A CONVENIENT SYNTHESIS OF CARBON LABELLED ADENINE NUCLEOTIDES: ADENOSINE-2-^{13}C 5'-PHOSPHATE

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SUMMARY

Treatment of adenosine 5'-phosphate 1-oxide with base gave 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamidoxime 5'-phosphate. Catalytic reduction of this amidoxime gave the versatile intermediate, 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamidine 5'-phosphate. Condensation of this nucleotide with formaldehyde-^{13}C in the presence of palladium provided a simple preparation of adenosine-2-^{13}C 5'-phosphate.

Key Words: Adenosine-2-^{13}C 5'-phosphate

INTRODUCTION

The versatility of 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamidine cyclic 3',5'-phosphate (1) for the preparation of a variety of 2-substituted derivatives of adenosine cyclic 3',5'-phosphate (cyclic AMP) has been amply demonstrated (2,3). We report here the preparation of the corresponding 5'-phosphate (1) by a convenient, large-scale procedure. The utility of this intermediate is demonstrated by the one-step synthesis of a carbon-labeled adenine nucleotide, adenosine-2-^{13}C 5'-phosphate (5'-AMP-2-^{13}C, 4).

The oxidation of 5'-AMP was originally described by Brown and co-workers (4). We have found that using the same conditions described for the oxidation of cyclic AMP (1), adenosine 5'-phosphate 1-oxide (2) crystallizes in 75% yield from the buffered two-phase system with m-chloroperbenzoic acid as the oxidant. Treatment of 2 with refluxing 1 N NaOH gives 33% of 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamidoxime 5'-phosphate (3) [previously characterized (4) by ultraviolet spectra and Pauley spot test]. Compound 3 is readily purified on a

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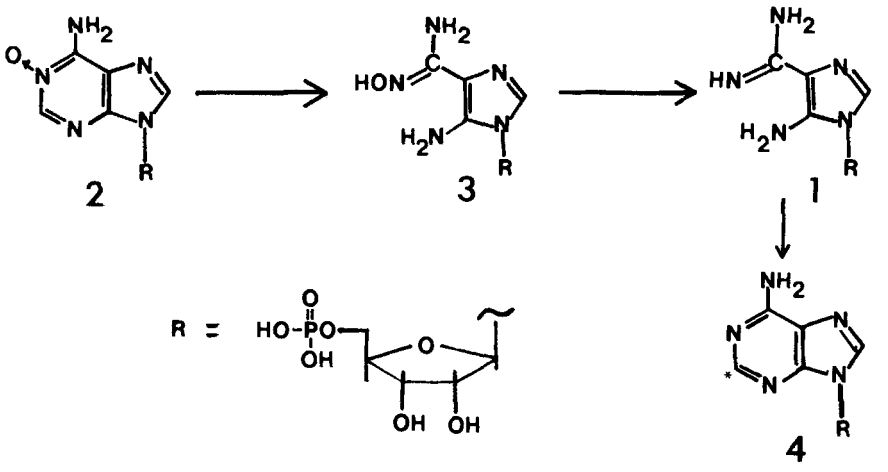

Dowex 1-X8 (formate) column. Catalytic hydrogenation with Raney nickel catalyst then gives 1 directly.

The only commercially available adenine nucleotides with a carbon label in the purine ring are labeled at C-8 with ^{14}C. The availability of imidazole nucleotide 1 allows the one step preparation of adenylic acid with a carbon label in the 2-position. To illustrate this, we have synthesized 5'-AMP-2-^{13}C (4) from 1 using the oxidative cyclization method previously developed in the synthesis of cyclic AMP derivatives (2,3). When 1 is treated with formaldehyde-^{13}C in refluxing aqueous methanol in the presence of palladium on carbon, 4 is produced in 40% yield. The ^{1}H-nmr spectra at 80 MHz of 4 gives a doublet for the H-2 with J = 214 Hz. The downfield half of the doublet at 9.77 ppm is out of the usual range of purine resonances and should be useful for observation of adenine proton resonances in polynucleotides. Another attractive aspect of this synthetic scheme is that the carbon label is introduced at the last step of the sequence, making it particularly useful for the preparation of radioactive carbon labeled adenine nucleotides. The availability of other glycosyl derivatives of aminoimidazole-carboxamidines (5) makes this method generally applicable to the preparation of a number of labeled adenine nucleoside also.

EXPERIMENTAL

Adenosine 5'-phosphate·H_2O was purchased from Sigma Chemical Co. (St. Louis) and activated Raney nickle was freshly prepared from Raney nickel alloy (Alfa

Products, Danvers, MA). Formaldehyde-^{13}C (20% solution 90 mol % ^{13}C) was purchased from Merck and Co., Canada. UV spectra were determined on a Cary 118 and NMR on a Varian FT-80 operating at 80 mHz. Tlc was run on Bakerflex Silica Gel 1B2-F plates developed in either solvent system A (i-PrOH:NH_4OH:H_2O 3:1:1) or B (MeCN:0.1 N NH_4Cl 2:1). Elemental analyses were performed by the Microanalytical Laboratory, University of California, Berkeley.

Adenosine-5'-Phosphate 1-Oxide (2).

Treatment of adenosine 5'-phosphate with m-chloroperbenzoic acid as previously described for the synthesis of the oxide of cyclic AMP (1) gave 2 in 75% yield as the sesquihydrate, which contained 7% AMP as determined by HPLC [C_{18}Lichrosorb reverse phase column (Altex), 4.6 x 250 mm; 0.01 M NaOAc buffer, pH 4, 1 mL/min]. This material was used for transformations.

Anal. Calc for $C_{10}H_{14}N_5O_8P \cdot 1.5\ H_2O$: C, 30.77; H, 4.39; N, 17.95. Found: C, 30.65; H, 4.40; N, 18.00.

5-Amino-1-β-D-ribofuranosylimidazole-4-carboxamidoxime 5'-Phosphate (3).

A solution of 15 g (38.5 mmol) of 2 in 150 mL 1 N NaOH was refluxed 1 hour. After cooling to room temperature, the solution was adjusted to pH 9 with Dowex 50-X8 (H^+, 20-50 mesh). The filtered solution was passed through a 2.5 x 30 cm column of Dowex 1-X8 (formate, 200-400 mesh) and the column was washed with 500 mL H_2O. The product was eluted with a linear gradient of 1 L H_2O in the mixing chamber and 1 L 0.5 N formic acid in the reservoir. Fractions containing product were identified by tlc and by a purple color with 2% $FeCl_3$. Lyophilization of fractions containing product gave 4.70 g (12.8 mmol, 33%): UV λ_{max} (pH 1) 277 nm (ε = 9100); (pH 13) 258 (11800); R_f (A) 0.25, (B) 0.07.

Anal. Calcd for $C_9H_{16}N_5O_8P \cdot 0.75\ H_2O$: C, 29.47; H, 4.81; N, 19.09. Found: C, 29.86; H, 4.80; N, 18.61.

5-Amino-1-β-D-ribofuranosylimidazole-4-carboxamidine 5'-Phosphate (1).

A solution of 3.5 g (9.5 mmol) of 3 in 150 mL H_2O was shaken with 2.5 g activated Raney nickel under 2-3 atm H_2 for 24 hours. The filtered mixture was lyophilized to give 2.50 g (73%): UV λ_{max} (pH 1) 282 nm (ε = 12100); (pH 13) 268 (9200); R_f (A) 0.093, R_f(B) 0.087.

Anal. Calcd for $C_9H_{16}N_5O_7P \cdot 1.5\ H_2O$: C, 29.67; H, 5.26; N, 19.23. Found: C, 29.62; H, 4.95; N, 18.98.

Adenosine-2-^{13}C 5'-Phosphate (4).

A mixture of 1.05 g 1 (2.9 mmol), 4.5 mL 2 N NaOH, 0.45 mL of 20% formaldehyde-^{13}C (3 mmol), and 0.30 g of 10% Pd/C was stirred at room temperature for 10 min, then refluxed 1 hour. The filtered solution was passed through a 2.5 x 15 cm column of Dowex 1-X8 (formate, 200-400 mesh). The column was washed with water and eluted with a linear gradient of 500 mL H_2O in the mixing chamber and 500 mL 0.3 N HCOOH in the reservoir. Fractions containing product were pooled and evaporated to dryness giving 0.416 g (40% as the hydrate) of product which was identical to 5'-AMP by chromatography and spectral data: R_f(A) 0.36, R_f(B) 0.23; NMR (D_2O) 8.42 (d, 0.9 H, J = 214 Hz), 8.42 (s, 0.1 H), 8.62 (s, 1H).

In another reaction using identical conditions and commercial reagent formaldehyde, a 95% yield of 5'-AMP was obtained.

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