The Stereochemical Course of Substitution of Sulfur by Oxygen Nucleophiles in Five-membered Cyclic Phosphorothioates

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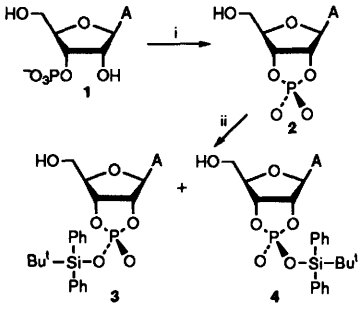
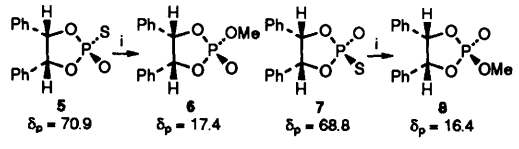
The replacement of non-bridging sulfur in five-membered cyclic phosphorothioates by oxygen nucleophiles in the presence of bromine occurs with retention of configuration and has allowed the ^{31}P NMR signals of the diastereoisomeric *tert*-butyldiphenyl silyl esters of 2',3'-cyclic adenosine monophosphate to be assigned.

Adenosine 5'-phosphosulfate kinase is the enzyme responsible for the conversion of adenosine 5'-phosphosulfate (APS) into 3'-phospho-adenosine 5'-phosphosulfate (PAPS), the principle sulfuryl donor in all biological systems.[1] The enzyme from *E. coli* obeys an ordered sequential kinetic mechanism in both the forward and reverse direction and yet it forms an isolable phospho-enzyme intermediate when incubated with MgATP which is both chemically and kinetically competent in the phosphorylation of APS and MgADP.[2,3] These observations are at variance with the view that enzymes which catalyse phosphoryl transfer by a sequential kinetic mechanism do so by direct 'in-line' phosphoryl transfer between enzyme-bound substrates and hence with inversion of configuration at phosphorus, whereas enzymes which catalyse phosphoryl transfer *via* a phospho-enzyme intermediate obey ping-pong kinetics and proceed with overall retention of configuration at phosphorus.[4,5] Consequently we have embarked upon a stereochemical investigation of the mechanism of phosphoryl transfer catalysed by APS kinase. For this purpose ATP-[γ(*S*)^{16}O,^{17}O,^{18}O],[6] will be used and the stereochemistry at phosphorus of 3'-[^{16}O,^{17}O,^{18}O]phospho-APS analysed after removing the 5'-phosphosulfate group. We now report an investigation which allows the stereochemistry at phosphorus of adenosine 3'-[^{16}O,^{17}O,^{18}O]phosphate to be determined.

The stereochemical analysis of adenosine 3'-[^{16}O,^{17}O,^{18}O]phosphate envisages cyclisation with diphenylphosphoryl chloride to the isotopomers of 2',3'-cyclicAMP followed by formation of diastereoisomeric 2',3'-cyclicAMP triesters, analogous to the method developed for the stereochemical analysis of adenosine 5'-[^{16}O,^{17}O,^{18}O]phosphate.[7] Cyclization of 3'-AMP **1** with diphenylphosphoryl chloride gave 2',3'-cyclicAMP **2** as expected. After exploring a number of reagents *tert*-butyldiphenylsilyl chloride, which gives the two diastereoisomeric silyl esters (**3** and **4**, Scheme 1), was selected. The ^{31}P NMR spectrum showed the two diastereoisomeric silyl esters to have δ_P 8.620 and 7.951,† but a means of assigning the resonances was required.



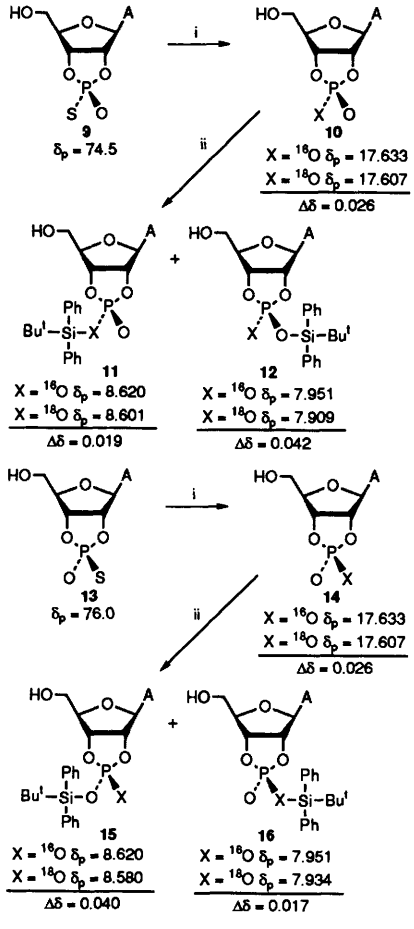
Scheme 1 *Reagents and conditions*: i, 1.1 equiv. of $(PhO)_2POCl$ in 1,4-dioxane; ii, 2 equiv. of *tert*-$BuPh_2SiCl$ and 2 equiv. of pyridine in DMF (A = adenine)



Scheme 2 *Reagents and conditions*: i, 3 equiv. of MeOH and 1.5 equiv. of Br_2 in DMF

Ludwig and Eckstein assigned the ^{31}P NMR signals of the *exo*- and *endo*-2',3'-cyclic adenosine phosphorothioates (2',3'-cAMPS)[8] by comparison with the ^{31}P chemical shifts of the *exo*- and *endo*-2',3'-cyclic uridine phosphorothioates (2',3'-cUMPS), the structure of the *endo*-diastereoisomer having been assigned by X-ray crystallography.[9] Substitution of sulfur by ^{18}O in the *exo*- and *endo*-2',3'-cAMPS by a process with established stereochemistry should make it possible to assign the ^{31}P resonances to the diastereoisomeric silyl esters from the isotope shift of the ^{31}P resonances, since the magnitude of the isotope shift is dependent on the bond order, ^{18}O in a P=O bond giving an isotope shift approximately double that in a P–O single bond.[10]

Thiophosphate esters may be converted to the corresponding ^{18}O-labelled phosphate esters by bromine,[11] cyanogen bromide,[12] or *N*-bromosuccinimide,[13] in ^{18}O-water. However, the stereochemical course of the replacement of sulfur in a



Scheme 3 *Reagents and conditions*: i, 1.5 equiv. of Br_2 and 10 equiv. of 50 atom% ^{18}O-water in DMF; ii, 2 equiv. of *tert*-$BuPh_2SiCl$ and 2 equiv. of pyridine in DMF

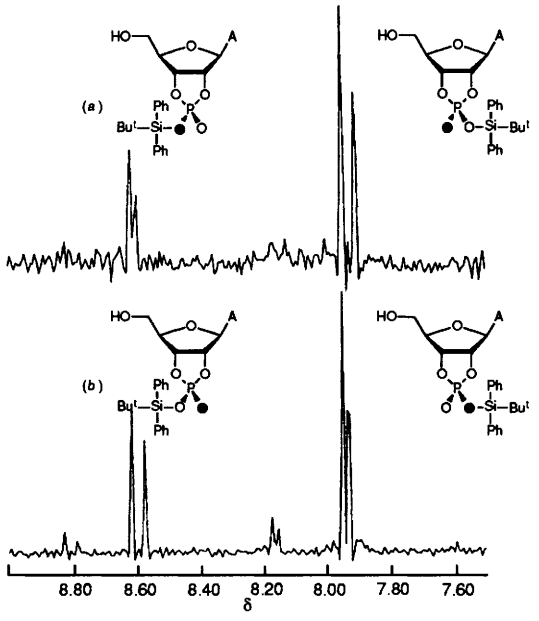


Fig. 1 The ^{31}P NMR spectra (101.256 MHz) of (*a*) the diastereoisomeric *tert*-butyldiphenyl silyl esters derived from endo-[^{18}O]cAMP (**10**, X = ^{18}O) and (*b*) the diastereoisomeric *tert*-butyldiphenyl silyl esters derived from *exo*-[^{18}O]cAMP (**14**, X = ^{18}O). The relative intensities of the ^{16}O to ^{18}O isotopomers indicates that a trace of water must have been present in the solvent before the addition of the 50 atom% ^{18}O-water. The two pairs of signals close to δ 8.2 and 8.8 are due to a small amount of the diastereoisomeric silyl triesters being additionally silylated at the 5′-hydroxy group.

cyclic five-membered phosphorothioate by an oxygen nucleophile has not been investigated. Since this replacement is likely to be unusual, in that retention of configuration is expected owing to the intervention of pseudorotation,[14] it was imperative that the stereochemical course of this displacement was investigated. The *cis*- and *trans*-diastereoisomers of 2-hydroxy-4,5-diphenyl-1,3,2-dioxaphospholane-2-thione **5** and **7**, whose stereochemistry had been unambiguously assigned,[15,16] were selected for this investigation. The two diastereoisomers were separately dissolved in dimethylformamide and 3 equiv. of methanol added followed by 1.5 equiv. of bromine (Scheme 2). The ^{31}P NMR spectrum of the reaction mixtures taken approximately 5 minutes after the addition of bromine showed that the phosphorothioates had completely disappeared. In the reaction with the *cis*-diastereoisomer **5** a resonance appeared at δ_P 17.4 which coincides with that of *cis*-2-methoxy-2-oxo-4,5-diphenyl-1,3,2-dioxaphospholane **6**,[17] together with a very minor resonance at δ_P 15.0 which was assigned to 2-hydroxy-2-oxo-4,5-diphenyl-1,3,2-dioxaphospholane, presumably arising from traces of water in the solvent. When the same reaction was performed on *trans*-2-hydroxy-4,5-diphenyl-1,3,2-dioxaphospholane-2-thione **7** the ^{31}P NMR spectrum showed that a single diastereoisomeric cyclic phosphate triester had formed with δ_P 16.4 corresponding to the *trans*-2-methoxy-2-oxo-4,5-diphenyl-1,3,2-dioxaphospholane **8**,[17] together with a trace of 2-hydroxy-2-oxo-4,5-diphenyl-1,3,2-dioxaphospholane (δ_P 15.0). Thus the replacement of sulfur occurs cleanly and with retention of configuration in five-membered cyclic phosphorothioates in accord with the Westheimer rules.[14]

The diastereoisomers of 2′,3′-cAMPS were prepared, and separated by HPLC.[8] The purified diastereoisomers were reacted separately with 1.5 equiv. of bromine in dimethylformamide to which had been added (50 atom%) ^{18}O-water. Each diastereoisomer of [^{18}O]cAMP was treated with *tert*-butyldiphenylsilyl chloride to give a mixture of the *exo*- and *endo*-silyl esters (Scheme 3) which were investigated by ^{31}P NMR spectroscopy (Fig. 1). Since we have established that the displacement of sulfur from five-membered cyclic phosphorothioates proceeds with retention of configuration, the product derived from *endo*-cAMPS (**9**, δ_P 74.5) with bromine/^{18}O-water should be the *endo*-[^{18}O]-cAMP (**10**, X = ^{18}O). The ^{31}P NMR spectrum [Fig. 1(*a*)] of the silyl esters (**11** and **12**, X = ^{18}O) derived from *endo*-[^{18}O]-cAMP (**10**, X = ^{18}O) shows that the low field signal (δ_P 8.620) has the smaller isotope shift (Δδ 0.019 ppm) corresponding to ^{18}O in a P–O and is therefore the *endo*-silyl ester **11**, whereas the high field signal (δ_P 7.951) shows an isotope shift (Δδ 0.042 ppm) corresponding to ^{18}O being in the P=O indicating that this diastereoisomer is the *exo*-silyl ester **12**. This was confirmed when *exo*-[^{18}O]cAMP (**14**, X = ^{18}O) derived from *exo*-2′,3′-cAMPS **13**, was treated with *tert*-butyldiphenylsilyl chloride and the low field signal (δ_P 8.620) had the larger isotope shift (Δδ 0.040 ppm) whereas the higher field signal (δ_P 7.951) shows an isotope shift (Δδ 0.017 ppm) expected for ^{18}O in a P–O single bond [Fig. 1(*b*)].

Now that the ^{31}P NMR signals have been assigned to the diastereoisomeric *tert*-butyldiphenylsilyl esters of 2′,3′-cAMP, the absolute configuration of adenosine 3′-[^{16}O,^{17}O,^{18}O]phosphate may be determined by cyclisation with diphenylphosphoryl chloride, followed by silylation with *tert*-butyldiphenylsilyl chloride and ^{31}P NMR analysis of the isotopomeric mixture of the diastereoisomeric silyl esters.

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Footnote

† The reference for all ^{31}P NMR spectra is 2% phosphoric acid in ^2H_2O. Positive chemical shifts (ppm) are downfield from the reference signal.

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