL), and *N*,*N*-dimethylformamide azine (3.92 g, 27.6 mmol), pyridine (20 mL), and TMSCl (1.60 g, 14.7 mmol) were added to the residue, and the resulting solution was heated (100 °C) for 24 h. After the reaction cooled to room temperature, an additional portion of TMSCl (0.7 mL) was added and the solution was stirred for 10 min at room temperature and then concentrated. The brown syrup was dissolved in cold CH_2Cl_2 (175 mL) and washed once with a cold solution of brine (70 mL) and saturated aqueous $NaHCO_3$(35 mL) and twice with a solution of brine (15 mL) and aqueous HCl (1 M, 15 mL). The organic phase was dried (Na_2SO_4) and concentrated, and the residue was dissolved in MeOH (50 mL) and stirred overnight. The resulting suspension was concentrated, suspended in cold CH_2Cl_2, filtered, and air-dried to afford 1.70 g (73%) of pure **7a**: mp 174−176 °C (lit.[32] mp ca. 180 °C (variable)); ^{1}H NMR (DMSO-d_6) δ 9.67 (s, 2H, N=CH), 9.03 (d, 1H, J = 12.5 Hz), 8.95 (d, 1H, J = 16.1 Hz,), 6.55 (t_{app}, 1 H, J = 6.5 Hz), 5.6−4.8 (br m, 2H), 4.49 (m, 1 H), 3.92 (m, 1 H), 3.7−3.5 (m, 2 H), 2.8−2.4 (m, 1 H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 153.5, 152.2, 146.2, 142.9, 141.1, 122.8, 88.5, 84.6, 70.8, 61.7, 40.3−39.5 (m); ^{15}N NMR (DMSO-d_6) δ 251.31 (d, J = 17.1 Hz, N7), 239.96 (d, J = 10.7 Hz, N1); MS (EI) m/z (relative intensity) 305 (M^+, 1.5), 189 (100); HRMS m/z 305.1040 (calcd for $C_{10}H_{13}O_3N_3{}^{15}N_2$ 305.1022). Anal. Calcd for $C_{12}H_{13}O_3N_5{}^{15}N_2$: C, 47.21; H, 4.29; N, 32.12 Found: C, 47.34; H, 4.29; N, 32.16.

[1,7,*NH*$_2$-^{15}N$_3$]-2′-Deoxyadenosine (8a). A mixture of [^{15}N]NH_4Cl (0.601 g, 11.0 mmol) and potassium *tert*-butoxide (1.15 g, 10.2 mmol) in DMSO (10 mL) was sealed in a 22 mL sample vial with a rubber top seal and placed in an oven at 80 °C for 30 min. The cooled (0 °C) reaction vial was opened, **7a** (1.57 g, 5.13 mmol) was added quickly, and the vial was resealed and placed in the oven at 80 °C for 48 h. The reaction

mixture was cooled (0 °C) and diluted with water (20 mL), and the mixture was purified by reversed phase preparative chromatography (0−20% CH_3CN/H_2O). The combined fractions of **8a** were concentrated to dryness and the solid dried in a vacuum desiccator over P_2O_5 for 24 h to afford 1.15 g (82%) of pure **8a** monohydrate: mp 189−190 °C (lit.[33] 189−190 °C); ^{1}H NMR (DMSO-d_6) δ 8.34 (d, 1 H, J = 12.0 Hz), 8.14 (d, 1 H, J = 15.6 Hz, H2), 7.33 (dd, $J_{H-^{15}N6}$ = 90.1, $J_{H-^{15}N1}$ = 2.9 Hz, NH_2), 6.31 (t_{app}, 1 H, J = 6.3 Hz) 5.30 (d, 1 H, J = 3.9 Hz), 5.22 (t, 1 H, J = 6.5 Hz), 4.40 (m, 1 H), 3.87 (m, 1 H), 3.7−3.4 (m, 2 H), 2.8−2.6 (m, 1 H), 2.3−2.1 (m, 1H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 156.5 (d, J = 19.9 Hz, C6), 152.8, 149.3, 140.0, 119.7, 88.5, 84.5, 71.4, 62.4, 40.3−39.5 (m); ^{15}N NMR (DMSO-d_6) δ 243.8 (d, J = 12.7 Hz, N7), 239.0 (m, N1), 84.70 (dt, $J_{H-^{15}N6}$= 90.1, $J15_{N6-^{15}N1}$ = 4.3 Hz, N6); MS (EI) m/z (relative intensity) 254 (M^+, 4), 138 (100, [b +2H]$^+$); HRMS m/z 254.0916 (calcd for $C_{10}H_{13}O_3N_3{}^{15}N_2$ 254.0959). Anal. Calcd for $C_{10}H_{13}O_3N_2{}^{15}N_3\cdot H_2O$: C, 44.12; H, 5.55 N, 25.72. Found: C, 44.33; H, 5.37; N, 25.95.

[7,*NH*$_2$-^{15}N$_2$]-Adenosine *N*^{1}-Oxide (2b). To a solution of **1b** hemihydrate (2.66 g, 9.56 mmol) in 80 mL of 50% aqueous methanol was added 3.3 g of 3-chloroperoxybenzoic acid (MCPBA). The reaction mixture was stirred at room temperature for 17 h, diluted with water (50 mL), and extracted with ether (3 × 100 mL). The aqueous phase was concentrated to 60 mL and the mixture was purified by reversed phase preparative chromatography (0−20% CH_3CN/H_2O). The combined fractions of **2b** were concentrated to dryness and the solid dried in a vacuum desiccator over P_2O_5 for 24 h to afford 2.69 g (97%) of pure **2b**·0.25H_2O: mp 235−237 °C (dec) (lit.[34] mp 155 °C, 160 °C (dec)); ^{1}H NMR (D_2O) δ 8.63 (s, 1 H,), 8.45 (d, 1 H, J = 11.4 Hz, H8), 6.10 (d, 1 H, J = 5.5 Hz), 4.8 (m, 1 H), 4.5 (m, 1 H), 4.3 (m, 2 H), 3.7−4.0 (m, 2 H); ^{13}C NMR (50.3 MHz, D_2O) δ 151.1 (m), 147.0, 146.0, 144.3 (d, J = 5.0 Hz, C5), 122.0, 91.3, 88.3, 76.7, 73.1, 64.1; ^{15}N NMR (D_2O) δ 231.59 (d, J = 10.6 Hz, N7), 74.0 (s, N6); MS (EI) m/z (relative intensity) 285 (M^+, 4.5), 137 (100, [b + 2H]$^+$); HRMS m/z 285.0861 (calcd for $C_{10}H_{13}O_5N_3{}^{15}N_2$ 285.0858). Anal. Calcd for $C_{10}H_{13}O_5N_3{}^{15}N_2\cdot 0.25\ H_2O$: C, 42.11; H, 4.59; N, 24.55. Found: C, 41.54; H, 4.65; N, 24.19.

[7,*NH*$_2$-^{15}N$_2$]-6-Amino-2-(*N*,*N*-dimethylamino)-*N*^{1}-methoxy-9-(β-D-ribofuranosyl)purine (4b). A mixture of **2b** (0.210 g, 0.736 mmol) and methyl iodide (0.682 g, 4.80 mmol) in *N*,*N*-dimethylacetamide (5 mL) was stirred at room temperature for 2 h. The reaction mixture was chilled (0 °C) and 5 mL of 1:1 dimethylamine:methanol was added slowly. The mixture was allowed to warm to room temperature, was stirred for 1 h, and was concentrated to dryness. The residue was diluted with water (10 mL) and purified by preparative reversed phase HPLC (0−20% CH_3CN/H_2O, 30 min). Concentration of the product fractions gave 0.200 g (80%) of nearly pure **4b** as a mixture of diastereomers (ratio = 4:1; estimated by integration of the diastereomeric proton): mp 73−82 °C; ^{1}H NMR (DMSO-d_6) δ 8.59 (s, 0.8 H), 8.31 (s, 0.2 H), 7.65 (d, 0.8 H, J = 11.6 Hz, H8), 7.63 (d, 0.2 H, J = 11.6 Hz, H8), 5.50 (d, 2 H, J = 86 Hz, NH_2), 5.63 (d, 1H, J = 5.1 Hz), 5.20 (d, 1H, J = 3.2 Hz), 5.04 (d, 1 H, J = 5.1 Hz), 4.92 (t, 1 H, J = 5.4 Hz), 4.25−4.17 (m, 1 H), 4.06−3.98 (m, 1 H), 3.80−3.76 (m, 1 H), 3.65 (s, 3H), 3.2−2.8 (4s, 6H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 158.3 and 156.6 (C2), 149.3 and 150.6 (dd, $J_{C-^{15}N6}$ = 12.3, $J_{C-^{15}N7}$ = 3.1 Hz, C5), 140.5 and 141.5 (s, C6), 130.6 and 130.4 (C8), 116.1 and 115.5 (C4), 87.1 and 86.9 (C1′), 84.8, 74.8, 70.6 and 70.4 (C3′), 61.8 and 61.7, 60.9, 40.0, 33.8; ^{15}N NMR (DMSO-d_6) δ 262.4 (d, J = 10.6 Hz, N7), 255.7 (d, J = 10.7 Hz, N7), 67.6 (t, J = 87.3 Hz, N6), 67.4 (t, J = 89.4 Hz, N6); MS (EI) m/z (relative intensity) 344 (M^+, 54); HRMS m/z 344.1557 (calcd for $C_{13}H_{22}0_5N_4{}^{15}N_2$ 344.1594).

[1,7-^{15}N$_2$]Adenosine (6b). One-Flask Procedure from 2b. A mixture of **2b** (2.69 g, 9.43 mmol) and methyl iodide (4.05 g, 28.5 mmol) in *N*,*N*-dimethylacetamide (41 mL) was stirred at room temperature for 2 h. The reaction mixture was chilled (0 °C), 20 mL of 1:1 dimethylamine:methanol was added slowly, and the mixture was allowed to warm to room temperature and stirred for 2 h. The volatiles were removed with gentle heating (45 °C, oil pump vacuum is required), the residue was dissolved in methanol (20 mL) and refluxed for 48 h. This mixture was concentrated, the crude **5b** was dissolved in NH_4OH (30%, 63 mL) and heated to 95 °C, Raney nickel (50% water suspension, 13.5 g) was slowly added, and the suspension heated (95 °C) for 4 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 the residue purified by reversed phase preparative HPLC (0−20% CH_3CN/H_2O). The combined fractions of **6b** were concentrated to dryness and the solid dried in a vacuum desiccator over P_2O_5 for 24 h to afford 2.09 g of pure **6b**·0.25H_2O (81% yield from **2b**): mp 234−236 °C (lit.[35] mp 233−234 °C); ^{1}H NMR (DMSO-d_6) δ 8.36 (d, 1 H, J = 12.0 Hz, H8), 8.15 (d, 1 H, J = 15.8 Hz, H2), 7.4 (br s, 2 H), 5.89 (d, 1 H, J = 6.2 Hz), 5.4 (m, 2 H), 5.15 (m, 1 H), 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 (100.6 MHz, DMSO-d_6) δ 156.6, 152.8, 149.5, 140.4, 119.8, 88.4, 86.4, 74.0, 71.2, 62.1; ^{15}N NMR (DMSO-d_6) δ 243.91 (d, J = 10.6 Hz, N7), 239.04 (d, J = 17.1 Hz, N1); MS (EI) m/z (relative intensity) 269 (M^+, 4.5), 137 (100, [b + 2H]$^+$); HRMS m/z 269.0903 (calcd for $C_{10}H_{13}O_3N_3{}^{15}N_2$ 269.0909). Anal. Calcd for $C_{10}H_{13}0_4N_3{}^{15}N_2\cdot 0.25H_2O$: C, 44.61; H, 4.87; N, 26.01. Found: C, 43.89; H, 4.80; N, 25.68.

[1,7-^{15}N$_2$]-9-(β-D-Ribofuranosyl)-6-(1,2,4-triazol-4-yl)purine (7b). The procedure of Robins was used.[32] Dry pyridine (30 mL) was concentrated from a mixture of **6b** (5.48 g, 20.4 mmol) and *N*,*N*-dimethylformamide azine dihydrochloride (15.31 g, 107.7 mmol). Pyridine (30 mL) and TMSCl (9.04 g, 83.2 mmol) were added to the residue, and the resulting solution was heated (100 °C) for 24 h. The reaction solution was concentrated, and the brown syrup was dissolved in cold CH_2Cl_2 (200 mL) and washed twice with a cold solution of brine (70 mL) and water (40 mL) and twice with brine (50 mL) and aqueous HCl (2 M, 50 mL). The aqueous layer was backwashed with 3 × 30 mL of CH_2Cl_2. The combined organic layers were dried (Na_2SO_4) and concentrated, and the residue was dissolved in methanol (150 mL) and stirred for 3 h. The resulting suspension was concentrated, suspended in cold ethyl ether (70 mL), chilled to 0 °C, and filtered, and the residue was dried in a vacuum desiccator over P_2O_5 for 3 days to afford 6.03 g of pure **7b** (93%): mp 220−221 °C (lit.[32] mp ca. 223 °C (variable)); ^{1}H NMR (DMSO-d_6) δ 9.64 (s, 2H), 9.05 (d, 1H, J = 12.3 Hz), 8.94 (d, 1H, J = 15.8 Hz), 6.10 (d, 1 H, J = 5.3 Hz), 5.60 (d, 1H, J = 5.8 Hz), 5.28 (d, 1 H, J = 5.2 Hz), 5.12 (m, 1 H), 4.67−4.59 (m, 1 H), 4.25−41.8 (m, 1 H), 4.03−3.98 (m, 1 H), 3.75−3.56 (m, 2H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 153.8, 152.3, 146.3, 143.0, 141.2, 122.9, 88.5, 86.1, 74.4, 70.5, 61.4; ^{15}N NMR (DMSO-d_6) δ 252.12 (d, J = 14.9 Hz, N7), 240.99 (d, J = 12.8 Hz, N1). Anal. Calcd for $C_{12}H_{13}O_3N_5{}^{15}N_2$: C, 44.86; H, 4.08; N, 30.52. Found: C, 44.84; H, 4.07; N, 30.42.

[1,7,*NH*$_2$-^{15}N$_3$]Adenosine (8b). A mixture of $^{15}NH_4Cl$ (2.42 g, 44.4 mmol) and potassium carbonate (6.14 g, 44.4 mmol) in DMSO (40 mL) was sealed in a 100 mL bottle with a cap and placed in an oven at 85 °C for 30 min. The cooled (0 °C) reaction bottle was opened, **7b** (1.43 g, 4.44 mmol) was added quickly, and the bottle was resealed and placed in the oven at 85 °C for 5 days. The reaction mixture was cooled (0 °C) and quickly transferred into a bottle with an inlet and outlet tube. The excess $^{15}NH_3$ was recovered as $^{15}NH_4Cl$ by sparging the solution with helium into a cooled (0 °C), aqueous HCl (10%) solution. The aqueous phase was concentrated to dryness, suspended in dry acetone, filtered, and dried 24 h over P_2O_5 (vacuum desiccator) to afford 1.65 g (30.3 mmol) (76% of the excess $^{15}NH_4Cl$ recovered) of pure $^{15}NH_4Cl$: mp 340 °C (subl).

The purged reaction mixture was diluted with water (50 mL), the suspension filtered, and the filtrate purified by

(33) Robins, M. J.; Basom, G. L. *Can. J. Chem.* **1973**, *51*, 3161−3169.

(34) Stevens, M. A.; Magrath, D. I.; Smith, H. W.; Brown, G. B. *J. Am. Chem. Soc.* **1958**, *80*, 2755−2758.

(35) Sober, H. A. *Handbook of Biochemistry*, 2nd ed.; The Chemical Rubber Co.: Cleveland, Ohio, 1970.

reversed phase preparative chromatography (0−20% CH_3CN/H_2O in 40 min). 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 1.01 g (81%) of pure **8b** hemihydrate: mp 225−227 °C (lit.[35] 226−228 °C); ^{1}H NMR (DMSO-d_6) δ 8.33 (d, 1 H, J = 12.0 Hz, H8), 8.12 (d, 1 H, J = 15.7 Hz, H2), 7.34 (dd, $J_{H-^{15}N6}$= 90.1, $J_{H-^{15}N1}$ = 2.90 Hz, NH_2), 5.87 (d, 1 H, J = 6.2 Hz), 5.4 (m, 2 H), 5.15 (m, 1 H), 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 (100.6 MHz, DMSO-d_6) δ 156.6 (d, J = 20.2 Hz, C6), 152.9, 149.5, 140.5, 119.8, 88.5, 86.4, 74.0, 71.2, 62.1; ^{15}N NMR (DMSO-d_6, 40.543 MHz, with H coupling) δ 242.9 (d, J = 12.2 Hz, N7), 238.0 (m, N1), 84.2 (dt, $J_{H-^{15}N6}$= 91.5, $J_{N6-^{15}N1}$ = 6.1 Hz, N6); ^{15}N NMR (DMSO-d_6, 40.543 MHz, without H coupling) δ 242.9 (s, N7), 238.1 (d, $J_{N6-^{15}N1}$=6.1 Hz, N1), 84.1 (d, $J_{N6-^{15}N1}$ = 6.1 Hz, N6); 270 (M^+, 6.6), 138 (100); HRMS m/z 270.0865 (calcd for $C_{10}H_{13}O_3N_3{}^{15}N_2$ 270.0878). Anal. Calcd for $C_{10}H_{13}0_4N_3{}^{15}N_2 \cdot 0.5H_2O$: C, 43.01; H, 5.22; N, 25.08. Found: C, 43.29; H, 4.99; N, 24.93.

Acknowledgment. This work was supported by grants from the National Institutes of Health (GM48802 and GM31483).

JO9718152