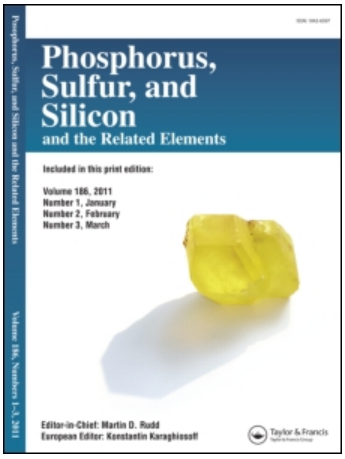


Phosphorus, Sulfur, and Silicon and the Related Elements
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713618290

A General Chemical Method for the Stereochemical Analysis of Nucleoside-5' [^{16}o,^{18}o] Phosphorothioates

Jane H. Cummins[a]; Barry V. L. Potter[a]
[a] Department of Chemistry, University of Leicester, Leicester, U. K

To cite this Article Cummins, Jane H. and Potter, Barry V. L.(1987) 'A General Chemical Method for the Stereochemical Analysis of Nucleoside-5' [^{16}o,^{18}o] Phosphorothioates', Phosphorus, Sulfur, and Silicon and the Related Elements, 30: 3, 589 — 592
To link to this Article: DOI: 10.1080/03086648708079134
URL: http://dx.doi.org/10.1080/03086648708079134

Phosphorus and Sulfur, 1987, Vol. 30, pp. 589-592
Photocopying permitted by license only

A GENERAL CHEMICAL METHOD FOR THE STEREOCHEMICAL ANALYSIS OF NUCLEOSIDE-5'[^{16}O,^{18}O] PHOSPHOROTHIOATES†

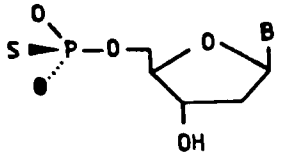
JANE H. CUMMINS AND BARRY V.L. POTTER*
Department of Chemistry, University of Leicester,
Leicester LE1 7RH, U.K.

Abstract A simple chemical method of configurational analysis is presented which will facilitate stereochemical investigation of any restriction endonuclease enzyme reaction.

INTRODUCTION

A stereochemical approach to understanding the mechanistic enzymology of transformations at phosphorus has recently been exploited, whereby a phosphorus centre is made chiral either by oxygen isotope substitution[1] or in a chiral phosphorothioate analogue[2]. Central to its success have been methods for the stereospecific synthesis of chiral biophosphates[1,2] and their stereochemical analysis[3]. Advances in oligonucleotide synthesis[4] now make this approach applicable to the large group of DNA restriction endonuclease enzymes[5]. However, although the synthetic side of the phosphorothioate method is well developed, present methods of stereochemical analysis limit its flexibility.

Cleavage of one diastereoisomer of an oligodeoxynucleotide phosphorothioate[6,7] in $H_2{}^{18}O$ by an enzyme and degradation to mononucleotides yields a 2'-deoxynucleoside 5'[^{16}O,^{18}O] phosphorothioate (1). Determination of the absolute configuration of (1) gives the stereo-

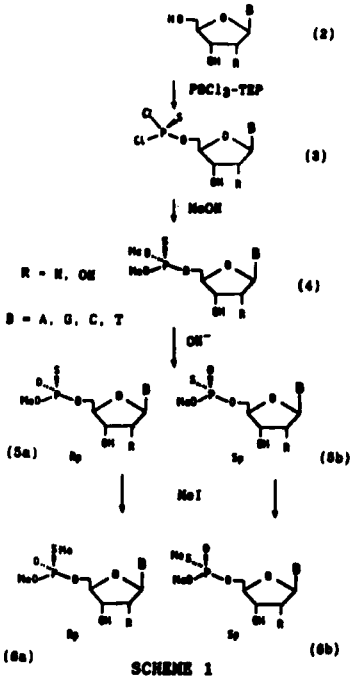


(1) ●=^{18}O

chemical course of the enzyme-catalysed reaction and provides important information as to the involvement or not of a phosphorylated enzyme intermediate. An enzymatic method for the stereospecific phosphorylation of adenosine 5'-phosphorothioate (AMPS) exists[8], which has been used for the configurational analysis of dAMPS by mass spectrometry and NMR spectroscopy[2,9], but this method rests on the specificity of adenylate kinase and is not applicable to other systems. Restriction enzymes can cleave next to any nucleoside residue in DNA, and we must therefore be able to determine the configuration of any such labelled 2'-deoxynucleoside 5'-phosphorothioate.

RESULTS AND DISCUSSION

Nucleoside 5'-O-methyl phosphorothioates (5a,b) have been synthesised from free nucleosides by a "one pot" procedure (SCHEME 1). The nucleoside (2) is thiophosphorylated and the resulting nucleoside 5'-thiophosphorodichloridate (3) converted to the corresponding O,O-dimethyl phosphorothioate triester (4). Base treatment yields diastereoisomers (5a,b), which are distinguishable by HPLC and ^{31}P NMR spectroscopy. Their configurations have been assigned by partial digestion with snake venom phosphodiesterase, which cleaves only one diastereoisomer of phosphorothioates[10,11]. We assign the S_p configuration to the hydrolysed diastereoisomer. Methylation of the now asymmetric mixture of (5a,b) using methyl iodide affords the corresponding triesters (6a, b), which can now be configurationally assigned by ^{31}P NMR spectroscopy (FIG. 1a, e.g. for B = Thymine).



SCHEME 1

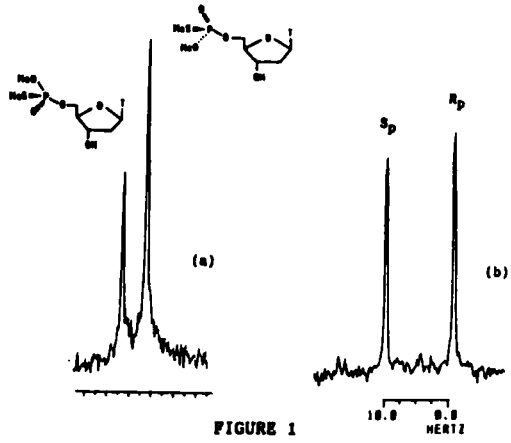


FIGURE 1

The general method of analysis is made possible since diastereoisomers (6a,b) are available directly from nucleoside 5'-phosphorothioates in almost quantitative yield by treatment with either diazomethane (e.g. FIGURE 1b) or dimethyl sulphate in DMF (both of these reagents give rise to varying types of base methylation according to the nucleoside used). For TMPS, AMPS, dAMPS and dCMPS either methyl-

ating agent can be used. For dGMPS, however, only dimethyl sulphate can be used, diazomethane giving rise to multiple methylation which complicates the ^{31}P NMR spectrum. Where stereochemical correlations for base-methylated material were required the appropriate asymmetric mixture of triesters (6a,b) was treated further with diazomethane or dimethyl sulphate.

We have established that, regardless of the nucleoside used (of the five tested) or the type of base methylation, or even if depurination occurs (for dGMPS), the S_p diastereoisomer of the nucleoside (or ribose) 5'-S-methyl-O-methyl phosphorothioate (6b) always resonates to lower field than the R_p diastereoisomer (6a) (FIGURE 1). It is now a simple matter to determine the position of an ^{18}O isotope in a nucleoside 5'-[^{16}O,^{18}O] phosphorothioate of unknown configuration by examining the magnitudes of the ^{18}O isotope shifts[12] in the ^{31}P NMR spectrum of the corresponding triester. These shifts are dependent on bond order[13]. In one diastereoisomer the ^{18}O atom will be in a bridging position and will exhibit a small isotope shift and in the other it will be in a doubly bonded position and will show a larger shift.

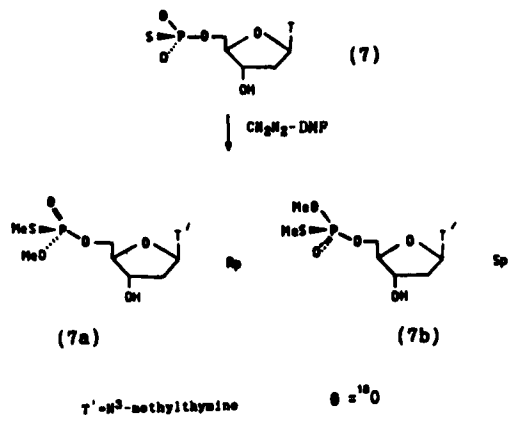


FIGURE 2

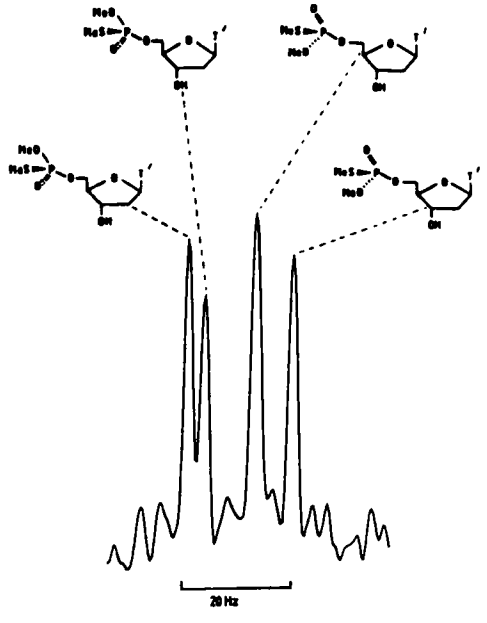


FIGURE 3

Methylation of a mixture of thymidine 5'[$^{16}O_2$]- and [^{16}O,^{18}O] phosphorothioates (7, FIGURE 2) (ca. 1.5μmol) of unknown absolute configuration obtained via the cleavage of 5'-O-thymidyl-3'-O-(2'-deoxyadenosyl) phosphorothioate catalysed by Phaseolus aureus

nuclease gave the ^{31}P NMR spectrum shown in FIGURE 3. Two isotope shifts are observable: a small one (2.5Hz) on the low field resonance (S_p-7b), and a larger one (5.7Hz) on the high field resonance (R_p-7a). We can now deduce that the configuration of (7) must have been S_p. Consequently, the enzyme-catalysed cleavage proceeds with inversion of configuration at phosphorus, presumably by means of a direct "in line" displacement mechanism[14].

We thank Dr M.R. Hamblin for the sample of [^{18}O]-TMPS and gratefully acknowledge financial support from the SERC and the Nuffield Foundation.

REFERENCES

1. S.L. Buchwald, D.E. Hansen, A. Hasset and J.R. Knowles, Meth. Enzymol., 87, 279 (1982); G. Lowe, Acc. Chem. Res., 16, 244 (1983).
2. P.A. Frey, Tetrahedron, 38, 1541 (1982); F. Eckstein, Angew. Chem. Int. Edn. Eng., 22, 423 (1983).
3. S.L. Buchwald and J.R. Knowles, J. Amer. Chem. Soc., 102, 6601 (1980); R.L. Jarvest, G. Lowe and B.V.L. Potter, J. Chem. Soc. Perkin Trans. I, 3186 (1981).
4. S.A. Narang, Tetrahedron, 39, 3 (1983).
5. P.A. Modrich, CRC Crit. Rev. Biochem., 13, 287 (1982).
6. B.A. Connolly, B.V.L. Potter, F. Eckstein, A. Pingoud and L. Grotjahn, Biochemistry, 23, 3443 (1984); J.E. Marugg, C. van den Bergh, M. Tromp, G.A. van der Marel, W.J. van Foest and J.H. van Boom, Nucl. Acids Res., 12, 9095 (1984); W.J. Stec, G. Zon, W. Egan and B. Stec, J. Amer. Chem. Soc., 106 6077 (1984).
7. B.A. Connolly, F. Eckstein and A. Pingoud, J. Biol. Chem., 259, 10760 (1984).
8. K.-F.R. Sheu and P.A. Frey, J. Biol. Chem., 252, 4445 (1977).
9. R.S. Brody and P.A. Frey, Biochemistry, 20, 1245 (1981).
10. P.M.J. Burgers, B.K. Sathyanarayana, W. Saenger and F. Eckstein, Eur. J. Biochem., 100, 585 (1979); P.M.J. Burgers and F. Eckstein, Biochemistry, 18, 592 (1979); B. Uznanski, W. Niewiarowski and W.J. Stec, Tet. Lett., 23, 4289 (1982).
11. F.R. Bryant and S.J. Benkovic, Biochemistry, 18, 2825 (1979).
12. G. Lowe and B.S. Sproat, J.C.S. Chem. Commun., 565 (1978); M. Cohn and A. Hu, Proc. Natl. Acad. Sci. U.S.A., 75, 200 (1978).
13. G. Lowe, B.V.L. Potter, B.S. Sproat and W.E. Hull, J.C.S. Chem. Comm., 733 (1979); M. Cohn and A. Hu, J. Amer. Chem. Soc., 102, 913 (1980).
14. M.R. Hamblin, J.H. Cummins and B.V.L. Potter, Biochem. J. (1986) submitted.

† A preliminary report for dAMPS has already appeared, see J.H. Cummins and B.V.L. Potter, J.C.S. Chem. Comm., 851-853 (1985).