Nitrogen-15-Labeled Deoxynucleosides. 2. Synthesis of [7-^{15}N]-Labeled Deoxyadenosine, Deoxyguanosine, and Related Deoxynucleosides

Barbara L. Gaffney, Pei-Pei Kung, and Roger A. Jones*

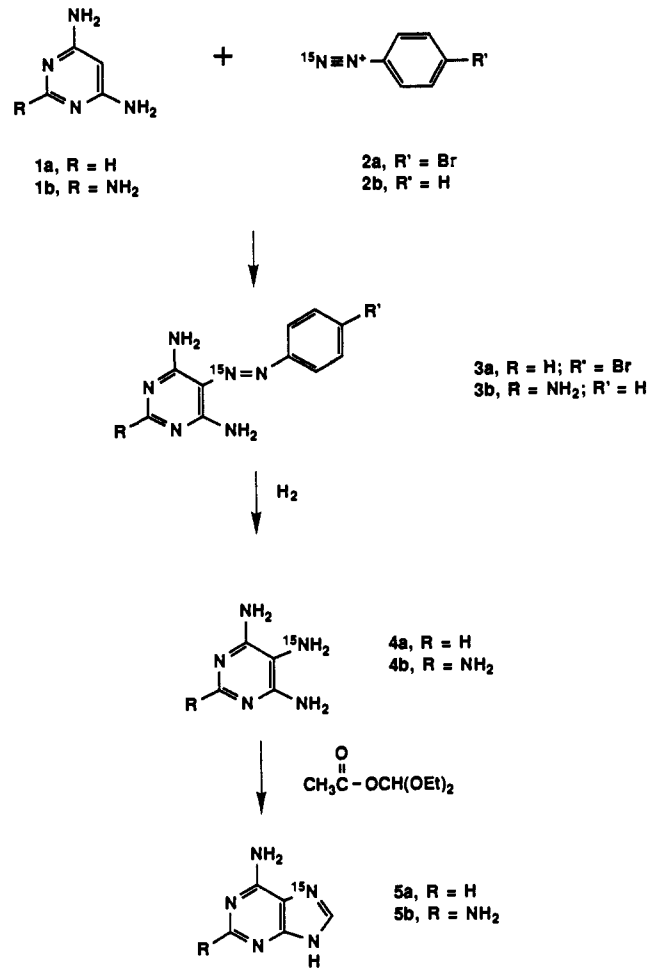
Department of Chemistry
Rutgers, The State University of New Jersey
Piscataway, New Jersey 08855

Received May 9, 1990

NMR studies employing oligonucleotides specifically labeled with ^{15}N may provide valuable information regarding nucleic acid structure, drug binding, and nucleic acid–protein interactions.[1,2] However, although appropriate spectrometers are widely available, as are adequate methods for synthesis and purification of oligonucleotides, the specifically labeled monomers required are largely unavailable. Recently we reported the first syntheses of ^{15}N-labeled purine deoxynucleosides, [6-^{15}N]- and [1-^{15}N]deoxyadenosines,[3] and showed that these ^{15}N labels could be used to monitor the helix-to-coil transition of d[CGTACG] by using either chemical shift or T_1.[4] Shortly thereafter an alternate synthesis of [6-^{15}N]deoxyadenosine and some ^{15}N NMR experiments were reported.[5] We chose the N^1 and N^6 positions for labeling because these are the nitrogens involved in Watson–Crick hydrogen bonding in the adenine·thymine base pairing present in DNA. Other positions, however, are also of interest. The N^7, for example, is potentially important in various ligand interactions and, in particular, in the Hoogsteen pairing thought to be present in triple and quadruple DNA helices.[6-14] We now report syntheses of the four [7-^{15}N]-labeled purine deoxynucleosides: [7-^{15}N]-2′-deoxyadenosine (**7a**); [7-^{15}N]-2′-deoxyinosine (**9a**); [7-^{15}N]-2′-deoxyguanosine (**9b**); and [7-^{15}N]-2,6-diamino-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine (**7b**).

The route we developed is a combination of chemical and enzymatic syntheses that emphasizes simple, high-yield procedures. In the first, chemical part (Scheme I), [7-^{15}N]adenine (**5a**) or [7-^{15}N]-2,6-diaminopurine (**5b**) is prepared in three steps in good to excellent overall yield. The synthesis of [7-^{15}N]adenine has been reported by two groups via nitrosation of 4,6-diaminopyrimidine followed by reduction to the [4,6-^{14}N,5-^{15}N]triaminopyrimidine using either sodium dithionite[15] or Raney nickel[16] and ring closure. We have explored introduction of the ^{15}N via an azo coupling reaction under the assumption that the coupling would proceed in high yield and that isolation of the azo derivative would be straightforward. In addition, we reasoned that the azo linkage might be cleavable under exceptionally mild conditions, thereby allowing high-yield formation of either the [4,6-^{14}N,5-^{15}N]triaminopyrimidine (**4a**) or the [2,4,6-^{14}N,5-^{15}N]tetraaminopyrimidine (**4b**).

Scheme I



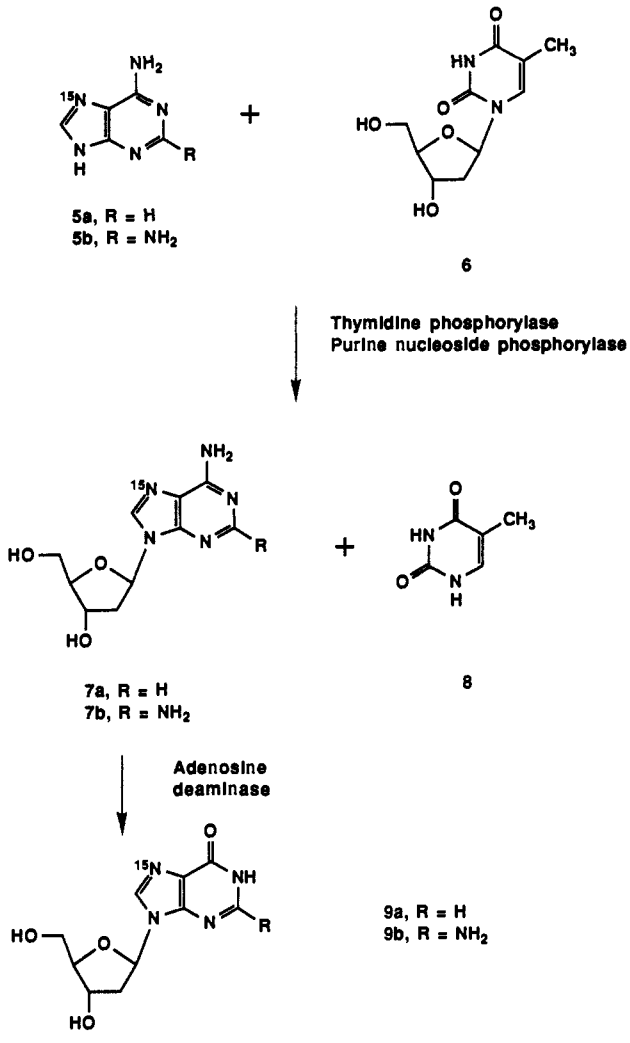
Diazotization of aniline (or other arylamines) using [^{15}N]-$NaNO_2$ gives the β-^{15}N diazonium ion.[17] Rearrangement of the ^{15}N to the α position generally occurs to the extent of only a few percent, at the most,[18] unless conditions specifically designed to promote rearrangement are employed.[19] Thus reaction of 4,6-diaminopyrimidine (**1a**) or 2,4,6-triaminopyrimidine (**1b**) with the β-^{15}N diazonium ion **2a** or **2b**, respectively, gives the azo derivative **3a** or **3b**. Hydrogenolysis of **3a/b** using 5% Pd/C or Raney nickel at 8–10 psi for 2–4 h cleaves the azo linkage cleanly. After filtration of the reaction mixture, the aniline also produced is removed by extraction into ethyl ether, leaving a remarkably homogeneous solution of **4a/b**. These aminopyrimidines degrade rapidly on handling in the air, as evidenced by pronounced yellowing of their solutions. Thus it is best if they are immediately converted to the more stable purines **5a/b**. We have found that this conversion can be effected rapidly and under quite mild conditions by treating a dimethylformamide solution of **4a/b** with diethoxymethyl acetate.[20] The cyclization is complete in 1–7 h at room temperature to give a mixture of, presumably, ethoxyethylidene derivatives of **5a/b**. These are hydrolyzed with dilute methanolic HCl to give **5a/b** quantitatively.

Conversion of **5a/b** to the deoxynucleosides **7a/b** is carried out in high yield by an enzymatic transglycosylation reaction (Scheme II) using thymidine as the glycosyl donor. Thymidine phospho-

(1) Kanamori, K.; Roberts, J. D. *Acc. Chem. Res.* **1983**, *16*, 35-41.
(2) Buchanan, G. W. *Tetrahedron* **1989**, *45*, 581-604.
(3) Gao, X.; Jones, R. A. *J. Am. Chem. Soc.* **1987**, *109*, 1275-1278.
(4) Gao, X.; Jones, R. A. *J. Am. Chem. Soc.* **1987**, *109*, 3169-3171.
(5) Kupferschmitt, G.; Schmidt, J.; Schmidt, T.; Fera, B.; Buck, F.; Rüterjans, H. *Nucleic Acids Res.* **1987**, *15*, 6225-6241.
(6) Mirkin, S. M.; Lyamichev, V. I.; Drushlyak, K. N.; Dobrynin, V. N.; Filippov, S. A.; Frank-Kamenetskii, M. D. *Nature (London)* **1987**, *330*, 495-497.
(7) Johnston, B. H. *Science* **1988**, *241*, 1800-1804.
(8) Sen, D.; Gilbert, W. *Nature (London)* **1988**, *334*, 364-366.
(9) Htun, H.; Dahlberg, J. E. *Science* **1989**, *243*, 1571-1576.
(10) Rajagopal, P.; Feigon, J. *Nature (London)* **1989**, *339*, 637-640.
(11) Williamson, J. R.; Raghuraman, M. K.; Cech, T. R. *Cell* **1989**, *59*, 871-880.
(12) Sundquist, W. I.; Klug, A. *Nature (London)* **1989**, *342*, 825-829.
(13) Santos, C. d. l.; Rosen, M.; Patel, D. *Biochemistry* **1989**, *28*, 7282-7289.
(14) Panyutin, I. G.; Kovalsky, O. I.; Budowsky, E. I.; Dickerson, R. E.; Rikhirev, M. E. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 867-870.
(15) 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.
(16) 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.
(17) Starkey, E. B. In *Organic Syntheses*; Wiley: New York, 1943; Collect. Vol. II, pp 225.
(18) Lewis, E. S.; Kotcher, P. G. *Tetrahedron* **1969**, *25*, 4873-4874.
(19) Ravenscroft, M. D.; Takagi, K.; Weiss, B.; Zollinger, H. *Gazz. Chim. Ital.* **1987**, *117*, 353.
(20) Montgomery, J. A.; Fitzgibbon, W. E. In *Nucleic Acid Chemistry, Improved and New Synthetic Procedures, Methods, and Techniques*; Townsend, L. B., Tipson, R. S., Eds.; Wiley-Interscience: New York, 1978; Vol. 2, pp 995-997.

0002-7863/90/1512-6748$02.50/0

Scheme II



rylase generates 2-deoxy-α-D-ribose 1-phosphate (dRib-1-P) from the thymidine, and bacterial purine nucleoside phosphorylase then couples the dRib-1-P with the purine derivative **5a/b**.[21,22] Using as little as a 5–10-fold excess of thymidine, we are able to get >95% conversion to **7a/b**. Since the product [7-^{15}N]-labeled deoxynucleosides do not have protons with pK's below 10, they are readily isolated from the enzymatic reaction mixture by using hydroxide form anion-exchange resin. All of the other components of the mixtures have sufficiently acidic protons that they are retained by the resin, while the product **7a/b** is eluted by using a simple water/methanol gradient.[23] This is therefore a highly efficient glycosylation procedure with regard to the [7-^{15}N]-labeled material. Moreover, as both deoxyadenosine and 2-amino-deoxyadenosine are excellent substrates for deamination by adenosine deaminase,[24] conversion of **7a/b** to **9a/b** proceeds in quantitative yield.

The purine syntheses reported above emphasize efficient use of ^{15}N, employ a minimal number of synthetic steps, and do not require complex isolation or purification procedures. The deoxynucleoside syntheses make use of high-yield transformations involving inexpensive and readily available enzymes. There are no protection or deprotection steps, and the only chromatography is a rapid, low-resolution ion-exchange column after the transglycosylation reaction step. These procedures, moreover, are applicable to the ribo series as well, simply by substituting uridine as a ribosyl donor along with uridine phosphorylase for the transglycosylation step.[21,22] This represents, therefore, a general route to synthesis of [7-^{15}N]-labeled nucleosides of the adenine and guanine families.

Acknowledgment. This work was supported by grants from the National Institutes of Health (GM31483), and Busch Memorial Fund, and an American Cancer Society Faculty Research Award to R.A.J.

Supplementary Material Available: A complete experimental section for compounds **3a/b–5a/b**, **7a/b**, and **9a/b** (3 pages). Ordering information is given on any current masthead page.

(21) Krenitsky, T. A.; Koszalka, G. W.; Tuttle, J. V. *Biochemistry* **1981**, *20*, 3615–3621.

(22) Krenitsky, T. A.; Rideout, J. L.; Chao, E. Y.; Koszalka, G. W.; Gurney, F.; Crouch, R. C.; Cohn, N. K.; Wolberg, G.; Vinegar, R. J. *J. Med. Chem.* **1986**, *29*, 138–143.

(23) The products (**7a/b**) are contaminated with a small, variable amount of material apparently derived from elimination of 2-deoxy-α-D-ribose 1-phosphate on the strongly basic anion-exchange resin. Pure materials may be obtained by crystallization, either before or after deamination. Alternatively, the products may be used as obtained for protection for oligonucleotide synthesis.

(24) Baer, H.-P.; Drummond, G. I.; Duncan, E. L. *Mol. Pharmacol.* **1966**, *2*, 67–76.

Additions and Corrections

Stereostructure of Pimaricin [*J. Am. Chem. Soc.* **1990**, *112*, 4060–4061]. JEAN-MARC LANCELIN and JEAN-MARIE BEAU*

Page 4060: In Figure 1 R = Me for structure **2** *should* read R = Ac.

Page 4061, left column, line 3: The 7*R* and 9*S* configurations should be the 7*S* and 9*R* configurations.

Page 4061, right column, line 5: The 4*S* configuration should be the 4*R* configuration. This change does not affect structure **10** (Figure 2) for which the correct chiral centers have been drawn.