Notes

Synthesis of [1-^{15}N]-Labeled 2′-Deoxyinosine and 2′-Deoxyadenosine†

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The potential of ^{15}N-labeled oligonucleotides in a variety of NMR studies[1] on nucleic acid structures and nucleic acid–protein interactions, in which it is important to have a correct and complete assignment of the exchangeable protons, justifies work on improving the synthesis of the requisite ^{15}N-labeled monomers. In the last years several groups have reported a number of syntheses of ^{15}N-labeled purine nucleosides.[1-8]

The 1-N and 6-N are the positions of choice for ^{15}N-labeling since they are directly involved in the Watson–Crick adenine–thymine base pairing in duplex DNA. Regarding the [1-^{15}N]-labeled purine nucleosides, the previously reported syntheses on an intact nucleoside follow essentially the same route, in which the [6-^{15}N]-labeled adenine derivative is transformed into [1-^{15}N]-labeled product *via* Dimroth rearrangement.[3,4] This rearrangement requires a positively charged substrate, such as 1-*N*-alkyladenine[5] or other 1-N-substituted adenine derivatives[6-8] to increase the rate of the initial ring opening, caused by attack of hydroxide ion at the 2-C position, followed by a fast ring reclosure.

It is well known that the basic treatment of an appropriate 1-N-substituted inosine leads, by a similar nucleophilic attack on 2-C, to ring opening to provide the aminoimidazole carboxamide derivative.[9] In a previous paper[10] we reported that the dimeric nucleoside **1**, having (1-N)–(6-C) linked hypoxanthine bases, treated with ammonia or alkylamine, did not lead to the imidazole carboxamide derivative but, by a reclosure of the six-membered ring, furnished inosine or 1-*N*-alkylinosine. **1**, reacted with $^{15}NH_3$, allowed us to obtain in one step [1-^{15}N]-2′-deoxyinosine. We reasoned that it might be possible to use an 1-*N* aryl derivative of 2′-deoxyinosine instead of the reported dimer as a more feasible intermediate to obtain the desired [1-^{15}N]-labeled compound. We report here that the 4-nitrophenyl moiety provides an appropriate electron-withdrawing group to induce in product **2** the requested increase in electrophilicity of the 2-C position. The 4-nitroaniline group also serves as a good leaving group upon ring closure of the formamidine intermediate **5**. In this approach (Scheme 1) the ^{15}N atom is incorporated in the hypoxanthine base by direct reaction of $^{15}NH_3$ with the purine derivative **4**.

Treating 3′,5′-di-*O*-acetyl-2′-deoxyinosine (**3**) with 4-nitrofluorobenzene (4-NFB, 2.5 equiv) and K_2CO_3 (2.5 equiv) in DMF, the 1-N-(4-nitrophenyl) derivative **4** was obtained as a sole product (92%). The reaction of **4** with aqueous $^{15}NH_3$ (3.3 N, 99% ^{15}N, 12 h, 50 °C) gave [1-^{15}N]-labeled 2′-deoxyinosine (**6**, 55%), as a result of the cited rearrangement and the concomitant removal of the sugar acetyl protecting groups. As a side product, the 5-amino-1-(2′-deoxy-β-D-ribofuranosyl)imidazole-4-[*N*-(4-nitrophenyl)carboxamide] (**7**) (43%) was also found, whose formation is thought to arise from the transient reactive intermediate **5** (not isolated), as a consequence of the loss of the formamidine group by aminolysis. The hypothesized mechanism was confirmed by reacting **4** with propylamine (12 h, 50 °C). In this case, the monocyclic intermediate **8** (25%) could be isolated, together with the expected 1-*N*-propyl derivative **9** (58%) and the imidazole carboxamide **7** (15%). Moreover, when pure **8** was heated in DMF (12 h, 50 °C), only the cyclization took place, affording **9** (90%), while on treatment with propylamine (12 h, 50 °C) the expected **7** (24%) and **9** (73%) were obtained. From all the reaction mixtures which led to **6** or **9**, the 4-nitroaniline could be isolated in the related percentages. The described procedure afforded [1-^{15}N]-2′-deoxyinosine (**6**) in only three steps in 48% overall yield, starting from 2′-deoxyinosine (**2**). This procedure is considerably more convenient than the route involving the dimer **1** on the basis of the higher final yields and the reduced consumption of the precious aqueous $^{15}NH_3$, inevitably also consumed in the removal of the four acetyl protecting groups.

The conversion of [1-^{15}N]-2′-deoxyinosine (**6**) to [1-^{15}N]-2′-deoxyadenosine (**12**) is not difficult and can be performed using previously reported procedures.[10-12] The reaction[10] of [1-^{15}N]-3′,5′-di-*O*-acetyl-2′-deoxyinosine (**10**) with the adduct triphenylphosphine/CCl_4 in CH_2Cl_2 in the presence of DBU afforded the [1-^{15}N]-6-chloro derivative **11** (38% yield), which, after aqueous concentrated ammonia treatment (8 h, 50 °C) gave [1-^{15}N]-2′-deoxyadenosine (**12**) in 89% yield.

All the synthesized compounds were purified by silica gel chromatography and the structures were confirmed by spectroscopic data (^{1}H and ^{13}C NMR, FAB MS, and UV) which agreed, for known compounds, with the literature values.

In conclusion we have developed an easy and convenient synthetic procedure for the preparation of [1-^{15}N]-2′-deoxyinosine and [1-^{15}N]-2′-deoxyadenosine. Starting from 2′-deoxyinosine, *via* the easily accessible key intermediate 1-*N*-(4-nitrophenyl) derivative **4**, the target compound [1-^{15}N]-2′-deoxyinosine, which can be consid-

† Dedicated to the memory of Prof. Ciro Santacroce.

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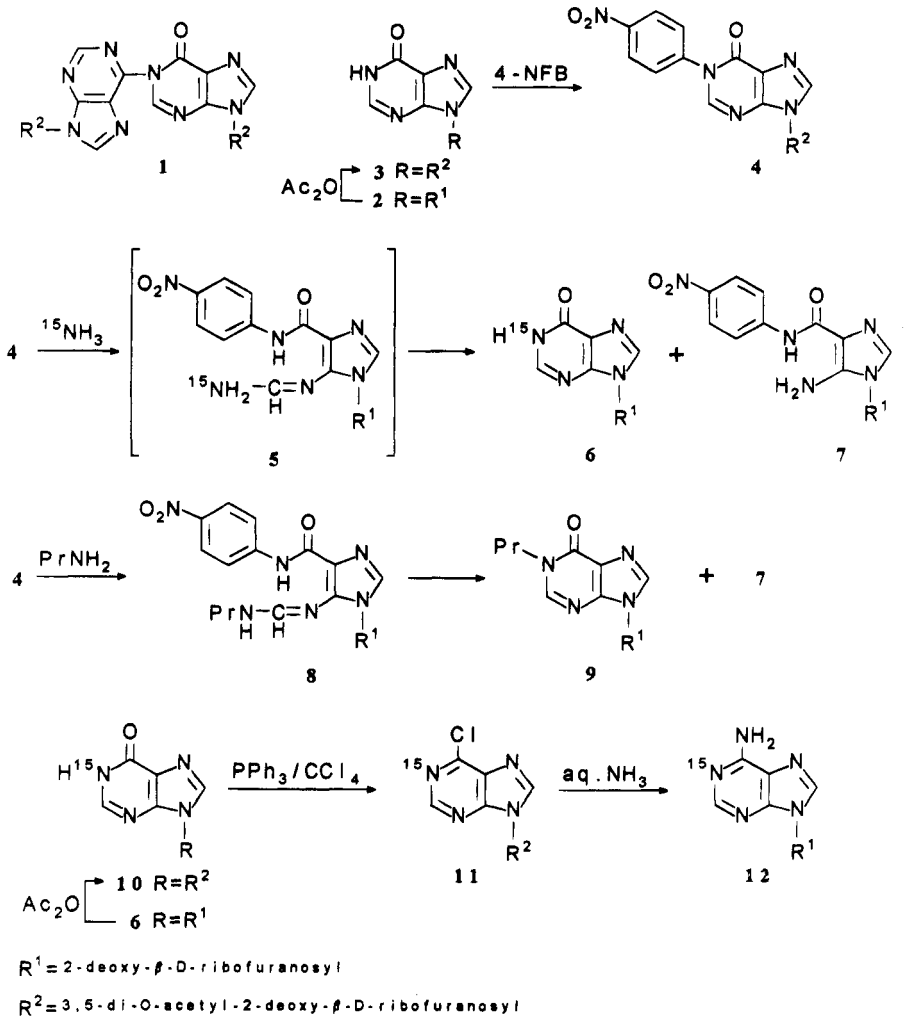
0022-3263/95/1960-2251$09.00/0

Scheme 1



ered also as a versatile labeled precursor in several purine ring transformations, was obtained in three steps in 48% overall yields.

Experimental Section

General Methods. ^{1}H NMR spectra were recorded at 270 and 400 MHz. The residual proton signals of chloroform, methanol, and water (assigned values of 7.26, 3.31, and 4.80 ppm) were used as references in these solvents. ^{13}C NMR spectra were obtained at 67.88 and 100.13 MHz; the $^{13}CDCl_3$, $^{13}CD_3OD$, and ^{13}C-DMSO signals (assigned values of 77.00, 49.00, and 39.70 ppm) were used as references in these solvents. Melting points are uncorrected. The [^{15}N]-NH_4OH was obtained from Cambridge Isotope Laboratories. The 2′-deoxyinosine was obtained from Fluka.

1-(4-Nitrophenyl)-3′,5′-di-*O*-acetyl-2′-deoxyinosine (4). A mixture of 0.37 g (1 mmol) of **3**, 0.26 mL (2.5 mmol) of 4-nitrofluorobenzene (4-NFB), and 0.35 g (2.5 mmol) of K_2CO_3 was suspended in anhydrous DMF (3 mL) at 80 °C under stirring for 5 h. After cooling the mixture, it was filtered and the solid washed with $CHCl_3$. The filtrate and washings, evaporated to dryness *in vacuo*, were purified on a silica gel column (3 cm × 50 cm), eluting with increasing amounts of CH_3OH in $CHCl_3$ (from 0 to 4%) to give **4** (0.42 g, 0.92 mmol, 92%).

4: mp 164–166 (CH_3OH); UV ($CHCl_3$) λ_{max} 253 nm (ϵ = 17 350); FAB MS *m/z* 458 ($M^+ + 1$); $[\alpha]_D = -16.3$ (*c* = 0.018, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 8.42 (d, 2H, *J* = 8.6 Hz); 8.06 (s, 1H), 8.01 (s, 1H); 7.63 (d, 2H, *J* = 8.6 Hz); 6.40 (dd, 1H, *J* = 6.8 and 6.8 Hz); 5.43 (m, 1H); 4.38 (m, 3H); 2.91 (m, 1H); 2.65 (m, 1H); 2.15 (s, 3H), 2.10 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 170.2, 170.0, 155.4; 147.6, 146.7 142.0, 146.0, 138.5, 128.3, 124.8, 124.7, 84.5, 82.5, 74.0, 63.4, 37.5, 20.7, 20.6.

Reaction of 4 with Aqueous $^{15}NH_3$. To 0.11 g (0.24 mmol) of **4** was added 3.5 mL of aqueous $^{15}NH_3$ (3.3 N, 99% ^{15}N), and the mixture was heated at 50 °C for 12 h under stirring. The resulting solution, dried *in vacuo*, was purified on silica gel plates (20 × 20 cm, 0.5 mm, Merck) and developed in $CHCl_3/CH_3OH$ (8:2, v/v). The bands at R_f 0.25 and 0.5, scraped from the plates and eluted with $CHCl_3/CH_3OH$ (1:1, v/v), afforded pure [1-^{15}N]-2′-deoxyinosine (**6**)[10] (0.033 g, 0.13 mmol, 55%) and 5-amino-1-(2′-deoxy-β-D-ribofuranosyl)imidazole-4-[*N*-(4-nitrophenyl)carboxamide] (**7**) (0.038 g, 0.10 mmol, 43%).

7: mp 204–210 ($CHCl_3$–CH_3OH); UV (H_2O) λ_{max} 347 nm (ϵ = 16 500); 267 nm (ϵ = 8400); FAB MS: *m/z* 364 ($M^+ + 1$); $[\alpha]_D = -20$ (*c* = 0.02, CH_3OH); ^{1}H NMR (CD_3OD) δ 8.20 (d, 2H, *J* = 8.7 Hz), 7.89 (d, 1H, *J* = 8.7 Hz), 7.41 (s, 1H), 6.02 (dd, 1H, *J* = 6.7 and 6.4 Hz), 4.51 (m, 1H), 3.98 (m, 1H), 3.76 (m, 2H), 2.63 (m, 1H), 2.27 (m, 1H); ^{13}C NMR (DMSO) δ 163.3, 146.4, 145.2, 141.5, 129.1, 124.9, 118.9, 112.0, 87.7, 84.0, 70.7, 61.5, 40.4 (partially submerged by DMSO).

Reaction of 4 with n-Propylamine. To 0.11 g (0.24 mmol) of **4** was added 5 mL of n-propylamine ($PrNH_2$), and the mixture was heated at 50 °C for 12 h under stirring. The resulting solution, dried *in vacuo*, was purified on silica gel plates (20 × 20 cm, 0.5 mm, Merck) developed in $CHCl_3/CH_3OH$ (8:2, v/v). The bands at R_f 0.5, 0.65, and 0.3, scraped from the plates and eluted with $CHCl_3/CH_3OH$ (1:1, v/v), afforded **7** (0.013 g, 0.036 mmol, 15%), **8** (0.026 g, 0.06 mmol, 25%), and **9**[10] (0.042 g, 0.14 mmol, 58%), respectively.

8: UV (H_2O) λ_{max} 350 nm (ϵ = 11 900); FAB MS *m/z* 433 ($M^+ + 1$); $[\alpha]_D = -3.03$ (*c* = 0.012, CH_3OH); ^{1}H NMR (CD_3OD) δ 8.34 (s, 1H), 8.17 (d, 2H, *J* = 9.5 Hz), 7.84 (d, 2H, *J* = 9.5 Hz), 7.75 (s, 1H), 6.18 (dd, 1H, *J* = 6.3 and 6.3 Hz), 4.46 (m, 1H), 3.94 (m, 1H), 3.73 (m, 2H), 3.36 (q, 2H), 3.39 (m, 2H), 1.66 (sextet, 2H),

1.00 (t, 3H); ^{13}C NMR (CD_3OD) δ 166.2,160.4, 151.2, 149.0, 146.2, 134.1, 128.2, 122.2, 113.0, 91.2, 87.2, 74.7, 65.5, 45.6, 44.4, 25.5, 14.3.

[1-^{15}N]-3′,5′-Di-*O*-acetyl-2′-deoxyinosine (10). To a solution of 0.10 g (0.41 mmol) of **6** in dry pyridine (2 mL) was added Ac_2O (1 mL), and the mixture was allowed to stand at room temp. After 2 h CH_3OH (1 mL) was added to the cooled solution which was successively dried *in vacuo* and purified on a silica gel column, eluted with $CHCl_3/CH_3OH$ (95:5, v/v), to give pure **10** (0.133 g, 0.39 mmol, 96%).

10: UV ($CHCl_3$) λ_{max} 252 nm (ϵ = 9000); FAB MS *m/z* 337 ($M^+ + 1$); $[\alpha]_D = -28.3$ (c = 0.015, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 8.26 [d, 1H, $J(H-^{15}N)$ = 7.5 Hz]; 8.03 (s, 1H), 6.38 (dd, 1H, J = 6.9 and 6.9 Hz), 5.41 (m, 1H), 4.35 (m, 3H), 2.89 (m, 1H), 2.62 (m, 1H), 2.12 (s, 3H), 2.08 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 170.4, 170.2, 158.9 [d, $J(C-^{15}N)$ = 10.9 Hz], 148.5, 145.4 [d, $J(C-^{15}N)$ = 9.1 Hz], 138.2, 125.0, 84.6, 82.6, 74.3, 63.6, 37.7, 20.8, 20.7.

[1-^{15}N]-6-Chloro-9-(2′-deoxy-3′,5′-di-*O*-acetyl-β-D-ribofuranosyl)purine (11). Amounts of 0.10 g (0.30 mmol) of **10** and 0.157 g (0.60 mmol) of triphenylphosphine were suspended in CH_2Cl_2/CCl_4 (1:1.4, v/v, 4 mL); the resulting mixture was kept at reflux under stirring and, after 30 min, DBU (5 μL, ca 0.03 mmol) was added. Two further portions of triphenylphosphine (0.078 g, 0.30 mmol each), dissolved in CCl_4 (0.3 mL), and of DBU (5 μl, 0.03 mmol) were then introduced in the reaction mixture during additional 2 h. The mixture was cooled, dried under reduced pressure and purified on a silica gel column eluting with increasing amounts of ethyl acetate in $CHCl_3$ (from 50 to 60%) to afford pure **11** (0.040 g, 0.11 mmol, 38%).

11: UV ($CHCl_3$) λ_{max} 263 nm (ϵ = 7000); FAB MS *m/z* 356 ($M^+ + 1$, ^{35}Cl); ^1H NMR ($CDCl_3$) δ 8.74 [d, 1H, $J(H-^{15}N)$ = 7.6 Hz], 8.30 (s, 1H), 6.48 (dd, 1H, J = 6.6 and 6.4 Hz), 5.43 (m, 1H), 4.39 (m, 3H), 2.98 (m, 1H), 2.68 (m, 1H), 2.13 (s, 3H), 2.09 (s, 3H).

[1-^{15}N]-2′-Deoxyadenosine (12). To 0.035 g (0.1 mmol) of **11** were added 2 mL of aqueous concd ammonia (32%) at 55 °C. After 8 h the dried solution was purified on silica gel plates developed with $CHCl_3/CH_3OH$ (7:3). The bands at R_f 0.35, scraped from the plates and eluted with $CHCl_3/CH_3OH$ (1:1, v/v), afforded 0.022 g of **12** (0.089 mmol, 89%).

12: UV (H_2O) λ_{max} 259 nm (ϵ = 14 000); FAB MS *m/z* 253 ($M^+ + 1$, ^{35}Cl); ^1H NMR (CD_3OD) δ 8.31 (s, 1H), 8.17 [d, 1H, $J(H-^{15}N)$ = 11.8 Hz]; 6.44 (dd, 1H, J = 6.5 and 6.4 Hz), 4.59 (m, 1H), 4.08 (m, 1H), 3.80 (m, 2H), 2.82 (m, 1H), 2.41 (m, 1H).

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Supplementary Material Available: ^1H and ^{13}C NMR assignments for compounds **4**, **7**, **10**, **11**, and **12** (2 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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