Synthesis of Adenosine 5′-[γ(*R*)-^{17}O,^{18}O-thio]triphosphate

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An efficient synthesis of adenosine 5′-[γ(*R*)-^{17}O,^{18}O-thio]triphosphate has been developed using a combined chemical and enzymic strategy.

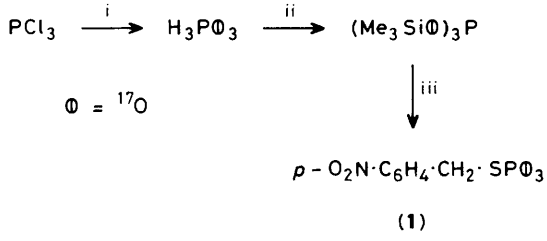
Adenosine 5′-triphosphate (ATP) is the universal currency of free energy in living systems and as such is involved in a great many enzyme-catalysed reactions. Stereochemical investigations of many of these reactions have provided important evidence concerning their mechanism of action.[1] Several of the enzymes, however, lead either directly or indirectly to the release of inorganic phosphate and with a view to studying the stereochemical course of such reactions a new method for the configurational analysis of chiral inorganic [^{16}O,^{17}O,^{18}O]thiophosphate has recently been developed.[2] We now report an efficient synthesis of adenosine 5′-[γ(*R*)-^{17}O,^{18}O-thio]triphosphate which is required as a substrate for these stereochemical investigations.

Several routes to *S*-nitrobenzyl [$^{17}O_3$]phosphorothioate (**1**) were investigated but the method of choice, in terms of overall yield and efficient use of isotopically enriched water, is outlined in Scheme 1. Phosphorus trichloride was hydrolysed quantitatively with a slight excess (1.5 equiv.) of [^{17}O]water to [$^{17}O_3$]phosphorous acid which was converted into its tris-(trimethylsilyl) ester with chlorotrimethylsilane. Bis(*S*-*p*-nitrobenzyl)disulphide, prepared by the method of Gladysz *et al.*,[3] on reaction with the P^{III} triester gave directly *S*-*p*-nitrobenzyl [$^{17}O_3$]phosphorothioate (**1**). This conversion was essentially quantitative (as determined by ^{31}P n.m.r. spectroscopy).

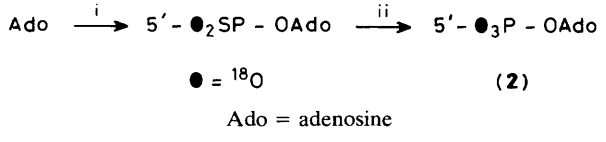
Although adenosine can be phosphorylated selectively at the 5′-hydroxy group by phosphoryl chloride in various solvents,[4] we and others[5] have found that 2′- and 3′-substituted adenosine monophosphates (AMP) are also formed (together with small amounts of adenosine bisphosphates) which are difficult to remove. In contrast adenosine is thiophosphorylated exclusively at the 5′-hydroxy group leading after hydrolysis to adenosine 5′-phosphorothioate. By hydrolysing the intermediate with [^{18}O]water, adenosine 5′-[$^{18}O_2$]phosphorothioate is formed,[6] which may be converted into adenosine 5′-[$^{18}O_3$]phosphate (**2**) by treatment with bromine in [^{18}O]water,[7] (Scheme 2). This proved to be the most efficient route of several investigated.

Adenosine 5′-[γ(*R*)-^{17}O,^{18}O-thio]triphosphate was now synthesized from *S*-*p*-nitrobenzyl [$^{17}O_3$]phosphorothioate (**1**) and adenosine 5′-[$^{18}O_3$]phosphate (**2**) as outlined in Scheme 3. Activation of *S*-*p*-nitrobenzyl [$^{17}O_3$]phosphorothioate (**1**) with diphenylphosphoryl chloride, followed by coupling with adenosine 5′-[$^{18}O_3$]phosphate using a modification of the Michelson procedure,[8] gave after reductive cleavage of the *S*-nitrobenzyl group with sodium in liquid ammonia, adenosine 5′-[α-$^{18}O_2$,αβ-^{18}O,β-$^{17}O_2$-thio]diphosphate (**3**). A preliminary study of the coupling of *S*-benzyl [$^{18}O_3$]phosphorothioate with adenosine 5′-phosphate had established that the bridge oxygen is derived exclusively from AMP.

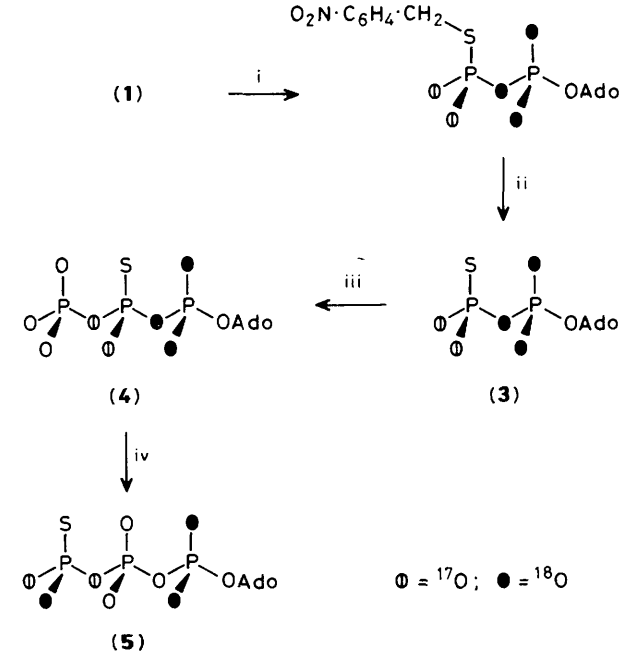
Pyruvate kinase phosphorylates adenosine 5′-β-thiodiphosphate (ADPβS) with phosphoenolpyruvate to give predominantly adenosine 5′-[(*S*)-β-thio]triphosphate [(S_p)-ATPβS] (*ca.* 80%) and some (R_p)-ATPβS (*ca.* 20%).[9] The (R_p)-ATPβS, however, is the diastereoisomer overwhelmingly preferred by yeast hexokinase and so by incubating the diastereoisomers with hexokinase and D-glucose, (S_p)-ATPβS and ADPβS are obtained which are readily separated.[9,10] Incubation of adenosine 5′-[α-$^{18}O_2$,αβ-^{18}O,β-$^{17}O_2$-thio]diphosphate (**3**) with pyruvate kinase and phosphoenolpyruvate gave a mixture of (S_p)-[$^{17}O_2$,$^{18}O_3$]ATPβS and (R_p)-[$^{17}O_2$,$^{18}O_3$]ATPβS which after incubation with hexokinase



Scheme 1. *Reagents:* i, [^{17}O]water; ii, Me_3SiCl, Et_3N; iii, (a) (p-$O_2N{\cdot}C_6H_4{\cdot}CH_2S^-$)$_2$, (b) H_2O, NaOH.



Scheme 2. *Reagents:* i, (a) $PSCl_3$, $(EtO)_3PO$; (b) [^{18}O]water, ii, Br_2, [^{18}O]water.



Scheme 3. *Reagents:* i, (a) $(PhO)_2POCl$, (b) [$^{18}O_3$]AMP; ii, Na, liquid NH_3; iii, (a) pyruvate kinase, phosphoenolpyruvate, (b) hexokinase, D-glucose; iv, methionyl-tRNA synthetase, methionine.

and D-glucose gave (S_p)-[$^{17}O_2$,$^{18}O_3$]ATPβS (**4**). The recovered [$^{17}O_2$,$^{18}O_3$]ADPβS was recycled using the combined action of pyruvate kinase (with phosphoenolpyruvate) and hexokinase (with D-glucose). By this means adenosine 5′-[α-$^{18}O_2$,αβ-^{18}O,β-$^{17}O_2$thio]diphosphate (**3**) was converted into adenosine 5′-[α-$^{18}O_2$,αβ-^{18}O,β(S)-$^{17}O_2$-thio]triphosphate (**4**) in 77% yield.

Most aminoacyl-tRNA synthetases will catalyse the activation of their specific amino acid in the absence of the cognate tRNA. The activating agent is MgATP and the product is the aminoacyl-adenylate and Mg inorganic pyrophosphate: the process is reversible.[11] Methionyl-tRNA synthetase accepts (S_p)-ATPβS as a substrate and in the presence of methionine converts it into adenosine 5′-(γ-thio)triphosphate (ATPγS), *via* the methionyl adenylate, this being the thermodynamically more stable nucleotide.[12] Incubation of adenosine 5′-[α-$^{18}O_2$,αβ-^{18}O,β(S)-$^{17}O_2$-thio]triphosphate (**4**) with methionine and methionyl-tRNA synthetase from *Bacillus stearothermophilus* gave adenosine 5′-[α-$^{18}O_2$,βγ-^{17}O,γ(R)-^{17}O,^{18}O-thio]triphosphate (**5**), referred to simply as adenosine 5′-[γ(R)-^{17}O,^{18}O-thio]triphosphate in the title. The isolated yield on this enzyme-catalysed rearrangement was 76%. Adenosine 5′-[γ(S)-^{17}O,^{18}O-thio]triphosphate has been synthesized previously,[13] but the incorporation of [^{17}O]water was much less efficient making the synthesis expensive if carried out on a substantial scale; moreover, difficulties in repeating the synthesis have been encountered.[14] The synthesis reported here started with 2 mmol of phosphorus trichloride which was hydrolysed with 9 mmol of [^{17}O]water (171 mg) to give 0.32 mmol of adenosine 5′-[γ(R)-^{17}O,^{18}O-thio]triphosphate (**5**).

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