inset of Figure 2 plots the pH dependence of the relative intensity of the 214- and 265-cm^{-1} peaks of beef enzyme. In the same way as in Figure 1, the 214-cm^{-1} line diminished around pH 10, while the 265-cm^{-1} and other lines exhibited no change until pH 11. Accordingly, the 214-cm^{-1} line of beef heart cytochrome oxidase is also assignable to the Fe–His stretching mode.

With regard to the spectral changes upon cyanide binding and pH variation, the Raman line of yeast cytochrome oxidase at 220 cm^{-1} and that of thermophilic bacterium PS3 enzyme at 213 cm^{-1} displayed very similar behavior to that of the 210-cm^{-1} line of the *T. thermophilus* cytochrome oxidase.[20] Consequently, these lines are also considered to involve mainly the Fe–His stretching vibration. In the parallel study of Raman spectra and the enzymatic activities of PS3 cytochrome oxidase, it was found that the incubation-temperature dependence of the intensity of the 213-cm^{-1} line was close to that of the proton-pump activity measured after incorporating the enzyme used for Raman experiments into phospholipid liposome, in contrast with the fact that the incubation-temperature dependence of the intensity of the 1667-cm^{-1} line of the a_3 formyl stretching mode was almost coincident with that of cytochrome *c* oxidase activity.[20] The present assignment will provide a new experimental base for more advanced interpretation of those Raman data.

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Registry No. Fe, 7439-89-6; His, 71-00-1; cytochrome oxidase, 9001-16-5.

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Facile Syntheses of Bridge-Oxygen-Labeled Pyrophosphates: The Preparation of Adenosine 5′-[β,γ-^{18}O]Triphosphate

Paul M. Cullis

Department of Chemistry, Leicester University
Leicester LE1 7RH, England

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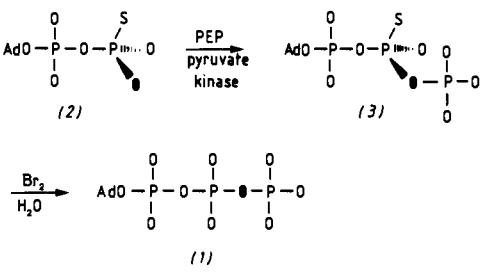
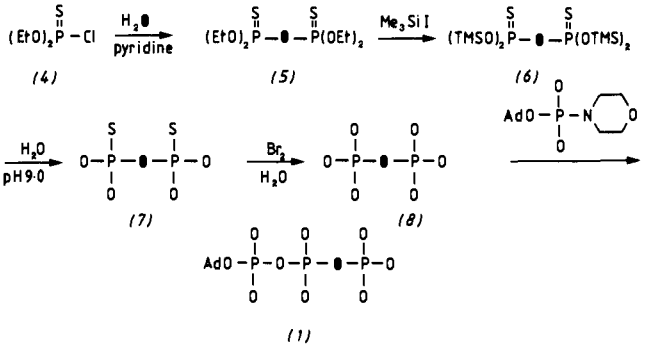
We report here the first syntheses of specifically bridge-oxygen-labeled pyrophosphates illustrated by two synthetic approaches to adenosine 5′-[β,γ-^{18}O]triphosphate (**1**) that exploit the facile displacement of sulfur from phosphorothioates using bromine in water.[1]

The synthesis of nucleotides stereospecifically enriched with the stable isotopes of oxygen has allowed detailed probing of the mechanisms of many important enzyme-catalyzed reactions at phosphorus; these studies include the positional isotope exchange analysis first reported by Midelfort and Rose[2] and the many stereochemical analyses that have recently been reported.[3] Hitherto there has been *no* method for specifically labeling the bridge position in pyrophosphates, despite the fact that such species would indeed be powerful mechanistic probes, simplifying the positional isotope exchange analysis and facilitating the measurement of heavy-atom kinetic isotope effects. The synthetic difficulties presumed to be inherent in this objective have meant that previous studies have been done with nucleoside triphosphates that are labeled in the *nonbridging* positions.[2,4] The syntheses described here overcome these difficulties.

Scheme I



Scheme II



Our first synthesis exploits the stereospecific enzyme-catalyzed phosphorylation of ADPβS (**2**).[5] Pyruvate kinase, for example catalyzes the phosphorylation of the *pro-S* position of P_β whereas acetate kinase catalyzes phosphorylation of the *pro-R* position.[6] The synthesis of (R_P)- and (S_P)-[β-^{18}O]ADPβS has been reported,[7] and by choosing the appropriate enzyme it was possible to phosphorylate exclusively the ^{18}O site. We synthesised (S_P)-[β,γ-^{18}O]ATPβS (**3**) in this way and converted it to [β,γ-^{18}O]ATP (**1**) by treatment with bromine in water[1] as shown in Scheme I. The yield of **1** based on [β-^{18}O]ADPβS (**2**) was 32%, and the material was shown to be identical with authentic ATP by enzyme assay. The location of ^{18}O in the $\beta\gamma$-bridge follows from the synthetic route and was confirmed by ^{31}P NMR (data not shown).

There are a number of objections to the route shown in Scheme I. The major drawback is that the synthesis and separation of the diastereoisomers of [β-^{18}O]ADPβS (**2**) is far from trivial, and it would seem excessive to generate isotopic chirality that is ultimately lost in the product. Other objections are that the method gives poor yield with respect to isotope, the level of enrichment in **1** is only ~80%, and the strategy lacks generality. These considerations led us to develop a second route that addresses these difficulties.

The synthesis is outlined in Scheme II. Diethoxythiophosphoryl chloride (**4**) when treated with 0.5 equiv. of water in the presence of base reacts to give tetraethyl dithiopyrophosphate in almost quantitative yield.[8] The reaction was carried out with $H_2{}^{18}O$ (98.3 atom %) to give **5**. The ethyl groups were removed by treatment with trimethylsilyl iodide[9] to give the tetrakis(trimethylsilyl) dithiopyrophosphate (**6**). The trimethylsilyl groups are extremely hydrolytically labile and were removed by stirring in sodium bicarbonate buffer, pH 9.5. The symmetrical dithiopyrophosphate (**7**)[10] was isolated by ion-exchange chromatography on DEAE sephadex in a yield of 54% based on $H_2{}^{18}O$.

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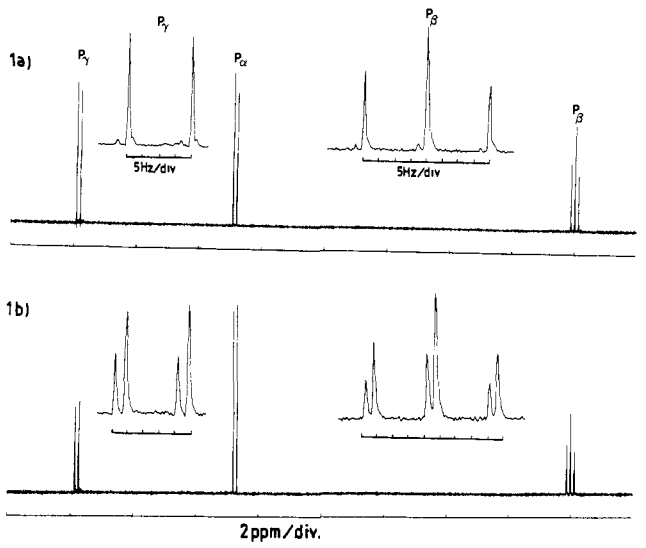

Figure 1. ^{31}P NMR spectrum (162 MHz) of (a) adenosine 5'-[β,γ-^{18}O]triphosphate with expansions of P_β and P_γ resonances and (b) adenosine 5'-[β,γ-^{18}O]triphosphate mixed with authentic ATP (ca. 2:1), in 0.1 M Tris buffer, pH 9.5, containing D_2O (50%) and ethylenediaminetetraacetic acid (10 mM). ^{31}P NMR parameters: offset 45 332 Hz; sweep width 3650 Hz; pulse width 14 μs; acquisition time 2.245 s; Guassian multiplication (no line broadening, Gaussian broadening 0.20 Hz).

The dithiopyrophosphate (**7**) when treated with excess bromine in water[1] gave bridge-^{18}O-labeled inorganic pyrophosphate (**8**) in almost quantitative yield. The location of ^{18}O in the bridging positions of **5**, **7**, and **8** was established by ^{31}P NMR, these intermediates all showed a small upfield shift, relative to the unlabeled materials, consistent with the isotope being singly bonded to phosphorus.[11] The mass spectrum of **5** demonstrated that only one ^{18}O had been incorporated and that the level of enrichment was 97 ± 2%, which was comparable to the enrichment of the $H_2{}^{18}O$ used. [β,γ-^{18}O]ATP (**1**) was synthesized in good yield (72%) from the bridge-labeled pyrophosphate (**8**) and adenosine 5'-phosphomorpholidate.[12] Although the procedure of Wehrli et al. requires the pyrophosphate to be used in approximately 5-fold excess, the unreacted pyrophosphate was readily recovered during the isolation of [β,γ-^{18}O]ATP (**1**) by ion exchange chromatography on DEAE sephadex.

The ^{31}P NMR of [β,γ-^{18}O]ATP is shown in Figure 1a. The spectrum is identical with that of authentic ATP; however, the expansions of P_β and P_γ reveal small duplicate resonances shifted to lower field[13] that correspond to 4% [β,γ-^{16}O]ATP that arises due to the residual ^{16}O in the $H_2{}^{18}O$ together with any dilutions that occurred during the synthesis. This assignment was confirmed by adding authentic ATP to the sample and rerecording the ^{31}P NMR spectrum (Figure 1b). The P_β and P_γ resonances are clearly split, the magnitudes of these shifts (2.63 and 3.37 Hz, respectively) being proof that the ^{18}O is in the $\beta\gamma$-bridge, while P_α remains a sharp doublet.

Although we report only the synthesis of [β,γ-^{18}O]ATP, since the nucleotide is introduced into the sequence in the last step and is coupled by a standard condensation reaction, this means that other nucleotides, deoxyribonucleotides, and nucleotide analogues can readily be incorporated. This synthesis is likely to be the preferred route to substrates for positional isotope exchange analysis. We are currently using such species to measure heavy-atom kinetic isotope effects for some the kinases.

Registry No. 1, 87191-03-5; **2**, 68973-41-1; **3**, 87883-26-9; **4**, 2524-04-1; **5**, 87883-27-0; **6**, 87883-28-1; **7**, 87883-29-2; **8**, 87883-30-5; adenosine 5'-phosphomorpholidate, 7331-13-7; pyruvate kinase, 9001-59-6.

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Soluble Metal Sulfides. Synthesis and Structures of $[M_6S_{17}]^{4-}$ (M = Nb, Ta): Icosahedral-Fragment Cages Containing Four Types of Coordinated Sulfide

Joan Sola,[1a] Youngkyu Do, Jeremy M. Berg,[1b] and R. H. Holm*

Department of Chemistry, Harvard University Cambridge, Massachusetts 02138

Received September 12, 1983

An emerging class of transition-element compounds consists of soluble salts of metal–sulfur anions $[M_xS_y]^{z-}$. Examples include $[MS_4]^{3-,2-,1-}$[2] (M = V, Mo, W, Re), $[MoS_9]^{2-}$,[3,4] $[Mo_2S_{10}]^{2-}$,[4,5] $[Mo_2S_{12}]^{2-}$,[4,6] $[M_3S_9]^{2-}$[7,8] (M = Mo, W), $[Mo_3S_{13}]^{2-}$,[9] and $[W_4S_{12}]^{2-}$.[10] The chemistry of $[MS_4]^{2-}$ (M = Mo, W) has been substantially elaborated, especially as ligands in heterometallic complexes.[2,11,12] Among group 5A thiometalates the derivative chemistry of tractable $(NH_4)_3[VS_4]$ has been initiated.[13] $[NbS_4]^{3-}$, $[NbOS_3]^{3-}$, and $[TaS_4]^{3-}$ are known only as insoluble salts synthesized at high temperatures,[14] and the preparation of $(Me_4N)_3[NbO_2S_2]$[15] has not been repeatable in our hands. Consequently, a search for soluble Nb and Ta sulfides was undertaken.

An anaerobic reaction mixture containing the mol ratio M(OEt)$_5$ (11 mmol):$(Me_3Si)_2S$:Et_4NCl = 1:6:3 in 350 mL of dry acetonitrile was stirred for 4–12 h at ~25 °C. Anaerobic recrystallization (acetonitrile) of the solids obtained by slow addition of ether to the reaction mixture filtrates afforded 1.75 g of black (M = Nb) or 1.25 g of dark brown (M = Ta) crystalline salts, which are sensitive to dioxygen and water. On the basis of data for $Tl_3[MS_4]$,[16] absorption spectra of these materials do not conform to those of anticipated compounds of $[MS_4]^{3-}$.[17] Values

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