Carbocyclic Adenosine Phosphates as Nucleotide Mimics in Some Enzyme-catalysed Reactions

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The carbocyclic nucleotides **1–4** have been synthesised and shown to be good mimics of adenosine phosphates in some enzyme-catalysed reactions.

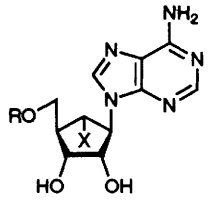
The chemistry and biological activity of carbocyclic nucleosides has attracted a good deal of attention.[1] Often a carbocyclic surrogate acts as a good mimic of the corresponding naturally occurring nucleoside. We were interested to investigate whether carbocyclic-AMP **1**, carbocyclic-ATP **2** and the 6′α-fluoro derivatives **3** and **4** could substitute for AMP or ATP in enzyme-catalysed reactions that utilize the latter species as substrates. In particular, we have studied transformations catalysed by hexokinase and glycerol kinase because of the intrinsic importance of these reactions in synthetic chemistry. Preliminary results are reported in this communication.

The monophosphate **1** was obtained from aristeromycin[2] by protection of the *cis*-diol unit as the acetonide and phosphorylation using the method of Johns and Perrich.[3] The fluoro-compound **5** was synthesised from cyclopentadiene by a reliable, albeit multi-stage, procedure.[4] Phosphorylation and deprotection using the prescribed methodology furnished the monophosphate **3**.

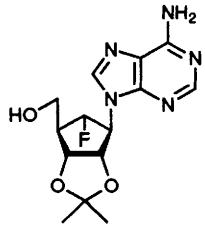
The monophosphates were converted into the corresponding triphosphates using adenylate kinase and pyruvate kinase working in tandem as illustrated in Scheme 1. This process is an adaptation of Whitesides' method[5] for the production of ATP.

Thus, the carbocyclic nucleoside monophosphates **1** and **3** (20 mg) were converted smoothly into the corresponding triphosphates **2** and **4** (*ca.* 70% isolated yield) over 15 h employing 0.5 mg of ATP to 'kick-start' the reaction and Mg^{II} ions as essential ingredients. The triphosphates were characterised by spectroscopy and some of the physical data for the fluoro-compound **4** are as follows: m.p. 202–204 °C, δ_H (250 MHz, D_2O), 8.53 (1H, s), 8.32 (1H, s), 5.45 (1H, ddd, *J* 54, 7.5 and 5 Hz), 5.30 (1H, m), 4.74 (1H, dd, *J* 9 and 5 Hz), 4.40 (3H, m), 2.72 (1H, dm, *J* 27 Hz); δ_F (235.3 MHz) 16.85 (1F, ddd, *J* 54, 27 and 21 Hz), δ_P (101.3 MHz) −9.15 (1P, br s), −10.38 (1P, d, *J* 20 Hz), −22.10 (1P, br s); Found: $M^+ + H$, 524.0130. $C_{11}H_{18}FN_5O_{12}P_3$ requires M + H, 524.0149. The carbocyclic nucleoside phosphates are obviously substrates for adenylate kinase and pyruvate kinase.

The triphosphate **4** (0.5 mg) was used to 'kick-start' a second 20 mg conversion of compound **3** to ensure that the 'second generation' batch of compound **4** contained an insignificant quantity of ATP (<0.04%) prior to its use in further studies.



1; X = H, R = P(O)(OH)$_2$
2; X = H, R = P(O)(OH)OP(O)(OH)OP(O)(OH)$_2$
3; X = F, R = P(O)(OH)$_2$
4; X = F, R = P(O)(OH)OP(O)(OH)OP(O)(OH)$_2$



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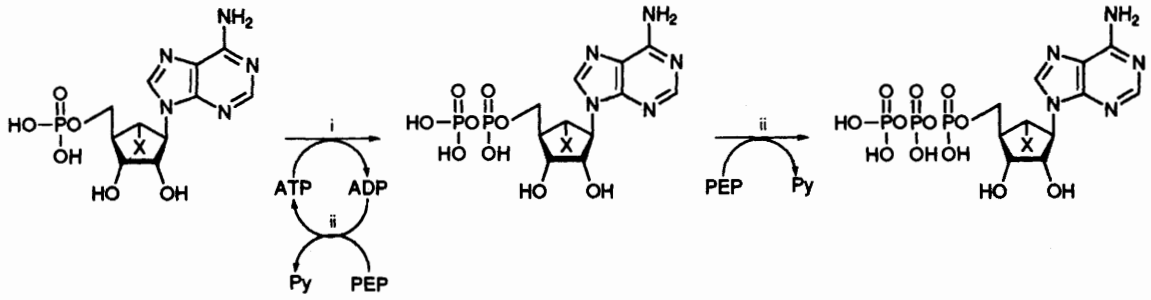
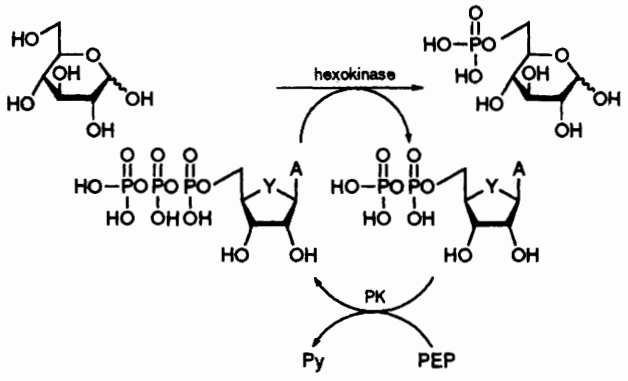


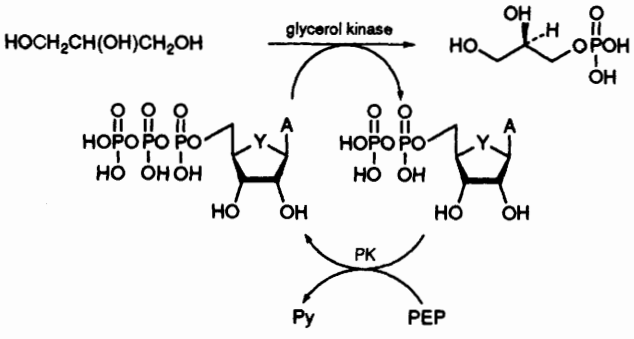
PEP = phosphoenol pyruvate
Py = pyruvate

Scheme 1 Enzymes: i, adenylate kinase; ii, pyruvate kinase



Pk = pyruvate kinase
Y = O ATP
Y = CH_2 **2**
Y = CHF **4**

Scheme 2



Y = O ATP
Y = CH_2 **2**
Y = CHF **4**

Scheme 3

One of the most important enzyme-catalysed reactions employing ATP as a substrate is the phosphorylation of glucose at the C-6 position using hexokinase as the catalyst (Scheme 2).[6] Early qualitative results show that nucleoside mimics **2** and **4** act as substrates in this conversion. Thus on using either ATP, compound **2** or compound **4**, a smooth transformation of glucose to glucose-6-phosphate was observed, indicating that the carbocyclic analogues could substitute for ATP in this reaction. In practice, to a solution of glucose (0.04 g) in distilled water (6 cm^3) was added phosphoenol pyruvate (0.068 g), magnesium chloride hexahydrate (0.018 g), mercaptoethanol (0.001 cm^3) and a catalytic amount of ATP (0.001 g) or compound **2** (0.001 g) or compound **4** (0.001 g). The solution was adjusted to pH 7.6 and degassed by passing a stream of nitrogen through the solution for 30 min. Glycerol kinase (3 U) and pyruvate kinase (10 U) were added and the mixture was stirred under nitrogen. Glucose triphosphate was formed steadily in all cases and all the reactions were complete after 48 h. A control reaction using the above conditions but employing ATP alone (0.0001 g) proceeded at a very slow rate and little glucose-6-phosphate was formed over a 48 h period. Similarly the conversion of glycerol into glycerol-3-phosphate using glycerol kinase could be effected using either ATP[7] (the natural substrate), aristeromycin triphosphate **2** or 6′-fluorocarbocyclic adenosine triphosphate **4**. In this case the reactions involving the carbocyclic compounds were marginally slower (by a factor of 3). In a typical experiment to a solution of glycerol (0.03 g) in distilled water (3 cm^3) was added phosphoenol pyruvate (0.074 g), magnesium chloride hexahydrate (0.013 g) and ATP (0.001 g) or compound **2** (0.001 g) or compound **4** (0.001 g). The solution was adjusted to pH 7.8 and degassed by passing a stream of nitrogen through the reaction mixture for 30 min. Glycerol kinase (3 U) and pyruvate kinase (10 U) were added and the mixture was stirred under nitrogen. The reaction containing ATP was complete after 12 h, while the conversions using compounds **2** and **4** were finished after 36 h. A control experiment using ATP (0.0001 g) as substrate progressed to only a small (<20%) extent over 36 h.

Quantitative data, included v_{max} and K_m values for the enzyme-catalysed reactions, are being gathered presently.

In conclusion the carbocyclic nucleotides participate as substrates in two synthetically important enzyme-catalysed reactions. The greater intrinsic stability of the non-natural substrates may be useful in some circumstances. The possibility of monitoring the triphosphate **4** by ^{19}F NMR is noteworthy.[8]

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