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A New and Efficient Synthetic Method for $^{15}N_3$-Labeled Cytosine Nucleosides: Dimroth Rearrangement of Cytidine N_3-Oxides

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Abstract: The treatment of $^{15}N^4$-labeled cytidine N_3-oxide and $^{15}N^4$-labeled 2′-deoxycytidine N_3-oxide, prepared from the appropriate unprotected uridines in three reaction steps, with benzyl bromide in the presence of excess lithium methoxide allowed the smooth occurrence of their Dimroth rearrangement even under mild conditions leading to the corresponding $^{15}N_3$-labeled uridine 4-*O*-benzyloximes which can easily undergo the reductive N−O bond cleavage to give the desirable $^{15}N_3$-labeled cytosine nucleosides in high total yields.

NMR spectroscopic studies of DNA and RNA oligonucleotides regiospecifically labeled with ^{15}N-enriched nitrogen atom(s) provide valuable information regarding the dynamic structural features of nucleic acids and their interactions with xenobiotics or proteins in the solution states.[1] Along this line, we have documented the convenient and efficient syntheses of $^{15}N^6$-labeled adenine nucleosides[2] and $^{15}N^4$-labeled cytosine nucleosides[3] using the silylation−benzylamination of the appropriate unprotected nucleosides, inosines and uridines, followed by the oxidative *N*-debenzylation with ammonium persulfate. Also, Ariza et al.[4] recently reported the first synthesis of the specifically $^{15}N_3$-labeled cytidine,[5] which involves the N_3-nitration of 2′,3′,5′-*O*-protected uridine and subsequent Dimroth rearrangement[6] occurring in the presence of $^{15}NH_4Cl$−KOH−Et_3N[7] at room temperature. Their method for the preparation of the $^{15}N_3$-labeled cytidine, however, required a long time for the completion of the rearrangement (e.g., one week), the introduction of an exocyclic amino group[8] to the $^{15}N_3$-labeled uridine derivative obtained after the intramolecular rearrangement, and also the *O*-deprotection in the sugar moiety as the final step. In this paper, we describe an alternative and efficient synthetic method for the $^{15}N_3$-labeled cytidine and the first synthesis of $^{15}N_3$-labeled 2′-deoxycytidine using the appropriate $^{15}N^4$-labeled cytosine nucleosides [3] as the starting material.

The oxidations of $^{15}N^4$-labeled cytidine[3,4] and $^{15}N^4$-labeled 2′-deoxycytidine[3] with excess *m*-chloroperbenzoic acid proceeded smoothly even at ambient temperature to give the corresponding cytidine N_3-oxides ($^{15}N^4$-labeled **1a**,**b**) in almost quantitative yields.[9] Analogous results were obtained with use of magnesium monoperoxyphthalate as an oxidant. The treatment of the $^{15}N^4$-labeled cytidine N_3-oxide ($^{15}N^4$-labeled **1a**) with a slight excess of benzyl bromide in the presence of excess lithium methoxide at 37 °C for 1 day followed by chromatographic separation with a short column allowed the isolation of the desired Dimroth-rearrangement product, the $^{15}N_3$-labeled uridine 4-*O*-benzyloxime ($^{15}N_3$-labeled **2a**), in 95% yield. The structure of the labeled **2a** was confirmed based on its spectral data, i.e., the observation of a molecular ion peak *m/z* 351.1328 [calcd for $C_{16}H_{20}N_2{}^{15}NO_6$ (MH^+) 351.1323] in its HR-FABMS spectrum, the appearance of a singlet signal at δ 4.93 (2H) assignable to the *O*-benzylmethylene protons and a doublet signal with a large coupling constant ($J = 95$ Hz) at δ 9.99 (1H) assignable to the $^{15}N_3H$-proton in the ^1H NMR spectrum, and the observation of the UV spectral change [272 and 224 nm for the starting *N*-oxide (cf. **1a**); 279, 249, and 208 nm for the uridine 4-*O*-benzyloxime **2a**]. Acid hydrolysis of the $^{15}N_3$-labeled **2a** under mild conditions leading to the $^{15}N_3$-labeled uridine[7] {HR-FABMS *m/z* 246.0753 [calcd for $C_9H_{13}N^{15}NO_6$ (MH^+) 246.0744]} provided further evidence supporting the structure of the labeled **2a**. In a sharp contrast to these results, the heating of the *N*-oxide **1a** in the absence of benzyl bromide caused the complete recovery of the starting *N*-oxide even in the presence of excess lithium methoxide, and the heating of **1a** in a mixed solvent of phosphate buffers (pH 7.5−8.5) with methanol containing benzyl bromide resulted in the formation of the N_3-*O*-benzylated product, N_3-*O*-benzyl-4-iminouridine (**3**), as the major product, together with a small amount of N_3-*O*-benzyluridine (**4**).[10] When the N_3-*O*-benzylated product **3** was

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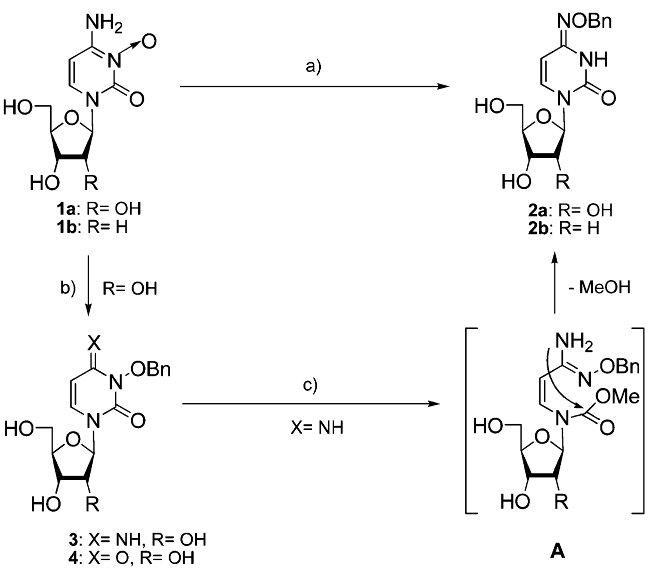
SCHEME 1. Dimroth Rearrangement of Cytidine *N*-Oxides 1a,b[a]



[a] Reagents and conditions: (a) BnBr, excess MeOLi, MeOH, 37 °C, 1 day; (b) BnBr, 0.1 M phosphate buffer (pH 7.5−8.5)−MeOH (4/1), 37 °C; (c) excess MeOLi, MeOH, 37 °C, overnight.

stirred in methanol containing lithium methoxide at 37 °C overnight, the almost quantitative conversion to the uridine 4-*O*-benzyloxime **2a** was observed. These results strongly suggest that the presence of alkyl halides and a strong base in the media are a prerequisite for the occurrence of the present rearrangement and that the N_3-*O*-benzyl-4-iminouridine **3** is a key intermediate for this reaction.

On the basis of these facts, the formation of the uridine 4-*O*-benzyloxime **2a** in this reaction can be reasonably explained by considering the transient formation of the intermediate carbamate (cf. **A**) by the initial attack of a methoxide ion on the C_2-carbonyl group in the N_3-*O*-benzyated 4-iminouridine **3** as illustrated in Scheme 1.[11]

Hydrogenation of the $^{15}N_3$-labeled uridine 4-*O*-benzyloxime ($^{15}N_3$-labeled **2a**) in the presence of a PtO_2 catalyst proceeded quickly to afford the corresponding $^{15}N_3$-labeled cytidine[4] in an almost quantitative yield.[12] Structural proof for this product rests upon the observation of a molecular ion peak *m/z* 245.0911 [calcd for $C_9H_{14}N_2{}^{15}NO_5$ (MH$^+$) 245.0904] in its HR-FABMS spectrum and the observation of ^{1}H NMR and UV spectral data identical with those of the nonlabeled cytidine. Analogous results were obtained when using the $^{15}N^4$-labeled 2′-deoxycytidine as the starting material that produced the $^{15}N_3$-labeled 2′-deoxycytidine {HR-FABMS: *m/z* 229.0949 [calcd for $C_9H_{14}N_2{}^{15}NO_4$ (MH$^+$) 229.0955]} as the desired compound.

Thus, the syntheses of the $^{15}N_3$-labeled cytosine nucleosides were accomplished by using the N_3-oxidation (cf. **1**) of the $^{15}N^4$-labeled cytidines, their Dimroth rearrangement in the presence of benzyl halides, and subsequent reductive cleavage of the N−O bond in the $^{15}N_3$-labeled uridine 4-*O*-benzyloximes (cf. **2**).

Characteristics of the present synthetic method are (1) the use of the $^{15}N^4$-labeled cytidine or $^{15}N^4$-labeled 2′-deoxycytidine, easily prepared from commercially available unprotected uridine or 2′-deoxyuridine, as the starting material, (2) the involvement of a novel type of Dimroth rearrangement in the cytidine N_3-oxides smoothly proceeding even under mild conditions, and (3) the reactions effectively proceeding even without protection of the primary and secondary alcohols in the sugar moiety. The present synthetic method, in principle, is applicable for the preparation of $^{15}N_3$-labeled cytidine derivatives having the protected hydroxyl groups in the sugar moiety, which are starting materials for the syntheses of DNA and RNA oligonucleotides, and the preparation of the $^{15}N_3$-labeled cytosine nucleosides with a modified sugar moiety.[13]

Experimental Section

4-Amino-1-(3,4-dihydroxy-5-hydroxymethyltetrahydrofuran-2-yl)-1*H*-pyrimidin-2-one 3-Oxide (Cytidine N_3-Oxide) (1a). To a suspension of cytidine (Aldrich, 99% purity; 491.4 mg, 2.0 mmol) in dry methanol (30 mL) was added *m*-chloroperoxybenzoic acid (Aldrich, 77% purity; 1.345 g, 6.0 mmol), and the mixture was stirred at ambient temperature overnight under an argon atmosphere. TLC analysis of the reaction mixture with use of chloroform−methanol−acetic acid (16/6/3) as the developing solvent showed almost the complete consumption of the starting cytidine and the formation of a more polar compound as the sole product. After removal of the solvent under reduced pressure, the resulting residue was triturated with acetone, collected by filtration, and then washed well with acetone to obtain the desired N_3-oxide (**1a**) [9] (447.8 mg, 86%) as a colorless powder in an almost pure state: mp 263−269 °C dec, from methanol; FABMS *m/z* 260 (MH$^+$), 244, and 128; UV (MeOH) 277 and 225 nm; IR (KBr) 3370, 1692, and 1653 cm^{-1}; ^{1}H NMR δ 3.57 and 3.68 (each 1H, each br d, $J = 12$ Hz), 3.87 (1H, m), 3.94 (1H, m), 3.99 (1H, m), 5.09 (1H, d, $J = 5$ Hz), 5.14 (1H, br t), 5.51 (1H, br s), 5.81 (1H, d, $J = 4$ Hz), 6.07 (1H, d, $J = 8$ Hz), 7.75 and 8.25 (each 1H, each br), and 7.79 (1H, d, $J = 8$ Hz) ppm; HR-FABMS *m/z* 260.0884 [calcd for $C_9H_{14}N_3O_6$ (MH$^+$) 260.0883].

Analogous results (the isolated yield of **1a**: 85% in the 2.0 mmol scale experiment) were obtained by the employment of magnesium monoperoxyphthalate hexahydrate (Ardrich, 80% purity) in place of *m*-chloroperoxybenzoic acid as the oxidant, though the column chromatographic purification with chloroform−methanol−acetic acid (16/6/3) as an eluting solvent was required to separate the phthalate concurrently formed in this case.

4-Amino-1-(4-hydroxy-5-hydroxymethyltetrahydrofuran-2-yl)-1*H*-pyrimidin-2-one 3-Oxide (2′-Deoxycytidine N_3-Oxide) (1b). In a manner similar to that of the cytidine N_3-oxide **1a**, the desired N_3-oxide **1b** [9d] (206.3 mg, 85%) was

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(10) No drastic changes were observed even after prolonged reaction times (e.g., 2 days).

(11) An analogous mechanism has been proposed for the conversion of acetic acid 3-acetoxy-5-(2,8-dimethyl-5-oxo[1,2,4]triazolo[1,5-*c*]pyrimidin-6-yl)tetrahydrofuran-2-ylmethyl ester to 6-(4-hydroxy-5-hydroxymethyltetrahydrofuran-2-yl)-3,8-dimethyl-6*H*-[1,2,4]triazolo[4,3-*c*]pyrimidin-5-one in the presence of a methoxide ion. Cf.: Loakes, D.; Brown, D. M.; Salisbury, S. A. *J. Chem. Soc., Perkin Trans. 1* **1999**, 1333−1337. Loakes, D.; Brown, D. M. *Recent Res. Dev. Org., Bioorg. Chem.* **2001**, *4*, 89−95.

(12) On the other hand, the Pd/C reduction of the uridine 4-*O*-benzyloxime **2a** caused the concurrent occurrence of *O*-debenzylation leading to the corresponding unsubstituted uridine 4-oxime (22%, by TLC densitometry).

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prepared by starting from 2′-deoxycytidine monohydrate (Aldrich, 99+% purity; 245.2 mg, 1.0 mmol): FABMS *m/z* 244 (MH^+), 228, and 128; UV (H_2O) 271 and 223 nm; IR (KBr) 3389, 1703, and 1657 cm^{-1}; ^1H NMR δ 2.02 and 2.22 (each 1H, each m), 3.54 and 3.59 (each 1H, each br d, $J = 12$ Hz), 3.83 (1H, dd, $J = 7$ and 3 Hz), 4.21 (1H, d, $J = 2$ Hz), 5.03 (1H, br), 5.27 (1H, br), 6.08 (1H, d, $J = 8$ Hz), 6.13 (1H, t, $J = 6$ Hz), 7.76 (1H, d, $J = 8$ Hz), 6.90 and 8.30 (each 1H, each br) ppm; HR-FABMS *m/z* 244.0925 [calcd for $C_9H_{14}N_3O_5$ (MH^+) 244.0934].

1-(3,4-Dihydroxy-5-hydroxymethyltetrahydrofuran-2-yl)-1*H*-pyrimidin-2,4-dione 4-*O*-Benzyloxime (Uridine 4-*O*-Benzyloxime) (2a). To a suspension of the N_3-oxide **1a** (77.7 mg, 0.30 mmol) and lithium hydride (Aldrich, 95% purity; 12.6 mg, 1.5 mmol) in dry methanol (5 mL) was added benzyl bromide (Tokyo Kasei, >98% purity; 40.0 μL, 0.33 mmol), and the mixture was stirred at 37 °C for 1 day under an argon atmosphere. TLC analyses of the reaction mixtures with chloroform−methanol−acetic acid (16/6/3) and chloroform−methanol (10/1) as the developing solvents showed complete consumption of the starting *N*-oxide **1a** for the almost quantitative conversion to a less polar compound. After being neutralized with 1 N HCl solution and subsequent removal of the solvent under reduced pressure, the resulting residue was subjected to a short silica gel column by eluting with chloroform−methanol (20/1) to isolate the desired oxime **2a** (triturated with diethyl ether, 99.5 mg, 95%) as a colorless amorphous powder: mp 123−125 °C (from methanol); FABMS *m/z* 350 (MH^+), 260, 218, 217, and 91; UV (MeOH) 279, 249, and 208 nm; IR (KBr) 3409 and 1691 cm^{-1}; ^1H NMR δ 3.34−3.63 (2H, m), 3.76 (1H, m), 3.90 (1H, m), 3.95 (1H, m), 4.93 (2H, s), 4.98 (1H, t, $J = 5$ Hz), 5.01 (1H, d, $J = 5$ Hz), 5.23 (1H, d, $J = 6$ Hz), 5.54 (1H, br d, $J = 8$ Hz), 5.72 (1H, d, $J = 6$ Hz), 7.12 (1H, d, $J = 8$ Hz), 7.24−7.38 (5H, m), and 9.99 (1H, br s); HR-FABMS *m/z* 350.1361 [calcd for $C_{16}H_{20}N_3O_6$ (MH^+) 350.1352].

$^{15}N_3$-Labeled Uridine 4-*O*-Benzyloxime ($^{15}N_3$-Labeled 2a). FABMS *m/z* 351 (MH^+), 261, 219, 218, and 91; ^1H NMR, identical with the above data described for the unlabeled oxime **2a**, except δ 9.99 (1H, d, $J = 95$ Hz) ppm; HR-FABMS *m/z* 351.1328 [calcd for $C_{16}H_{20}N_2{}^{15}NO_6$ (MH^+) 351.1323].

1-(4-Hydroxy-5-hydroxymethyltetrahydrofuran-2-yl)-1*H*-pyrimidin-2,4-dione 4-O−Benzyloxime (2′-Deoxyuridine 4-*O*-Benzyloxime) (2b). In a manner similar to that of the uridine 4-*O*-benzyloxime **2a**, the desired oxime **2b** (301 mg, 90%) was obtained by starting from the 2′-deoxycyctidine N_3-oxide (**1b**) (243 mg, 1.0 mmol): mp 119−122 °C; FABMS *m/z* 334 (MH^+), 218, 217, and 91; UV (MeOH) 279, 240, and 204 nm; IR (KBr)′ 3352, 1724, and 1674 cm^{-1}; ^1H NMR δ 1.95 and 2.45 (each 1H, each m), 3.50 (2H, br s), 3.70 (1H, m), 4.18 (1H, br s), 4.89 (1H, br s), 4.93 (2H, s), 5.18 (1H, br s), 5.53 (1H, br d, $J = 8$ Hz), 6.13 (1H, t, $J = 7$ Hz), 7.11 (1H, d. $J = 8$ Hz), 7.24−7.38 (5H, m), and 9.96 (1H, br s); HR-FABMS *m/z* 334.1398 [calcd for $C_{16}H_{20}N_3O_5$ (MH^+) 334.1403].

$^{15}N_3$-Labeled 2′-Deoxyuridine 4-*O*-Benzyloxime ($^{15}N_3$-Labeled 2b). FABMS *m/z* 335 (MH^+), 219, 218, and 91; ^1H NMR, identical with the above data described for the 2′-deoxyuridine 4-*O*-benzyloxime **2b** except δ 9.96 (1H, d, $J = 94$ Hz) ppm; HR-FABMS *m/z* 335.1385 [calcd for $C_{16}H_{20}N_2{}^{15}NO_5$ (MH^+) 335.1373].

Independent Syntheses of the Uridine 4-*O*-Benzyloximes (2a,b).[14]To a suspension of cytidine (102.2 mg, 0.42 mmol) or 2′-deoxycytidine (monohydrate, 100.0 mg, 0.42 mmol) in methanol (5 mL) containing sodium methoxide (2.0 mmol) was added *O*-benzylhydroxylamine HCl (Aldrich, 99% purity; 319.2 mg, 2.0 mmol), and the mixture was then stirred at ambient temperature for 3 days. After removal of the solvent under reduced pressure, the resulting residue was subjected to a silica gel column by eluting with chloroform−methanol (5/1) to isolate the desired oximes **2a**,**b** in 21% and 10% yields, respectively. The structures of the products **2a**,**b** were confirmed by comparison with the ^1H NMR spectra of the authentic compounds described above.

Reduction of $^{15}N_3$-Labeled Uridine 4-*O*-Benzyloxime ($^{15}N_3$-Labeled 2a) or $^{15}N_3$-Labeled 2′-Deoxyuridine 4-*O*-Benzyloxime ($^{15}N_3$-Labeled 2b). To a suspension of the ^{15}N-labeled uridine 4-oxime ($^{15}N_3$-labeled **2a**; 25.0 mg, 0.07 mmol) in dry methanol (1.0 mL) was added platinum(IV) oxide (PtO_2, Aldrich) (2.5 mg), and the mixture was stirred vigorously at 60 °C for 1 h under a hydrogen atmosphere. TLC analyses of the reaction mixture with chloroform−methanol−acetic acid (16/6/3) and chloroform−methanol (10/1) as the developing solvents showed the complete conversion of the starting **2a** to a more polar compound. After removal of the solvent under reduced pressure, the resulting residue was subjected to a silica gel short column by eluting with chloroform−methanol (5/1 to 3/1) to isolate the desired $^{15}N_3$-labeled cytidine (triturated with acetone, 15.7 mg, 92%):[4] UV (MeOH) 273 and 224 (sh) nm; ^1H NMR, identical with the data for the unlabeled cytidine; HR-FABMS *m/z* 245.0911 [calcd for $C_9H_{14}N_2{}^{15}NO_5$ (MH^+) 245.0904].

In a similar manner, the PtO_2 reduction of the $^{15}N_3$-labeled 2′-deoxyuridine 4-*O*-benzyloxime ($^{15}N_3$-labeled **2b**; 0.1 mmol) was accomplished to obtain the desired $^{15}N_3$-labeled 2′-deoxycytidine in 95% yield after silica gel column chromatographic purification with chloroform−methanol (5/1 to 3/1): UV (MeOH) 273 and 232 (sh) nm; ^1H NMR, identical with the data for the unlabeled 2′-deoxycytidine; HR-FABMS *m/z* 229.0949 [calcd for $C_9H_{14}N_2{}^{15}NO_4$ (MH^+) 229.0955].

Reactions of Cytidine N_3-Oxide (1a) with Benzyl Bromide in a Mixed Solvent of Phosphate Buffers (pH 7.5 and 8.5) with Methanol. A solution of cytidine N_3-oxide (**1a**; 26.0 mg, 0.1 mmol) in 0.1 mol phosphate buffer (pH 7.5 or 8.5)−methanol (4/1) (2.5 mL) containing benzyl bromide (36 μL, 0.3 mmol) was stirred at 37 °C overnight. TLC analyses of the reaction mixtures with chloroform−methanol−acetic acid (16/6/3) and chloroform−methanol (10/1) as the developing solvents showed the complete consumption of the starting *N*-oxide **1a** and the formation of a less polar product as the major product, together with a small amount of more less-polar product. The product ratios in these reactions were almost equal. After removal of the solvent under reduced pressure, the resulting residue was subjected to a silica gel column by eluting with chloroform−methanol (10/1 to 3/1) to isolate 3-benzyloxy-1-(3,4-dihydroxy-5-hydroxymethyltetrahydrofuran-2-yl)-4-imino-3,4-dihydro-1*H*-pyrimidin-2-one (**3**) (triturated with diethyl ether, 31.5 mg, 90%) {IR (KBr) 1729 and 1660 cm^{-1}; UV (MeOH) 281, 229, and 206 nm; ^1H NMR δ 3.59 and 3.72 (each 1H, each br dd, $J = 12$ and 5 Hz), 3.93 (1H, br s), 3.96 (1H, m), 4.08 (1H, m), 5.14 (1H, d, $J = 6$ Hz), 5.18 (2H, s), 5.21 (1H, t, $J = 5$ Hz), 5.59 (1H, d, $J = 5$ Hz), 5.71 (1H, d, $J = 3$ Hz), 6.10 (1H, d, $J = 8$ Hz), 7.42 (3H, m), 7.59 (2H, m), 8.14 (1H, d, $J = 8$ Hz), and 9.56 (1H, br); HR-FABMS *m/z* 350.1365 [calcd for $C_{16}H_{20}N_3O_6$ (MH^+) 350.1352]} as a colorless amorphous powder and 3-benzyloxy-1-(3,4-dihydroxy-5-hydroxymethyltetrahydro- furan-2-yl)-1*H*-pyrimidine-2,4-dione (**4**) (2.0 mg, 6%) {IR (KBr) 1719 and 1674 cm^{-1}; UV (MeOH) 263 and 205 nm; ^1H NMR δ 3.56 and 3.66 (each 1H, each br dd, $J = 12$ and 4 Hz), 3.87 (1H, m), 3.97 (1H, dd, $J = 10$ and 5 Hz), 4.06 (2H, m), 5.02 (2H, s), 5.09 (1H, d, $J = 5$ Hz), 5.13 (1H, t, $J = 5$ Hz), 5.45 (1H, d, $J = 5$ Hz), 5.77 (1H, d, $J = 5$ Hz), 5.83 (1H, d, $J = 8$ Hz), 7.39 (3H, m), 7.53 (2H, m), and 7.96 (1H, d, $J = 8$ Hz); HR-FABMS *m/z* 351.1200 [calcd for $C_{16}H_{19}N_2O_7$ (MH^+) 351.1192]} as an oily product.

Supporting Information Available: Experimental procedures for the acid-hydrolysis of the $^{15}N_3$-labeled **2a** leading to the $^{15}N_3$-labeled uridine and for the chemical conversion of the N_3-*O*-benzylated 4-iminouridine **3** to **2a**, and IR, UV, ^1H NMR, and/or MS spectra for the compounds **1a**,**b**, **2a**,**b**, **3**, and **4**, $^{15}N_3$-labeled cytidine, $^{15}N_3$-labeled 2′-deoxycytidine, and $^{15}N_3$-labeled uridine. This material is available free of charge via the Internet at http://pubs.acs.org.

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