Syntheses of [1-^{15}N]-2′-Deoxyinosine, [4-^{15}N]-2′-DeoxyAICAR, and [1-^{15}N]-2′-Deoxyguanosine

Bruno Catalanotti,[a] Lorenzo De Napoli,[b] Aldo Galeone,[a] Luciano Mayol,*[a] Giorgia Oliviero,[a] Gennaro Piccialli,[c] and Michela Varra[a]

Dedicated to the memory of Prof. Giacomino Randazzo

Keywords: Labeled deoxyinosine / labeled deoxyAICAR / labeled deoxyguanosine / purine rearrangement / labeled nucleosides

A high-yield synthesis of [1-^{15}N]-2′-deoxyinosine (**5**), [4-^{15}N]-2′-deoxyAICAR (**7**), and [1-^{15}N]-2′-deoxyguanosine (**10**) from 2′-deoxyinosine (**1**) using relatively low expensive $^{15}NH_3$ as ^{15}N source is described. The method exploits 2-C reactivity of 2′-deoxyinosine (**1**) to obtain its ^{15}N-labelled counterpart, through [1-^{15}N]-2′-deoxyinosine (**5**), and successively, [4-^{15}N]-2′-deoxyAICAR (**7**). [1-^{15}N]-2′-Deoxyguanosine (**10**) can be prepared as well, through an improved cyclization procedure. No protection of sugar hydroxyl groups is required at any stage.

In recent years the synthesis of ^{15}N-labeled nucleosides and 2′-deoxynucleosides has attracted the interest of several researchers, since such molecules are powerful tools especially for NMR structural studies. In fact, oligomers containing labeled nucleosides are employed in NMR studies of protein-DNA and drug-DNA interaction[1−3] as well as nucleic acids structures and investigation of hydrogen bonds both in Watson−Crick[4−9] and other base pairing patterns.[10−16] For these applications the preferred ^{15}N-labeling positions are exocyclic amino groups of cytidine, adenosine and guanosine, 3-N of pyrimidine- and 1-N and/or 7-N of purine-nucleosides. ^{15}N labeling of exocyclic amino groups can be achieved through activation of 4-C position for pyrimidine, 2-C and/or 6-C positions of purine and subsequent reaction with aqueous $^{15}NH_3$ or other ^{15}N nucleophiles from which amino groups can be generated.[17−22] As for labeling of endocyclic positions, two general approaches have been so far developed: i) total syntheses of the heterocyclic base, from a labeled precursor, followed by condensation with an activated sugar[2,23−25] ii) procedures involving rearrangement of nucleoside purine or pyrimidine systems.[19,20,26−29] Recently, the above approaches have been used for the synthesis of ^{15}N and ^{15}N/^{13}C multilabeled nucleosides.[30][31]

Two routes for the troublesome synthesis of the [1-^{15}N]-2′-deoxyguanosine have been proposed. Jones et al.[19] achieved the goal molecule starting from [6-^{15}N]-2′-deoxyadenosine synthesized according to their previously proposed procedure.[20] The whole synthetic pathway included a number of reactions, some of which have been successively revised by the same authors.[31] In any case, the key step is a Dimroth rearrangement through which the 6-^{15}N atom moves to the endocyclic 1-^{15}N purine position. On the other hand, Bleasdale et al.[32] involved the well-known cyclization of 5-amino-1-(β-D-ribofuranosyl)imidazole-[4-^{15}N]-carboxamide[33][34] ([4-^{15}N]-AICAR) prepared from the unlabeled counterpart following the Srivatava[35] procedure. Additional steps, however, are required to convert the [1-^{15}N]-guanosine into the corresponding 2′-deoxynucleoside.

In this paper we describe a convenient synthetic approach to [1-^{15}N]-2′-deoxyinosine (**5**) along with its conversion to [4-^{15}N]-2′-deoxyAICAR (**7**) which, in turn, is of use to prepare [1-^{15}N]-2′-deoxyguanosine (**10**) by a slight modification of the above cyclization procedure.[33]

Previously, we reported[36] the synthesis of [1-^{15}N]-2′-deoxyinosine (**5**) through reaction of 3′,5′-diacetyl-1-*N*-(4-nitrophenyl)-2′-deoxyinosine with aqueous $^{15}NH_3$. In this 2′-deoxyinosine derivative the presence of a strong electron-withdrawing group, attached to the 1-nitrogen of the hypoxantine ring, makes the 2-C so electrophilic to react with labeled ammonia, thus leading to the opened formamidine **3** (not isolated). This compound, through a fast ring reclosure favoured by the loss of 4-nitroaniline, gives [1-^{15}N]-2′-deoxyinosine (**5**) (48% overall yield from 2′-deoxyinosine **1**) with concomitant removal of the sugar acetyl protecting groups and unfavorable $^{15}NH_3$ consumption. This procedure strictly matches a recently reported procedure for the synthesis of [1-^{15}N]-2′-deoxyinosine which uses a nitro group attached to 1-*N* to induce a similar reactivity in the purine 2-carbon.[28]

[a] Dipartimento di Chimica delle Sostanze Naturali, Università degli Studi di Napoli "Federico II",
Via D. Montesano 49, I-80131 Napoli, Italy
Fax: (internat.) +39-081/7486552
E-mail: mayoll@unina.it
[b] Dipartimento di Chimica Organica e Biologica, Università degli Studi di Napoli "Federico II",
Via Mezzocannone 16, I-80134 Napoli, Italy
[c] Facoltà di Scienze, Università degli Studi del Molise,
Via Mazzini 8, I-86170 Isernia, Italy

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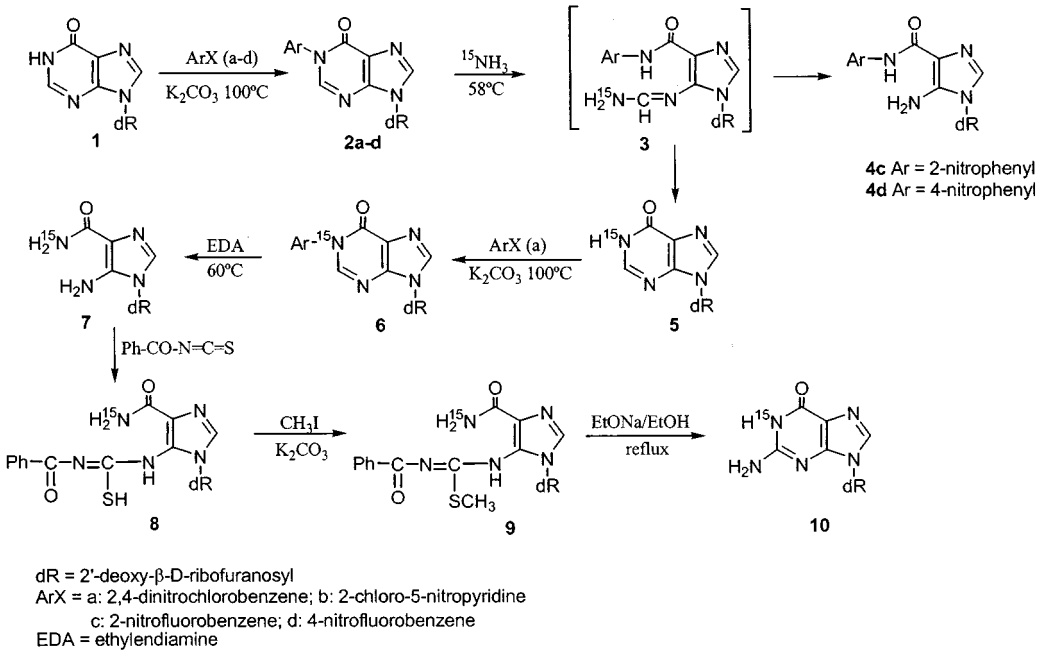

Aiming to develop a more efficient synthetic procedure to obtain [1-^{15}N]-2′-deoxyinosine (**5**), we explored both the introduction of several electron-withdrawing groups on the 1-*N* position without sugar hydroxyls protection and the consequent reactivity towards aqueous $^{15}NH_3$. We found that 2′-deoxyinosine (**1**) reacts regioselectively with 2,4-dinitrochlorobenzene (1.2 equiv.) and K_2CO_3 (2.5 equiv.) in DMF at 100 °C yielding 1-*N*-(2,4-dinitrophenyl) derivative **2a** (98% yield). Similar results were obtained with 2-chloro-4-nitropyridine leading to **2b** (97% yield). On the other hand, with 2-(or 4-)nitrofluorobenzene, the more time consuming reaction lowered yields of 1-*N*-arylated derivatives [**2c** (81%) and **2d** (83%)] and a number of side products were observed. Treatment of purified **2a-b** with aqueous $^{15}NH_3$ (3.3 M, 99% ^{15}N, 3.0 equiv.) led to **5** in 86% and 82% yields, respectively, (83% overall yield from **1** via **2a**), whereas the same reaction on **2c** and **2d** gave, as the main product, the 2′-deoxyAICAR derivative **4** with poor yields of the cyclization product **5** (5−10%).

The proposed synthesis of [4-^{15}N]-2′-deoxyAICAR (**7**) closely follows our previous route[37] to the unlabeled counterpart based on degradation of hypoxantine ring of **2a** by treatment with ethylenediamine (EDA). Introduction of 2,4-dinitrophenyl group on 1-^{15}N labeled **5**, as above described, afforded the intermediate **6** (98% yield), whose treatment with absolute EDA led to the target compound **7** in 94% yield (76% overall yield from **1**).

Conversion of **7** into [1-^{15}N]-2′-deoxyguanosine (**10**) was performed through three consecutive steps, without intermediate isolation, following the previously reported procedure for [1-^{15}N]-guanosine[34]. Treatment of **7** with benzoylisothiocyanate (1.2 equiv.) yielded **8** almost quantitatively (by TLC and ^{1}H-NMR analysis). The described treatment of the latter compound with methyl iodide (1.2 equiv.), surprisingly afforded only traces of the expected sulfur methylated intermediate **9** (by TLC and ^{1}H-NMR analysis). Complete sulfur methylation, however, could be achieved performing the reaction in the presence of K_2CO_3 (2.5 equiv.). Treatment of **9** with sodium ethoxyde in ethanol (2 h, reflux) yielded, after purification by reverse phase HPLC, the target product [1-^{15}N]-2′-deoxyguanosine (**10**) in 89% yield (68% overall yield from **1**). It is to be noted that cyclization step performed at room temperature (48 h) failed to give the expected N^2-benzoyl[1-^{15}N]-2′-deoxyguanosine.[32][33]

In conclusion, we developed a convenient synthetic approach to [1-^{15}N]-2′-deoxyinosine exploiting a regioselective introduction of 2,4-dinitrophenyl group on 1-*N* position of 2′-deoxyinosine and using the relatively low expensive $^{15}NH_3$ as a ^{15}N source. [1-^{15}N]-2′-deoxyinosine has been shown to be a convenient precursor for the synthesis of the new labeled nucleoside [4-^{15}N]-2′-deoxyAICAR, which, in turn, can be cyclized to [1-^{15}N]-2′-deoxyguanosine in high yields.

Experimental Section

General Methods: ^{1}H and ^{13}C, ^{1}H-decoupled NMR were recorded at 500 MHz. The residual proton and carbon signals of deuterated solvents ([D_6]DMSO: δ = 2.55 and 39.70; 3.31 and 49.3) were used as references in these solvents. − Preparative HPLC separations were carried out using Waters μ BONDAPAK C_{18} (7.8 mm × 30 cm) column. Preparative TLC separations were performed with plates 20 × 20 cm, 0.5 mm MERCK. Mass spectra were recorded by a Fisons Prospec FIB instrument. − U.V. spectra were recorded by a Philips PU 8740 spectrometer. − Polarimetric data were obtained by a Perkin-Elmer 243 B polarimeter. − General reagents were purchased from Sigma-Aldrich. $^{15}NH_3$ aqueous solution 3.3 M was purchased from Cambridge Isotope Laboratories.

1-*N*-(2,4-Dinitrophenyl)-2′-deoxyinosine (2a): A mixture of 0.5 g (1.98 mmol) of 2′-deoxyinosine, 0.694 g (4.95 mmol) of K_2CO_3 and

0.561 g (2.77 mmol) of 2,4-dinitrochlorobenzene was suspended in anhydrous DMF (10 mL) at 100°C for 15 min under stirring. After cooling, the mixture was filtered and the solution dried in vacuo. The solid product dissolved in 5 mL of MeOH, was adsorbed on 10 g of silica gel (Merck Kieselgel, 70−230 mesh), and dried. This dry material was added to the top of a silica gel column (100 g, 100 × 2 cm, i.d.). The column, eluted with $CHCl_3$/MeOH, 85:15 (v/v), afforded 0.81 g of **2a** (98% yield) as a diastereoisomeric mixture, due to hindered rotation around the *N*-2-phenyl bond[37].

^{1}H NMR (CD_3OD) δ = 9.04 (s, 1 H, 3-H 2,4-dinitrophenyl) 8.78 (dd, 1 H, 5-H 2,4-dinitrophenyl), 8.50 (s, 1 H, 8-H), 8.45 (d, 1 H, 2-H), 8.02 (m, 1 H, 6-H 2,4-dinitrophenyl), 6.51 (dd, 1 H, 1′-H), 4.60 (m, 1 H, 3′-H), 4.10 (m, 1 H, 4′-H), 3.82 (m, 2 H, 5′-H_2), 2.77 (m, 1 H, 2′-H_a), 2.54 (m, 1 H, 2′-H_b). − ^{13}C NMR (CD_3OD) δ = 157.1 (C-6), 149.8 (C-2 2,4-dinitrophenyl), 148.0 (C-4 2,4-dinitrophenyl), 147.3 (C-3 2,4-dinitrophenyl), 141.6 (C-8), 136.0 (C-2), 133.5 (C-6 2,4-dinitrophenyl), 130.2 (C-4), 124.7 (C-1 2,4-dinitrophenyl), 122.1 (C-5), 89.7 (C-4′), 86.4 (C-1′), 72.5 (C-3′), 63.2 (C-5′), 42.0 (C-2′). − FAB$^+$ MS *m/z* = 419 [MH$^+$]; HRMS *m/z* = 418.0895 (calcd. for $C_{16}H_{14}N_6O_8$ 418.0873). − U.V. (MeOH) 244.5 nm (ε = 19300); crystallized from acetone/MeOH, 9:1. − mp > 234 °C (dec.); $[\alpha]_D^{25}$ (MeOH)= −5.200.

1-*N*-(5-Nitropyridin-2-yl)]-2′-deoxyinosine (2b): A mixture of 0.5 g (1.98 mmol) of 2′-deoxyinosine, 0.694 g (4.95 mmol) of K_2CO_3 and 0.394 g (2.77 mmol) of 2-chloro-5-nitropyridine was suspended in anhydrous DMF (10 mL) at 100 °C for 25 min under stirring. After cooling the mixture was filtered and the solution was dried in vacuo. After purification (see **2a**), 0.7 g of **2b** were obtained (98% yield).

^{1}H NMR (CD_3OD) δ = 9.46 (d, 1 H, 6-H 5-nitropyridinyl) 8.83 (dd, 1 H, 4-H 5-nitropyridinyl), 8.70 (s, 1 H, 2-H), 8.44 (s, 1 H, 8-H), 8.18 (d, 1 H, 3-H 5-nitropyridinyl), 6.50 (dd, 1 H, 1′-H), 4.60 (m, 1 H, 3′-H), 4.08 (m, 1 H, 4′-H), 3.80 (m, 2 H, 5′-H_2), 2.80 (m, 1 H, 2′-H_a), 2.50 (m, 1 H, 2′-H_b). − ^{13}C NMR ([D_6]DMSO) δ 156.7 (C-2 5-nitropyridinyl), 154.7 (C-6), 148.2 (C-4 and C-2 5-nitropyridinyl), 146.2 (C-4 5-nitropyridinyl), 140.7 (C-8), 136.4 (C-5 5-nitropyridinyl), 126.2 (C-5 and C-3 5-nitropyridinyl), 89.2 (C-4′), 85.2 (C-1′), 71.7 (C-3′), 62.7 (C-5′), overlapped by [D_6]DMSO (C-2′). − FAB$^+$ MS *m/z* = 375 [MH$^+$]. − HRMS *m/z* = 374.0957 (calcd. for $C_{15}H_{14}N_6O_6$ 374.0957). − UV (MeOH) λ_{max} = 246.8 nm (ε = 12500), shoulder 276.2 nm (ε = 8300); crystallized from H_2O/MeOH, 8:2; mp 110−112 °C; $[\alpha]_D^{25°}$(MeOH)= −12.100.

1-*N*-(4-Nitrophenyl)-2′-deoxyinosine (2c): A mixture of 0.500 g (1.98 mmol) of 2′-deoxyinosine, 0.694 g (4.95 mmol) of K_2CO_3, and 293 μL (2.77 mmol) of 4-nitrofluorobenzene was suspended in anhydrous DMF (10 mL) at 100 °C for 2 h under stirring. After cooling the mixture was filtered and the solution was dried in vacuo. The solid product dissolved in 5 mL of MeOH, was adsorbed on 10 g of silica gel (Merck Kieselgel, 70−230 mesh) and dried in vacuo. This dry material was added to the top of a silica gel column (100 g, 100 × 2 cm, i.d.).The column, eluted with $CHCl_3$/MeOH, 85:15 (v/v) afforded 0.598 g (81% yield) of pure **2c**.

^{1}H NMR ([D_6]DMSO) δ = 8.62 (s, 1 H, 8-H), 8.61 (s, 1 H, 2-H), 8.59 (d, 2 H, 2-H and 6-H 4-nitrophenyl) 8.01 (d, 2 H, 3-H and 5-H 4-nitrophenyl), 6.52 (dd, 1 H, 1′-H), 5.53 (d, 1 H, 3′-OH), 5.19 (t, 1 H, 5′-OH) 4.60 (m, 1 H, 3′-H), 4.03 (m, 1 H, 4′-H), 3.73 (m, 2 H, 5′-H_2), 2.83 (m, 1 H, H_a2′), 2.52 (m, 1 H, 2′-H_b). ^{13}C NMR ([D_6]DMSO) δ = 155.8 (C-6), 147.9, 147.5, 147.2 (C-1 4-nitrophenyl, C-4 4-nitrophenyl and C-2), 143.1 (C-4), 139.6 (C-8), 129.6 (C-3 and C-5 4-nitrophenyl), 124.5 (C-2 and C-6 4-nitrophenyl), 123.8 (C-5), 88.2 (C-4′), 83.9 (C-1′), 70.8 (C-3′), 61.7 (C-5′), overlapped by DMSO (C-2′). − FAB$^+$ MS *m/z* = 374 [MH$^+$]. − HRMS *m/z* = 373.1007 (calcd. for $C_{16}H_{15}N_5O_6$ 373.1022). − UV (MeOH) λ_{max} = 263.6 nm (ε = 24200); crystallized from ethyl acetate mp 195°C; $[\alpha]_D^{25}$ (DMSO) = −8.500

1-*N*-(2-Nitrophenyl)-2′-deoxyinosine (2d): A mixture of 0.500 g (1.98 mmol) of 2′-deoxyinosine, 0.694 g (4.95 mmol) of K_2CO_3, and 293 μL (2.77 mmol) of 2-nitrofluorobenzene was suspended in anhydrous DMF (10 mL) at 100°C for 2.5 h under stirring After cooling the mixture was filtered and the solution dried in vacuo. The solid product dissolved in 5 mL of MeOH, was adsorbed on 10 g of silica gel (Merck Kieselgel, 70−230 mesh) and dried. This dry material was added to the top of a silica gel column (100 g, 100 × 2 cm, i.d.). The column, eluted with $CHCl_3$/MeOH, 85:15 (v/v), afforded 0.613 g (83% yield) of pure **2d**.

^{1}H NMR (CD_3OD) δ = 8.44 (s, 2 H, 2-H, 8-H), 8.31 (d, 1 H, 3-H 2-nitrophenyl), 7.95 (dd, 1 H, 5-H 2-nitrophenyl) 7.70 (d, 6-H 2-nitrophenyl), 6.53 (dd, 1 H, 1′-H), 4.60 (m, 1 H, 3′-H), 3.85 (m, 1 H, 4′-H), 3.82 (m, 2 H, 5′-H_2), 2.80 (m, 1 H, 2′-H_a), 2.54 (m, 1 H, 2′-H_b). − ^{13}C NMR ([D_6]DMSO) δ = 155.0 (C-6), 148.0 (C-2), 147.5 (C-4), 145.5 (C-2 2-nitrophenyl), 139.5 (C-8), 135.5 (C-5 2-nitrophenyl), 131.0 (C-6 2-nitrophenyl), 130.5 (C-1, 2-nitrophenyl), 125.3 (C-5 and C-3 2-nitrophenyl), 123.5 (C-5), 87.5 (C-4′), 84.3 (C-1′), 70.1 (C-3′), 60.3 (C-5′), overlapped by DMSO (C-2′). − FAB$^+$ MS: *m/z* = 374 [MH$^+$]. − HRMS *m/z* = 373.1037 (calcd. for $C_{16}H_{15}N_5O_6$ 373.1022). − UV (CH_3OH) λ_{max} = 266.8 nm (ε = 35400); crystallized from $CHCl_3$/MeOH, 9:1; mp 185 °C; $[\alpha]_D^{25}$ (MeOH) = −4.228.

5-Amino-1-(2′-deoxy-β-D-ribofuranosyl)imidazole-4-[*N*-(2-nitrophenyl)carboxamide] (4c): 0.2 g (0.53 mmol) of **2c** were treated with 0.7 mL of a solution of aqueous $^{15}NH_3$ (3.3 M) and the mixture was stirred at 55 °C in a capped viall. After 24 h, the mixture was dried in vacuo and the residue, dissolved in 5 mL of methanol, was adsorbed on 10 g of silica gel (Merck Kieselgel, 70−230 mesh) and dried in vacuo. The dry material was added to the top of a silica gel column (70 g, 100 × 2 cm, i.d.). The column, eluted with $CHCl_3$/MeOH, 95:5 (v/v), afforded 0.165 g (86% yield) of pure **4**.

^{1}H NMR ([D_6]DMSO) δ = 11.2 (s, 1 H, NH), 8.83 (d, 1 H, 3-H 2-nitrophenyl), 8.22 (d, 1 H, 6-H 2-nitrophenyl), 7.78 (dd, 1 H, 4-H 2-nitrophenyl), 7.57 (s, 1 H, 8-H), 7.25 (dd, 1 H, 5-H 2-nitrophenyl), 6.45 (s, 2 H, NH_2), 6.06 (dd, 1 H, 1′-H), 5.40 (bs, 1 H, 3′-OH), 5.29 (bs, 1 H, 5′-OH), 4.39 (m, 1 H, 3′-H), 3.89 (m, 1 H, 4′-H), 3.61 (m, 2 H, 5′-H_2), 2.49 (m, 1 H, 2′-H_a), 2.23 (m, 1 H, 2′-H_b). − ^{13}C NMR ([D_6]DMSO) δ 162.7 (CO), 145.0 (C-2 2-nitrophenyl), 136.3, 135.9, 135.4 (C-4, C-1 and C-6 2-nitrophenyl), 129.5 (C-2), 125.9 (C-5 2-nitrophenyl), 122.3 (C-6 2-nitrophenyl), 121.3 (C-3 2-nitrophenyl), 112.2 (C-5), 87.8 (C-4′), 83.9 (C-1′), 70.9 (C-3′), 61.5 (C-5′), overlapped by [D_6]DMSO (C-2′). − FAB$^+$ MS *m/z* = 363 [MH$^+$]. − HRMS *m/z* = 363.1199 (calcd. for $C_{15}H_{17}N_5O_6$ 363.1179). − U.V. (MeOH) λ_{max} = 282.6 nm (ε = 11000), λ = 380.8 nm (ε = 3000); crystallized from $CHCl_3$/MeOH, 9:1; mp 208−209 °C; $[\alpha]_D^{25}$ (DMSO) = −10.70.

5-Amino-1-(2′-deoxy-β-D-ribofuranosyl)imidazole-4-[*N*-(4-nitrophenyl)carboxamide] (**4d**)[36] was obtained by treating **2d** with aqueous $^{15}NH_3$ as described for **2c** (85% yield).

[1-^{15}N]-2′-Deoxyinosine (5): 0.5 g of **2a** (1.20 mmol) or **2b** (1.34 mmol) were treated with 1.8 mL of aqueous $^{15}NH_3$ (3.3 M). The mixture was stirred at 55 °C for 40 h in a capped vial. Crude material, diluted with 10 mL of water, was filtered and the solid washed with water. The filtrate and washings were dried in vacuo. After dissolving in water, the crude material was purified by preparative HPLC (eluent: H_2O/MeOH, 8:2, v/v), thus obtaining 0.260 g of pure **5** (86% yield starting from **2a**) and 0.248 g (82% yield starting from **2b**).

^{1}H NMR ([D$_6$]DMSO) δ = 8.35 (s, 1 H, 8-H), 8.09 [d, 1 H, *J*(H-^{15}N) = 11.8 Hz, 2-H], 6.34 (dd, 1 H, 1′-H), 4.43 (m, 1 H, 3′-H), 3.91 (m, 1 H, 4′-H), 3.59 (m, 2 H, 5′-H), 2.67 (m, 1 H, 2′-H), 2.36 (m, 1 H, 2′-H). − ^{13}C NMR ([D$_6$]DMSO) δ = 158.2 [d, *J*(^{13}C-^{15}N) = 11.2 Hz, C-6], 149.3 (C-4), 147.2 [d, *J*(^{13}C-^{15}N) = 9.4 Hz, C-2], 139.7 (C-8), 125.7 (C-5), 89.2 (C-4′), 84.7 (C-1′), 71.7 (C-3′), 62.7 (C-5′), overlapped by [D$_6$]DMSO (C-2′). ^{1}H and ^{13}C NMR data in agreement with literature values[27]. − FAB$^+$ MS: *m/z* = 254 [MH$^+$]. − HRMS *m/z* = 253.0837 (calcd. for $C_{10}H_{12}N_3{}^{15}NO_4$ 253.0829); crystallized from MeOH/H$_2$O, 1:1; mp 221−224 °C.

[1-^{15}N]-1-*N*-(2,4-Dinitrophenyl)-2′-deoxyinosine (6): 0.324 g (98% yield) of **6** were obtained, after purification, starting from 0.2 g (0.79 mmol) of **5** through the same procedure described for **2c**.

^{1}H NMR (CD$_3$OD) δ = 9.06 (s, 1 H, 3-H 2,4-dinitrophenyl) 8.78 (dd, 1 H, 5-H 2,4-dinitrophenyl), 8.50 [d, 1 H, *J*(H-^{15}N) = 6.56 Hz, 2-H], 8.46 (s, 1 H, 8-H), 8.04 (m, 1 H, 6-H 2,4-dinitrophenyl), 6.53 (dd, 1 H, 1′-H), 4.62 (m, 1 H, 3′-H), 4.09 (m, 1 H, 4′-H), 3.80 (m, 2 H, 5′-H$_a$), 2.80 (m, 1 H, 2′-H$_a$), 2.56 (m, 1 H, 2′-H$_b$). − ^{13}C NMR (CD$_3$OD) δ = 157.1 [d, *J*(^{13}C-^{15}N) = 8.7 Hz, C-6], 149.6 (C-2 2,4-dinitrophenyl), 148.7 (C-4), 146.9 [d, *J*(^{13}C-^{15}N) = 13.4 Hz, C-2], 147.5 (C-4 2-4-dinitrophenyl), 141.4 (C-8), 136.5 [d, *J*(^{13}C-^{15}N) = 17.4 Hz, C-1 2,4-dinitrophenyl], 133.4 (C-6 2,4-dinitrophenyl), 130.3 (C-5 2,4-dinitrophenyl), 124.8 [d, *J*^{1}(C-^{15}N) = 8.3 Hz, C-5], 122.1 (C-3 2,4-dinitrophenyl), 89.7 (C-4′), 86.4 (C-1′), 72.5 (C-3′), 63.2 (C-5′), 42.0 (C-2′). − FAB$^+$ MS *m/z* = 420 (MH$^+$). − HRMS *m/z* = 419.0892 (calcd. for $C_{16}H_{14}N_5{}^{15}NO_8$ 419.0873). − U.V.(MeOH) λ_{max} = 244.5 nm (ε = 19300); $[\alpha]_D^{25}$ (MeOH)= −5.200.

5-Amino-1-(β-D-2′-deoxyribofuranosyl)imidazole-[4-^{15}N]-carboxamide (7): 6 (0.3 g, 0.72 mmol) was dissolved in pure ethylenediamine and solution stirred at 80 °C for 12 h. The solution was evaporated to dryness in vacuo, and the resulting residue was co-evaporated with methanol three times. The solid product dissolved in 2 mL of MeOH, was adsorbed on 5 g of silica gel (Merck Kieselgel, 70−230 mesh) and dried in vacuo. The dry material was added to the top of a silica gel column (70 g, 100 × 2 cm, i.d.) and the column eluted starting from CHCl$_3$/MeOH, 85:15 (v/v) to CHCl$_3$/MeOH, 60:40 (v/v) furnished 0.163 g of pure **7** (94% yield).

^{1}H NMR ([D$_6$]DMSO) δ = 7.39 (s, 1 H, 8-H), 6.84 [d, 1 H, *J*(H-^{15}N) = 86.7 Hz, ^{15}NH′], 6.67 [d, 1 H, *J*(H-^{15}N) = 86.7 Hz, ^{15}NH′′], 5.98 (dd, 1 H, 1′-H), 5.35 (bs, 1 H, OH), 5.18 (bs, 1 H, OH), 4.35 (m, 1 H, 3′-H), 3.83 (m, 1 H, 4′-H), 3.58 (m, 2 H, 5′-H), 2.44 (m, 1 H, 2′-H), 2.18 (m, 1 H, 2′-H). − ^{13}C NMR (CD$_3$OD) δ = 169.3 [d, *J*(^{13}C-^{15}N) = 17.4 Hz, CO], 145.0 (C-5), 130.6 (C-2), 113.4 [d, *J*(^{13}C-^{15}N) = 6.7 Hz, C-4], 89.0 (C-4′), 86.4 (C-1′), 72.4 (C-3′), 62.7 (C-5′), 40.3 (C-2′). − ^{15}N NMR ([D$_6$]DMSO) δ 95.0 [t, *J*(^{15}N-H) = 86.7 Hz], ref. $^{15}NH_4Cl$ in 10% HCl. − FAB$^+$ MS *m/z* = 244 (MH$^+$). − HRMS *m/z* = 243.1002 (calcd. for $C_9H_{14}N_3{}^{15}NO_4$ 243.0985). − UV (H$_2$O) λ_{max} = 267 nm (ε = 11500); crystallized from CHCl$_3$/MeOH, 9:1; mp 175−177 °C; $[\alpha]_D^{25}$ (MeOH)= −13.1.

[1-^{15}N]-Deoxyguanosine (10): 7 (0.15 g, 0.62 mmol) was dissolved in 2 mL of dry DMF under nitrogen and benzoyl isothiocyanate (140 μL, 1.14 mmol) was added. The reaction mixture was stirred at room temperature for 20 min and then dried under reduced pressure. The solid was dissolved in 2 mL of dry ethanol and iodomethane (45 μL, 0.72 mmol) and K_2CO_3 (0.157 g, 1.12 mmol) were added. The mixture was stirred at room temperature for 70 min under nitrogen. After addition of sodium ethoxide (0.67 g, 11.6 mmol), the solution was refluxed for 3 h, neutralized with HCl 0.1 N and dried under reduced pressure. The solid material was dissolved in water and purified by preparative HPLC (eluent: H$_2$O/MeOH, 8:2, v/v) obtaining 0.147 g (89% yield).

^{1}H NMR ([D$_6$]DMSO) δ 7.9 (s, 1 H, 8-H), 6.74 (s, 2 H, NH$_2$), 6.16 (dd, 1 H, 1′-H), 5.34 (s, 1 H, 3′OH), 5.23 (s, 1 H, 5′OH), 4.38 (m, 1 H, 3′-H), 3.86 (m, 1 H, 4′-H), 3.57 (m, 2 H, 5′-H$_2$), overlapped by [D$_6$]DMSO (1 H, 2′-H$_a$), 2.22 (m, 1 H, 2′-H$_b$). ^{13}C NMR ([D$_6$]DMSO) δ = 158.7 [d, *J*(^{13}C-^{15}N) = 13.4 Hz, C-6], 155.2 [d, *J*(^{13}C-^{15}N) = 13.7 Hz, C-2], 151.1 (C-4), 135.1 (C-8), 117.2 [d, *J*(^{13}C-^{15}N) = 8.3 Hz, C-5], 87.7 (C-4′), 82.7 (C-1′), 71.2 (C-3′), 61.7 (C-5′), overlapped by DMSO (C-2′). ^{1}H-, ^{13}C-, and ^{15}N-NMR in agreement with literature values[19]. − ^{15}N NMR ([D$_6$]DMSO) δ = 240.1 [d, *J*(^{15}N-H) = 17.2 Hz]. − FAB$^+$ MS *m/z* = 269 [MH$^+$]. − HRMS *m/z* = 268.0921 (calcd. for $C_{10}H_{13}N_4{}^{15}NO_4$ 268.0938); crystallized from H$_2$O/MeOH, 1:1; mp > 240 °C (dec.).

Acknowledgments

This work is supported by Italian M.U.R.S.T. and C.N.R. The authors are grateful to "Centro Ricerche Interdipartimentale di Analisi Strumentali", C.R.I.A.S., for supplying NMR facilities.

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Received February 2, 1999
[O99039]