(13) VII may also exhibit a transannular interaction between the nitrogen and sulfur atoms.
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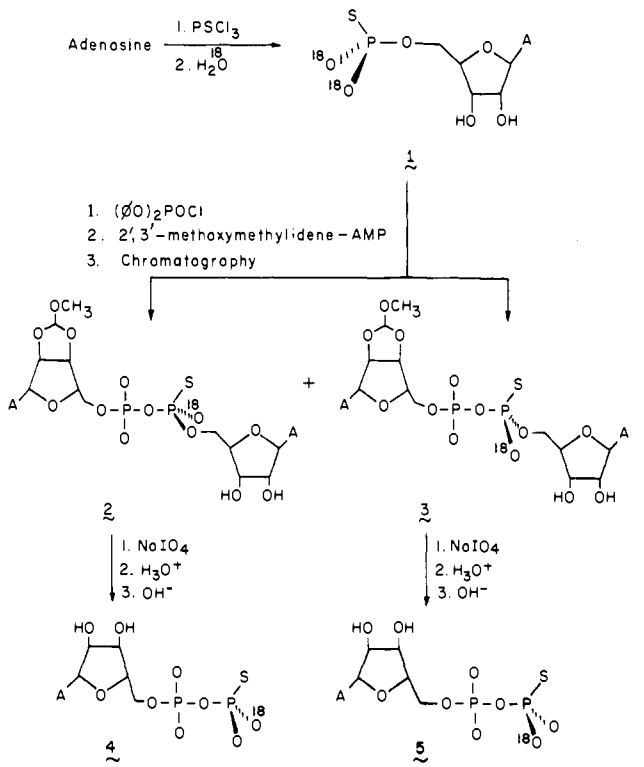
Synthesis of Nucleoside [^{18}O]Pyrophosphorothioates with Chiral [^{18}O]Phosphorothioate Groups of Known Configuration. Stereochemical Orientations of Enzymatic Phosphorylations of Chiral [^{18}O]Phosphorothioates

Sir:

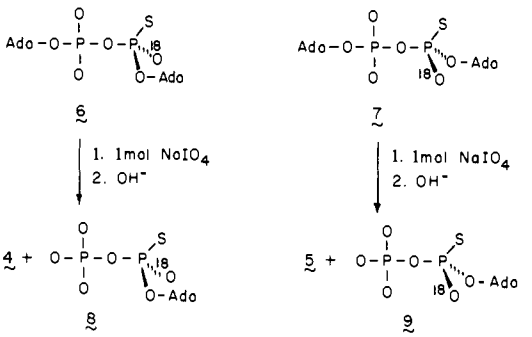
The study of enzymatic transformations of chiral phosphorothioates is recognized as a means for delineating the stereochemical courses of reactions catalyzed by phosphotransferases and nucleotidyltransferases.[2] The capability to synthesize chiral [^{18}O]phosphorothioates of known configuration will facilitate these investigations. We report here syntheses of **4** and **5**, the diastereomers of ADPβS,β^{18}O[3] which are epimeric at P_β.

The synthesis is outlined in Scheme I. $H_2{}^{18}O$ (0.5 g, 99% enriched) is introduced in aqueous workup after reacting adenosine with thiophosphoryl chloride.[4] The yield is 50% relative to adenosine, and the enrichment of ^{18}O at each thiophosphoryl oxygen is 80–90%. Activation with diphenyl phosphorochloridate[5] followed by coupling to methoxymethylidene-AMP[6] yields **2** and **3** in 50–60% total yields. These are separated in 80–90% isomeric purity[7] by column chromatography on DEAE-Sephadex A-25. Cleavage of unblocked ribosyl rings in **2** and **3** with $NaIO_4$, deblocking in acid, and alkaline elimination of the cleaved nucleoside fragments yield **4** and **5** in ~75% yield.[8]

Scheme I



Scheme II



Scheme III

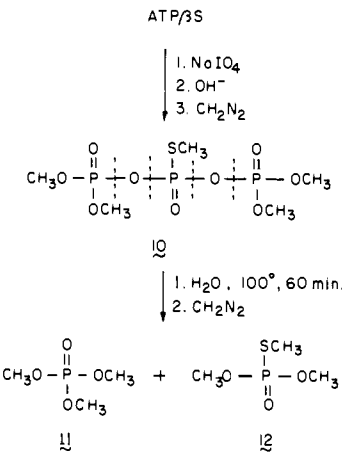


Table I. Enzymatic and Mass Spectral Analyses of [^{18}O]-Thiophosphoryl Groups in **4** and **5**

compd phosphorylated	phosphorylating[a] system	mass % ^{18}O[b] trimethyl phosphate	mass % ^{18}O[b] trimethyl phosphorothioate
4	acetate kinase	20.7 ± 1.1	40.5 ± 1.8
	pyruvate kinase	7.4 ± 2.0	71.6 ± 0.1
5	acetate kinase	1.4 ± 0.5	78.6 ± 4.2
	pyruvate kinase	17.4 ± 1.0	46.6 ± 1.8

[a] The phosphorylating systems acting on **4** or **5** were either acetyl phosphate and *E. coli* acetate kinase (Sigma Chemical Co.) or phosphoenol pyruvate and rabbit muscle pyruvate kinase (Sigma Chemical Co.). [b] Trimethyl phosphate and trimethyl phosphorothioate obtained in the degradation outlined in Scheme III were subjected to gas chromatographic–mass spectral analysis using a Du Pont 21-490 spectrometer coupled to a Perkin-Elmer 990 gas chromatograph. Oxygen-18 enrichments were calculated from the relative parent ion and m + 2 intensities after correcting for the natural abundances of ^{18}O and ^{34}S.

Since compounds **3, 5,** and **7** (or **2, 4,** and **6**)[9] are identical in configuration at the chiral phosphorus, determination of this configuration in one defines it in the other two. Scheme II shows how **6** or **7**[9] derived from **2** or **3** may be converted to mixtures of ADPαS,α^{18}O and ADPβS,β^{18}O. Treatment of **6** (containing no ^{18}O) led to **8** which, by ^{31}P NMR,[10] was shown to have the *S* configuration at P_α.[11] Similar treatment of **7** led to **9** with the *R* configuration. Inasmuch as maximum yields of ADPβS from **6** or **7** are low, **2** and **3** were the compounds actually used as precursors for chiral ADPβS,β^{18}O. Therefore, the configurations at P_α of **2** and **3** had to be correlated with those of **6** and **7**. This was done by deblocking **2** and **3** in dilute acid[8] and comparing ^{31}P NMR spectra of the products with those of **6** and **7**, whose configurations had been determined. The spectrum of the compound obtained from **2** was super-

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imposable on that of **6**. A similar correlation was made between **3** and **7**.

Acetate kinase catalyzes stereoselective phosphorylation of one of the oxygen atoms at P_β of ADPβs and pyruvate kinase stereoselectively phosphorylates the other.[12] To confirm the chiral purity at P_β of **4** and **5** prepared synthetically and to establish the orientations of phosphorylation by these enzymes, **4** and **5** were enzymatically phosphorylated to ATPβS,β^{18}O. Scheme III outlines our analytical procedure for determining whether ^{18}O in ATPβS,β^{18}O is bridging or nonbridging. Hydrolysis of **10** in Scheme III occurs with nearly equal partitioning of bridging oxygens into both **11** and **12** (53.1 ± 2.8% into **12** and 46.8 ± 2.8% into **11**). Therefore, if ^{18}O is bridging in ATPβS,β^{18}O, both **11** and **12** isolated according to Scheme III will be enriched in ^{18}O. If it is nonbridging, no ^{18}O will be found in **11**. Table I gives relevant mass spectral data. The ^{18}O enrichment in **4** and **5** was 81.3%; so Table I confirms the ^{31}P NMR data on chiral purity of these compounds. The data also show that acetate kinase catalyzes phosphorylation of the *pro-R* oxygen in ADPβS, i.e., ^{18}O in **4**, and pyruvate kinase catalyzes phosphorylation of the *pro-S* oxygen, i.e., ^{18}O in **5**.

Jaffe and Cohn have recently employed a different approach and reached the same conclusion regarding the absolute configurations at P_β in ATPβS diastereomers.[13]

References and Notes

(1) Supported by Grant No. GM 24390 from the National Institute of General Medical Sciences.
(2) (a) D. A. Usher, E. S. Erenrich, and F. Eckstein, *Proc. Natl. Acad. Sci. U.S.A.*, **69**, 115–118 (1972); K.-F. R. Sheu, and P. A. Frey, *J. Biol. Chem.*, **253**, 3378–3380 (1978); (c) F. Eckstein, V. W. Armstrong, and H. Sternbach, *Proc. Natl. Acad. Sci. U.S.A.*, **73**, 2987–2990 (1976); (d) G. A. Orr, J. Simon, S. R. Jones, G. J. Chin, and J. R. Knowles, *ibid.*, **75**, 2230–2233 (1978).
(3) The abbreviations are ADPβS, adenosine 5′-(2-thiodiphosphate); ATPβS, adenosine 5′-(2-thiotriphosphate); ADPαS, adenosine 5′-(1-thiodiphosphate); ATPαS, adenosine 5′-(1-thiotriphosphate).
(4) W. A. Murray, and M. R. Atkinson, *Biochem. J.*, **7**, 4025–4029 (1968). In hydrolytic workup the unreacted thiophosphoryl chloride was removed by vacuum distillation and sodium acetate (0.50 g) and $H_2{}^{18}O$ (0.5 g, 99% enriched in ^{18}O) were added in place of 10% aqueous barium acetate.
(5) A. M. Michaelson, *Biochim. Biophys. Acta*, **91**, 1–13 (1964).
(6) J. L. Darlix, H. P. M. Fromageot, and P. Fromageot, *Biochim. Biophys. Acta*, **145**, 517–519 (1967).
(7) **2**, $\delta(P_2)$ 43.175 ppm downfield from H_3PO_4; **3**, $\delta(P_2)$ 43.56 ppm downfield from H_3PO_4; both compounds, $\delta(P_1)$ 12.20 ppm upfield from H_3PO_4 ($J_{P_1-P_2}$ = 28.08 Hz).
(8) Acid, pH 2.0 for 20 min at room temperature; base, pH 10.5 for 30 min at 50 °C.
(9) **6** and **7** were synthesized according to Scheme I, substituting AMP for methoxymethylidene-AMP. They were separated by chromatography on a DEAE-Sephadex A-25 column using a $(C_2H_5)_3NH^+HCO_3^-$ gradient.
(10) K.-F. R. Sheu, and P. A. Frey (1977) *J. Biol. Chem.*, **252**, 4445–4448 (1977). ADPαS and ADPβS are well separated by $(C_2H_5)_3NH^+HCO_3^-$ gradient elution from DEAE-Sephadex A-25 columns.
(11) S. J. Benkovic and F. Eckstein (personal communications) have independently assigned the *S* configuration to the α-phosphorus of the diastereomers previously designated ADPαS (A) and ATPαS (A). P. M. J. Burgers and F. Eckstein, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
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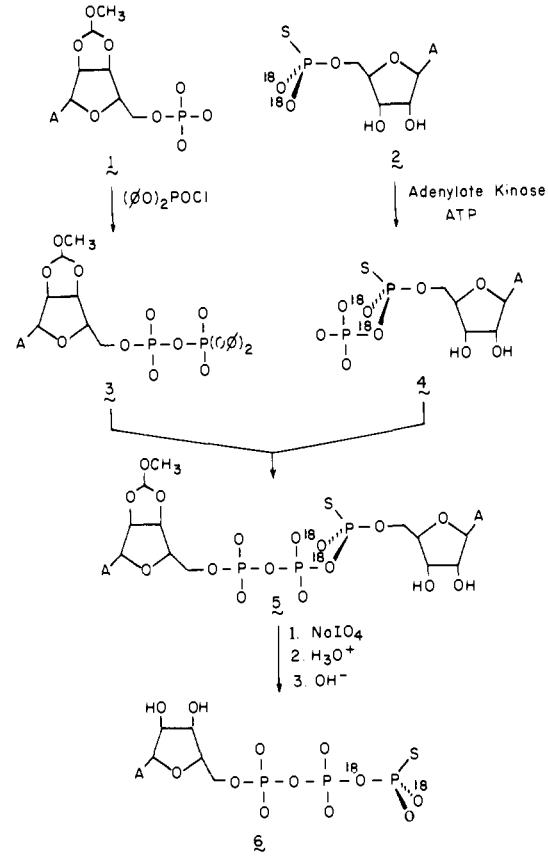
Stereochemical Course of Thiophosphoryl Group Transfer Catalyzed by Adenylate Kinase

Sir:

In recent years the mechanisms of phosphotransferase action have been studied intensively by such techniques as kinetics, radiochemical tracers, and magnetic resonance. These have produced valuable mechanistic information; however, the findings in such experiments are generally determined by the kinetics of the catalytic pathway, including the kinetics for desorption of products. Therefore, for example, the detection of a catalytic intermediate such as a covalent phosphoryl enzyme may be difficult if it exists at a small steady-state concentration.

Scheme I



Stereochemical data on phosphotransferases can give important mechanistic information which is independent of the kinetics. When the phosphate group is chiral and its configurations in the substrate and product can be related, the stereochemical course of the phosphoryl group transfer can be established. Net inversion of configuration is indicative of a single displacement of the phosphoryl group, and net retention is indicative of a double displacement, possibly via a covalent phosphoryl enzyme intermediate. In this paper we report on the synthesis of ATPγS,$\gamma^{18}O^2$ with a chiral γ-[^{18}O]phosphorothioate group of known configuration and on its use in showing that [^{18}O]thiophosphoryl group transfer catalyzed by rabbit muscle adenylate kinase occurs with net inversion of configuration of the [^{18}O]phosphorothioate group.

The synthesis of ATPγS,γ^{18}O having the *R* configuration at P_γ, **6**, is outlined in Scheme I. ADPαS,α^{18}O, **4**, having the *S* configuration at P_α is prepared by rabbit muscle adenylate kinase catalyzed phosphorylation of **2** by ATP.[3] **1**[4] was activated to **3** by reaction with diphenyl phosphochloridate, and **3** and **4** reacted smoothly in dimethylformamide–pyridine to produce **5**. The latter compound was not routinely purified but was converted directly to **6** by periodate cleavage of the unblocked ribosyl ring, acid deblocking of the other ribosyl ring, and alkaline elimination of the cleaved nucleoside as described in the preceding paper.[5] The overall yield of **6** from **4** was 55%. In one experiment **5** was purified by DEAE-Sephadex column chromatography, and it gave a ^{31}P NMR spectrum consisting of a P_α doublet 11.44 ppm upfield from H_3PO_4 ($J_{\alpha,\beta}$ = 18.31 Hz), a P_γ doublet 43.21 ppm downfield from H_3PO_4 ($J_{\beta,\gamma}$ = 25.64 Hz), and a P_β doublet of doublets at 24.13 ppm upfield from H_3PO_4.

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