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Use of ^{13}C as an Indirect Tag in ^{15}N Specifically Labeled Nucleosides. Syntheses of [8-^{13}C-1,7,NH_2-$^{15}N_3$]Adenosine, -Guanosine, and Their Deoxy Analogues

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We have previously reported the use of a ^{13}C tag at the C2 of ^{15}N-multilabeled purine nucleosides to distinguish the adjacent-labeled ^{15}N atoms from those in an untagged nucleoside. We now introduce the use of an *indirect* tag at the C8 of $^{15}N7$-labeled purine nucleosides. This tag allows unambiguous differentiation between a pair of $^{15}N7$-labeled purines in which only one is $^{13}C8$ labeled. Although the very small C8−N7 coupling (<1 Hz) precludes its direct detection in 1D ^{15}N spectra, 2D $^{1}H-^{15}N$ NMR experiments display the large C8−H8 coupling (>200 Hz) because H8 is coupled to both N7 and C8. The $^{13}C8$ atom is introduced by means of a ring closure of the exocyclic amino groups of a pyrimidinone using [^{13}C]sodium ethyl xanthate. Here, we present methods for the syntheses of [8-^{13}C-1,7,NH_2-$^{15}N_3$]adenosine, -guanosine, and their deoxy analogues.

Introduction

^{15}N NMR of labeled DNA and RNA fragments is a valuable method for probing a variety of interactions at specific nitrogen atoms since ^{15}N chemical shifts are quite sensitive to effects such as protonation,[1,2] hydrogen bonding,[3−8] metal binding,[9] and aromatic stacking.[10] A significant advantage of this use of ^{15}N as a probe is that it does not perturb the structure while giving straightforward spectra.[11] In a related but different method, uniformly ^{15}N-labeled nucleic acid samples have proven to be highly effective for structure determination by ^{1}H NMR,[12] although nonhelical regions and larger molecules can leave assignment ambiguities for which specific labeling by chemical synthesis provides a clear resolution.[13−15] Further, multilabeled nucleosides and nucleic acids also provide useful mass tags for mass spectroscopic work.[16−20]

To facilitate ^{15}N NMR studies of specifically labeled DNA and RNA, we have developed methods for efficient synthesis of a variety of ^{15}N-multilabeled purine nucleosides.[21−24] To permit spectral differentiation of two multilabeled nucleotides, we introduced the use of a ^{13}C tag at the purine C2 position to distinguish the neighboring $^{15}N1$, $^{15}N3$, and the guanine $^{15}NH_2$ from those in an untagged nucleotide based on the substantial $^{13}C-^{15}N$ couplings (6−23 Hz).[21,24] Use of this $^{13}C2$ tag allowed us to probe local hydrogen bonding and stacking in a series of specifically labeled RNA molecules with a minimum of synthetic effort.[10,25,26]

We now extend this concept to the purine C8 position. A preliminary account of this work with no synthetic

(1) Live, D. H.; Radhakrishnan, I.; Misra, V.; Patel, D. J. *J. Am. Chem. Soc.* **1991**, *113*, 4687−4688.
(2) Wang, C.; Gao, H.; Gaffney, B. L.; Jones, R. A. *J. Am. Chem. Soc.* **1991**, *113*, 5486-5488.
(3) Davis, D. R.; Yamaizume, Z.; Nishimura, S.; Poulter, C. D. *Biochemistry* **1989**, *28*, 4105−4108.
(4) Gaffney, B. L.; Goswami, B.; Jones, R. A. *J. Am. Chem. Soc.* **1993**, *115*, 12607−12608.
(5) Gaffney, B. L.; Kung, P.-P.; Wang, C.; Jones, R. A. *J. Am. Chem. Soc.* **1995**, *117*, 12281−12283.
(6) Gao, X.; Jones, R. A. *J. Am. Chem. Soc.* **1987**, *109*, 3169−3171.
(7) Kupferschmitt, G.; Schmidt, J.; Schmidt, T.; Fera, B.; Buck, R.; Rüterjans, H. *Nucleic Acids Res.* **1987**, *15*, 6225−6241.
(8) Massefski, W., Jr.; Redfield, A.; Sarma, U. D.; Bannerji, A.; Roy, S. *J. Am. Chem. Soc.* **1990**, *112*, 5350−5351.
(9) Tanaka, Y.; Kojima, C.; Morita, E. H.; Kasai, Y.; Yamasaki, K.; Ono, A.; Kainosho, M.; Taira, K. *J. Am. Chem. Soc.* **2002**, *124*, 4595−4601.
(10) Zhang, X.; Gaffney, B. L.; Jones, R. A. *J. Am. Chem. Soc.* **1998**, *120*, 615−618.
(11) Levy, G. C. *Nitrogen-15 Nuclear Magnetic Resonance Spectroscopy*; John Wiley & Sons: New York, 1979.
(12) Varani, G.; Aboul-ela, F.; Allain, F. H.-T. *Prog. Nucl. Magn. Reson. Spectrosc.* **1996**, *29*, 51−127.
(13) Jiang, F.; Patel, D. J.; Zhang, X.; Zhao, H.; Jones, R. A. *J. Biomol. NMR* **1997**, *9*, 55−62.
(14) Kettani, A.; Bouazin, S.; Gorin, A.; Zhao, H.; Jones, R. A.; Patel, D. J. *J. Mol. Biol.* **1998**, *282*, 619−636.
(15) Lin, C. H.; Wang, W.; Jones, R. A.; Patel, D. J. *Chem. Biol.* **1998**, *5*, 555−572.
(16) Abdi, F. A.; Mundt, M.; Doggett, N.; Bradbury, E. M.; Chen, X. *Genome Res.* **2002**, *12*, 1135−1141.
(17) Gu, F.; Stillwell, W. G.; Wishnok, J. S.; Shallop, A. J.; Jones, R. A.; Tannenbaum, S. R. *Biochemistry* **2002**, *41*, 7508−7518.
(18) Tretyakova, N.; Matter, B.; Jones, R.; Shallop, A. *Biochemistry* **2002**, *41*, 9535−9544.
(19) Veldhuyzen, W. F.; Shallop, A. J.; Jones, R. A.; Rokita, S. E. *J. Am. Chem. Soc.* **2001**, *123*, 11126−11132.
(20) Ziegel, R.; Shallop, A.; Jones, R.; Tretyakova, N. *Chem. Res. Toxicol.* **2003**, *16*, 541−550.
(21) Abad, J. L.; Gaffney, B. L.; Jones, R. A. *J. Org. Chem.* **1999**, *64*, 6575−6582.
(22) Pagano, A. R.; Lajewski, W. M.; Jones, R. A. *J. Am. Chem. Soc.* **1995**, *117*, 11669−11672.
(23) Pagano, A. R.; Zhao, H.; Shallop, A.; Jones, R. A. *J. Org. Chem.* **1998**, *63*, 3213−3217.
(24) Zhao, H.; Pagano, A. R.; Wang, W.; Shallop, A.; Gaffney, B. L.; Jones, R. A. *J. Org. Chem.* **1997**, *62*, 7832−7835.
(25) Zhang, X.; Gaffney, B. L.; Jones, R. A. *J. Am. Chem. Soc.* **1997**, *119*, 6432−6433.
(26) Zhang, X.; Gaffney, B. L.; Jones, R. A. *J. Am. Chem. Soc.* **1998**, *120*, 6625−6626.

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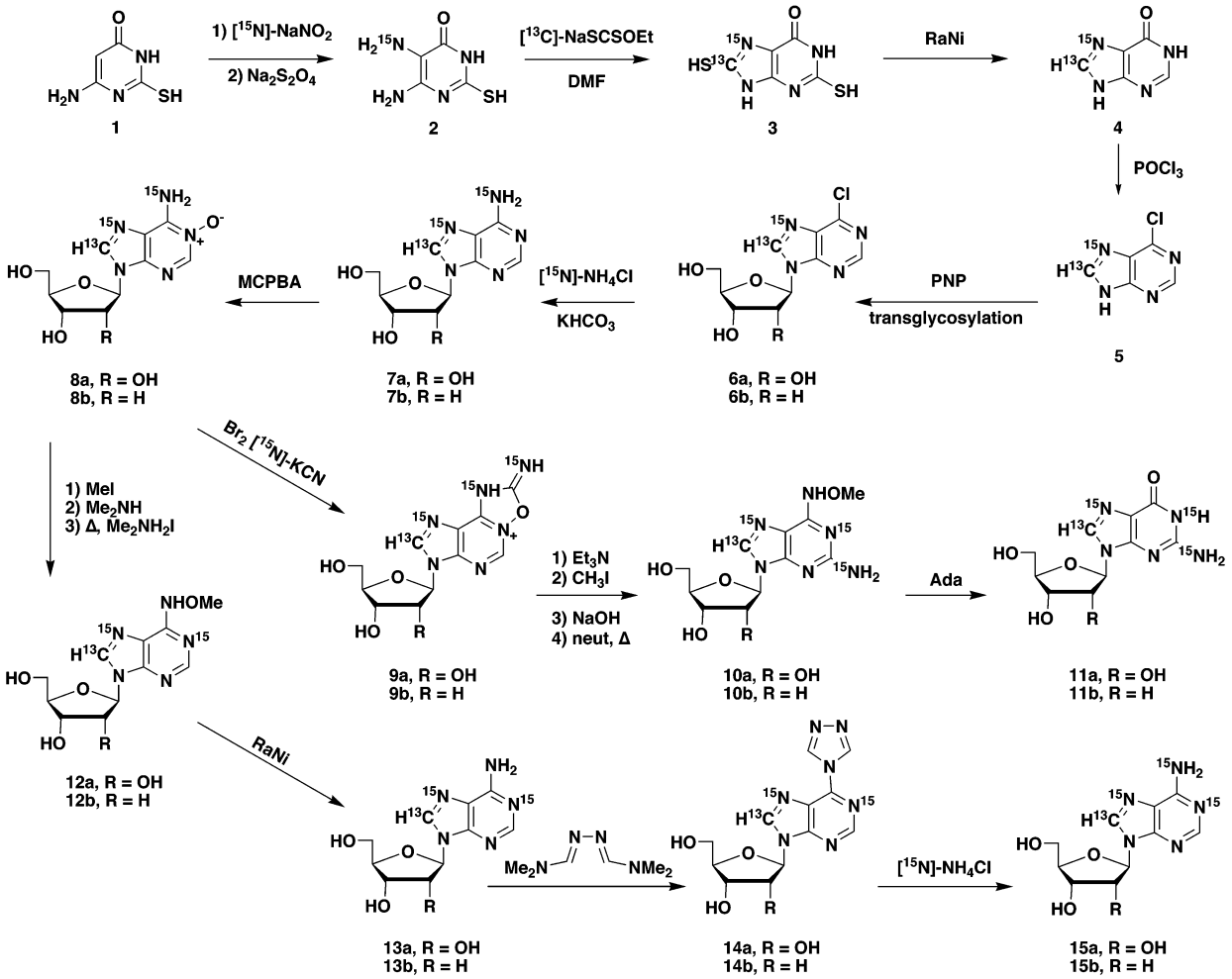
SCHEME 1



details was reported earlier as part of an overview of our use of ^{13}C tags with specifically ^{15}N labeled nucleosides.[27] Because the $^{13}C8-^{15}N7$ coupling often is small (<1 Hz), the $^{13}C8$ more conveniently serves as an *indirect* tag to differentiate one of a pair of $^{15}N7$ labeled purines through the combination of the $^{13}C8-H8$ coupling (>200 Hz) and the $H8-^{15}N7$ coupling (12 Hz). We present here procedures for the syntheses of [8-^{13}C-1,7,NH$_2$-^{15}N$_3$]adenosine, -guanosine, and their deoxy analogues.

A number of methods have previously been reported for incorporation of ^{13}C at the purine C8 position, either as the only label,[28−32] or along with some ^{15}N, such as [8-$^{13}C-NH_2$,9-$^{15}N_2$]adenosine.[33] However, none of these reports includes ^{15}N at the biologically significant N7 in the major groove. In addition, although the synthesis of [8-^{13}C-1,3,7,NH$_2$-^{15}N$_4$]adenine from [8-^{13}C-1,3,7,NH$_2$-^{15}N$_4$]-xanthine has recently been reported,[34] a procedure to prepare the latter is not readily available.

Results and Discussion

The synthetic focus of the work reported here is the incorporation of ^{13}C at the C8 position of adenosine and guanosine. We have previously introduced ^{13}C at the C2 position of purines either by a set of transformations using [^{13}C]cyanogen bromide[24] or by closure of the pyrimidine ring using [^{13}C]sodium ethyl xanthate.[21] The latter procedure is directly applicable to closure of the imidazole ring. The [^{13}C]NaSCSOEt is easily synthesized from [^{13}C]CS_2, and the resulting thiol can conveniently be removed along with the thiol in the original starting material.

As shown in Scheme 1, the $^{15}N7$ can be incorporated easily and in very high yield into the inexpensive pyrimidinone **1** by a nitrosation using [^{15}N]$NaNO_2$, followed by reduction of the nitroso group to the ^{15}N-labeled amino group.[22] The ring closure to incorporate the $^{13}C8$ occurs in 90% yield by refluxing **2** in dimethylformamide (DMF) with [^{13}C]NaSCSOEt, which is preformed by reacting [^{13}C]CS_2 with NaOH/EtOH. Both thiols in **3** are cleaved with Raney nickel in 82% yield. Chlorination of **4** in

(27) Abad, J.-L.; Shallop, A. J.; Gaffney, B. L.; Jones, R. A. *Biopolymers (NAS)* **1998**, *48*, 57−63.
(28) Kang, P.; Foote, C. S. *J. Am. Chem. Soc.* **2002**, *124*, 4865.
(29) LaFrancois, C. J.; Fujimoto, J.; Sowers, L. C. *Nucleosides Nucleotides* **1999**, *18*, 23−37.
(30) Poe, M.; Germershausen, J. I.; MacCoss, M. *Anal. Biochem.* **1987**, *164*, 450−456.
(31) SantaLucia, J., Jr.; Shen, L. X.; Cai, Z.; Lewis, H.; Tinoco, I., Jr. *Nucleic Acids Res.* **1995**, *23*, 4913−4921.
(32) Sharma, M.; Alderfer, J. L.; Box, H. C. *J. Labelled Compd. Radiopharm.* **1983**, *20*, 1219−25.
(33) Orji, C. C.; Michalczyk, R.; Silks, L. A. P., III. *J. Org. Chem.* **1999**, *64*, 4685−4689.
(34) Chiriac, M.; Axente, D.; Palibroda, N.; Craescu, C. T. *J. Labelled Compd. Radiopharm.* **1999**, *42*, 377−385.

TABLE 1. NMR Chemical Shifts and Coupling Constants for Base Protons and Labeled Atoms for 11a and b and 15a and b[a]

compd	$^{13}C8^b$	$^{15}N1^b$	$^{15}N7^b$	$^{15}NH_2^b$	H1	H2	H8	$^{15}NH_2$
11a	135.3 (s) <1:C8−N7	148 (s)	244 (s)	75 (s)	10.62 (d) 89:H1−N1		7.92 (dd) 214:H8−C8 12:H8−N7	6.44 (d) 90:N**H$_2$**-N**H**$_2$
11b	135.2 (s) <1:C8−N7	148 (s)	249 (s)	74 (s)	10.63 (d) 89:H1−N1		7.92 (dd) 213:H8−C8 12:H8−N7	6.44 (d) 90:N**H$_2$**-N**H**$_2$
15a	139.9 (s) <1:C8−N7	237 (d) 5:N1-N**H**$_2$	242 (s)	83 (d) 5:N**H**$_2$−N1		8.13 (d) 16:H2−N1	8.34 (dd) 213:H8−C8 12:H8−N7	7.34 (dd) 90:N**H$_2$**-N**H**$_2$ 3:N**H$_2$**−N1
15b	139.5 (s) <1:C8−N7	237 (d) 5:N1-N**H**$_2$	242 (s)	82 (d) 5:N**H**$_2$−N1		8.12 (d) 16:H2−N1	8.31 (dd) 213:H8−C8 12:H8−N7	7.28 (dd) 90:N**H$_2$**-N**H**$_2$ 3:N**H$_2$**−N1

[a] Each entry shows chemical shift (δ) and splitting pattern in parentheses, followed by coupling constants (Hz) and designation of coupled atoms. [b] Proton decoupled.

refluxing $POCl_3$ to give **5** works well,[22] but hydrolysis back to **4** during workup of this reaction can be a problem. We have found that care in removing excess $POCl_3$ and having a pH > 10 avoid this hydrolysis.

Enzymatic transglycosylation to give **6a** and **b** occurs in high yield[22] with purine nucleoside phosphorylase and either 7-methylguanosine for the ribonucleoside or thymidine and thymidine phosphorylase for the deoxynucleoside. The second ^{15}N label is then readily incorporated in 85−90% yield by ammination of **6a** and **b** to give the adenosine products **7a** and **b**.[22] Their oxidation gives the N1-oxides **8a** and **b** in 92% yield.[24]

We have further optimized our previously reported[24] adenosine to guanosine transformation. In the first step, the N1-oxides **8a** and **b** are reacted with cyanogen bromide, generated in situ by addition of [^{15}N]KCN to Br_2, to give **9a** and **b**. In our previous procedure, the reverse order of addition (Br_2 to KCN) may have generated some cyanogen, $(CN)_2$, resulting in the loss of the ^{15}N label. In the next steps, treatment of **9a** and **b** with Et_3N opens the oxazolidine ring so that the N1-oxide can be methylated with CH_3I. Neutralization followed by heating results in deformylation and ring closure to give **10a** and **b**. The time required for some of these steps has been significantly reduced, resulting in cleaner reaction mixtures and higher yields of **10a** and **b** (∼80%). Enzymatic deamination completes the transformation to give **11a** and **b** in 89−95% yield.

The triply labeled adenosine N1-oxides **8a** and **b** can also be converted to quadruply labeled adenosine **15a** and **b** by our reported methods.[23] After an N^1-alkoxy-mediated Dimroth rearrangement transposes the labeled amino to the N1 position in **12a** and **b**, the resulting N^6-methoxy group is removed with Raney Ni to give **13a** and **b** in an overall yield of 81% from **8a** and **b**. The final label is introduced by converting the 6-NH_2 to a 6-triazole group in **14a** and **b**, based on a high-yield procedure developed by Robins.[35,36] The displacement of the 6-triazole with [^{15}N]NH_3 gives **15a** and **b** in 81−83% yield.

NMR chemical shifts and coupling constants for the $^{15}N/^{13}C$ atoms and selected base protons in the final four nucleosides are shown in Table 1, and their full spectra are included in the Supporting Information. As expected, the $^{13}C8-H8$ coupling constants are very large (>200 Hz) and the $^{15}N7-H8$ constants are moderate (12 Hz) but the $^{13}C-^{15}N$ constants are small (<1 Hz). Thus, while use of the $^{13}C8$ as a direct tag for the adjacent $^{15}N7$ atom may be difficult, it serves most conveniently as an *indirect* tag, via the larger H8 coupling to both the $^{13}C8$ (>200 Hz) and the $^{15}N7$ (12 Hz). A heteronuclear $^1H-^{15}N$ 2D NMR spectrum displays a H8, N7 cross-peak that is split by >200 Hz along the ^1H dimension (see Supporting Information). With this approach, a tagged $^{15}N7$ resonance from a DNA or RNA fragment can be distinguished readily from an untagged one.

Experimental Section

[8-^{13}C-7-^{15}N]-2,8-Dithioxohypoxanthine (3). Dry **2**[22] (11.3 g, 71 mmol) and [^{13}C]−NaSCSOEt[21] (11.3 g, 78 mmol) were suspended in anhydrous DMF (200 mL) and refluxed for 2.5 h. The reaction mixture was cooled in an ice bath, and the product was precipitated by addition of cold CH_3CN. The crude product was filtered, washed with cold CH_3CN, and dried to give 10.5 g. The filtrate was concentrated and purified by reversed phase C18 chromatography using water to give an additional 5.69 g. The crude products were combined and used without further purification.

[8-^{13}C-7-^{15}N]Hypoxanthine (4). To a suspension of **3** (4.00 g, 17.8 mmol) in 250 mL of water was added 50% aqueous Raney Ni (16.0 g) and 96% formic acid (7.0 mL). The resulting suspension was refluxed for 15 min, ethylenediaminetetraacetic acid and dipotassium salt (EDTA) (6.30 g, 15.6 mmol) was added, and refluxing was continued for 2 h. The hot reaction mixture was filtered, and the solid was washed with boiling water. The filtrate was concentrated and the residue purified on a C18 column using a gradient of water to 40% CH_3CN in water over 30 min to give 2.23 g (16.1 mmol, 92% from **2**) of **4**.

[8-^{13}C-7-^{15}N]-6-Chloropurine (5). A mixture of **4** (4.00 g, 29.0 mmol), $POCl_3$ (100 mL, 1.07 mol), and *N*,*N*-dimethylaniline (9.0 mL) was refluxed for 45 min until a homogeneous black solution was obtained. The solution was cooled and concentrated to a black gum to remove all traces of $POCl_3$. The gum was dissolved with cooling in 45 mL of 30% aqueous NH_3 and washed first with ethyl acetate and then ether, taking care to keep the pH above 10. The aqueous layer was then concentrated to dryness, and the residue was dissolved in water (100 mL) and acidified with cooling to pH 2 using 1 M HCl. This solution was then extracted with ether for 4 days using a liquid/liquid continuous extractor. The ether was concentrated to dryness, and the residue was dissolved in water and purified on a C18 column using a gradient of water to 20% CH_3CN in water over 30 min to give 3.98 g (25.4 mmol, 88%) of **5**.

(35) Samano, V.; Miles, R. W.; Robins, M. J. *J. Am. Chem. Soc.* **1994**, *116*, 9331−9332.
(36) Miles, R. W.; Samano, V.; Robins, M. J. *J. Am. Chem. Soc.* **1995**, *117*, 5951−5957.

[8-^{13}C-7-^{15}N]-6-Chloro-9-(β-D-ribofuranosyl)purine (6a). A suspension of **5** (4.75 g, 30.3 mmol) and 7-methylguanosine (13.7 g, 46.0 mmol) in aqueous K_2HPO_4 (120 mL, 0.02 M) was adjusted to pH 7.4 with 6 N aqueous NaOH. To this mixture was added purine nucleoside phosphorylase (1500 units). The resulting mixture was kept at 30 °C with gentle agitation for 2 days. DMF (50 mL) was then added, and the mixture was stirred at room temperature for 5 h. This mixture was filtered to remove most of the 7-methylguanine. The filtrate was concentrated to a small volume and the residue purified on a C18 column using a gradient of water to 20% CH_3CN in water over 40 min to give 8.17 g (28.3 mmol, 93%) of **6a**.

[8-^{13}C-7-^{15}N]-6-Chloro-9-(2′-deoxy-β-D-*erythro*-pentofuranosyl)purine (6b). A suspension of **5** (3.32 g, 21.2 mmol) and thymidine (15.4 g, 63.7 mmol) in aqueous K_2HPO_4 (100 mL, 0.02 M) was adjusted to pH 7 with 6 N aqueous NaOH. To this mixture was added purine nucleoside phosphorylase (716 units) and thymidine phosphorylase (714 units). The resulting mixture was kept at 37 °C with gentle agitation for 2 days and then extracted with CH_2Cl_2 for 2 days using a liquid/liquid continuous extractor. The organic layer was concentrated to dryness, and the residue was dissolved in water and purified as for **6a** to give 4.77 g (17.5 mmol, 83%) of **6b**.

[8-^{13}C-7,NH_2-$^{15}N_2$]Adenosine (7a). A mixture of **6a** (3.05 g, 10.6 mmol), [^{15}N]NH_4Cl (1.16 g, 21.3 mmol), and $KHCO_3$ (3.25 g, 32.5 mmol) in dimethyl sulfoxide (DMSO) (16 mL) was sealed in a bomb and kept at 80 °C for 3 days. The cooled bomb (−20 °C) was carefully opened, and the contents were diluted with water (25 mL) and adjusted to pH 7 with acetic acid. The product was purified on a C18 column using a gradient of water to 20% CH_3CN in water over 30 min to give 2.61 g (9.3 mmol, 88%) of **7a**.

[8-^{13}C-7,NH_2-$^{15}N_2$]-2′-Deoxyadenosine (7b). A mixture of **6b** (4.00 g, 14.7 mmol), [^{15}N]NH_4Cl (1.73 g, 31.8 mmol), and $KHCO_3$ (4.72 g, 47.1 mmol) in DMSO (24 mL) was sealed in a bomb and kept at 80 °C for 4 days. The product was treated as for **7a** to give 3.64 g (13.4 mmol, 91%) of **7b**.

[8-^{13}C-7,NH_2-$^{15}N_2$]Adenosine N^1-Oxide (8a). A mixture of **7a** (3.00 g, 10.7 mmol) and *m*-chloroperoxybenzoic acid (MCPBA) (3.70 g, 21.4 mmol) in 50% aqueous CH_3OH (90 mL) was stirred at room temperature for 18 h. The mixture was diluted with water and then washed with ether. The aqueous layer was concentrated to a small volume and the residue purified on a C18 column using a gradient of water to 20% CH_3CN in water over 30 min to give 2.97 g (10.4 mmol, 97%) of **8a**.

[8-^{13}C-7,NH_2-$^{15}N_2$]-2′-Deoxyadenosine N^1-Oxide (8b). A mixture of **7b** (1.59 g, 5.8 mmol) and MCPBA (3.89 g, 22.5 mmol) in 50% aqueous CH_3OH (200 mL) was stirred at room temperature for 3 h. The reaction mixture was concentrated, giving a suspended white solid, and washed with ether. The aqueous layer was concentrated and the residue purified as for **8a** to give 1.50 g (5.6 mmol, 95%) of **8b**.

[8-^{13}C-1,7,NH_2-$^{15}N_3$]-2-Amino-6-(methoxyamino)-9-(β-D-ribofuranosyl)purine (10a). To bromine (2.09 g, 13.1 mmol) in water (3.8 mL) cooled to 0 °C was slowly added [^{15}N]KCN (0.83 g, 12.6 mmol) in water (35 mL). After 30 min of stirring, this solution was added to **8a** (2.56 g, 8.9 mmol) dissolved in water (90 mL). After 1.5 h, the reaction mixture was concentrated to a small volume from 50% DMF/CH_3CN three times. DMF (67 mL) and Et_3N (4.7 mL, 34 mmol) were added under nitrogen. The mixture was stirred at room temperature for 45 min, after which CH_3I (4.7 mL, 75.5 mmol) was slowly added. Stirring was continued in darkness for 2 h, and the reaction mixture was then concentrated to a yellow oil. This oil was dissolved in 0.25 M NaOH (150 mL) and stirred at room temperature for 10 min. The pH was adjusted to 7.4 with 1 M HCl, and ethanol (160 mL) was added. The mixture was heated at 60 °C for 4 h and then concentrated to 50 mL. The product was purified on a C18 column using a gradient of water to 20% CH_3CN in water over 30 min to give 2.20 g (7.0 mmol, 78%) of **10a**.

[8-^{13}C-1,7,NH_2-$^{15}N_3$]-2-Amino-6-(methoxyamino)-9-(2′-deoxy-β-D-erythro-pentofuranosyl)purine (10b). To bromine (1.05 g, 6.6 mmol) in water (2.0 mL) cooled to 0 °C was slowly added [^{15}N]KCN (0.45 g, 6.8 mmol) in water (10 mL). After 30 min of stirring, this solution was added to **8b** (1.30 g, 4.8 mmol) in 45 mL of water and the mixture was treated as for **10a**, using 2.5 mL (40.2 mmol) of CH_3I and 82 mL of 0.25 M NaOH. The product was purified on a C18 column using a gradient of 0.1 M NH_4HCO_3 (pH 7) to 10% CH_3CN in 0.1 M NH_4HCO_3 over 1 h to give 1.16 g (3.9 mmol, 81%) of **10b**.

[8-^{13}C-1,7,NH_2-$^{15}N_3$]Guanosine (11a). To **10a** (2.87 g, 9.1 mmol) dissolved in 190 mL of 0.1 M aqueous K_2HPO_4 (pH 7.4) was added adenosine deaminase (546 units). The solution was kept at 37 °C with gentle agitation for 5 days during which time the product crystallized. The mixture was cooled to 0 °C and filtered, and the product was recrystallized from water to give 2.33 g (8.10 mmol, 89%) of **11a**.

[8-^{13}C-1,7,NH_2-$^{15}N_3$]-2′-Deoxyguanosine (11b). To **10b** (0.92 g, 3.1 mmol) dissolved in 60 mL of 0.1 M triethylammonium acetate (TEAA) (pH 7.4) was added adenosine deaminase (211 units). The mixture was kept at 37 °C with gentle agitation for 6 days. The solution was purified on a C18 column using a gradient of water to 12% CH_3CN in water over 40 min to give 0.75 g (2.6 mmol, 85%) of pure **11b**.

[8-^{13}C-1,7-$^{15}N_2$]-6-(Methoxyamino)-9-(β-D-ribofuranosyl)-purine (12a). A mixture of **8a** (1.50 g, 5.2 mmol) and CH_3I (1.0 mL, 16.1 mmol) in 25 mL of *N*,*N*-dimethylacetamide was stirred at room temperature for 2.5 h. The reaction mixture was chilled in an ice bath, 2 M dimethylamine in CH_3OH (14.5 mL) was added slowly, and stirring was continued at room temperature for 1 h. The mixture was concentrated, dissolved in 20 mL of CH_3OH, and refluxed for 48 h. The mixture was again concentrated to give crude **12a**, which was used without further purification.

[8-^{13}C-1,7-$^{15}N_2$]-6-(Methoxyamino)-9-(2′-deoxy-β-D-*erythro*-pentofuranosyl)purine (12b). A mixture of **8b** (1.50 g, 5.6 mmol) and CH_3I (1.1 mL, 16.9 mmol) in 24 mL of *N*,*N*-dimethylacetamide was stirred at room temperature for 24 h and then treated as for **12a** to give crude **12b**.

[8-^{13}C-1,7-$^{15}N_2$]Adenosine (13a). Crude **12a** was dissolved in 35 mL of 30% aqueous NH_3 and heated to 95 °C, whereupon 50% aqueous Raney Ni (8.00 g) was added slowly, and the suspension was maintained at 95 °C for 2.5 h. The hot mixture was filtered, the Raney Ni was washed with boiling water, the filtrate was concentrated to a small volume, and the residue was purified on a C18 column using a gradient of water to 20% CH_3CN in water over 30 min to give 1.23 g (4.4 mmol, 84% from **8a**) of **13a**.

[8-^{13}C-1,7-$^{15}N_2$]-2′-Deoxyadenosine (13b). Crude **12b** was dissolved in 60 mL of 30% aqueous NH_3, heated to 95 °C, treated with Raney Ni, and purified as for **13a** to give 1.24 g (4.6 mmol, 82%) of **13b**.

[8-^{13}C-1,7-$^{15}N_2$]-9-(β-D-Ribofuranosyl)-6-(1,2,4-triazol-4-yl)-purine (14a). A mixture of **13a** (0.97 g, 3.5 mmol) and *N*,*N*-dimethylformamide azine dihydrochloride (2.69 g, 12.5 mmol) was dried by evaporation from pyridine three times to a final volume of 10 mL. Trimethylsilyl chloride (TMSCl) (1.75 mL, 13.8 mmol) was added to the solution, which was then heated at 100 °C for 22 h. The solution was concentrated and the residue dissolved in cold CH_2Cl_2 (35 mL). This solution was washed twice with cold brine/saturated $NaHCO_3$ and then twice with cold brine/2 M HCl. The organic layer was concentrated and the residue dissolved in CH_3OH (27 mL). After 3 h with stirring, the suspension was cooled (0 °C) and filtered. The solid was resuspended in cold ether, filtered again, and dried to give 0.87 g of **14a** without further purification.

[8-^{13}C-1,7-$^{15}N_2$]-9-(2′-Deoxy-β-D-*erythro*-pentofuranosyl)-6-(1,2,4-triazol-4-yl)-purine (14b). A mixture of **13b** (1.93 g, 7.1 mmol) and *N*,*N*-dimethylformamide azine (4.39 g, 30.9 mmol) was dried by evaporation from pyridine three times to a final volume of 30 mL. TMSCl (2.0 mL, 16.6 mmol) was added to the solution, which was treated as for **14a**, except

that the final product was suspended in cold CH_2Cl_2 rather than ether, to give 1.61 g of **14b** without further purification.

[8-^{13}C-1,7,NH$_2$-^{15}N$_3$]Adenosine (15a). A mixture of [^{15}N]-NH_4Cl (1.49 g, 27.4 mmol) and K_2CO_3 (3.73 g, 27.0 mmol) in 24 mL of DMSO was sealed in a bomb and kept at 85 °C for 30 min. The cooled bomb (−20 °C) was carefully opened, and crude **14a** (0.85 g) was quickly added. The resealed bomb was heated at 85 °C for 5 days. The cooled bomb was carefully opened and the contents diluted in 40 mL of water and adjusted to pH 7 with acetic acid. The product was purified on a C18 column using a gradient of water to 20% CH_3CN in water over 30 min to give 0.60 g (2.1 mmol, 81%) of **15a**.

[8-^{13}C-1,7,NH$_2$-^{15}N$_3$]-2′-Deoxyadenosine (15b). A mixture of [^{15}N]NH_4Cl (0.28 g, 5.1 mmol) and potassium *tert*-butoxide (0.52 g, 4.6 mmol) in 7.0 mL of DMSO was sealed in a bomb and kept at 85 °C for 30 min. The cooled bomb (−20 °C) was carefully opened, and **14b** (0.70 g, 2.3 mmol) was quickly added. The resealed bomb was heated at 85 °C for 2 days. The cooled bomb was carefully opened, and the contents were diluted with 20 mL of water. The product was purified as for **15a** to give 0.47 g (1.9 mmol, 83%) of **15b**.

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Supporting Information Available: General methods, characterization of compounds (UV, mp, ^{1}H, ^{13}C, ^{15}N NMR chemical shifts and coupling constants, HRMS, and elemental analysis), ^{1}H, ^{13}C, ^{15}N 1D NMR spectra for selected intermediates and final compounds, and 2D HSQC ^{1}H-^{15}N NMR for final compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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