The Reactivity of Adenosine 5′-*O*-(*S*-methyl-1-thiotriphosphate): A Facile Way of generating cyclo-Diphosphate Dianion

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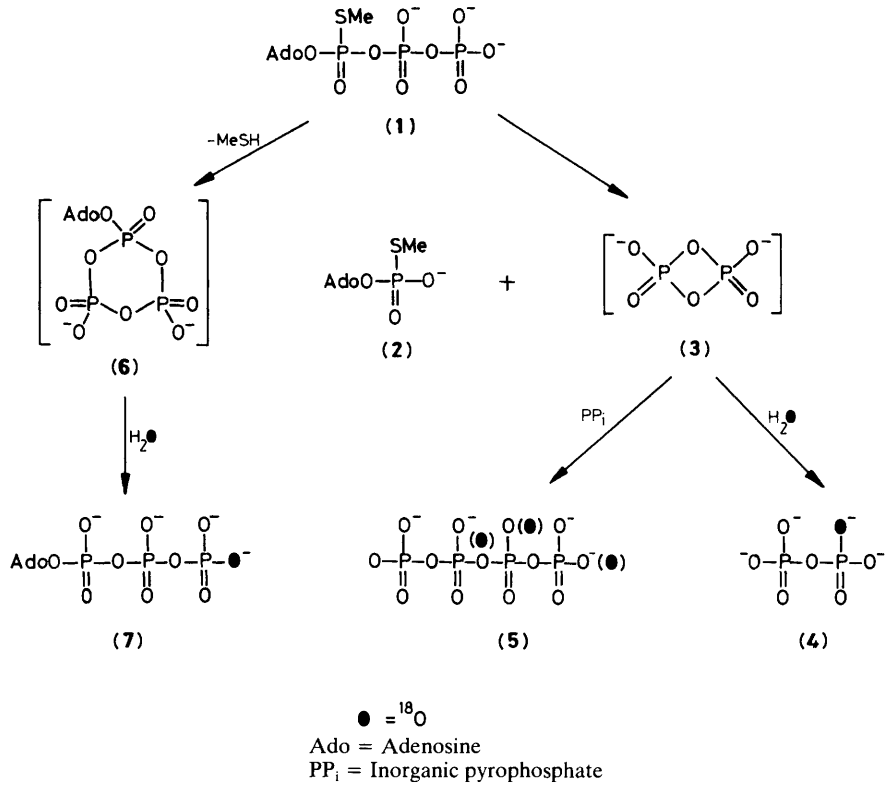
Adenosine 5′-*O*-(*S*-methyl 1-thiotriphosphate) (**1**) hydrolyses in [^{18}O] water to give adenosine 5′-*O*-(*S*-methyl thiophosphate) (**2**) and [^{18}O]inorganic pyrophosphate (**4**) as the major products, together with [^{18}O]inorganic tetraphosphate (**5**) as a minor product, each of which can be accommodated by a mechanism involving cyclo-diphosphate dianion (**3**) as an intermediate; the related reaction in anhydrous acetonitrile gives rise to a transient species tentatively assigned to the cyclo-diphosphate.

In some previous work we have studied in detail the reactivity of P^1-*O*-alkyl P^1-*S*-alkyl thiodiphosphate, including adenosine 5′-*O*-(*S*-methyl-1-thiodiphosphate).[1] The dianion shows very high reactivity *via* a dissociative mechanism and we have exploited this as a facile way of generating monomeric metaphosphate. The related P^1-*O*-alkyl P^1-*S*-alkyl thiotriphosphate is less reactive than the corresponding diphosphate, with the trianion decomposing in aqueous solution to give inorganic pyrophosphate and the corresponding *O*-alkyl *S*-alkylthiophosphate diester. Adenosine 5′-*O*-(*S*-methyl 1-thiotriphosphate) (**1**) (ATPαSMe) has been previously synthesised and used as a probe of the metal–nucleotide binding sites of phosphotransferase enzymes.[2] The hydrolysis of ATPαSMe to give adenosine 5′-*O*-(*S*-methyl thiophosphate) (AMPSMe) and inorganic pyrophosphate (PP_i) was briefly studied and it was concluded that the likely mechanism for the reaction involves S_N2 attack by water or hydroxide at the triesterified α phosphorus. We have studied this reaction in detail using [^{18}O]water and high field ^{31}P n.m.r. spectroscopy and conclude that the reaction is most likely to proceed by way of a cyclo-diphosphate intermediate (dioxadiphosphetane) (**3**).

Adenosine 5′-*O*-(*S*-methyl 1-thiotriphosphate) (**1**) (as a mixture of R_p and S_p isomers) was prepared by methylation of ATPαS tris(triethylammonium) salt with methyl iodide in methanol.[2] ATPαSMe (150 μmol) tris(triethylammonium) salt (**1**) was dissolved in [^{18}O]water (0.4 cm^3, *ca.* 50 atom%) and the reaction monitored by ^{31}P n.m.r. spectroscopy. After 24 h the products detectable in the crude reaction mixture were AMPSMe (**2**), inorganic pyrophosphate (**4**), ATP (**7**) and an unidentified material with ^{31}P n.m.r. resonances at *ca.* −6.1 and −21.7 p.p.m. The ^{18}O contents of each of the isolated products were established by high field ^{31}P n.m.r. spectroscopy.[3] Surprisingly, the inorganic pyrophosphate (**4**)



Scheme 1. The mechanism of the hydrolysis of adenosine 5′-*O*-(*S*-methyl-1-thiotriphosphate) (**1**).

contained a single ^{18}O in a non-bridging position,[4]† whilst no ^{18}O could be detected in AMPSMe (**2**). This would *not* be the expected result if the hydrolysis had occurred by direct S_N2 displacement of pyrophosphate by attack at P_α. In line with expectation the small amount of ATP (**7**) generated contained a single non-bridging ^{18}O at P_γ, *via* a mechanism involving ring opening of the intermediate cyclic triphosphate (**6**), Scheme 1. This mechanism is well established for a number of related reactions involving displacement of sulphur from ATPαS and is in agreement with the previous proposals.[2,5] The additional product was identified as the linear tetraphosphate (**5**) on the basis of the following observations: it eluted from the DEAE Sephadex column towards the end of the gradient (suggesting >5 charges); contained no nucleoside moiety; and showed a complex highly symmetric ^{31}P n.m.r. spectrum with no proton coupling. The spectrum could be well simulated by an AA′BB′ spin system with J_{AB} and $J_{BB'}$ 20 Hz and Δδ AB 10, AA′ and BB′ 0 p.p.m. (Brucker PANIC programme), Figure 1 (*cf.* n.m.r. spectra reported for Ap4A analogues[6]).

The α phosphorus is the most electrophilic centre and therefore direct S_N2 attack by hydroxide on ATPαSMe might be expected to occur at this centre leading to ^{18}O incorporation into AMPSMe, which is not observed. Direct attack of hydroxide on P_β although not as favourable in terms of the relative electrophilicities of the α and β phosphorus centres would be favoured when considering the relative pK_a's of the respective leaving groups. Displacement at P_α involves inorganic pyrophosphate as the leaving group and depending on the pH this will involve the 3rd or 4th pK_a's of this leaving group (*ca.* 6 and 9), whereas displacement at P_β involves AMPSMe as the leaving group with a pK_a *ca.* 1. A mechanism involving hydroxide displacement at P_β however, does not fully accord with the pH rate profile reported.[2] The $k_{obs.}$ (the pseudo first order rate constant) for such a mechanism contains the hydroxide ion concentration and would therefore be expected to continue to rise with pH, which is not observed. The data presented by Eckstein can be better accounted for in terms of the 3rd pK_a of ATPαSMe. At high pH the trianion predominates and the major mechanism for hydrolysis involves nucleophilic participation by P_γ to form a cyclo-diphosphate intermediate (**3**) (dioxadiphosphetane), which is trapped by water to give inorganic pyrophosphate containing a single ^{18}O label in the non-bridging position. Such a mechanism can also account for the formation of tetraphosphate (**5**) through trapping of the reactive cyclo-diphosphate (**3**) by inorganic pyrophosphate (**4**). It seems less likely that pyrophosphate will attack P_β directly. A cyclo-diphosphate intermediate has recently been proposed by Frey to account for labelling pattern obtained in the displacement of sulphur from ADPαS promoted by cyanogen bromide.[7]

In support of these conclusions we have also looked at the reactivity of ATPαSMe (**1**) in organic solvent. As the tris(tetrabutylammonium) salt in anhydrous acetonitrile, ATPαSMe decomposes rapidly ($t_{\frac{1}{2}}$ *ca.* 1—2 h), to give AMPSMe (**2**) and products with ^{31}P resonances centred around −7 and −21 p.p.m. which probably correspond to various polyphosphates. Early in the reaction a sharp singlet is detectable at −16.7 p.p.m., which is distinct from pyrophosphate and shows no phosphorus coupling. This is tentatively assigned to the intermediate dioxadiphosphetane (**3**) on the

† The ^{31}P n.m.r. spectrum for pyrophosphate ^{18}O-labelled in the non-bridging position is an apparent singlet upfield of the unlabelled material. However, because the symmetry of the molecule is removed on introduction of the non-bridging isotope the spectrum is in fact an extreme AB system with the central resonances overlapping.

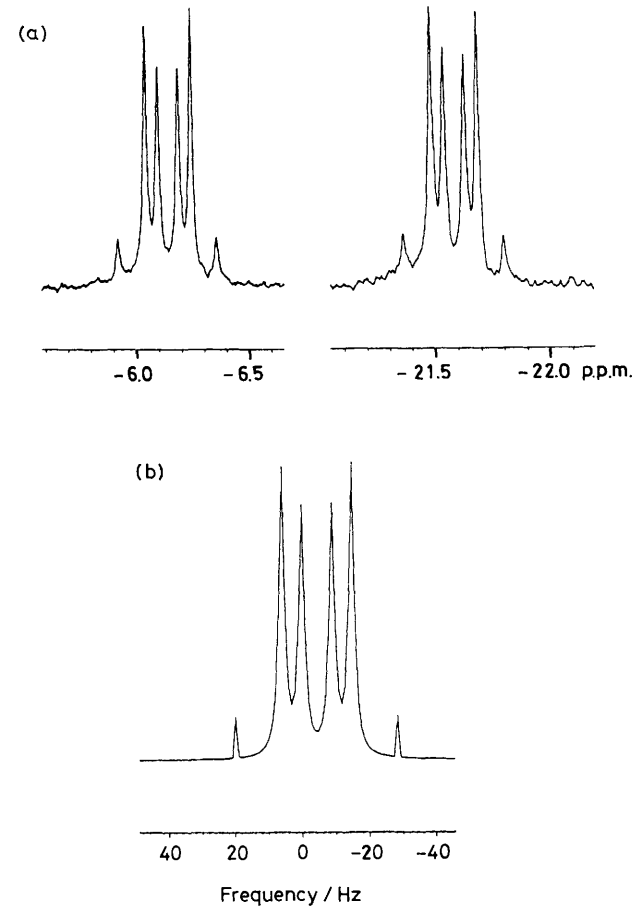


Figure 1. (a) The ^{31}P n.m.r. spectrum of the linear tetraphosphate (**5**) in Tris(hydroxymethyl)aminomethane buffer (0.1 M, pH 9.5), recorded on a Bruker AM-300 spectrometer at 121.5 MHz. (b) Simulation of one branch of the spectrum in (a) assuming an AA'BB' spin system with $\Delta\delta$ 10 p.p.m., J_{AB} and J_{BB} 20 Hz, $\delta_A = \delta_{A'}$, and $\delta_B = \delta_{B'}$, using the Bruker PANIC programme.

basis of its transient appearance and on the observed chemical shift.‡ Although diphosphetanes with other heteroatoms such as nitrogen and sulphur are well known, *e.g.* Lawesson's reagent, this may represent the first direct detection of a dioxadiphosphetane. The alkylation of a P^1-*O*-alkyl P^1-thiotriphosphate thus represents a facile method of generating the cyclo-diphosphate dianion *in situ*.

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‡ Cyclic trimetaphosphate appears at *ca.* −23 p.p.m. and it is known that the introduction of a small ring (4- or 5-membered) leads to a significant downfield shift (D. G. Gorenstein, 'Phosphorus ^{31}P n.m.r. Principles and Applications,' Academic Press, London, 1984) thus −16.7 p.p.m. is a reasonable chemical shift for the cyclo-diphosphate dianion.