# Synthesis of Methyl (*R*)- and (*S*)-[$^{18}$O]Phosphorothioates and Determination of the Absolute Configuration at Phosphorus of the Diastereoisomers of Adenosine 5′-(1-thiotriphosphate)

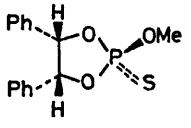
By RICHARD L. JARVEST and GORDON LOWE*

(*The Dyson Perrins Laboratory, Oxford University, South Parks Road, Oxford* OX1 3QY)

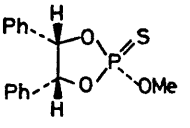
*Summary* A general route for the synthesis of enantiomeric [$^{18}$O]phosphorothioate esters of known absolute configuration has been developed, and isomer A of adenosine 5′-(1-thiotriphosphate) shown to have the (*S*)-configuration at $P_\alpha$.

A STEREOCHEMICAL approach to the mechanism of action of nucleotidyl transferases has been made possible by the demonstration that diastereoisomeric nucleoside phosphorothioates (*e.g.*, ATPαS)† which are enantiomeric at phosphorus, are selectively utilised by these enzymes.[1] This approach has been exploited recently and the stereochemical course of several enzymes established.[2] However in order to determine the stereochemical fate of a terminal thiophosphoryl group (*e.g.*, in ATPγS)† transferred by a phosphokinase, it is necessary to use chiral [$^{18}$O]phosphorothioates (*e.g.*, ATPγSγ$^{18}$O).[3] The first synthesis of nucleoside [$^{18}$O]phosphorothioates of known chirality has been achieved recently by a combination of chemical and enzymic reactions;[4] the method however is not applicable to the synthesis of [$^{18}$O]phosphorothioate esters in general. We report now a general route for the synthesis of enantiomeric [$^{18}$O]phosphorothioate esters of known chirality and its application to the determination of the absolute configuration of the diastereoisomers of ATPαS.

*cis*-2-Methoxy-4,5-diphenyl-1,3,2-dioxaphospholan-2-thione (**1**) and its *trans*-diastereoisomer (**2**) have been well characterised,[5] the *cis*-diastereoisomer being formed stereospecifically from *trans*-2-methoxy-4,5-diphenyl-1,3,2-dioxaphospholan whose structure has been established by *X*-ray crystallography.[6] The *trans*-diastereoisomer (**2**) was obtained by treating *meso*-hydrobenzoin with thiophosphoryl bromide in the presence of pyridine followed by methanolysis of the resulting product in the presence of triethylamine. Although there was evidence of both diastereoisomers (**1**) and (**2**) in the reaction product, (**2**) predominated (ratio 1 : 4) and was the only isolated product.[5] Reinvestigation of this reaction confirmed that when 2 equiv. of pyridine were used, the diastereoisomer (**2**) was the predominant product after methanolysis. However when pyridine was used in excess or as solvent, with thiophosphoryl bromide or chloride, the predominant product was the diastereoisomer (**1**) after methanolysis, the ratio of (**1**) : (**2**) being $>8:1$. These observations cannot be explained by exocyclic nucleophilic substitution at phosphorus for it is expected[7] and has been demonstrated[5] that they



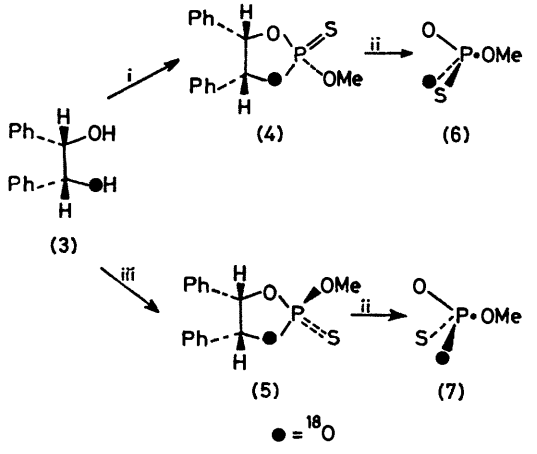

(1)




(2)

† ATPαS and ATPγS are the abbreviations for adenosine 5′-(1-thiotriphosphate) and adenosine 5′-(3-thiotriphosphate), respectively.

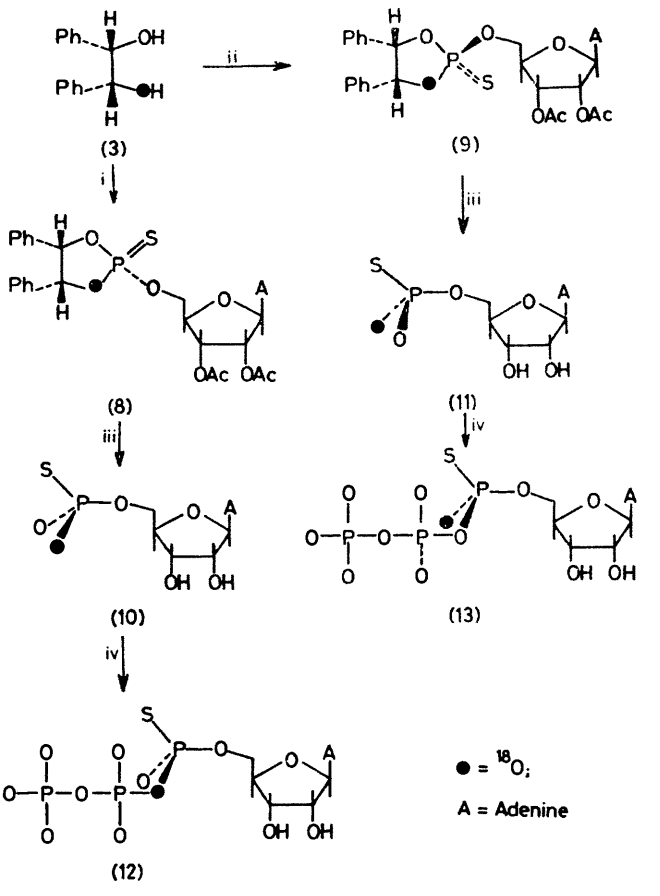
occur with retention of configuration. In the presence of an excess of pyridine, reversible ring opening of the cyclic phosphorochloridate (or bromidate) is possible, allowing the kinetically favoured product to be transformed into the thermodynamically more stable one. This interpretation was supported by monitoring the reaction between *meso*-hydrobenzoin and thiophosphoryl chloride in pyridine by $^{31}P$ n.m.r. spectroscopy. After 30 min peaks were observed at 78·8 and 78·1 p.p.m. [2·9 : 1, from $(MeO)_3PO$] which were assigned to the cyclic phosphorochloridates. After a further 20 min the upfield signal had virtually disappeared. The reaction was repeated and quenched with methanol after 30 min; the ratio of the diastereoisomers (**1**) and (**2**) established that the precursor of (**2**) is the kinetically favoured product.




SCHEME 1. i, (a) $PSBr_3$–$C_5H_5N$ (2 equiv.), $C_6H_6$, (b) MeOH–$Et_3N$; ii, Na–liq. $NH_3$; iii, (a) $PSCl_3$–$C_5H_5N$ (solvent), (b) MeOH.

(1*R*,2*S*)-1,2-[1-$^{18}O$]-Dihydroxy-1,2-diphenylethane (**3**)[8] was converted into the diastereoisomeric thiophosphate triesters as shown in Scheme 1. The crystalline triesters (**4** and **5**) however possess chirality due to isotopic substitution. Reductive cleavage of the triesters with sodium in liquid ammonia gave 1,2-diphenylethane and the enantiomeric methyl [$^{18}O$]phosphorothioates which were purified by ion-exchange chromatography on DEAE-Sephadex A25 with triethylammonium bicarbonate buffer (pH 8·1). From the mode of synthesis, the triester (**4**) gives methyl (*R*)-[$^{18}O$]phosphorothioate (**6**) and the triester (**5**) gives methyl (*S*)-[$^{18}O$]phosphorothioate (**7**).

Treatment of (**3**)[8] with thiophosphoryl bromide under kinetic and thermodynamic control as above, followed by reaction of the cyclic thiophosphorobromidates with 2′,3′-diacetyladenosine gave the *trans*-cyclic triester (**8**) and the *cis*-cyclic triester (**9**) respectively (Scheme 2). The stereochemistry of the triesters was confirmed by their relative $^{31}P$ chemical shifts and the coupling constant between the phosphorus atom and ring protons [(**8**):$\delta_P(CDCl_3)$ −77·27 p.p.m., $^3J_{HP}$ 9·4 Hz, and (**9**): $\delta_P(CDCl_3)$ −75·82 p.p.m., $^3J_{HP}$ 7·1 Hz].[5] Reduction of the cyclic triesters with sodium in liquid ammonia gave adenosine 5′(*R*)-[$^{18}O$]-phosphorothioate (**10**) and adenosine 5′(*S*)-[$^{18}O$]phosphorothioate (**11**).




SCHEME 2. i, (a) $PSBr_3$–$C_5H_5N$ (2 equiv.), tetrahydrofuran (THF), (b) 2′,3′-diacetyladenosine–$Et_3N$; ii, (a) $PSBr_3$–$C_5H_5N$ (excess), THF, (b) 2′,3′-diacetyladenosine–$Et_3N$; iii, Na–liq. $NH_3$; iv, adenylate kinase, pyruvate kinase, lactate dehydrogenase, ATP, phosphoenolpyruvate, NADH, $K^+$, $Mg^{2+}$.

Adenosine 5′-phosphorothioate can be converted into a single diastereoisomer of ATPαS (isomer A, designated ATPαS-A) enzymically.[9] Similar treatment of (**10**) and (**11**) will give samples of [$^{18}O$]ATPαS-A, one of which will contain $^{18}O$ in the $P_\alpha$–O–$P_\beta$ bridge and the other in the non-bridging position at $P_\alpha$. Since $^{18}O$ directly bonded to phosphorus causes an isotope shift in the $^{31}P$ n.m.r. spectrum,[10] the position of the $^{18}O$ can be readily established and hence the absolute configuration of ATPαS-A determined. The $^{31}P$ n.m.r. spectrum (36·43 MHz) of the [$^{18}O$]ATPαS-A (in the presence of ATPαS-A) derived from (**10**) showed isotope shifts on both $P_\alpha$ (0·6 ± 0·2 Hz) and $P_\beta$ (0·6 ± 0·2 Hz) whereas the [$^{18}O$]ATPαS-A (in the presence of ATPαS-A) derived from (**11**) showed an isotope shift only on $P_\alpha$ (1·1 ± 0·2 Hz). It follows therefore that the sample derived from (**10**) has structure (**12**) and that derived from (**11**) has structure (**13**). ATPαS-A has therefore the (*S*)-configuration at $P_\alpha$ and its diastereoisomer ATPαS-B the (*R*)-configuration at $P_\alpha$. This conclusion is in agreement with an independent assignment reported recently.[11]

It is noteworthy that the isotope shift on $P_\alpha$ and $P_\beta$ in [$\alpha\beta$-$^{18}$O]ATP$\alpha$S-A (**12**) is smaller than the isotope shift on $P_\alpha$ in [$\alpha$-$^{18}$O]ATP$\alpha$S-A (**13**), indicating that the $^{18}$O isotope shift on a $^{31}$P resonance is significantly different in bridging and non-bridging positions.

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