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SYNTHESIS OF 6-^{15}N AND 1-^{15}N LABELED ADENOSINE MONOPHOSPHATES

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Abstract: A chemical synthesis of 6-^{15}N and 1-^{15}N AMPs from 5'-O-acetyl-2',3'-O-isopropylideneinosine is reported.

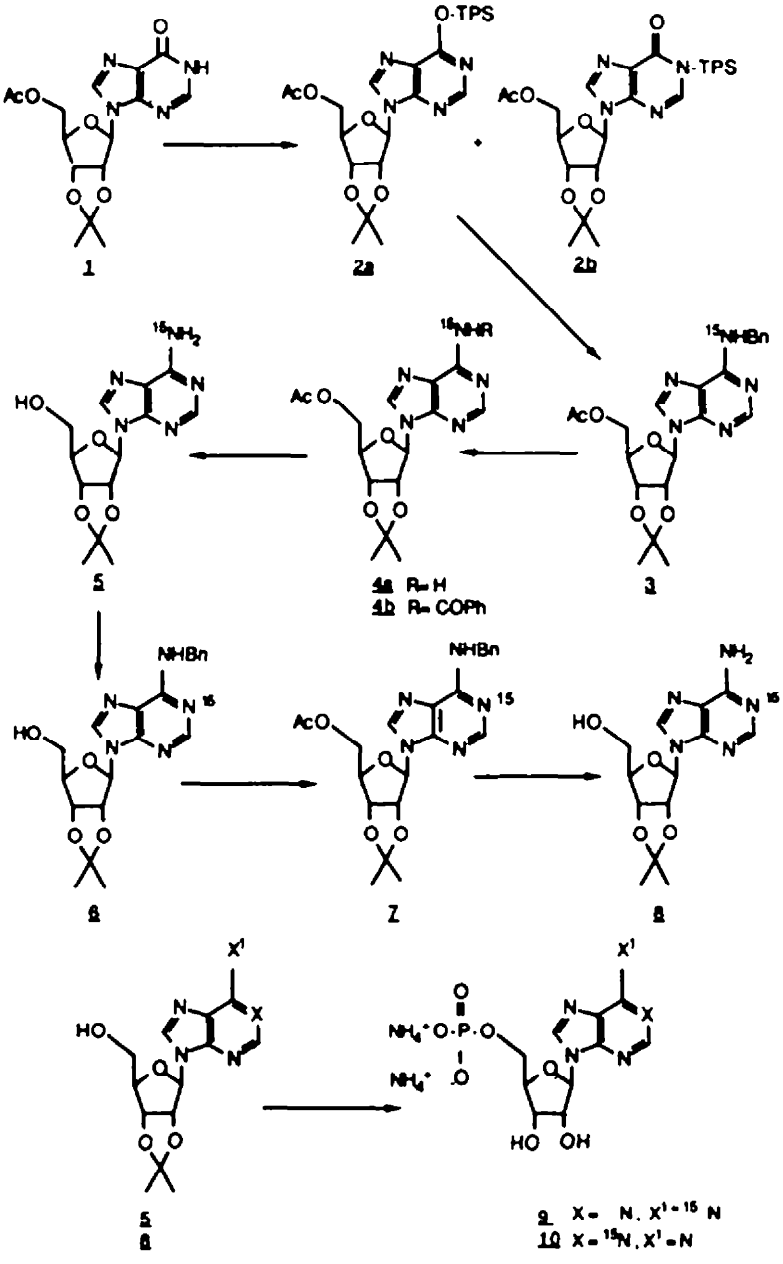
Two approaches have been employed for the synthesis of ^{15}N labeled nucleosides. In the first approach, the appropriately ^{15}N labeled heterocycles have been synthesized and condensed with appropriate sugars to furnish the desired ^{15}N labeled nucleosides. Pyrimidonucleosides[1-3] labeled with ^{15}N have been prepared using this approach. No purinic nucleoside has been synthesized using this method. However, Leonard et al.[4-5] have reported the synthesis of variously substituted adenines but have not transformed them to the corresponding nucleosides. Recently, a second approach have been developed by Jones et al.[6] who have transformed the intact nucleoside, eg-deoxyadenosine and deoxyinosine to 6-^{15}N and 1-^{15}N deoxyadenosines. These ^{15}N labeled nucleosides have been used to develop potential ^{15}N NMR probes[7-10]. In this paper we report for the first time, the synthesis of 6-^{15}N and 1-^{15}N labeled adenosine monophosphates which we require to study their interactions with adenylate kinase[11], using the second methodology developed by Jones et al.

For the synthesis of 6-^{15}N and 1-^{15}N labeled AMPs, 5'-O-acetyl-2',3'-O-isopropylideneinosine 1 has considered as a starting material of judicious choice, since at the end of the synthesis, we require 2',3' protected adenosine to phosphorylate 5' hydroxyl group selectivity.

Reaction of 1 with triisopropylbenzenesulfonyl chloride (TPSCl) in presence of triethylamine and catalytic amount of DMAP[12-14] furnished a mixture of O-sulfonated and N-sulfonated 2a and 2b respectively in the ratio of 3:7 which were separated on a column of silica gel using a mixture of diethyl ether and hexane (7:3) as eluent. Nucleophilic displacement of sulfonyl group in 2a with ^{15}N labeled benzylamine[15] in CH_2Cl_2 yielded 3 (71%). Debenzylation of 3 was performed using a mixture of $NaIO_4$ (4 eq.) and RuO_2 x H_2O (.02 eq.) as an oxidant in a mixture of CH_2Cl_2: CH_3CN: H_2O (2:2:3)[16] to give a mixture of 4a (64%) and 4b (25%). However, earlier workers[6] reported uniquely the amide formation during a similar oxidation of N-benzylated derivatives. Formation of 4a in these conditions could be explained by concieving the metal assisted hydrolysis of the benzamide 4b formed during the oxidation. It is noteworthy that the acyl group

present at 5' position in 3 is not hydrolysed in these conditions. Mixture of 4a and 4b, on treatment with aqueous NH_3 resulted in the required intermediate 5 (80%) for the synthesis of 6-^{15}N labeled AMP.

5 was transformed to 1-^{15}N labeled AMP 10 as shown in scheme 1. Quaternization of 1-N in 5 with benzyl bromide followed by Dimroth rearrangement[4] using a mixture of MeOH and $(CH_3)_2NH$ (1:1) furnished 6 (82.5%). Selective protection of the 5'-hydroxyl group in 6 with acetic anhydride in CH_3CN using DMAP as a catalyst[17] gave 7 in quantitative yield. 7 was then converted to 8, following the same sequences of the reactions described for the conversion of 3 to 5.



Scheme-1

5 and 8 were converted to 6-^{15}N and 1-^{15}N AMPs 9 and 10 respectively by the sequential treatment with cyanoethyl bispyridinium phosphate and DCC in pyridine for 12 hrs, ammonium hydroxide at 60°C for 2 hrs and acetic acid (80%) at 100°C for 2 hrs[18].

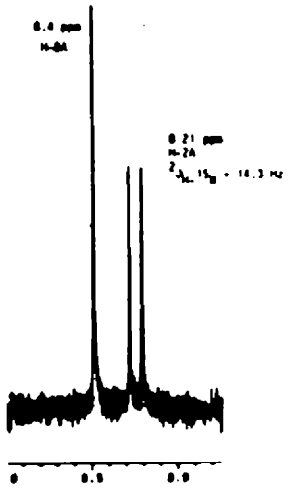


Figure-1 ^{1}H NMR(200 MHz, D_2O) of the adenine part of 10 showing $^2J_{H-N}$ coupling of 14.3 Hz

In the ^{15}N-NMR spectra of 9, the 6-^{15}N signal appears at-77.5 ppm in ammonium carbonate buffer (pH = 7.9), however the amino protons in ^{1}H-NMR appears at 6.83 ppm and split into a doublet with a coupling constant of 92 Hz due to the spin value of 1/2 of the isotope ^{15}N. In the case of each of the 1-^{15}N labeled nucleosides 6-8 and AMP 10, the proton present at position 2 of adenine in ^{1}H-NMPR splits into a doublet with a coupling constant of 14-16 Hz, a characteristic of 1-^{15}N labeled adenine[19]. The ^{1}H-NMR spectrum of the adenine moiety of 10 is shown in Fig. 1. The 1-^{15}N labeled spectra of the adenosine derivatives are also useful in unambiguous assignment of chemical shift of each adenine proton.

In conclusion, in this article, we have presented a straightforward synthesis of 6-^{15}N and 1-^{15}N AMPs. Using this approach multigrams quantity of these compounds could be obtained. Presently, the applicability of these molecules as ^{15}N NMR probes for studying their interaction with biomolecules is under progress and will be published elsewhere.

EXPERIMENTAL SECTION

Mass spectra under chemical ionisation (CI) conditions with NH_3, 90 ev were measured on mass spectrometer Nermag R10-10C. Fast atom bombardment (FAB) mass spectra were recorded on V.G. 70-250 Instrument.

^{1}H NMR spectra were obtained on Varian 90 and Brucker SP 200, which were measured in an appropriate solvent with TMS or the chemical shift of the deuterated solvent as standard. Chemical shifts are expressed in ppm downfield from TMS.

Progress of the reactions were monitered on Merck silica gel plates (60 F_{254}). Chromatographic separations were carried out on 230-400 mesh Merck silica gel (Kieselgel-60) and Sephadex G-10. When the products were not soluble in an eluating solvent, solid pack of the products was prepared prior to deposite on the column. The purity of each product was checked by spectroscopic methods. High Performance Liquid Chromatography (HPLC) was performed on Perkin Elmer series 3B, liquid chromatography, using a gradient of triethylammonium acetate (TEAA) and CH_3CN (5%-50%) in 20 minutes. A U.V. detector spectrometer, operating at 254 nm was used to detect AMPs.

5'-O-Acetyl-2',3'-O-isopropylideneinosine 1 was synthesized from inosine using litterature procedure[20]. ^{15}N labeled benzamide was a generous gift from Dr. D. Cowburn.

9-(5'-O-acetyl-2',3'-O-isopropylidene)-6-(triisopropylbenzenesulfonoyloxy-β-D-ribofuranosyl)purine (2a)

To a solution of 1 (7.05 g, .02 M) in dimethoxyethane (150 ml) were added triethylamine (4 ml), triisopropylbenzenesulfonyl chloride (9.06 g, .03 M) and dimethylaminopyridine (.150 g, 1.23 mmol) successively at room temperature with stirring. Reaction mixture was stirred for another period of 30 minutes. The solvent was removed in vacuo and the residue partitioned between CH_2Cl_2 (3 x

150 ml) and water (3 x 100 ml). The organic layer was separated and dried over anhydrous Na_2SO_4 and concentrated to dryness. The resulting residue was purified on a column of silica gel using a gradient of hexane: diethyl ether (20-50%) as eluent to give 2a (3.30 g, 26.7%) and 2b (7.80 g, 63%).
2a ^1H NMR ($CDCl_3$, 90 MHz): 1.23 (m, 18H, 6X-CH_3), 1.40 (s, 3H, -CH_3), 1.63 (s, 3H, -CH_3), 2.0 (s, 3H, -$COCH_3$), 2.90 (m, 1H, -CH), 3.96-4.7 (m, 5H, H-4', 5'-CH_2 & CH), 5.0 (dd, 1H, $J_{3',4'}$ = 3 Hz, $J_{2',3'}$ = 7 Hz, H-3'), 5.28 (dd, 1H, $J_{3'4'}$ = 2 Hz, $J_{2',3'}$ = 7 Hz, H-2'), 6.15 (d, 1H, J = 2 Hz, H-1'), 7.23 (s, 2H, Ar-H), 7.90 (s, 1H, H-8A), 8.96 (s, 1H, H-2A); MS (CI, M^+ = 616) m/e: 617 $(M + 1)^+$.
2b ^1H NMR ($CDCl_3$, 90 MHz): 1.26 (m, 21H, 7X-CH_3), 1.57 (s, 3H, CH_3), 1.90 (s, 3H, $COCH_3$), 2.83 (m, 1H, Ar-3H), 4.0-4.67 (m, 5H, 2XAr-CH, 5'-CH_2 & H-4'), 4.95 (dd, 1H, $J_{3',4'}$ = 3 Hz, $J_{3',2'}$ = 7 Hz, H-3'), 5.36 (dd, 1H, $J_{1',3'}$ = 1.5 Hz, $J_{2',3'}$ = 7.5 Hz, H-2'), 6.13 (d, 1H, J = 1.5 Hz, H-1'), 7.26 (s, 2H, Ar-H), 8.16 (s, 1H, H-8A), 8.60 (s, 1H, H-2A); MS (CI, M^+ = 616) m/e: 617 $(M + 1)^+$.

5'-O-Acetyl-2',3'-O-isopropylidene-6-^{15}N benzyladenosine (3)

A solution of ^{15}N labeled benzylamine (296 mg, 2.74 mmol) in dioxane (.5 ml) was added to a solution of 2a (850 mg, 1.38 mmol) in dry dioxane (2 ml) dropwise with stirring at room temperature. Reaction mixture was stirred at this temperature for three hours. Solvent was removed in vacuo and the resulting solid was dissolved in CH_2Cl_2 (50 ml) and washed with water. Organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to give impure 3 which was purified on a column of silica gel using diethyl ether as eluant to yield 3 (430 mg, 71%).
^1H NMR($CDCl_3$, 90 MHz): 1.42 (s, 3H, CH_3), 1.63 (s, 3H, CH_3), 2.0 (s, 3H, -$COCH_3$), 4.33 (m, 2H, 5'-CH_2), 4.48 (m, 1H, H-4'), 4.93 (d, 2H,$PhCH_2$), 5.1 (dd, 1H, $J_{3',4'}$ = 3 Hz, $J_{3',2'}$ = 7 Hz, H-3'), 5.5 (dd, 1H, $J_{1',2'}$ = 1.5 Hz, $J_{2',3'}$ = 7 Hz, H-2'), 6.10 (d, 1H, J = 1.5 Hz, H-1'), 7.36 (m, 5H, Ar-H), 7.73 (s, 1H, H-8A), 8.43 (s, 1H, H-2A); MS (CI, M^+ = 440) m/e: 441 $(M + 1)^+$.

5'-O-Acetyl-2',3'-O-isopropylidene-6-^{15}N-adenosine (4a)

Sodium metaperiodate (1.640 g, 7.66 mmol) and catalytic amount of RuO_2 x H_2O (10 mg) were added to a biphasic solution of 3 (.842g, 1.91 mmol) in CH_2Cl_2:CH_3CN: H_2O (42 ml, 10:10:1) at room temperature. Stirring was continued at this temperature for 18 hrs. Reaction mixture was diluted with CH_2Cl_2 (150 ml). Organic layer was washed with H_2O (50 ml), followed by $NaHCO_3$ solution (5%, 50 ml) and brine (50 ml), separated and dried over anhydrous Na_2SO_4. Usual work-up of the organic layer furnished the mixture of 4a & 4b which were separated on a column of silica gel using CH_2Cl_2: MeOH as eluant to yield 4a (430 mg, 64.3%) and 4b (214 mg, 24.7%).
4a ^1H NMR ($CDCl_3$, 90 MHz): 1.38 (s, 3H, CH_3), 1.61 (s, 3H, CH_3), 1.96 (s, 3H, -$COCH_3$), 4.26 (m, 2H, 5'-CH_2), 4.43 (m, 1H, H-4'), 5.03 (dd, 1H, $J_{3',4'}$ = 3 Hz, $J_{3',2'}$ = 7 Hz, H-3'), 5.47 (dd, 1H, $J_{2',1'}$ = 1.5 Hz, $J_{3',2'}$ = 7 Hz, H-2'), 6.03 (d, 1H, 1.5 Hz, H-1'), 7.86 (s, 1H, H-8A), 8.30 (s, 1H, H-2A); MS (CI, M^+ = 350) m/e: 351 $(M + 1)^+$.
4b ^1H NMR ($CDCl_3$, 90 MHz): 1.40 (s, 3H, CH_3), 1.62 (s, 3H, CH_3), 1.97 (s, 3H, $COCH_3$), 4.27 (m, 2H, 5'-CH_2), 4.47 (m, 1H, H-4'), 5.03 (dd, 1H, $J_{3',4'}$ = 3 Hz, $J_{3',2'}$ = 7 Hz, H-3'), 5.47 (dd, 1H, $J_{2',1'}$ = 1.5 Hz, $J_{2',3'}$ = 7 Hz, H-2'), 6.13 (d, 1H, J = 1.5 Hz, H-1'), 7.45 (m, 3H, Ar-H), 8.03 (dd, 2H, J_o = 9 Hz, J_m = 2 Hz, Ar-H), 8.13 (s, 1H, H-8A), 8.73 (s, 1H, H-2A); MS (CI, M^+ = 454) m/e: 455 $(M + 1)^+$.

2',3'-O-Isopropylidene-6-^{15}N-adenosine (5)

The mixture of 4a + 4b obtained after the oxidation of 3 without purification was treated with aq. NH_3 (25%, 50 ml) at room temperature for 12 hrs. The reaction mixture was extracted with CH_2Cl_2 (3 x 50 ml). Usual work-up of the organic layer gave a solid which was purified by column

chromatography to furnish 5 (80%) as white crystalline solid.

^{1}H NMR ($CDCl_3$, 200 MHz): 1.40 (s, 3H, CH_3), 1.67 (s, 3H, CH_3), 3.81 (dd, 1H, J_{Vic} = 1.9 Hz, J_{Gem} = 12.7 Hz, 5'-CH), 3.99 (dd, 1H, J_{Vic} = 1.6 Hz, J_{Gem} = 12.7 Hz, 5'-CH), 4.56 (m, 1H, H-4'), 5.13 (dd, 1H, $J_{3',4'}$ = 1.2 Hz, $J_{3',2'}$ = 6 Hz, H-3'), 5.26 (dd, 1H, $J_{2',1'}$ = 4.7 Hz, $J_{2',3'}$ = 6 Hz, H-2'), 5.90 (d, 1H, J = 4.7 Hz, H-1'), 7.89 (s, 1 H, H-8A), 8.30 (s, 1 H, H-2A); MS (CI, M^+ = 308) m/e: 309 $(M + 1)^+$.

2',3'-O-Isopropylidene-6-N-benzyl-1-^{15}N-adenosine (6)

Benzyl bromide (.267 g, 1.5 mmol) was added to a solution of 5 (.145 g, .46 mmol) in dry DMF (2 ml) and stirred at 40°C for 24 hrs. DMF was removed in vacuo. The resulting residue was dissolved in CH_2Cl_2 (1 ml) and precipitated with petroleum ether (15 ml). The so obtained semisolid was dissolved in a mixture of CH_3OH: $(CH_3)_2NH$ (1:1, 10 ml) and stirred at room temperature for 3 hrs. Solvent was evaporated to dryness. The resulting viscous solid was co-evaporated with methanol (3 x 10 ml) and purified on a column of silica gel using a gradient of CH_2Cl_2: MeOH (0-1%) to furnish 6 (.150 g, 82.5%).

^{1}H NMR ($CDCl_3$: 90 MHz): 1.40 (s, 3H, CH_3), 1.67 (s, 3H, CH_3), 3.8 (dd, J_{Vic} = 1 Hz, J_{Gem} = 13 Hz, 5'-CH), 4.06 (dd, 1H, J_{Vic} = 1 Hz, J_{Gem} = 13 Hz, 5'-CH), 4.57 (m, 1H, H-4'), 4.92 (d, 2H, Ph-CH_2), 5.22 (m, 2H, H-3' & H-2'), 5.83 (d, 1H, J = 4 Hz), 7.42 (m, 5 H, Ar-H), 7.7 (s, 1H, H-8A), 8.36 (d, 1H, $^2J_{H-^{15}N}$ = 16 Hz, H-2A); MS (CI, M^+ = 398) m/e: 399 $(M + 1)^+$.

5'-O-Acetyl-2',3'-O-isopropylidene-6-N-benzyl-1-^{15}N-adenosine (7)

To a solution of 6 (.206 g, .519 mmol) in acetonitrile (5 ml), Et_3N (0.1 ml), Ac_2O (0.075 ml) and DMAP (.062 g, .5 mmol) were added successively at 0°C. Reaction mixture was stirred at this temperature for 10 minutes. CH_3OH (5 ml) was added to the reaction mixture and evaporated to dryness. The resulting syrup was dissolved in CH_2Cl_2 (50 ml) and washed with H_2O. The usual work-up of organic layer and the chromatographic purification of the resulting product on silica gel using CH_2Cl_2 followed by diethyl ether furnished pure 7 (.222 g, 97%) as a white crystalline solid.

^{1}H NMR ($CDCl_3$, 90 MHz) is similar to 3 except that H-2A appears as doublet ($^2J_{H-^{15}N}$ = 16 Hz) at 8.40; MS (CI, M^+ = 440) m/e: 441 $(M + 1)^+$.

2',3'-O-Isopropylidene-1-^{15}N-adenosine (8)

Debenzylation of 7 was performed as for 3 to yield 9 (80%).

^{1}H NMR ($CDCl_3$, 200 MHz) of 8 is similar to 5 except that H-2A gives a doublet ($^2J_{H-^{15}N}$ = 16 Hz) instead of singlet at 8.34 ppm; MS (CI, M^+ = 308), m/e: 309 $(M + 1)^+$.

General synthesis of 6-^{15}N and 1-^{15}N-adenosine-5' monophosphates (9 and 10)

5 and 8 were converted to corresponding 6-^{15}N and 1-^{15}N AMPs 9 and 10 respectively by the procedure described by Tener[18].

MS (FAB): 449 $(M + H)^+$.

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