



Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 3093–3096

BIOORGANIC &  
MEDICINAL  
CHEMISTRY  
LETTERS

# A General Synthesis of Specifically Deuterated Nucleotides for Studies of DNA and RNA

Bingzi Chen, Elizabeth R. Jamieson and Thomas D. Tullius\*

*Department of Chemistry, Boston University, Boston, MA 02215, USA*

Received 19 March 2002; accepted 1 August 2002

**Abstract**—An efficient procedure is described for the preparation of ribonucleotides and deoxyribonucleotides with deuterium incorporated at the 1', 4', or 5' position. Three intermediates—[1-<sup>2</sup>H]-D-ribose, [4-<sup>2</sup>H]-D-ribose, and [5-<sup>2</sup>H<sub>2</sub>]-D-ribose—were prepared by chemical synthesis and subsequently converted to ribonucleotides and deoxyribonucleotides via enzymatic reactions. Milligram quantities of the desired products were obtained with an average deuterium content of 96±1%.

© 2002 Elsevier Science Ltd. All rights reserved.

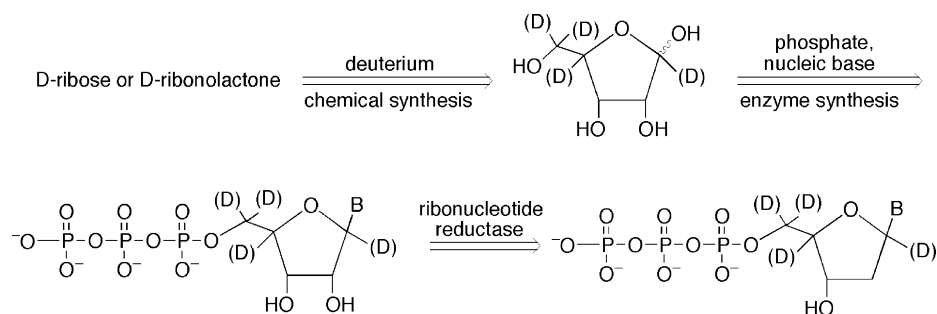
Specifically deuterated ribonucleotides and deoxyribonucleotides have important applications in biochemistry and molecular biology. Deuterated nucleotides can be incorporated into DNA and RNA molecules to suppress nonessential proton resonances in NMR structural studies.<sup>1,2</sup> Another use for these molecules has been in atom transfer and kinetic isotope effect experiments with various DNA damaging agents, such as calicheamicin,<sup>3</sup> bleomycin<sup>4</sup> and the hydroxyl radical.<sup>5</sup> While these types of experiment provide valuable information, their application to biochemical problems has been limited due to the difficulty in synthesizing specifically labeled nucleotides. Here we describe a convenient scheme for preparing specifically deuterated deoxyribonucleotides and ribonucleotides using a combination of chemical and enzymatic syntheses.

Synthesis of a series of nucleotides with deuterium incorporated at the 1', 4', or 5' position of the deoxyribose or ribose ring was accomplished in stages (Scheme 1). Chemical transformation of D-ribose or D-ribonolactone was carried out through a series of reactions<sup>6</sup> to incorporate the isotope at a specific position. One-pot enzymatic phosphorylation and coupling of the deuterated ribose sugar to a base<sup>7</sup> allowed the direct formation of the desired ribonucleotides. Deoxyribonucleotides were produced by enzymatic reduction of the corresponding ribonucleotide.<sup>8</sup>

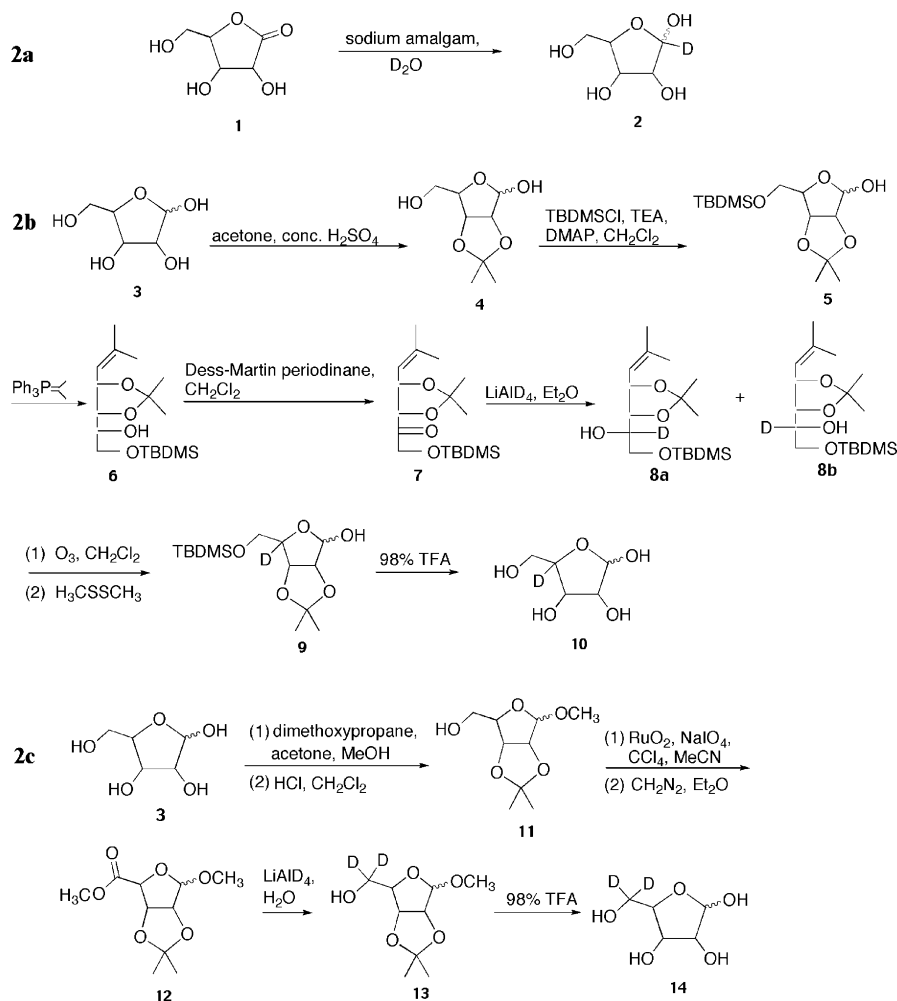
Using a combination of chemical and enzymatic methods greatly simplifies the synthesis, in particular eliminating the need for multistep chemical coupling, deoxygenation, and phosphorylation to produce the desired (deoxy)-nucleoside triphosphates. A further advantage of this scheme is the ease of preparation of a large variety of nucleotide products from a relatively small number of chemically synthesized precursors. While this work describes the synthesis of specifically deuterated purine nucleotides, this procedure could easily be extended to produce pyrimidine nucleotides.<sup>7</sup> The nucleoside triphosphates made by this method are suitable for synthesizing larger RNA and DNA molecules, as we show here by their use in an *in vitro* transcription reaction to produce specifically deuterated L-21 Sca ribozyme.

We prepared [1-<sup>2</sup>H]-D-ribose **2** by reduction of D-ribonolactone with sodium amalgam in D<sub>2</sub>O, with a yield of 89% (Scheme 2a). We followed the synthetic scheme developed by Townsend and coworkers<sup>6</sup> to prepare ribose specifically deuterated at either the 4' or 5' position. The syntheses of [4-<sup>2</sup>H]- and [5-<sup>2</sup>H<sub>2</sub>]-D-ribose **10** and **14** (Scheme 2b and c) began with protection of the 2' and 3' hydroxyl groups of D-ribose. After further protection of either the 5' or 1' hydroxyl, the protected ribose was oxidized. Reduction of the resulting ketone **7** or ester **12** by LiAlD<sub>4</sub> introduced deuterium at either the C-4 or the C-5 position. <sup>1</sup>H NMR spectroscopy indicated that deuterium was incorporated into **9** and **13**, as the signal for the 4' proton or the 5' protons completely disappeared, compared to the corresponding non-deuterated compounds. The protected deuterated ribose was hydrolyzed

\*Corresponding author. Fax: + 1-617-353-3535; e-mail: tullius@bu.edu



**Scheme 1.** Chemical/enzymatic synthesis of specifically deuterated nucleotides.



**Scheme 2.** Synthesis of [1-<sup>2</sup>H]-, [4-<sup>2</sup>H]-, and [5-<sup>2</sup>H<sub>2</sub>]-D-ribose.

with aqueous TFA to obtain deuterated D-ribose that was suitable for enzymatic attachment to a purine base.

The chemical synthesis of nucleosides has been troublesome because of the difficulty of obtaining regio- and stereospecificity in the coupling of base to sugar. A typical approach involves condensation of protected and activated base and sugar.<sup>9–12</sup> In a typical coupling reaction, D-ribose is protected as 1,2,3,5-tetra-*O*-acetyl-, acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranoside, or other similar species. In our initial trials, coupling of an anomeric mixture of isopropyl-protected D-ribose with a silylated purine base resulted in complicated mixtures

containing only trace quantities of the desired N-9-β nucleoside. The necessity of carrying out several additional reactions to change the isopropyl protecting group to acetyl groups on the sugar ring rendered this approach unappealing.

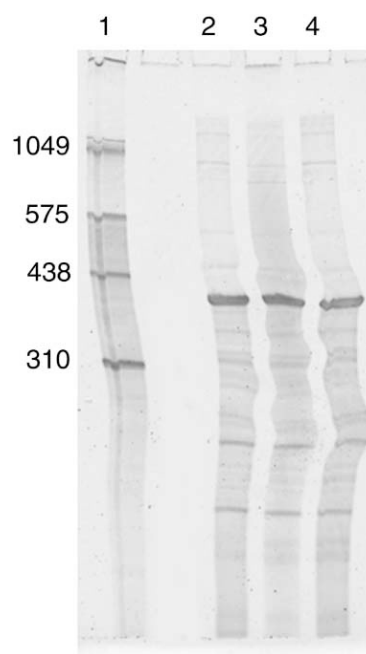
We decided instead to use the procedure developed by Tolbert and Williamson<sup>7</sup> to phosphorylate and couple deuterated ribose to a base. This one-pot method uses an enzyme cocktail<sup>13</sup> (ribokinase, PRPP synthetase, myokinase, pyruvate kinase, enolase, 3-phosphoglycerate mutase, and either adenine phosphoribosyltransferase, or xanthine-guanine phosphoribosyltransferase plus guanylate

kinase) to convert unprotected D-ribose into a purine nucleoside triphosphate under mild conditions with good yield. Analytic HPLC using a Vydac 302IC4.6 HPLC column (anion exchange, 10  $\mu\text{m}$ , 4.6 mm id $\times$ 250 mm) was used to monitor the progress of the enzymatic reaction and to identify products. Purification of the deuterated ribonucleoside triphosphate from the enzymatic reaction was performed by chromatography on Affi-gel 601 (BioRad) boronate-derivatized polyacrylamide according to a published method.<sup>14</sup> NMR and mass spectrometry were used to characterize the deuterated NTP's.<sup>15</sup> For example, proton NMR spectra of [ $1'-^2\text{H}$ ]-, [ $4'-^2\text{H}$ ]- or [ $5'-^2\text{H}_2$ ]-nucleotides showed a complete absence of the resonance for the  $1'$ -hydrogen ( $\delta$  5.73 or 5.93), the  $4'$ -hydrogen ( $\delta$  4.15 or 4.21), or the  $5'$ -hydrogens ( $\delta$  4.06 or 4.05), respectively, demonstrating excellent incorporation of deuterium.

We used ribonucleotide reductase<sup>8,16,17</sup> to transform a specifically deuterated ribonucleotide into a  $2'$ -deoxyribonucleotide that is suitable for incorporation into DNA via the polymerase chain reaction. Formation of the dNTP occurred relatively quickly. After 1.5 h, only a trace of the unreacted starting material could be detected. The reaction was stopped after 3 h. An aliquot (5  $\mu\text{L}$ ) was removed and analyzed using a YMC ODS-AQ HPLC column with a mobile phase of 0.1 M phosphate (pH 6.5), at a flow rate of 0.7 mL/min, and detection at 260 nm (retention times: ATP, 11.60 min; dATP, 25.63 min; GTP, 6.09 min; dGTP, 12.35 min). The products were purified on DEAE-Sephadex A-25 to afford deuterated deoxyribonucleotides in yields of 74–76%. Deuterium content was determined by mass spectrometry.

Our aim in synthesizing specifically deuterated ribonucleotides was to incorporate them into large RNA molecules. To demonstrate this application, we carried out *in vitro* transcription of the gene for the ScaI *Tetrahymena* ribozyme using either [ $1'-^2\text{H}$ ]-ATP or [ $5'-^2\text{H}_2$ ]-ATP, along with the other three natural nucleoside triphosphates. As shown in Figure 1, a  $\sim$ 390 nt product was obtained with either deuterated NTP (lanes 2 and 3). The yield of deuterated RNA from the transcription reaction was similar to that obtained when the reaction was run with commercially available, all-protio ATP (lane 4). These results clearly demonstrate the utility of specifically deuterated ribonucleotides in making isotopically labeled RNA molecules.

In conclusion, we have synthesized purine nucleotides with specific deuterium labels at C- $1'$ , C- $4'$ , and C- $5'$  by a simple and short route. With slight modifications this procedure could be used to produce pyrimidine nucleotides, and it is easily adaptable to the synthesis of nucleotides with labels in other positions of the ribose or base moiety. Limited chemical synthesis was required to accomplish isotopic substitution at the various sugar positions.<sup>18</sup> Enzymatic reactions efficiently coupled isotopically labeled ribose to a purine base to yield the desired NTP. An enzymatic transformation was used to convert ribonucleotides to deoxyribonucleotides. The method presented here allows for the synthesis of a variety of specifically labeled deuterated nucleotides in



**Figure 1.** Denaturing polyacrylamide gel electrophoresis of the products of *in vitro* transcription of the gene for the L-21 Sca ribozyme. Lane 1, RNA molecular weight markers. Lane 2, transcription of the ribozyme gene was performed with [ $1'-^2\text{H}$ ]-ATP. Lane 3, transcription of the ribozyme gene was performed with [ $5'-^2\text{H}_2$ ]-ATP. Lane 4, transcription of the ribozyme gene was performed with commercially available, all-protio ATP (control). The intense band in lanes 2–4 corresponds to the expected 390 nt L-21 Sca ribozyme.

quantities useful for RNA transcription or DNA synthesis.

### Acknowledgements

This research was supported by PHS grant R01 GM40894 (TDT). National Research Service Award F32 GM20475 to E.R.J. is gratefully acknowledged. We thank Professor JoAnne Stubbe and Professor Jamie Williamson for generously supplying materials. We thank Professor C. A. Townsend, Dr. J. J. Hangeland, and Dr. L. G. Scott for helpful discussions, and Dr. D. Young for the deuterium content determination. Mass spectral data were provided by the Boston University School of Medicine Mass Spectrometry Resource, which is supported by NIH/NCRR Grant Nos. P41-RR10888 and S10-RR10493 to Professor C. E. Costello.

### References and Notes

1. Yamakage, S.; Maltseva, T. V.; Nilson, F. P.; Foldesi, A.; Chattopadhyaya, J. *Nucleic Acids Res.* **1993**, *21*, 5005.
2. Puglisi, J. D.; Wyatt, J. R.; Tinoco, I. *J. Mol. Biol.* **1990**, *214*, 437.
3. De Voss, J. J.; Townsend, C. A.; Ding, W.-D.; Morton, G. O.; Ellestad, G. A.; Zein, N.; Tabor, A. B.; Schreiber, S. L. *J. Am. Chem. Soc.* **1990**, *112*, 9669.
4. Kozarich, J. W.; Worth, L.; Frank, B. L.; Christner, D. F.; Vanderwall, D. E.; Stubbe, J. *Science* **1989**, *245*, 1396.
5. Balasubramanian, B.; Pogozelski, W. K.; Tullius, T. D. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 9738.

6. De Voss, J. J.; Hangeland, J. J.; Townsend, C. A. *J. Org. Chem.* **1994**, *59*, 2715.
7. Tolbert, T. J.; Williamson, J. R. *J. Am. Chem. Soc.* **1996**, *118*, 7929.
8. Wu, J. C.; Kozarich, J. W.; Stubbe, J. *Biochemistry* **1985**, *24*, 7562.
9. Vorbruggen, H.; Krolkiewicz, K.; Benua, B. *Chem. Ber.* **1981**, *114*, 1234.
10. Lichtenthaler, F. W.; Voss, P.; Heerd, A. *Tetrahedron Lett.* **2141**, 1974.
11. Niedballa, U.; Vorbruggen, H. *J. Org. Chem.* **1974**, *39*, 3660.
12. Wright, G. E.; Dudycz, L. W. *J. Med. Chem.* **1984**, *27*, 175.
13. 3-Phosphoglycerate mutase was purchased from Boehringer Mannheim. Enolase, myokinase, pyruvate kinase, and guanylate kinase were purchased from Sigma. We obtained as the generous gifts of Jamie Williamson bacterial expression strains for ribokinase, PRPP synthetase, adenine phosphoribosyltransferase, and xanthine-guanine phosphoribosyltransferase. We expressed and purified these enzymes using the procedures described in ref 7.
14. Batey, R. T.; Inada, M.; Kujawinski, E.; Puglisi, J. D.; Williamson, J. R. *Nucleic Acids Res.* **1992**, *20*, 4515.
15. [ $1^2\text{H}$ ]-Adenosine-5'-triphosphate: yield, 78%;  $^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ )  $\delta$  8.35 (s, 1H), 8.08 (s, 1H), 4.58 (d, 1H,  $J=5.2$  Hz), 4.39 (m, 1H), 4.21 (m, 1H), 4.01–4.12 (m, 2H); MS  $m/z$  507.2 (507.15 calcd for  $\text{C}_{10}\text{H}_{14}\text{DN}_5\text{O}_{13}\text{P}_3$ ); deuterium content 94%. [ $4^2\text{H}$ ]-Adenosine-5'-triphosphate: yield, 81%;  $^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ )  $\delta$  8.35 (s, 1H), 8.08 (s, 1H), 5.93 (d, 1H,  $J=6.0$  Hz), 4.58 (d×d, 1H,  $J=6.0, 5.6$  Hz), 4.38 (d, 1H,  $J=5.6$  Hz), 4.03–4.11 (m, 2H); MS  $m/z$  507.0 (507.15 calcd for  $\text{C}_{10}\text{H}_{14}\text{DN}_5\text{O}_{13}\text{P}_3$ ); deuterium content 98%. [ $5^2\text{H}_2$ ]-Adenosine-5'-triphosphate: yield, 75%;  $^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ )  $\delta$  8.36 (s, 1H), 8.10 (s, 1H), 5.93 (d, 1H,  $J=6.0$  Hz), 4.58 (m, 1H), 4.38 (m, 1H), 4.21 (d, 1H,  $J=2.8$  Hz); MS  $m/z$  508.1 (508.12 calcd for  $\text{C}_{10}\text{H}_{13}\text{D}_2\text{N}_5\text{O}_{13}\text{P}_3$ ); deuterium content 94%. [ $1^2\text{H}$ ]-Guanosine-5'-triphosphate: yield, 74%;  $^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ )  $\delta$  7.94 (s, 1H), 4.58 (d, 1H,  $J=6.8$  Hz), 4.39 (m, 1H), 4.16 (m, 1H), 3.98–4.10 (m, 2H); MS  $m/z$  523.0 (523.15 calcd for  $\text{C}_{10}\text{H}_{14}\text{DN}_5\text{O}_{14}\text{P}_3$ ); deuterium content 94%. [ $4^2\text{H}$ ]-Guanosine-5'-triphosphate: yield, 79%;  $^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ )  $\delta$  7.96 (s, 1H), 5.73 (d, 1H,  $J=6.0$  Hz), 4.58 (d×d, 1H,  $J=6.0, 4.8$  Hz), 4.37 (d, 1H,  $J=4.8$  Hz), 3.99–4.10 (m, 2H); MS  $m/z$  523.2 (523.15 calcd for  $\text{C}_{10}\text{H}_{14}\text{DN}_5\text{O}_{14}\text{P}_3$ ); deuterium content 100%. [ $5^2\text{H}_2$ ]-Guanosine-5'-triphosphate: yield, 72%;  $^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ )  $\delta$  7.94 (s, 1H), 5.73 (d, 1H,  $J=5.6$  Hz), 4.58 (m, 1H), 4.36 (m, 1H), 4.15 (m, 1H); MS  $m/z$  524.0 (524.12 calcd for  $\text{C}_{10}\text{H}_{13}\text{D}_2\text{N}_5\text{O}_{14}\text{P}_3$ ); deuterium content 99%.
16. We obtained the ribonucleotide reductase expression plasmid pSquire as a generous gift from JoAnne Stubbe. We expressed and purified the enzyme as described in ref 17.
17. Booker, S.; Stubbe, J. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 8352.
18. To obviate the need to use chemical synthesis to produce specifically deuterated riboses, the following deuterated riboses may be obtained from Omicron Biochemicals (South Bend IN; <http://www.omicronbio.com>): D-[ $1^2\text{H}$ ]ribose, D-[ $2^2\text{H}$ ]ribose, D-[ $3^2\text{H}$ ]ribose, D-[ $4^2\text{H}$ ]ribose, and D-[ $5,5^2\text{H}$ ]ribose.