A Facile Synthetic Method for $^{15}N^4$-Labeled Cytosine Nucleosides

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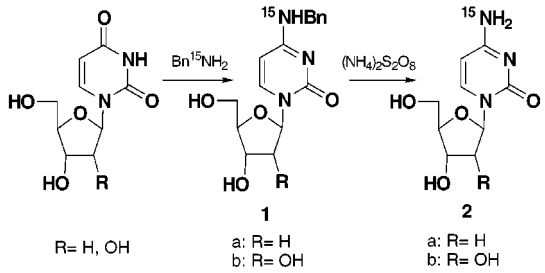
Recent advances in heteronuclear NMR spectroscopic techniques on isotopically labeled DNA and RNA oligonucleotides have made it possible to obtain valuable information regarding the dynamic structural features of nucleic acids and their interactions with proteins or xenobiotics in the solution state.[1] Along this line, a variety of methods for the regioselective ^{15}N-labeling of purine nucleosides has been developed.[2] The preparative methods for regioselectively ^{15}N-labeled pyrimidine nucleosides, however, are still unsatisfactory to use.[3]

The exocyclic amino group in a cytosine moiety is a good candidate for the ^{15}N-labeling of nucleic acids, because this functional group plays very important roles to form hydrogen bonds with suitable acceptors in the nucleic acids, proteins, or xenobiotics. The synthetic methods for the $^{15}N^4$-labeled cytosine nucleosides previously reported involve the introduction of a good leaving group such as SMe, SO_3H, and tetrazolyl groups into the C_4-position of the uridine derivatives and subsequent nucleophilic displacement with ^{15}N-enriched ammonia or phthalimide (followed by alkaline hydrolysis).[4] These methods, however, required many steps from commercially available starting materials because of the necessity of protecting the primary and secondary alcohols in the sugar moiety during the reactions for the preparation of the desired $^{15}N^4$-labeled cytosine nucleosides.

In a previous paper,[5] we have documented a convenient synthesis of $^{15}N^6$-labeled adenosine and 2′-deoxyadenosine involving the silylation−benzylamination of inosine

Scheme 1



derivatives followed by oxidative *N*-debenzylation with ammonium persulfate under thermal conditions. We report herein the successful application of this synthetic method which provides a simple method for the preparation of $^{15}N^4$-labeled cytosine nucleosides starting from the appropriate uridine derivatives.

Treatment of 2′-deoxyuridine with ^{15}N-enriched benzylamine in hexamethyldisilazane (HMDS) containing excess α-picoline and a catalytic amount of chlorotrimethylsilane at 140 °C for 2 days[6a] afforded $^{15}N^4$-labeled N^4-benzyl-2′-deoxycytidine (**1a**) in 74% yield, together with small amounts of ^{15}N-labeled N^4-benzylcytosine and $^{15}N^{2,15}N^4$-labeled N^2,N^4-dibenzylaminopyrimidine. The structure of the labeled compound (**1a**) was confirmed based on its spectral data, i.e., the observation of a molecular ion peak *m*/*z* 318.1335 [calcd for $C_{16}H_{19}{}^{15}N_1{}^{14}N_2O_4$ (M^+) 318.1345] in its HRMS spectrum and the appearance of broad double triplet signals with a large $^{15}N-^1H$ coupling constant ($J = 93$ Hz) at δ 8.14 (1H) and a doublet signal at δ 4.51 (2H, $J = 6$ Hz) assignable to the ^{15}N-labeled benzylamino group in the ^1H NMR spectrum. Under analogous conditions,[6b] the thermal reaction of uridine with ^{15}N-enriched benzylamine in HMDS afforded $^{15}N^4$-labeled N^4-benzylcytidine (**1b**) in 84% yield (Scheme 1).

Heating a solution of the N^4-benzyl-2′-deoxycytidine (**1a**) and a slight excess of ammonium persulfate in a mixed solvent of 0.5 mol phosphate buffer (pH 7.0) with acetonitrile at 80 °C for 30 min followed by chromatographic separation allowed the isolation of $^{15}N^4$-labeled 2′-deoxycytidine (**2a**)[4b] in 79% yield, together with $^{15}N^4$-labeled N^4-benzylcytosine, $^{15}N^4$-labeled cytosine,[7] and benzaldehyde. Structural proof for the product (**2a**) rests upon the observation of a molecular ion peak: *m*/*z* 228.0883 [calcd for $C_9H_{13}{}^{15}N_1{}^{14}N_2O_4$ (M^+) 228.0876] in its HRMS spectrum and the observation of two broad doublet signals with large coupling constants (each $J =$ 89 Hz) at δ 7.08 and 7.19 (each 1H) assignable to the ^{15}N-labeled primary amino group in its ^1H NMR spectrum. Analogous results were obtained for the oxidation of the N^4-benzylcytidine (**1b**) with ammonium persulfate that produced $^{15}N^4$-labeled cytidine (**2b**).

Thus, the syntheses of the desired $^{15}N^4$-labeled cytosine nucleosides (**2**) were accomplished using the silylation−benzylamination of appropriate uridine derivatives fol-

(1) (a) Moore, P. B. *Acc. Chem. Res.* **1995**, *28*, 251−256. (b) Lynch, S. R.; Tinoco, I., Jr. *Nucleic Acids Res.* **1998**, *26*, 980−987. (c) Zhang, X.; Gaffney, B. L.; Jones, R. A. *J. Am. Chem. Soc.* **1998**, *120*, 6625−6626. (d) Leitner, D.; Schroeder, W.; Weisz, K. *J. Am. Chem. Soc.* **1998**, *120*, 7123−7124. (e) Dingley, A. J.; Grzesiek, S. *J. Am. Chem. Soc.* **1998**, *120*, 8293−8297. (f) Pervushin, K. V.; Ono, A.; Fernandez, C.; Szyperski, T.; Kainosho, M.; Wuthrich, K. *Proc. Natl. Acad. Sci., USA*, **1998**, *95*, 14147−14151. (g) Wuthrich, K. *Nat. Struct. Biol.* **1998**, 492−495. (h) Masse, J. E.; Allain, F. H.-T.; Yen, Y.-M.; Johnson, R. C.; Feigon, J. *J. Am. Chem. Soc.* **1999**, *121*, 3547−3548.

(2) (a) Grenner, G.; Schmidt, H.-L. *Chem. Ber.* **1977**, *110*, 373−375. (b) Leonard, N. J.; Henderson, T. R. *J. Am. Chem. Soc.* **1975**, *97*, 4990−4999. (c) Kieper, I.; Schmidt, T.; Fera, B.; Ruterjans, H. *Nucleosides Nucleotides*, **1988**, *7*, 821−825. (d) De Napoli, L.; Messere, A.; Montesarchio, D.; Piccialli, G. *J. Org. Chem.* **1995**, *60*, 2251−2253. (e) Orji, C. C.; Kelly, J.; Ashburn, D. A.; Silks, L. A., III *J. Chem. Soc., Perkin Trans. 1* **1996**, 595−597. (f) Zhao, H.; Pagano, A. R.; Wang, W.; Shallop, A.; Gaffney, B. L.; Jones, R. A. *J. Org. Chem.* **1997**, *62*, 7832−7835 and references therein.

(3) (a) Sako, M. *J. Synth. Org. Chem., Jpn.* **1996**, *54*, 54−61 and references therein. (b) Amantea, A.; Henz, M.; Strazewski, P. *Helv. Chimica. Acta* **1996**, *79*, 244−254.

(4) (a) Niu, C.-H. *Anal. Biochem.* **1984**, *139*, 404−407. (b) Kupferschmitt, G.; Schmidt, J.; Schmidt, T.; Fera, B.; Buck, F.; Ruterjans, H. *Nucleic Acids Res.* **1987**, *15*, 6225−6241. (c) Kamaike, K.; Takahashi, M.; Utsugi, K.; Tomizuka, K.; Ishido, Y. *Tetrahedron Lett.* **1995**, *36*, 91−94.

(5) Sako, M.; Ishikura, H.; Hirota, K.; Maki, Y. *Nucleosides Nucleotides*, **1994**, *13*, 1239−1246.

(6) (a) Vorbruggen, H.: Krolikiewicz. K.; Niedballa, U. *Liebigs Ann. Chem.* **1975**, 988−1002. (b) Vorbruggen, H.: Krolikiewicz. K. *Liebigs Ann. Chem.* **1976**, 745−761. (c) Vorbruggen, H. *Acc. Chem. Res.* **1995**, *28*, 509−520.

(7) Wempen, I.; Brown, G. B.; Ueda, T.; Fox, J. J. *Biochemistry* **1965**, *4*, 54−57.

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lowed by a single-electron oxidation of the resulting $^{15}N^4$-labeled N^4-benzylcytidine derivatives (**1**) with a generated sulfate anion radical in the pH 7.0 buffer solution. Advantages of the present synthetic method are (1) the use of ^{15}N-enriched benzylamine easily prepared from the relatively inexpensive ^{15}N-enriched benzamide as a source of ^{15}N-labeling, (2) the deprotection of the N^4-benzyl group smoothly proceeded even under mild conditions, and (3) the reactions proceeding effectively even without protection of the hydroxyl groups in the sugar moiety. The present synthetic method is, in principle, applicable to the preparation of $^{15}N^4$-labeled cytidine derivatives having the protected hydroxyl groups in the sugar moiety, which are starting materials for the syntheses of DNA and RNA oligonucleotides.

Experimental Section

^1H NMR spectra were obtained at 400 MHz. Column chromatography was performed on silica gel (Cica Merck No. 9385-5B; silica gel 60). ^{15}N-Enriched benzylamine was prepared by the $LiAlH_4$ reduction of ^{15}N-enriched benzamide (99 atom % ^{15}N, Isotec Inc.), according to a previously reported procedure.[8] Unless otherwise noted, materials obtained from commercial suppliers were used without further purification.

Preparation of $^{15}N^4$-Labeled N^4-Benzyl-2′-deoxycytidine (1a). A mixture of 2′-deoxyuridine (Aldrich, 99+% purity) (218 mg, 0.95 mmol) and ^{15}N-enriched benzylamine (205 mg, 1.90 mmol) in HMDS (Aldrich, 99.9% purity) (606 μL, 2.87 mmol) containing α-picoline (250 μL, 2.53 mmol) and chlorotrimethylsilane (12 μL, 0.1 mmol) was heated at 140 °C under an argon atmosphere for 2 days. After treatment with 10% aqueous methanol at 60 °C overnight, the resulting mixture was evaporated to dryness and subjected to a silica gel column eluting with chloroform−methanol (10/1) to isolate the desired $^{15}N^4$-labeled N^4-benzyl-2′-deoxycytidine (**1a**) (223 mg, 74%), $^{15}N^4$-labeled N^4-benzylcytosine (14 mg, 7%), and $^{15}N^{2,15}N^4$-labeled N^2,N^4-dibenzylaminopyrimidine (11 mg, 4%).

For $^{15}N^4$-labeled N^4-benzyl-2′-deoxycytidine (1a): mass *m*/*z* 318 (M^+), 287, 244, 229, 202 (base peak), 107, 91; HRMS *m*/*z* 318.1335 [calcd for $C_{16}H_{19}{}^{15}N_1{}^{14}N_2O_4$ (M^+) 318.1345]; ^1H NMR (DMSO-d_6) δ 1.96 and 2.13 (each 1H, each m), 3.57 (2H, m), 3.78 (1H, m), 4.22 (1H, br), 4.51 (2H, d, J = 6 Hz), 4.96 (1H, t, J = 5 Hz), 5.19 (1H, d, J = 4 Hz), 5.84 (1H, d, J = 7 Hz), 6.18 (1H, dd, J = 6 and 7 Hz), 7.26−7.38 (5H, m), 7.80 (1H, d, J = 7 Hz), 8.14 (1H, br dt, J = 93 and 6 Hz).

For $^{15}N^4$-labeled N^4-benzylcytosine: mass *m*/*z* 202 (M^+), 107, 91; HRMS *m*/*z* 202.0864 [calcd for $C_{11}H_{11}{}^{15}N_1{}^{14}N_2O$ (M^+) 202.0873]; IR (KBr) 1641, 1606, 1512 cm^{-1}.

For $^{15}N^2$,$^{15}N^4$-labeled N^2,N^4-dibenzylaminopyrimidine: mass *m*/*z* 292 (M^+), 201, 186, 107, 91; HRMS *m*/*z* 292.1477 [calcd for $C_{18}H_{18}{}^{15}N_2{}^{14}N_2$ (M^+) 292.1472]; ^1H NMR ($CDCl_3$) δ 4.49 (2H, d, J = 6 Hz), 4.58 (2H, d, J = 5 Hz), 5.04 (1H, br d, J = 90 Hz), 5.30 (1H, br d, J = 90 Hz), 5.71 (1H, d, J = 6 Hz), 7.2−7.3 (10H, m), 7.81 (1H, d, J = 6 Hz).

Preparation of $^{15}N^4$-Labeled N^4-Benzylcytidine (1b). A mixture of uridine (Aldrich, 99% purity) (234 mg, 0.95 mmol) and ^{15}N-labeled benzylamine (205 mg, 1.90 mmol) in HMDS (810 μL, 3.84 mmol) containing ammonium sulfate (13 mg, 0.10 mmol) was heated at 140 °C under an argon atmosphere for 1 day. The after-treatment was similar to the case of **1a** followed by chromatographic separation that allowed the isolation of the desired ^{15}N-labeled cytidine derivative (**1b**) (267 mg, 84%), together with $^{15}N^{2,15}N^4$-labeled N^2,N^4-dibenzylaminopyrimidine (36 mg, 13%) and $^{15}N^4$-labeled N^4-benzylcytosine (2 mg, 1%).

For $^{15}N^4$-labeled N^4-benzylcytidine (1b): mass *m*/*z* 334 (M^+), 303, 261, 242, 231, 202 (B), 107, 91; HRMS *m*/*z* 334.1300 [calcd for $C_{16}H_{19}{}^{15}N_1{}^{14}N_2O_5$ (M^+) 334.1295]; IR (KBr) 1649, 1570 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.60 (2H, m), 3.84 (1H, br s), 3.96 (2H, br s), 4.51 (2H, d, J = 6 Hz), 4.99 (1H, d, J = 5 Hz), 5.05 (1H, b), 5.30 (1H, d, J = 5 Hz), 5.79 (1H, br s), 5.84 (1H, d, J = 7 Hz), 7.3−7.4 (5H, m), 7.87 (1H, d, J = 7 Hz), 8.02 (1H, br dt, J = 93 and 6 Hz).

Preparation of $^{15}N^4$-Labeled 2′-Deoxycytidine (2a). A solution of $^{15}N^4$-benzyl-2′-deoxycytidine (**1a**) (170 mg, 0.53 mmol) and ammonium persulfate (Wako, 98% purity) (130 mg, 0.56 mmol) in 0.5 mol phosphate buffer (pH 7.0)−acetonitrile (1/2) (3 mL) was heated at 80 °C for 30 min. After removal of the solvent under reduced pressure, the resulting residue was subjected to a silica gel column eluting with chloroform−methanol (5/1) to isolate the desired compound (**2a**) (96 mg, 79%), $^{15}N^4$-labeled cytosine (2 mg, 4%), and $^{15}N^4$-labeled N^4-benzylcytosine (3 mg, 3%), together with the starting (**1a**) (20 mg, 12%). The ^1H NMR spectrum of the first fraction showed the presence of a small amount of benzaldehyde.

For $^{15}N^4$-labeled 2′-deoxycytidine (2a): mass *m*/*z* 228 (M^+), 154, 139, 112; HRMS *m*/*z* 228.0883 [calcd for $C_9H_{13}{}^{15}N_1{}^{14}N_2O_4$ (M^+) 228.0877]; ^1H NMR (DMSO-d_6) δ 1.95 and 2.11 (each 1H, each m), 3.57 (2H, m), 3.78 (1H, m), 4.22 (1H, m), 5.00 (1H, br), 5.22 (1H, br d, J = 4 Hz), 5.75 (1H, br d, J = 7 Hz), 6.17 (1H, dd, J = 6 and 7 Hz), 7.08 and 7.19 (each 1H, each br d, J = 89 Hz), 7.81 (1H, d, J = 7 Hz).

For $^{15}N^4$-labeled cytosine: mass *m*/*z* 112 (M^+); HR-MS *m*/*z* 112.0398 [calcd for $C_4H_5{}^{15}N_1{}^{14}N_2O$ (M^+) 112.0403]; IR (KBr) 1656 cm^{-1}; ^1H NMR (DMSO-d_6) δ 5.59 (1H, d, J = 7 Hz), 6.89 and 7.12 (each 1H, each br d, J = 90 Hz), 7.33 (1H, d, J = 7 Hz).

Preparation of $^{15}N^4$-labeled cytidine (2b). The labeled cytidine (**2b**) was prepared by a procedure similar to the case of **2a**. The column chromatographic separation of the reaction mixture obtained from **1b** (49 mg, 0.15 mmol) allowed the isolation of **2b** (19 mg, 53%), $^{15}N^4$-labeled cytosine (2.6 mg, 16%), and $^{15}N^4$-labeled N^4-benzylcytosine (1.8 mg, 6%), together with the starting **1b** (10 mg, 20%). The ^1H NMR spectrum of the first fraction showed the presence of a small amount of benzaldehyde.

For $^{15}N^4$-labeled cytidine (2b): mass *m*/*z* 245 (M^+), 207, 185 (B); HRMS *m*/*z* 245.0911 [calcd for $C_9H_{14}{}^{15}N_1{}^{14}N_2O_5$ (M^+) 245.0904]; ^1H NMR (DMSO-d_6) δ 3.56 and 3.67 (each 1H, each d, J = 10 Hz), 3.84 (1H, m), 3.96 (2H, br s), 5.07, 5.11, and 5.30 (each 1H, each br), 5.76 (1H, dd, J = 6 and 7 Hz), 5.78 (1H, d, J = 8 Hz), 7.20 and 7.35 (each 1H, each br d, J = 95 Hz), 7.89 (1H, d, J = 8 Hz).

Under the conditions employed above, the deglycosylation of cytidine to cytosine proceeded more smoothly (in 56% yield) compared with the case (in 14% yield) of 2′-deoxycytidine. Thus, the prolonged reaction time for the oxidative debenzylation of the ^{15}N-labeled cytidine (**1b**) caused a decrease in the isolated yield of the labeled cytidine (**2b**).

Supporting Information Available: ^1H NMR and MS data for 1a,b and 2a,b. This material is available free of charge via the Internet at http://pubs.acs.org.

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(8) (a) Nystrom, R. F.; Brown, W. G. *J. Am. Chem. Soc.* **1948**, *70*, 3738−3740. (b) Brown, H. C.; Heim, P. *J. Org. Chem.* **1973**, *38*, 912−916. (c) Horneman, U. *Carbohydr. Res.* **1973**, *28*, 171−174.