Synthesis of 2′β-Deoxy-[8-^{13}C;amino,9-$^{15}N_2$]adenosine: Unusual Annulation Conditions To Assemble the Purine Core

Charles C. Orji,† Ryszard Michalczyk, and Louis A. "Pete" Silks III*

Los Alamos National Laboratory, Chemical Sciences and Technology Division, CST-4, The Bioscience and Biotechnology Group, MS; E529, Los Alamos, New Mexico 87545

Received December 21, 1998

Synthesis of 2′β-Deoxy-[8-^{13}C;amino,9-$^{15}N_2$]adenosine has been accomplished using a five-step process which employs a novel annulation of [^{13}C]formamide [*N*-(4-[^{15}N]amino-6-chloro-5-pyrimidinyl)] **2**. To effect this dehydration, a complex of triethyl phosphite and $TiCl_2(i\text{-}OPr)_2$ was used. 6-Chloro-[8-^{13}C; 9-^{15}N]purine was then converted in two steps to 2′β-deoxy-[8-^{13}C;amino,9-$^{15}N_2$]-adenosine.

Introduction

Heteronuclear multidimensional nuclear magnetic resonance (NMR) spectroscopy is a very attractive tool for the study of the structure and dynamics of oligomeric nucleic acids.[1] The capacity of NMR spectroscopy to provide exquisitely detailed structural information for oligonucleotides is significantly enhanced if the requisite monomeric units are labeled with magnetically active nuclei, especially ^{2}H, ^{13}C, and ^{15}N.[2] Uniformly labeled ribonucleic acids (RNAs) are now routinely obtained in milligram quantities by microbiological synthesis. As a result, NMR methods have become highly developed and have been extensively applied to study the solution structure of many biologically important RNA sequences.[3] However, the determination of the solution structure and dynamics of DNA have been hampered by the unavailability of labeled DNA at a reasonable cost. Despite recent advances in the enzymatic synthesis of DNA oliognucleotides, chemical synthesis of the nucleoside precursors is the method of choice.[2c]

Our efforts to develop straightforward chemoenzymatic methods for labeling DNA have resulted in a number of ^{15}N and ^{2}H singly and multiply labeled DNAs.[4] These labels have been proven to be useful in the study of a number of biologically relevant sequences of DNA, many of which contain triplet repeats. The expansion of tandemly repeated DNA triplets is associated with many human genetic disorders, including the fragile X syndrome,[5] myotonic dystrophy, Huntington's disease,[6] and Friedreich's ataxia.[7] Fragile X syndrome has a GCC/GGC sequence which has been found to be anomalously expanded and hypermethylated, while for Friedreich's ataxia, the GAA/TTC triplet is expanded anomalously. On the other hand, the $(CAG)_n$ of the Huntington's gene is susceptible to length polymorphism, and this attribute is associated with genetic instability. Using site-specific ^{15}N-labeled oligomeric nucleic acids, the extraction of structural and base pairing information for these DNA and DNA triplets by NMR spectroscopy has been accomplished.[8] In addition, the use of ^{2}H labeling of DNAs has allowed us to observe experimentally the effects of interference between *J* coupling and dipolar relaxation in ^{1}H COSY spectra of oligomeric DNA. This observation may have significant relevance to the accuracy of solution structures of oligomeric DNA determined by NMR spectroscopy.[9]

* Corresponding author. e-mail: pete-silks@lanl.gov.
† Current address for C. C. O. (CEO), Paradigm Organics, Inc., Partners Building II, Suite 3900, 840 Main Canopus Drive, Raleigh, NC, 27606. Tel: 919-838-9040, e-mail: charles@paradigmorganics.com.

(1) Kainosho, M**.** *Nat. Struct. Biol.* **1997**, *4*, 858. Griffey, R. H.; Poulter, C. D.; Bax, A.; Hawkins, B. L.; Yamaizumi, Z.; Nishimura, S. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80 (19)*, 5895. Legault, P.; Farmer, B. T.; Mueller, L.; Pardi, A. *J. Am. Chem. Soc.* **1994**, *116 (5)*, 2203. Hines, J. V.; Landry, S. M.; Vereni, G.; Tinoco, I., Jr. *J. Am. Chem. Soc.* **1994**, *116*, 6 (13), 5823.

(2) (a) Nikonowicz, E. P.; Sirr, A.; Legault, P.; Jucker, F. M.; Baer, L. M.; Pardi, A *Nucleic Acids Res.* **1992**, *20 (17)*, 4507. Santalucia, J.; Shen, L. X.; Cai, Z. P.; Lewis, H.; Tinoco, I., Jr. *Nucleic Acids Res.* **1995**, *23 (23)*, 4913. Davis D. R.; Yamaizumi, Z.; Nishimura, S.; Poulter, C. D. *Biochemistry* **1989**, *28 (9)*, 4105. Rhee, V. S.; Wang, C.; Gaffney, B. L.; Jones, R. A. *J. Am. Chem. Soc.* **1993**, *115 (19)*, 8742. Batey, R. T.; Inada, M.; Kujawinski, E.; Puglisi, J. D.; Williamson, J. R. *Nucleic Acids Res.* **1992**, *20(17)*, 4515. (b) For a recent example of site-specific labeling of β-2′-deoxyribonucleic acids, see Pagano, A. R.; Zhao, H.; Shallop, A.; Jones, R. A. *J. Org. Chem.* **1998**, *63 (10)*, 3213. (c) For the enzymatic synthesis of oligomeric DNA, see Zimmer, D. P.; Carothers, D. M. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92 (8)*, 3091. Mer, G.; Chazin, W. J. *J. Am. Chem. Soc.* **1998**, *120*, 607. Louis, J. M.; Martin, R. G.; Clore, G. M., Gronenborn, A. M. *J. Biol. Chem.* **1998**, *273 (4)*, 2374.

(3) Pardi, A. *Methods. Enzymol.* **1995**, 261, 350 and references therein. Tolbert, T. J.; Williamson, J. R. *J. Am. Chem. Soc.* **1997**, *119* (*50*), 12100.

(4) a. Kelly, J.; Ashburn, D. A.; Michalczyk, R.; Silks, L. A. *J. Labelled Compd. Radiopharm.* **1995**, 36 (7), 631. Orji, C. C.; Silks, L. A. *J. Labelled Compd. Radiopharm* **1996**, 38 (9), 625. b. Wu, R.; Orji, C. C.; Michalczyk, R.; Schmidt, J. G.; Li, Z.; Stocking, E.; Ashburn, D. A.; Kelly, J. A.; Khalsa, O.; Martinez, R. A.; Mariappan, S. V. S.; Unkefer, C. J.; Silks, L. A. *Recent Research Developments in Organic and Bioorganic Chemistry*; Pandalai, S. G., Ed.; Transworld Research Network: Trivandrum, India, 1998; Vol. 3, p 1.

(5) Ross, C. A.; McInnis, M. G.; Margolis, R. L.; Li, S.-H. *Trends Neurosci.* **1993**, *16 (7)*, 254. Heitz, D.; Rousseau, F.; Devys, D.; Saccone, S.; Abderrahim, H.; Le Paslier, D.; Cohen, D.; Vincent, A.; Toniolo, D.; Dellavalle G.; Johnson, S.; Schlessinger, D.; Oberle, I.; Mandel, J. L. *Science* **1991**, *251*, 1236.

(6) The Huntington's Disease Collaborative Research Group. *Cell* **1993**, *72*, 971.

(7) Campuzano, V.; Montermini, L.; Molto, M. D.; Pianese, L.; Cossee, M.; Cavalcanti, F.; Monros, E.; Rodius, F.; Duclos, F.; Monticelli, A.; Zara, F.; Canizares, J.; Koutnikova, H.; Bidichandani, S. I.; Gellera, C.; Brice, A.; Trouillas, P.; Demichele, G.; Filla, A.; Defrutos, R.; Palau, F.; Patel, P. I.; Didonato, S.; Mandel, J. L.; Cocozza, S.; Koenig, M.; Pandolfo, M. *Science* **1996**, *271*, 1423.

(8) Michalczyk, R.; Silks, L. A.; Russu, I. M. *Magn. Reson. Chem.* **1996**, *34*, 97. Mariappan, S. V. S.; Silks, L. A.; Chen, X.; Springer, P. A.; Wu, R. L.; Moyzis, R. K.; Bradbury, E. M.; Garcia, A. E.; Gupta, G. *J. Biomol. Struct. Dyn.* **1998**, *15 (4)*, 723. Edwards, C. E.; Tung, C.-S.; Silks, L. A.; Gatewood, J. M.; Fee, J. B.; Mariappan, S. V. S. *J. Biomol. Struct. Dyn.* **1998**, *16 (1)*, 13. Mariappan, S. V. S.; Silks, L. A.; Bradbury, E. M.; Gupta, G *J. Mol. Biol.* **1998**, *283 (1)*, 111. Mariappan, S. V. S.; Catasti, P.; Silks, L. A.; Bradbury, E. M.; Gupta, G *J. Mol. Biol.* **1999**, 284 (5), 2035.

(9) Kennedy, M. A.; Yang, J.; Silks, L. A.; Wu, R.; Isern, N.; Unkefer, C. J. *J. Am. Chem. Soc.* **1998**, in press. Also see, Yang, J.; Silks, L.; Wu, R.; Isern, N.; Unkefer, C.; Kennedy, M. A. *J. Magn. Res.* **1997**, *129 (2)*, 212.

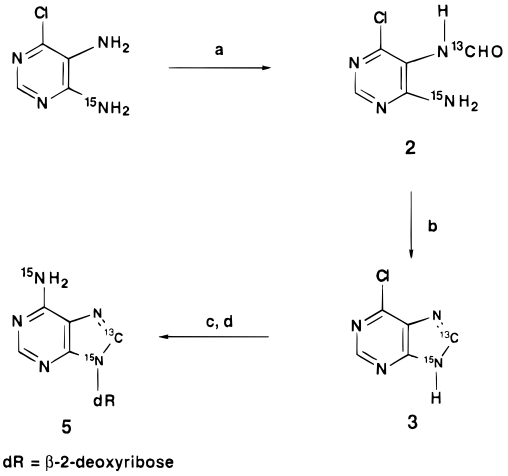
10.1021/jo9824753 CCC: $18.00
Published on Web 05/27/1999

Our initial labeling efforts for DNA have primarily focused on the base region. In that regard, one of our major goals has been to develop a common pathway to both adenine (A) and guanine (G). The advantage of a unified approach to labeling these bases is that chemistry will be developed that would allow for isotope incorporation in any desired position and in any desired combination. Thus, a new synthesis would not have to be developed each time a position needs to be labeled. Central to this approach has been the development of a critical annulation process giving rise to the purines with the C8 position labeled.

4,5,6-Triaminopyrimidine or 2,4,5,6-tetraaminopyrimidine have enjoyed significant use for the synthesis of A or G,[10] respectively. Their use for selective labeling of C4, C6, and N9 of G or A or the 6-amino group is precluded because of the C_2 symmetry of the starting pyrimidine. As a solution to this problem, we have developed a synthetic labeling strategy in which the 6-chloro-2,4,5-triaminopyrimidine was viewed as a pivotal intermediate for the synthesis of both A and G. In a model study using 6-chloro-2,4,5,-triaminopyrimidine it was determined that the major bottleneck of this strategy was finding a useful method to efficiently label C8. Interestingly, although the synthesis of 6-chloropurine is well documented, to the best of our knowledge there is not a report on the synthesis of 6-chloro-[8-^{13}C]purine. Traditionally, 6-chloropurine has been synthesized by the reaction of 6-chloro-4,5-diaminopyrimidine with the formic acid equivalents diethoxylmethyl acetate (DEMA) or triethyl orthoformate (TEOF).[11] However,^{13}C-labeled TEOF or DEMA are expensive reagents, and greater than stoichiometric amounts of these reagents are required for these reactions. Valsborg and co-workers reported using 1 equiv of [^{14}C]TEOF for the annulation of 2,6-dichloro-4,5-diaminopyrimidine, but our efforts to apply their conditions to 6-chloro-4,5-diaminopyrimidine were not successful.[12] Their result may be a consequence of the greater solubility of the 2,6-dichloro-4,5-diaminopyrimidine in acetonitrile. In addition, the use of either formic acid or formamide did not give good yields of 6-chloropurine because of a competing hydrolysis reaction at C6.[13]

Herein, we report a novel annulation process which allows for the synthesis of 6-chloro-[8-^{13}C, 9-^{15}N]purine which efficiently introduces the 8-^{13}C. The reaction conditions are applicable to the synthesis of 2-amino-6-chloropurine allowing access to guanine.[14] In addition, 6-chloro-[8-^{13}C, 9-^{15}N]purine has been carried forward in two steps giving rise to good yields of the 2′β-deoxy-[8-^{13}C;amino,9-$^{15}N_2$]adenosine. To the best of our knowledge this represents the first synthesis of the site specific triply labeled 2′β-deoxy-[8-^{13}C;amino,9-$^{15}N_2$]adenosine.

Scheme 1[a]



[a] Reagents: (a) **1**, CH_2Cl_2 (97.5%); (b) $TiCl_2[OCH(CH_3)_2]_2$ and $P(OEt)_3$ (88%); (c) 32% $^{15}NH_4OH$, 140 °C (100%); (d) thymidine, thymidine phosphorylase, nucleoside phosphorylase (91.7%).

Results and Discussion

We have synthesized 6-chloro-[9-^{15}N]purine by reaction of 6-chloro-4[amino-^{15}N],5-diaminopyrimidine using diethoxymethyl acetate (DEMA) as the solvent and reactant.[15] Since it is known that annulation of 6-chloro-4,5-diaminopyrimidine to 6-chloropurine using DEMA is a difficult process with stoichiometric amounts of label, we systematically examined a number of methods and reagents to effect this process.[16] Many of those methods failed to give either the product or the product in good yield. During these efforts it was evident that the formamide **2** (Scheme 1) had formed, and the major obstacle in the closure step was the poor nucleophilicity of the 4-amino group. On the basis of this observation, a two-step annulation via the formamide **2** was attempted. The use of a number of stoichiometric formylating agents for 6-chloro-4[amino-^{15}N],5-diaminopyrimidine such as formic acid and a variety of formic acid equivalents failed to produce good results. We were intrigued with a recent report of the use of formic trimethyl acetic anhydride (**1**) for this task.[17] Although, there are a number of reports that indicate mixed formic anhydrides are prone to disproportionations and/or decomposition, it was attractive to us because stoichiometric amount of this formyl transfer agent is used. Using this procedure we obtained a 56% yield of the distilled material. We believe the difficulty in extricating the mixed anhydride from the reaction matrix might account for our low yield (lit. 95%[17]). Examination of the ^{1}H NMR spectra before and after distillation indicated the reaction was nearly complete. On the basis of this observation, the reaction was simply filtered after it was determined to be complete by ^{1}H NMR. The solution was then used directly in the subsequent reaction. In addition, the methylene chloride

(10) Gaffney, B. L.; Kung, P.-P.; Jones, R. A. *J. Am. Chem. Soc.* **1990**, *112* (*18*), 6748. For labeling C8 using a ^{13}C labeled formyl transfer agent see Gmeiner, W. H.; Poulter, C. D. *J. Org. Chem.* **1988**, *53* (*6*), 1322. For use of H^{13}COOH for C8 labeling of adenine, see the following mention of the Silks et al. procedure, SantaLucia, J.; Shen, L. X.; Cai, Z. P.; Lewis, H.; Tinoco, I. Nucleic Acids Research **1995**, *23 (13)*, 4913.

(11) Robins, R. K.; Christensen, B. E. *J. Org. Chem.* **1951**, *16*, 324. Robins, R. K.; Christensen, B. E. *J. Am. Chem. Soc.* **1952**, *74*, 3624.

(12) Valsborg, J. S.; Knutsen, L. J. S.; Lundt, I.; Foged, C. *J. Labelled Compd. Radiopharm.* **1995**, *36 (5)*, 457.

(13) Robins, R. K.; Sille, K. J.; Willits, C. H.; Christensen, B. E. *J. Am. Chem. Soc.* **1953**, *75*, 263.

(14) Orji, C. C.; Silks, L. A. *Abstracts of Papers, Part 2*, 212th National Meeting of the American Chemical Society, Orlando, FL, Aug 25−29, 1996; American Chemical Society: Washington, D.C.; Abstr. No. 457-ORGN. Also, see ref 4b for a more detailed discussion.

(15) Orji, C. C.; Kelly, J.; Ashburn, D. A.; Silks, L. A *J. Chem. Soc., Perkin Trans. 1* **1996**, *7*, 595.

(16) Sekiya, M.; Suzuki, J.; Terao, Y. *Chem. Pharm. Bull.* **1976**, *24*, (*6*), 1331. Bendich, A.; Tinker, J. F.; Brown, G. B. *J. Am. Chem. Soc.* **1948**, *70*, 3109. Robins, R. K.; Dille, K. J.; Willits, C. H.; Christensen, B. E. *J. Am. Chem. Soc.* **1953**, *75*, 263. Robins, R. K.; Dille, K. L.; Christensen, B. E. *J. Org. Chem.* **1954**, *19*, 930. Daly, J. W.; Christensen, B. E. *J. Org. Chem.* **1956**, *21*, 177.

(17) Vlietstra, E. J.; Zwikker, J. W.; Nolte, R. J. M.; Drenth, W. *Recl. Trav. Chim. Pays-Bas* **1982**, *101 (12)*, 460.

solution of the mixed anhydride can be stored for extended periods of time below 0 °C without observable decomposition. Treatment of a suspension of 6-chloro-4[amino-^{15}N],5-diaminopyrimidine in methylene chloride with 1.1 equiv of the ^{13}C-labeled mixed anhydride at room temperature gave rise to a 95−97% yield of the formamide **2**. This reaction has been performed on a 5 g scale giving similar yields of **2**. While the synthesis of **2** was clearly straightforward, the assignment of regiochemistry of the formylation was ultimately proven by X-ray crystallography. While it is well-known that the 5-amino group of 4,5,6-triaminopyrimidine is the ultimate acceptor of the formyl group,[18] it was less clear that formylation of 6-chloro-4[amino-^{15}N],5-diaminopyrimidine would occur at the 5-amino group. The ^{1}H and ^{13}C NMR data at room temperature indicated the presence of a pair of rotational conformers in approximately 1:6.7 ratio (calculated from ^{13}C integration). More importantly, **2** did not exhibit detectable $^{1}J_{^{13}C-^{15}N}$'s. In addition, the ORTEP of the unlabeled **2** illustrates that indeed the formylation occurred at the 5-amino group. Interestingly, the C(2)−Hc(2)···N(2) intermolecular bond distance was determined to be 2.550 Å and possessed a bond angle of 143.2° (Figure 1, structure B). Considering that the sum of van der Waals radii of H and N (1.2 + 1.5 = 2.7 Å) is significantly longer that the distance found, it may signal the potential for significant CH···N hydrogen bonding. Cotton and co-workers have detailed criteria for a definition of relatively important hydrogen bonding interactions.[19] In their example, the CH···N distance was determined to be 2.44 Å and an angle of 177°, which was interpreted as possessing significant hydrogen bonding interaction. In addition, for the formamide **2**, the formyl hydrogen interaction, C(5a)Hc(5a)···O(1) as shown in Figure 1, structure A has a CH···O distance of 2.732 Å. The importance of these types of interactions can be found in Corey's formyl hydrogen bond model which has recently provided a useful basis for rationalizing the facial selectivity in a number of asymmetric processes.[20]

With **2** in hand, we next examined the ability of this compound to undergo annulation. A large number of reaction conditions and reagents to effect this annulation process was evaluated. Some of the more successful include refluxing **2** in nitrobenzene, P_2O_5 in DMF, and heating neat at elevated temperatures (mp = 199.5−200.0 °C). Eventually, the most consistent and good-yielding method used both $TiCl_2(iOPr)_2$ and triethyl phosphite. There is some evidence in the literature that combinations of phosphites and titanium Lewis acids form complexes in solution.[21] However, the reported use of these complexes for dehydration or annulation reactions was not evident. In addition, the annulation reaction does not proceed when only titanium tetrachloride

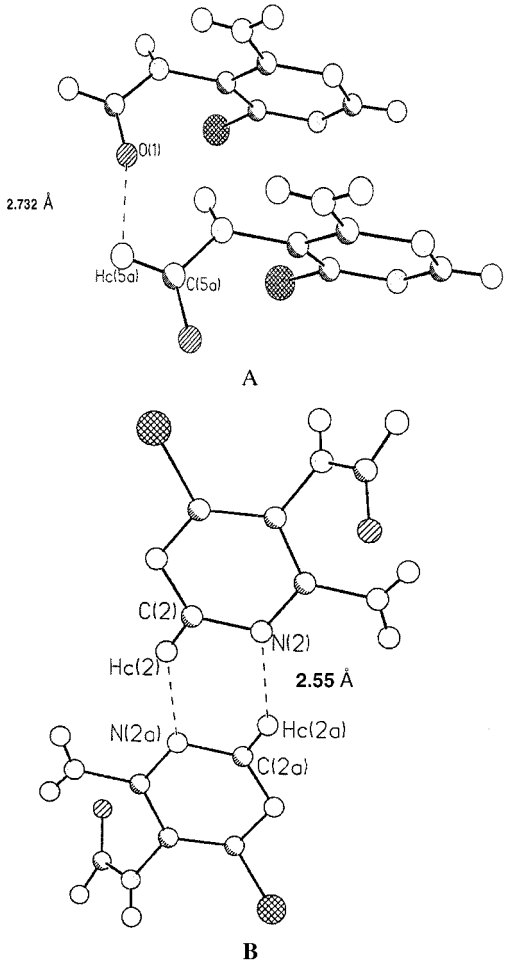


Figure 1. ORTEP of the solid state "dimer" of [^{13}C]formamide [*N*-(4-[^{15}N]amino-6-chloro-5-pyrimidinyl)]. Panel A illustrates the formyl C−H···O interaction. Panel B illustrates the C−H···N interaction.

or triethyl phosphite was used. Using this procedure, an 88% yield was obtained. The 6-chloropurine was then reacted with $^{15}NH_4OH$ followed by enzymatic ribosylation giving rise to 2′β-deoxy-[8-^{13}C;amino,9-$^{15}N_2$]adenosine, **5**.[22] The ^{1}H NMR spectrum of **5** (Figure 2) clearly illustrates the coupling of H8 proton to both the C8 ^{13}C and N9 ^{15}N nuclei. In addition, the signal of the ^{15}N amino hydrogens is a clear doublet (J = 90.1 Hz) centered around 7.3 ppm. The ^{13}C proton coupled spectrum of **5** (Figure 3) illustrates the coupled nuclei H8, N9, and H1′ to C8. Table 1 contains most of the extractable coupling data. Of some interest is the C8 H1′ ^{3}J, used in assignment of nucleic acid resonances, which was determined to be 4.1 Hz.

In summary, the synthesis of 6-chloro-[8-^{13}C]purine can be accomplished using formic [^{13}C]trimethyl acetic anhydride and a novel annulation method promoted by the unusual combination of triethyl phosphite and $TiCl_2(iOPr)_2$. In addition, to the best of our knowledge, this represents the first report of the synthesis of the triply labeled 2′β-deoxy-[8-^{13}C; amino,9-$^{15}N_2$]adenosine. This may find use in the studies of solution structure, function, and dynamics of oligomeric DNA by NMR and vibrational spectroscopies.

(18) Sharma, M.; Alderfer, J. L.; Box, H. C. *J. Labelled Compd. Radiopharm.* **1983**, *20 (10)*, 1219. Gmeiner, W. H.; Poulter, C. D. *J. Org. Chem.* **1988**, *53*, (*6*), 1322.

(19) Cotton, F. A.; Daniels, L. M.; Jordan, G. T.; Murillo, C. A. *J. Chem. Soc., Chem. Commun.* **1997**, *17*, 1673.

(20) Corey, E. J.; Rohde, J. J.; Fisher, A.; Azimioara, M. D. *Tetrahderon Lett.* **1997**, *38 (1)*, 33. Corey, E. J.; Barnes-Seeman, D.; Lee, T. W. *Tetrahedron Lett.* **1997**, *38 (25)*, 4351. For other formyl C−H···O solid-state interaction determinations see, Chaney, J. D.; Goss, C. R.; Folting, K.; Santarsiero, B. D.; Hollingsworth, M. D. *J. Am. Chem. Soc.* **1996**, *118*, (*39*), 9432. For an application of the Corey model for (*E*)-enol borinates aldols see, Paterson, I; Wallace, D. J.; Cowden, C. J. *Synthesis* **1998**, 639.

(21) Akhmadullina, A. G.; Yarkova, E. G.; Mukmeneva, N. A.; Kirpichnikov, P. A.; Muratova, A. A.; Pudovik, A. N. *Zh. Prikl. Khim. (Leningrad)* **1973**, *46 (6)*, 1291.

(22) Krenitsky, T. A.; Koszalka, G. W.; Tuttle, J. V. *Biochemistry* **1981**, *20*, 3615.

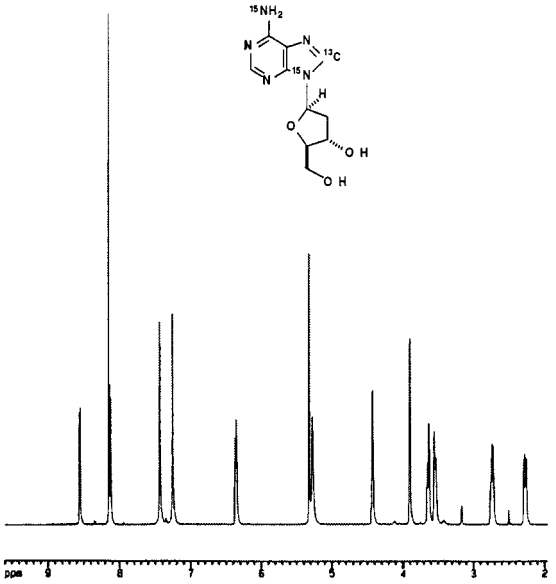


Figure 2. Proton spectrum for β-2′-deoxy-[8-^{13}C; amino,9-$^{15}N_2$]-adenosine in [$^2H_6$]DMSO.

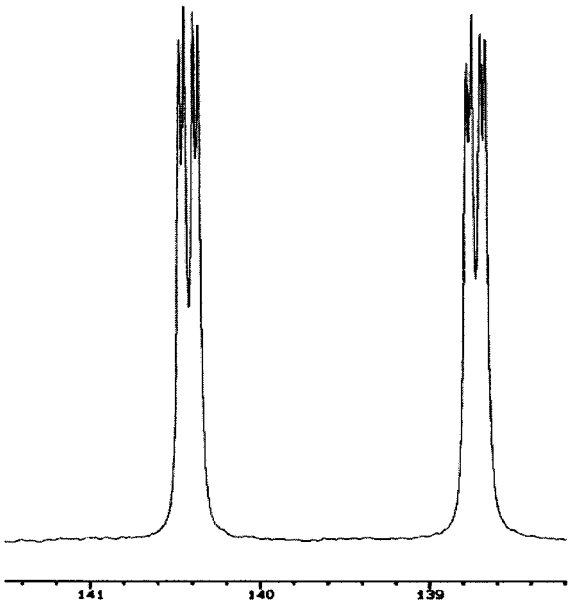


Figure 3. Proton-coupled ^{13}C spectrum for the C8 ^{13}C resonance of β-2′-deoxy-[8-^{13}C; amino,9-$^{15}N_2$]adenosine.

Experimental Section

General. δ_H and δ_C values are expressed relative to the internal [$^2H_6$]DMSO (δ_H 2.5; δ_C 39.5) unless otherwise noted. ^{15}N NMR chemical shifts are referenced with respect to KNO_3 ($\delta_N = 0$ ppm) unless otherwise noted. Positive chemical shifts denote resonances deshielded with respect to the reference. Measurements were made at or near ambient probe temperature in 5 mm NMR tubes using the solvent as an internal lock. Microanalyses were performed on a C,H,N,S elemental analyzer, CST-11, Los Alamos National Laboratory or by commercial vendors. Analytical thin-layer chromatography (TLC) was carried out on glass plates (silica gel 60 Å, 250 μm thickness). TLC visualization was accomplished with a UV lamp, I_2 staining, and an ethanolic solution of phosphomolybdic acid (PMA). The columns were handpacked with silica gel 60 (230−400 mesh). Pressures used were between 5 and 8 psi, and fractions were monitored by thin-layer chromatography (TLC). Moisture-sensitive reactions were performed in flame-dried glassware under a positive pressure of argon. Melting points obtained are uncorrected. Sodium[^{13}C]formate was dried

Table 1.[a] Coupling Constants for β-2′-deoxy-[8-^{13}C; amino,9-$^{15}N_2$]adenosine

δ (ppm)	position	C2	H2	C4	C5	C6	N6	H6	C8	H8	N9	C1′	H1′	C2′	H2′	H2″	C3′	H3′	C4′	H4′	C5′	H5′	H5″	5′-OH	3′-OH
152.4	C2		199.2				2.3				2.3														
8.14	H2	199.2				11.6																			
148.9	C4						1.0		9.7		3.0														
119.4	C5						3.0		8.6		3.0														
156.1	C6		11.6				20.4		7.0																
81.13	NH_2	2.3		1.0	3.0	20.4		90.1																	
7.33	NH_2						90.1																		
139.6	C8			9.7	8.6	7.0				213.0	10.5	1.8	4.1												
8.34	H8								213.0																
172.7	N9			19.1	3.0				10.5			10.8	1.6			2.6									
84.0	C1′	2.3							1.8		10.8		162.5												
6.35	H1′								4.1		1.6	162.5			6.1	7.8									
39.6	C2′												6.1			13.2		2.9							
2.72	H2′												7.8		13.2			5.7							
2.27	H2″																	151.4							
71.0	C3′																			2.8					
4.42	H3′														2.9	5.7	151.4			147.6					
88.1	C4′																		147.6						
3.90	H4′																	2.8				4.2	4.2		
61.95	C5′																					139.8	139.8		
3.63	H5′																			4.2	139.8		11.9	4.3	
3.53	H5″																			4.2	139.8	11.9		4.3	
5.34	5′-OH																					4.3	4.3		
5.26	3′-OH																								

[a] Error for chemical shift is 0.01 ppm. Errors for coupling constants are 0.1−0.2 Hz. ^{1}H and ^{13}C spectra referenced to TMS at 0.00 ppm through DMSO at 39.51 ppm. ^{15}N spectra referenced to liquid NH_3 at 0.00 ppm through lock frequency.

at 80 °C under high vacuum for 12 h and subsequently transferred to a drybox. All manipulations were then carried out in the drybox or under a dry inert atmosphere. X-ray structure determination was done on crystals obtained from methanol solutions. Details of data collection and structural refinement and tables of atomic coordinates and bond lengths can be obtained in the Supporting Information.

We have previously reported the synthesis of 6-chloro-4[amino-^{15}N],5-diaminopyrimidine.[14] Addition of $^{15}NH_4OH$ to commercially available 4,6-dichloro-5-aminopyrimidine gave rise to excellent yields of the monolabeled pyrimidine.

[^{13}C]Formic trimethyl acetic anhydride (1). Sodium [^{13}C]formate (1.25 g, 18.2 mmol) was dried overnight at 80 °C under high vacuum. It was finely divided, weighed, and transferred into a solid addition funnel in a drybox. It was added over 20 min to an ice-cooled, vigorously stirred solution of freshly distilled trimethylacetyl chloride (1.978 g, 16.40 mmol) and 15-crown-5 (0.233 g, 1.057 mmol) in methylene chloride (82 mL). The mixture was stirred for 5 h and filtered under an argon atmosphere. The filtrate was used as such in the subsequent reaction, no attempt being made to isolate the mixed anhydride. ^{1}H NMR ($CDCl_3$) δ_H 1.30 (s, 9H), 9.09 (d, J = 238 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ_C 156.5. Elemental analysis could not be performed since it is prone to decomposition at ambient temperature and highly moisture sensitive.

[^{13}C]Formamide [*N*-(4-[^{15}N]amino-6-chloro-5-pyrimidinyl)] (2). The solution of the mixed anhydride **1** was added by cannula to a stirred suspension of 6-chloro-4[amino-^{15}N],5-diaminopyrimidine (1.89 g, 13.0 mmol) in CH_2Cl_2 (26 mL) and the mixture was stirred at ambient temperature for 24 h. After removal of the solvent under reduced pressure, the resulting crude reaction mixture was dissolved in DMF. Silica gel was added to the resulting clear solution, and the DMF was removed under reduced pressure to afford the coated silica gel. This was applied to the top of the solvated silica gel column, and flash column chromatographic separation was performed using methanol/methylene chloride (20:80 v/v) as the eluant. Purification gave 2.19 g (97.5%) of **2** as a white solid. An analytical sample was prepared by recrystallization from methanol. mp 199.5−200.0 °C (dec); ^{1}H NMR signals from the *E*/*Z* isomers were not assigned: δ_H 7.28 (br s, 2H), 8.18 (d, J = 198 Hz, 1H), 8.12 (s, 1H), 9.55 (s, 1H); ^{13}C NMR δ_C 160.5, 164.8 (formyl ^{13}C tautomer); ^{15}N NMR δ_N −286.8 (major isomer), −286.3 (minor isomer); HRMS *m*/*z* calcd for $^{12}C_4{}^{13}C_1H_5Cl^{14}N_3{}^{15}N_1O$ (M^+) 174.0156, found 174.0149. Anal. Calcd for $^{12}C_4{}^{13}C_1H_5Cl^{14}N_3{}^{15}N_1O$: C, 34.51; H, 2.90; N, 32.20. Found: C, 34.45; H, 3.06; N, 31.84.

6-Chloro-[8-^{13}C, 9-^{15}N]purine (3). A solution of $TiCl_2(iOPr)_2$ was prepared by adding $TiCl_4$ (22 mL of a 1 M solution in CH_2Cl_2) to an ice-cooled solution of $Ti(iOPr)_4$ (6.26 g, 22.0 mmol) in CCl_4 (24 mL) and stirring the resulting mixture at ambient temperature for 10 min. The resulting solution was quickly added, via syringe, to a refluxing mixture of **2** (1.28 g, 7.34 mmol) and triethyl phosphite (3.66 g, 22.0 mmol) in CCl_4 (50 mL). The reaction immediately turned wine red. Reflux was continued until a light orange-red clear solution remained (approximately 16 h). Methanol was added and the solution turned yellow. The solvent was removed in vacuo, to give a syrupy material, which was dissolved in water, and the pH adjusted to 7 (at pH 4 a precipitation of a white solid occurred). After removal of the solvent in vacuo the resulting solid was loaded on a silica gel flash chromatography column which was eluted with methanol/methylene chloride (10:90 v/v) to give 0.84 g (88%) of 6-chloro-[8-^{13}C, 9-^{15}N]purine, **3**: mp 300 °C (dec); ^{1}H NMR (D_2O/DCl) δ_H 8.89 (s, 1H), 9.26 (dd, J = 220, 6 Hz, 1H); ^{13}C NMR δ_C 146.5 (d, J = 15.7 Hz); ^{15}N NMR δ_N −200.9 (d, J = 15.7 Hz); HRMS *m*/*z* calcd for $^{12}C_4{}^{13}C_1H_3Cl^{14}N_3{}^{15}N_1$ (M^+) 156.0050, found 156.0054.

[8-^{13}C; Amino,9-$^{15}N_2$]adenine (4). A 50 mL Parr stainless steel reactor was charged with 6-chloro-[8-^{13}C, 9-^{15}N]purine (0.82 g, 5.20 mmol) **3**, aqueous $^{15}NH_3$ (1.18 g of 32% aqueous solution, 21 mmol), and EtOH (16 mL). The reactor was sealed and heated at 140 °C for 6 h. The excess $^{15}NH_4OH$ was trapped with HCl.[14] The resulting crude mixture was adsorbed on silica gel, placed on a silica gel column, and eluted with methanol/methylene chloride (30:70 v/v) to give a solid. To the solid was added 1 M HCl (50 mL) to give a clear solution. Removal of the excess HCl afforded 1.08 g (100%) of material which contained the labeled adenine hydrochloride (and calculated 2 H_2O's). This was directly carried onto the next step. ^{1}H NMR (D_2O/NaOD) δ_H 7.89 (dd, J = 198, 12.6 Hz, 1H), 8.03 (s, 1H); ^{13}C NMR (D_2O/NaOD) δ_C 153.8 (d, J = 12 Hz); ^{15}N NMR (D_2O/NaOD) δ_N −307.2; −149.4 (d, J = 12 Hz); HRMS *m*/*z* calcd for $^{12}C_4{}^{13}C_1H_5Cl^{14}N_3{}^{15}N_2$ (M^+) 138.0519, found 138.0517.

2′β-Deoxy-[8-^{13}C;amino,9-$^{15}N_2$]adenosine (5). To the adenine hydrochloride (1.08 g) **4** were added thymidine (9.66 g, 39.9 mmol) and 10 mM KH_2PO_4 (125 mL). After adjusting the pH of the mixture to 7.5 with KOH, the reaction was placed in a heating bath at 40−44 °C. Thymidine phosphorylase (322 units) and nucleoside phosphorylase (215 units) were added, and the reaction was allowed to stir at 40−44 °C for 4 days. We have found the rate of the reaction is unpredictable, and that monitoring the reaction using HPLC can give reliable reaction end points. After removal of solvent under reduced pressure, the resulting solid was triturated with methanol/methylene chloride 100 mL 20:80 v/v) and filtered. The residue was washed with an additional 20 mL of the solvent. The combined solution was evaporated in vacuo and the process repeated four times with 50 mL of solvent each time. The resulting crude **5** was dissolved in the minimum amount of solvent and applied to a column. Elution and subsequent evaporation of solvent gave 1.22 g (91.7% **3**) of 2′β-deoxy-[8-^{13}C; amino,9-$^{15}N_2$]adenosine **5**; HRMS *m*/*z* calcd for $^{12}C_9{}^{13}C_1H_{13}{}^{14}N_3{}^{15}N_2O_3$ (M^+) 254.0993, found 254.0986.

Acknowledgment. We gratefully acknowledge financial support from the National Stable Isotopes Resource, NIH Division of Research Resources (RR 02231) and the grants X15C, XL2G, and XAB2 of the Los Alamos National Laboratory Directed Research and Development Fund.

Supporting Information Available: ^{1}H and ^{13}C spectra for compound **1**; ^{1}H, ^{13}C, HRMS spectra, elemental analysis, and X-ray data for **2**; ^{1}H, ^{13}C, ^{15}N spectra and elemental analysis for **3**, ^{1}H, ^{13}C, ^{15}N spectra and elemental analysis for **4**, ^{1}H, ^{13}C, ^{15}N spectra and HRMS for **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO9824753