Notes

A Facile Synthesis of [N1,NH_2-$^{15}N_2$]-, [N3,NH_2-$^{15}N_2$]-, and [N1,N3,NH_2-$^{15}N_3$]-Labeled Adenine

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The ^{15}N labeling of oligonucleotides at specific sites has proven to be very useful as a probe in NMR studies to elucidate nucleic acid structures[1−4] and nucleic acid interactions with protein, ligands, or drugs[5−9] as well as to provide direct evidence for H bonding in Watson−Crick, Hoogsteen, or non-Watson−Crick base pairs.[10−14] The recent direct detection of H bonds by spin−spin scalar coupling between nuclei of H-donor and -acceptor moieties in RNA as well as DNA provides a new approach for probing H bonds.[15−19] These new parameters are particularly valuable for studying nucleic acid polymorphism, in which interproton nuclear Overhauser effects (NOEs) are often severely underdetermined.

The synthesis of nucleosides with ^{15}N at relevant sites can be achieved either by chemical transformations of an intact nucleoside or by total synthesis, i.e., labeling of the base followed by its glycosylation with an appropriately functionalized sugar derivative. By the use of the first approach, N1 labeling of adenosine was carried out by reacting activated hypoxanthine, i.e., N1-aryl[20] or -nitro[21] derivatives, with $^{15}NH_3$ and also by N1-benzyl-mediated[22] or N1-methoxy-mediated[23] Dimroth rearrangement of [6-$^{15}NH_2$]adenosine derivatives. However, the second approach is more appropriate to achieve N3 labeling of the purines. The earlier method reported by several groups[24−26] to prepare N3-labeled adenine involves nitration of 4-bromoimidazole with $H^{15}NO_3$, whereas another strategy used by Rhee and Jones[27] involves bromination of ethyl imidazole-4,5-dicarboxylate followed by its azo coupling to introduce ^{15}N label at the C-5 position in its key steps. However, both methods require either protection of the imidazole nitrogen (earlier method) or blocking of the C-2 position to force the azo coupling at the required site (the later case). The Jones group further explored their methodology to prepare doubly labeled 5-amino-4-imidazolecarboxamide (AICA), **4c**, and to convert it to [N1,N3,NH_2-$^{15}N_3$]adenosine and [1,2,3-$^{15}N_3$]guanosine.[28] Chiriac et al. have recently reported synthesis of [8-^{13}C-1,3,7-$^{15}N_3$]xanthine from ^{15}N urea and its conversion to [8-^{13}C-1,3,7-NH_2-$^{15}N_4$]adenine.[29] However, their method involved many steps, and the overall yield is not satisfactory. Because the N3 site of purine is not involved in Watson−Crick H bonding or Hoogsteen pairing, it is available as a minor groove DNA-binding site for several small molecules acting as antiviral, antibiotic, and antineoplastic agents,[30] including Netropsin[31,32] and Distamycin.[33−35] Therefore, a practical

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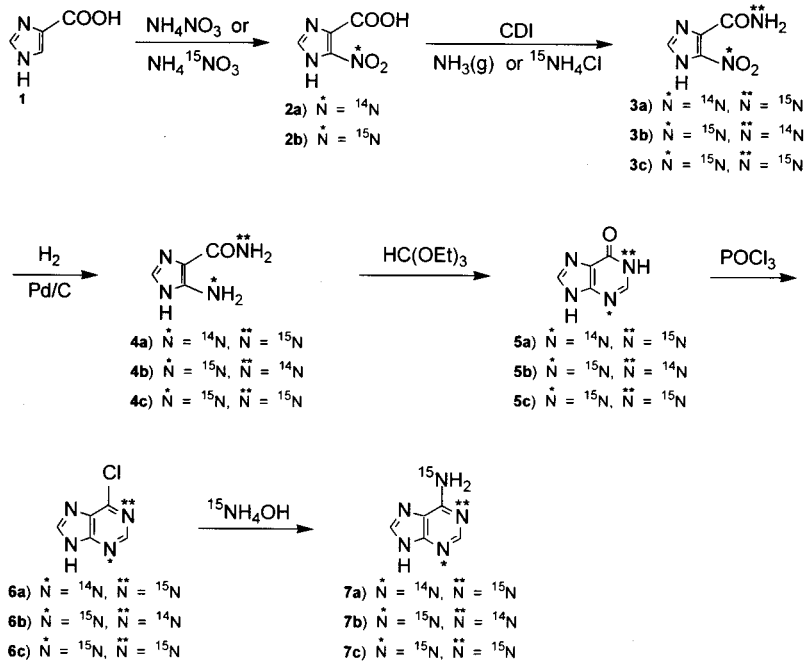
Scheme 1



and more convenient route for N3 labeling of purine is highly desired. Besides this, our major goal was to develop a common pathway for both adenine and guanine in such a way that labeling can be done at the desired position and in the desired combination. AICA is an ideal intermediate because it can be converted easily to both adenine and guanine[36] and can also afford the corresponding desired labeled nucleosides.[28,37]

We envisioned that nitration of commercially available 4-imidazolecarboxylic acid (**1**) can provide a simple route to introduce an ^{15}N label at the required C-5 position and can shorten the overall synthesis of this labeled key intermediate **4**. Herein, we report a facile synthesis of **4a**−**c** from **1** in excellent overall yields and their conversion to [N1,NH_2-$^{15}N_2$]adenine (**7a**), [N_3,NH_2-$^{15}N_2$]adenine (**7b**), and [N1,N3,NH_2-$^{15}N_3$]adenine (**7c**), respectively (Scheme 1).

The literature method to prepare 5-nitro-4-imidazolecarboxylic acid (**2a**) involves conversion of 4-methyl-5-nitroimidazole to its styryl derivative followed by oxidation,[38] resulting in poor overall yields. Surprisingly, a survey of the literature showed scanty information regarding nitration of the acid **1**. Our initial approach for nitration of **1** under different conditions using trifluoroacetic anhydride/ammonium nitrate[39] and potassium nitrate together with sulfuric acid[40] resulted in either failure or poor yield of the desired product. However, reaction conditions were optimized to use a solid nitrating agent (NH_4NO_3) instead of HNO_3 so that the corresponding cheaper and easy to handle $NH_4{}^{15}NO_3$ can be used to prepare its labeled derivative. Thus, heating **1** with NH_4NO_3 or $NH_4{}^{15}NO_3$ in sulfuric acid for 12 h at 100 °C resulted in smooth nitration of **1** at the required C-5 site to afford **2a** or its labeled analogue **2b** in 76% and 73% yield, respectively. The introduction of $^{15}NO_2$ functionality in **2b** was indicated from its ^{13}C NMR spectrum, which showed a doublet for C-5 (J_{C-N} = 26.4 Hz), and also from its ^{15}N NMR spectrum exhibiting a signal at δ 364.2.

Conversion of acid **2b** to its carboxamide **3b** via dimeric lactum using $SOCl_2$[41,42] followed by its reaction with ammonia gas afforded only 40% of the desired product. However, excellent yield (95%) was obtained by in situ activation of the acid **2b** using 1,1′-carbonyldiimidazole (CDI)[43] in DMF and subsequent bubbling of excess ammonia into it. To introduce a label at the amide position, the reaction was carried out using $^{15}NH_3$ (generated in situ with $^{15}NH_4Cl/K_2CO_3$) in CH_3CN giving **3a** or doubly labeled amide **3c** in 90% and 92% yields, respectively. The amide protons in the ^1H NMR of these carboxamides appeared as two singlets for **3b** and two doublets ($J_{N-H} \approx 90$ Hz) for **3a** and **3c** because of the proximity of the adjacent nitro group at the C-5 position. The ^{13}C NMR spectrum of **3c** showed two doublets at δ 143.8 (J_{C-N} = 26.8 Hz) and δ 159.3 (J_{C-N} = 18.6 Hz) as expected, and also its ^{15}N NMR spectrum displaying signals at δ 113.0 and 362.9 for amino and nitro functionalities, respectively, further supported its structure.

Catalytic reduction of **3a**−**c** using 10% Pd/C in MeOH/AcOH (9:1) at 45 psi for 6 h afforded singly (**4a**,**b**) or doubly labeled (**4c**) key intermediate AICAs, which were isolated as their HCl salts in 85−88% yields. The overall yields of **4a**−**c** from **1** were 57−61%. Ring closure of **4a**−**c**

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with triethyl orthoformate[44] in DMF at 140 °C to hypoxanthines **5a**−**c**, followed by their chlorination using $POCl_3$ and dimethylaniline,[28] gave the corresponding ^{15}N-labeled 6-chloropurines **6a**−**c**. Finally, ammonolysis[45] of **6a**−**c** with $^{15}NH_4OH$ in MeOH afforded the corresponding desired doubly labeled (**7a** and **7b)** or triply labeled adenine (**7c**). All of the adenines showed a doublet ($J_{N-H} \approx 90$ Hz) in their ^1H NMR spectrum, and also, their ^{15}N NMR spectrum exhibited a signal at δ 79.5 confirming the presence of the $^{15}NH_2$ functionality, besides signals at δ 237.8, 234.9, or both in the case of **7a**, **7b**, or **7c**, respectively. The overall yields of **7a**−**c** from **1** were 43−48%.

In summary, the labeling at N1 and N3 (using 1.5 equiv of cheap and solid regents, $^{15}NH_4Cl$ and $NH_4{}^{15}NO_3$, as labeling source) and also at the amino group in any required combination for adenine was achieved from 4-imidazolecarboxylic acid via a facile and straightforward synthesis of AICA in high yields.

Experimental Section

The melting points were recorded on a Gallenkamp melting point apparatus and are uncorrected. ^1H NMR spectra were acquired at 500 or 600 MHz, and ^{13}C NMR spectra were acquired at 125.7 or 150.9 MHz. ^{15}N NMR spectra were acquired at 50.6 or 60.8 MHz, and chemical shifts are reported relative to NH_3 using external 1 M [^{15}N]urea in DMSO (77.0 ppm) as a reference. The δ_H and δ_C values are expressed relative to the internal DMSO-d_6 (δ_H 2.5 ppm; δ_C 39.5 ppm), unless otherwise noted. Solvents were dried and distilled before use.

The $^{15}NH_4Cl$, $NH_4{}^{15}NO_3$, and $^{15}NH_4OH$ were purchased from Cambridge Isotope Laboratories Inc. General reagents and chemicals were purchased from commercial sources and used without further purification.

General Procedure for Preparation of 5-Nitro-4-imidazolecarboxylic Acid. To a solution of 4-imidazolecarboxylic acid (**1**, 10 mmol) in concentrated H_2SO_4 (8.0 mL) at 100 °C, NH_4NO_3 or $NH_4{}^{15}NO_3$ (15 mmol) was added slowly with stirring, and the mixture was further heated at 100 °C for 12 h. The reaction mixture was cooled and poured into crushed ice. After its pH was adjusted to 2 using NH_4OH, the mixture was extracted continuously with ethyl acetate (1.5 L) for 24 h. The organic layer was separated, washed with water, and dried over anhydrous $MgSO_4$. Concentration of the organic layer afforded the desired product, which was recrystallized from CH_3CN to afford the pure nitro compound.

5-Nitro-4-imidazolecarboxylic Acid (2a). Prepared from **1** by following the general procedure using NH_4NO_3 in 73% yield; mp >300 °C (lit.[38] mp 302−303 °C); ^1H NMR (DMSO-d_6) δ 7.91 (s, 1H), 14.03 (br s, 1H); ^{13}C NMR (DMSO-d_6) δ 119.3, 135.8, 147.2, 159.1.

[NO_2-^{15}N]-5-Nitro-4-imidazolecarboxylic Acid (2b). Prepared from **1** by following the general procedure using $NH_4{}^{15}NO_3$ in 76% yield; mp >300 °C; ^1H NMR (DMSO-d_6) δ 7.92 (s, 1H), 14.02 (br s, 1H); ^{13}C NMR (DMSO-d_6) δ 119.3, 135.8 (d, J = 3.9 Hz), 147.21 (d, J = 26.4), 159.1; ^{15}N NMR (DMSO-d_6) δ 364.1; HRMS m/z 158.0094 (calcd for $C_4H_3O_4{}^{14}N_2{}^{15}N$, 158.0094).

General Procedure for Preparation of 5-Nitro-4-imidazolecarboxamide. To a mixture of **2a** or **2b** (4.0 mmol) and 1,1′-carbonyldiimidazole (4.0 mmol), anhydrous DMF (6.0 mL) was added under N_2. After 4 h, the reaction mixture was diluted with 10 mL of aqueous THF (5% H_2O), and $^{15}NH_4Cl$ (6.0 mmol) and K_2CO_3 (8.0 mmol) were subsequently added to it (in the case of **3a** and **3c**), or ammonia gas was bubbled through it for 30 min (in the case of **3b**). After being stirred further for 48 h at room temperature, the reaction mixture was concentrated to dryness, diluted with water, and acidified with 1 N HCl to pH 4. The solid obtained was filtered, washed with water, and recrystallized from ethanol to afford pure desired product.

[$CONH_2$-^{15}N]-5-Nitro-4-imidazolecarboxamide (3a). Prepared from **2a** by following the general procedure in 90% yield; mp >300 °C; ^1H NMR (DMSO-d_6) δ 7.86 (s, 1H), 8.12 (d, J_{N-H} = 89.5 Hz, 1H), 8.16 (d, J_{N-H} = 89.0 Hz, 1H), 13.83 (br s, 1H); ^{13}C NMR (DMSO-d_6) δ 126.1 (d, J = 9.9 Hz), 135.6, 144.3, 159.8 (d, J = 18.8 Hz); ^{15}N NMR (DMSO-d_6) δ 112.9; HRMS m/z 157.0262 (calcd for $C_4H_4O_3{}^{14}N_3{}^{15}N$, 157.0254).

[NO_2-^{15}N]-5-Nitro-4-imidazolecarboxamide (3b). Prepared from **2b** by following the general procedure in 95% yield; mp >300 °C; ^1H NMR (DMSO-d_6) δ 7.84 (s, 1H), 8.11 (s, 1H), 8.16 (s, 1H), 13.83 (br s, 1H); ^{13}C NMR (DMSO-d_6) δ 126.1, 135.5, 144.4 (d, J = 25.0 Hz), 159.8; ^{15}N NMR (DMSO-d_6) δ 362.9; HRMS m/z 157.0249 (calcd for $C_4H_4O_3{}^{14}N_3{}^{15}N$, 157.0254).

[NO_2,$CONH_2$-$^{15}N_2$]-5-Nitro-4-imidazolecarboxamide (3c). Prepared from **2c** by following the general procedure in 92% yield; mp >300 °C; ^1H NMR (DMSO-d_6) δ 7.86 (s, 1H), 8.12 (d, J_{N-H} = 89.5 Hz, 1H), 8.16 (d, J_{N-H} = 88.6 Hz, 1H), 13.84 (br s, 1H); ^{13}C NMR (DMSO-d_6) δ 126.1, 135.5, 144.3 (d, J = 26.8 Hz), 159.8 (d, J = 18.6 Hz); ^{15}N NMR (DMSO-d_6) δ 113.0, 362.9; HRMS m/z 158.0218 (calcd for $C_4H_4O_3{}^{14}N_2{}^{15}N_2$, 158.0224).

General Procedure for Preparation of 5-Amino-4-imidazolecarboxamide (AICA). A suspension of **3** (4.0 mmol) in MeOH (18.0 mL) containing AcOH (2.0 mL) was purged with nitrogen before the catalyst (0.312 g of 10% activated Pd/C) was added and hydrogenated in a Parr hydrogenator with shaking at 45 psi for 6 h. The color of the solution changed from yellow to red during the reaction. The mixture was filtered through Celite, and dry HCl(g) was bubbled into the filtrate for 15 min at 0 °C. After the solvent was evaporated, the residue was triturated with ethanol, filtered, and dried to afford the desired product as its hydrochloride salt.

[$CONH_2$-^{15}N]-5-Amino-4-imidazolecarboxamide (4a). Prepared from **3a** by following the general procedure in 85% yield; mp 268−270 °C (dec); ^1H NMR (DMSO-d_6) δ 6.40 (br s, 2H), 7.56 (d, J = 85.8 Hz, 2H), 8.54 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 102.4 (d, J = 8.2 Hz), 128.2, 143.2, 161.2 (d, J = 16.5 Hz); ^{15}N NMR (DMSO-d_6) δ 104.0; HRMS m/z 127.0512 (calcd for $C_4H_6O^{14}N_3{}^{15}N$, 127.0512).

[NH_2-^{15}N]-5-Amino-4-imidazolecarboxamide (4b). Prepared from **3b** by following the general procedure in 86% yield; mp 268−270 °C (dec); ^1H NMR (DMSO-d_6) δ 6.36 (br s, 2H), 7.52 (br s, 2H), 8.48 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 102.4, 128.2, 143.1 (d, J = 17.8 Hz), 161.8; ^{15}N NMR (DMSO-d_6) δ 52.6; HRMS m/z 127.0511 (calcd for $C_4H_6O^{14}N_3{}^{15}N$, 127.0512).

[NH_2,$CONH_2$-$^{15}N_2$]-5-Amino-4-imidazolecarboxamide (4c). Prepared from **3c** by following the general procedure in 88% yield; mp 268−271 °C (dec); ^1H NMR (DMSO-d_6) δ 6.40 (br s, 2H), 7.57 (d, J = 87.6 Hz); ^{15}N NMR (DMSO-d_6) δ 53.0, 103.9. Its other spectroscopic characteristics were identical to those reported for its formate salt.[28]

[N1,^{15}N]Hypoxanthine (5a). Prepared from **4a** by following the literature procedure[44] in 84% yield; mp >300 °C; ^1H NMR (DMSO-d_6) δ 7.95 (d, J = 7.0 Hz, 1H), 8.10 (s, 1H), 12.9 (d, J = 90.0 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 119.2, 140.1, 144.8 (d, J = 7.5 Hz), 153.2, 155.3 (d, J = 10.5 Hz); ^{15}N NMR (DMSO-d_6) δ 172.6; HRMS m/z 137.0358 (calcd for $C_5H_4O^{14}N_3{}^{15}N$, 137.0355).

[N3,^{15}N]Hypoxanthine (5b). Prepared from **4b** by following the literature procedure[44] in 88% yield; mp >300 °C; ^1H NMR (DMSO-d_6) δ 7.95 (d, J = 13.0 Hz, 1H), 8.09 (s, 1H), 12.3 (br s, 1H); ^{13}C NMR (DMSO-d_6) δ 118.9, 140.1, 144.9 (d, J = 7.3 Hz), 153.2, 155.8; ^{15}N NMR (DMSO-d_6) δ 225.9; HRMS m/z 137.0357 (calcd for $C_5H_4O^{14}N_3{}^{15}N$, 137.0355).

[N1,N3,$^{15}N_2$]Hypoxanthine (5c). Prepared from **4c** by following the literature procedure[44] in 87% yield. Its physical properties and spectroscopic characteristics were identical to those reported.[28]

[N1,^{15}N]-6-Chloropurine (6a). Prepared from **5a** by following the literature procedure[28] in 93% yield; mp >300 °C; ^1H NMR (DMSO-d_6) δ 8.67 (s, 1H), 8.73 (d, J = 15.0 Hz, 1H), 13.91 (br s, 1H); ^{13}C NMR (DMSO-d_6) δ 130.5, 145.8, 147.7, 151.5 (d, J = 3.2 Hz), 152.8 (br s); ^{15}N NMR (DMSO-d_6) δ 255.2; HRMS m/z 155.0012 (calcd for $C_5H_3{}^{14}N_3{}^{15}NCl$, 155.0017).

[N3,^{15}N]-6-Chloropurine (6b). Prepared from **5b** by following the literature procedure[28] in 91% yield; mp >300 °C; ^1H NMR (DMSO-d_6) δ 8.66 (s, 1H), 8.73 (d, J = 15.0 Hz), 13.89 (br s, 1H);

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^{13}C NMR (DMSO-d_6) δ 130.6, 145.8, 147.7 (br s), 151.5 (d, J = 3.2 Hz), 152.8; ^{15}N NMR (DMSO-d_6) δ 273.5; HRMS m/z 155.0013 (calcd for $C_5H_3{}^{14}N_3{}^{15}NCl$, 155.0017).

[N1,N3,$^{15}N_2$]-6-Chloropurine (6c). Prepared from **5c** by following the literature procedure[28] in 88% yield. Its physical properties and spectroscopic characteristics were identical to those reported.[28]

[N1,NH_2-$^{15}N_2$]Adenine (7a). Prepared by following the literature procedure[45] using **6a** in 98% yield; mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 7.08 (d, J = 89.6 Hz, 2H), 8.07 (s, 1H), 8.10 (d, J = 15.5 Hz), 12.37 (br s, 1H);^{13}C NMR (DMSO-d_6) δ 118.3, 139.2, 150.2, 152.4 (br s), 155.5 (br s);^{15}N NMR (DMSO-d_6) δ 79.5, 234.8; HRMS m/z 137.0489 (calcd for $C_5H_5{}^{14}N_3{}^{15}N_2$, 137.0486).

[N3, NH_2-$^{15}N_2$]Adenine (7b). Prepared from **6b** by following the literature procedure[45] in 94% yield; mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 7.10 (d, J = 89.7 Hz, 2H), 8.08 (s, 1H), 8.10 (d, J = 14.8 Hz), 12.85 (br s, 1H);^{13}C NMR (DMSO-d_6) δ 118.3, 139.3, 150.2, 152.4 (br s), 155.5 (br s); ^{15}N NMR (DMSO-d_6) δ 79.5, 227.9; HRMS m/z 137.0482 (calcd for $C_5H_5{}^{14}N_3{}^{15}N_2$, 137.0486).

[N1,N3,NH_2-$^{15}N_3$]Adenine (7c). Prepared from **5c** by following the literature procedure[45] in 92% yield; mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 7.09 (d, J = 89.7 Hz, 2H), 8.09 (s, 1H), 8.11 (t, J = 15.4 Hz, 1H), 12.85 (br s, 1H);^{13}C NMR (DMSO-d_6) δ 118.3, 139.2, 150.4, 152.4 (br s), 155.5 (br s); ^{15}N NMR (DMSO-d_6) δ 79.5, 227.8, 234.9; HRMS m/z 138.0461 (calcd for $C_5H_5{}^{14}N_2{}^{15}N_3$, 138.0456).

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Supporting Information Available: ^{1}H and ^{15}N NMR spectra for compounds **2b**, **3c**, and **7c**. This material is available free of charge via the Internet at http://pubs.acs.org.

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