Stereochemistry of Oxidation of Diastereoisomeric d(T*p*A) Phosphonates with Sulphur and Iodine–[^{18}O]Water†

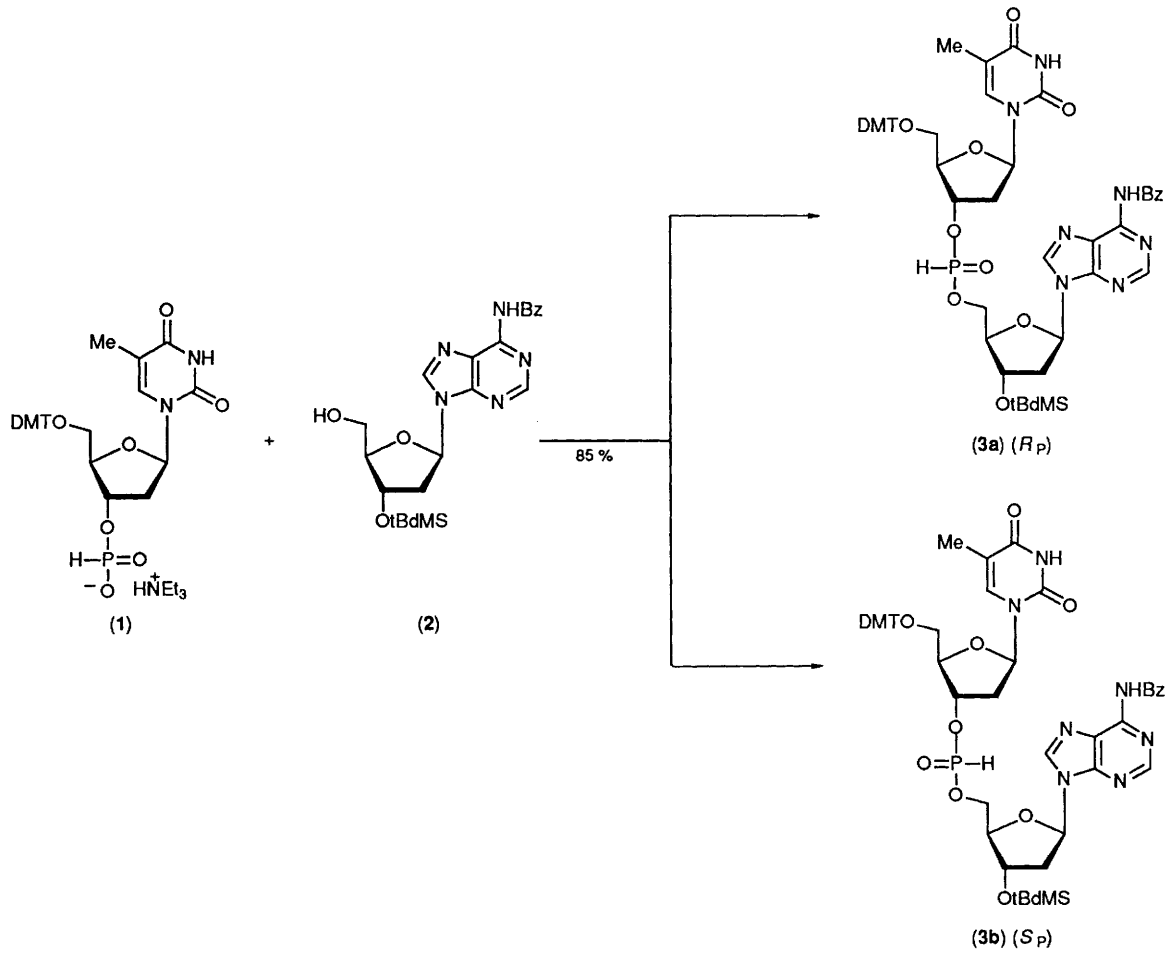
Frank Seela* and Uwe Kretschmer

Laboratorium für Organische und Bioorganische Chemie, Fachbereich Biologie/Chemie, Universität Osnabrück, Barbarastr. 7, D-4500 Osnabrück, West Germany

Oxidation of the diastereoisomeric phosphonate diesters of d(T*p*A) (**3a**) or (**3b**) with sulphur showed that the reaction is stereospecific and proceeds with retention of configuration; oxidation with I_2–$H_2{}^{18}O$, however, leads to (S_p)-[^{18}O]d(T*p*A), preferentially, regardless of the diastereoisomeric phosphonate diester used.

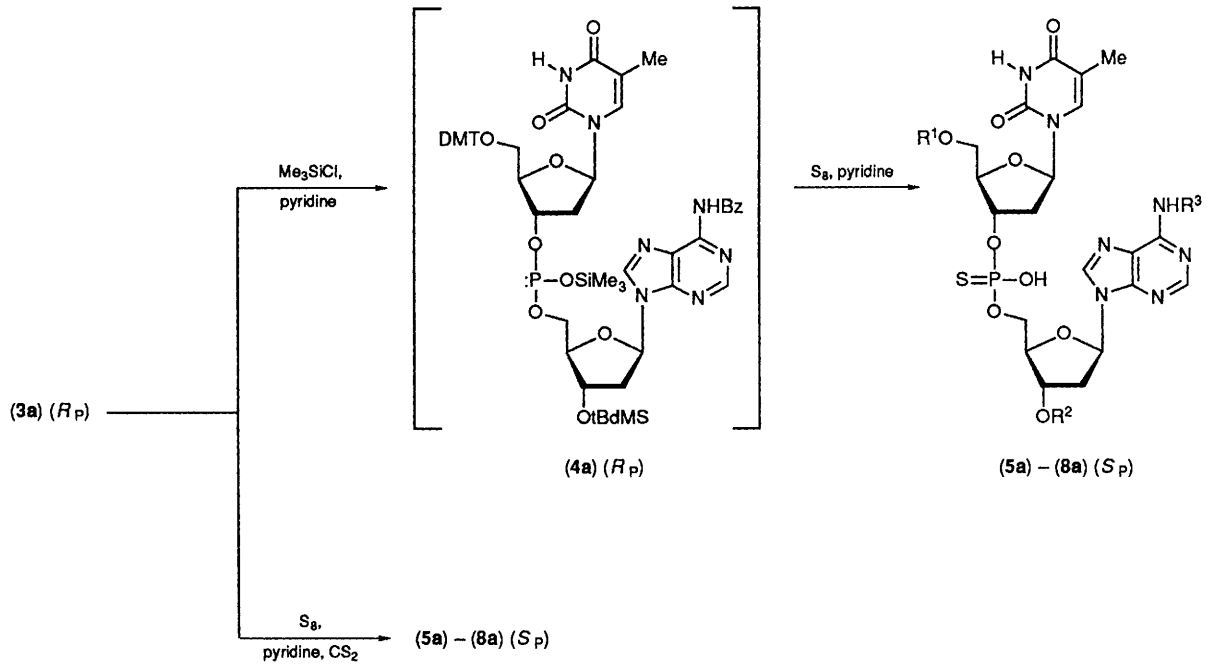
Currently phosphonate chemistry is employed in oligonucleotide synthesis[1] and also on solid supports.[2] Mechanistic[3] and stereochemical aspects[4] of this reaction have been investigated. As nucleoside phosphonate diesters are chiral the stereochemical course of the oxidation is of interest. We now describe the separation of *R*p and *S*p phosphonates of T*p*A and report on the stereochemical course of their oxidation with sulphur or I_2–$H_2{}^{18}O$.

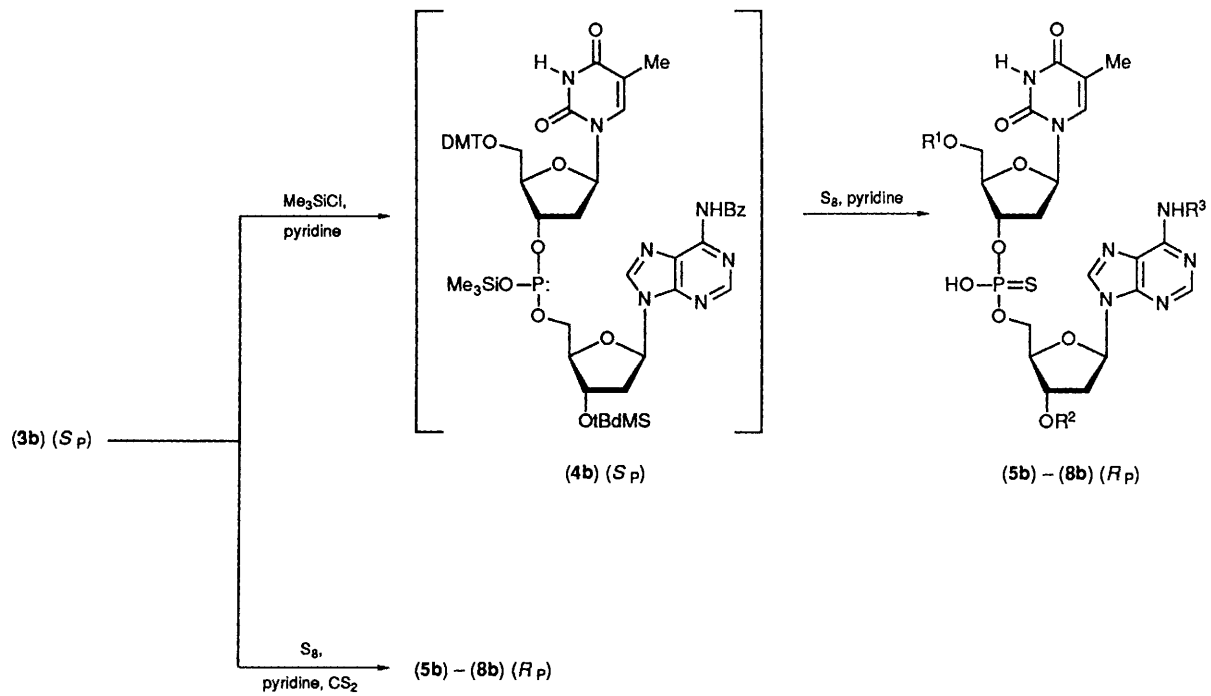
The thymidine-3′-phosphonate (**1**)[5] and the 3′-silylated 2′-deoxyadenosine derivative (**2**)[6] (1.0 mmol each) were condensed in anhydrous pyridine in the presence of pivaloyl chloride (3 mmol).[1] A mixture of the diastereoisomeric phosphonate esters (**3a**) and (**3b**) was obtained in 85% yield and in a 1 : 1 ratio determined by UV–TLC scanning (EtOAc–AcOH, 998 : 2; 260 nm). The diastereoisomers, later assigned as (**3a**) and (**3b**), were separated by preparative column



tBdMS = t-butyldimethylsilyl; DMT = dimethoxytrityl; Bz = PhC(:O)–.

† *p* represents the phosphonate moiety.





	R^1	R^2	R^3
(5)	DMT	tBdMS	Bz
(6)	DMT	H	Bz
(7)	H	H	Bz
(8)	H	H	H

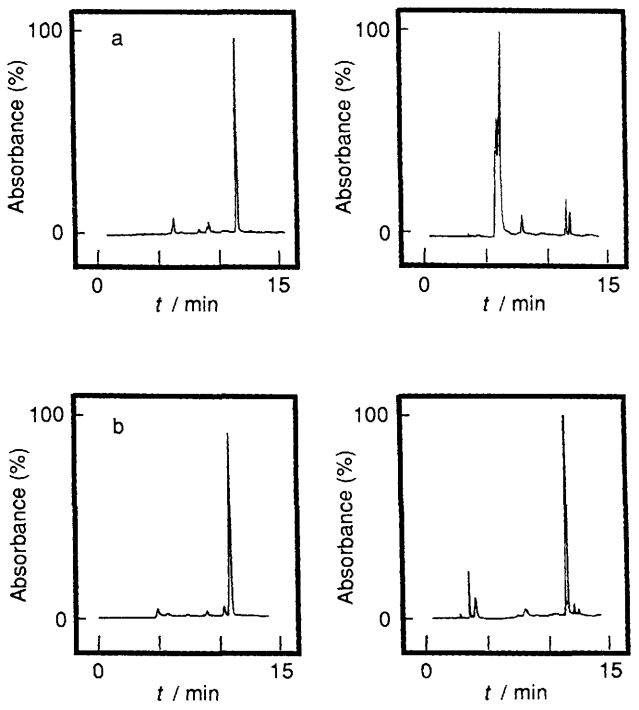


Figure 1. HPLC profile of *Sp*- and *Rp*-d[Tp(S)A] upon treatment with nuclease PI (ammonium acetate; pH 5.3; 37 °C). Column: LiChrosorb RP-18; gradient: triethylammonium acetate–acetonitrile; 5—25% MeCN, 20 min). a: (**8a**) (*Sp*-diastereoisomer); b: (**8b**) (*Rp*-diastereoisomer); left, initially; right, after 48 h.

chromatography (silica gel 60 H; EtOAc–AcOH, 998:2). Compound (**3a**) with R_F 0.37 was isolated as an amorphous foam exhibiting a ^{31}P NMR signal at 9.4 ppm (CD_3SOCD_3). The isomer with R_F 0.35 (**3b**) exhibited a signal at lower field (10.3 ppm). Both compounds showed identical $J(^{31}P, ^{1}H)$ coupling constants [^{1}J(P, H) 720, ^{3}J(P, H-3′) 16.5, and ^{3}J(P, H-5′) 8.2 Hz].

Phosphite diesters exists in the tetrahedral phosphonate form. Their oxidation or sulphurization requires more vigorous conditions than phosphite triesters.[7] We have carried out the oxidation of (**3a**) and (**3b**) as well as the *in situ*-formed trimethylsilyl triesters (**4a**) and (**4b**) separately with octameric sulphur. Sulphurization[8] of (**4a**) and (**4b**) was carried out in pyridine; pyridine–CS_2 (1:1)[9] was used in case of the diesters (**3a**) and (**3b**). Starting with either (**3a**) or (**4a**) the reaction (4 h; room temp.) furnished (**5a**). Compound (**5b**) was obtained by using (**3b**) or (**4b**). Without isolation of intermediates, compounds (**5a**) or (**5b**) were deprotected by (i) desilylation (**6a**) or (**6b**) (1 M Bu_4NF in tetrahydrofuran, THF), (ii) detritylation (**7a**) or (**7b**) (80% acetic acid), and (iii) deacylation (**8a**) or (**8b**) (25% aq. ammonia; 12 h; 50 °C). The phosphorothioates (**8a**) or (**8b**) were purified by ion exchange chromatography (Sephadex A-25, TBK-buffer, gradient 0—500 mM).

HPLC analysis (Figure 1) indicated that each diastereoisomeric phosphonate diesters formed only one stereochemically pure phosphorothioate [(**8a**) from (**3a**) and (**8b**) from (**3b**)]. This demonstrated the stereospecific course of the reaction. The configuration of the diastereoisomeric phosphorothioates (**8a**) and (**8b**) has already been established.[10] The *Sp*-isomer is hydrolysed by nuclease P I exclusively.[11,12] We have carried out this reaction with the sulphurization products of (**3a**) and (**3b**). As only the product from (**3a**) was hydrolysed the configuration of (**8a**) was assigned as *Sp*. Compound (**8b**) was resistant (Figure 1).

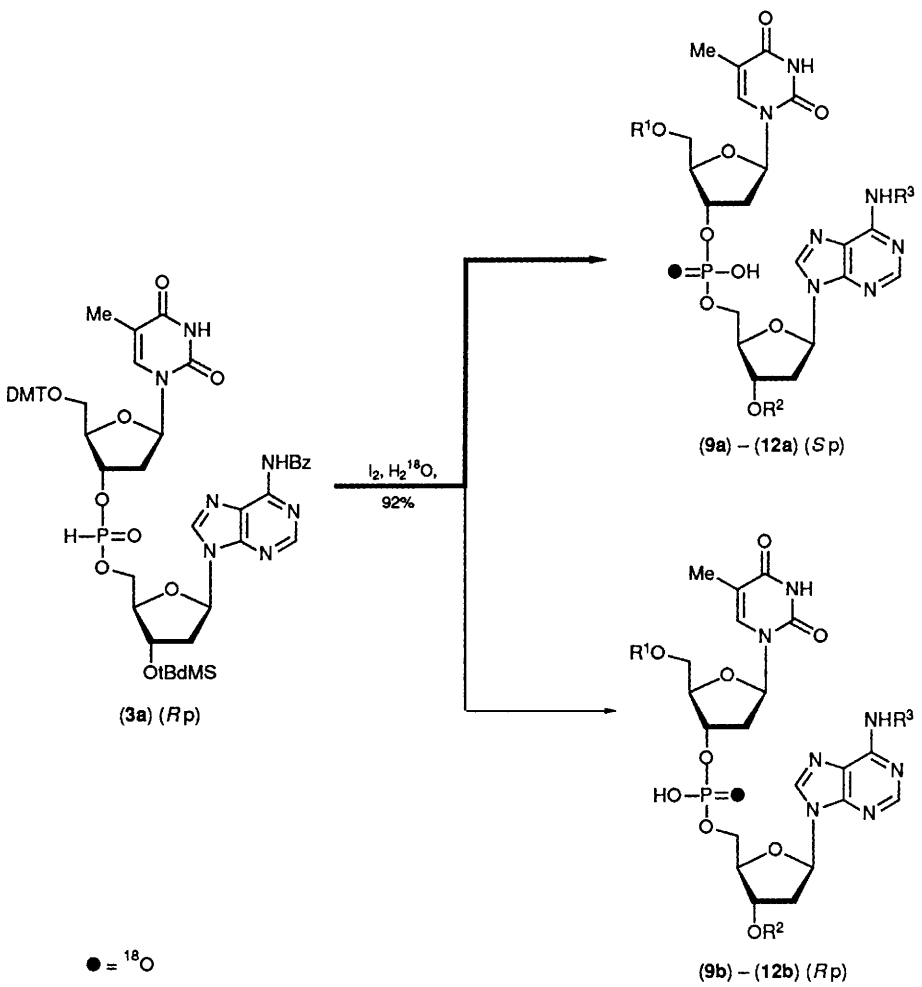
As identical reaction products were obtained by direct oxidative sulphurization or upon transient silylation the stereochemistry at phosphorus is retained during formation of the phosphite triesters (**4a**) or (**4b**). On the other hand, it has been shown for alkylphosphine derivatives that oxidation with sulphur proceeds with retention of configuration.[13,14] As this can be considered for phosphonate diesters, too, the fast migrating isomer (**3a**) (*Rp*) must have the same configuration at phosphorus as the phosphorothioates (**8a**) (*Sp*) and *vice versa*. The change in notation from *Rp* in (**3a**) to *Sp* in (**8a**) is a consequence of the Cahn–Ingold–Prelog rules, and does not indicate a change in stereochemistry.

The diastereoisomeric phosphonate diesters (**3a**) and (**3b**) (600 mg, 0.57 mmol of each) were also oxidized separately with I_2–$H_2{}^{18}O$ (isotopic purity 90%) similar to phosphite triesters.[15,16] Oxidation was carried out in absolute pyridine (15 min). Excess of I_2 was removed by aq. Na_2SO_3 (1% solution). For configurational assignment the reaction mixtures were (i) desilylated (1 M Bu_4NF in THF; room temp.; 30 min), (ii) detritylated (80% acetic acid), (iii) chromatographically purified (silica gel 60 H; CH_2Cl_2–MeOH–Et_3N, 85:10:5). Debenzoylation (25% aq. ammonia; 12 h; 50 °C) followed by chromatographic purification (Sephadex A-25; TBK buffer, gradient 0—200 mM) afforded the (*Rp*)-[^{18}O]d(TpA) (**9b**) and (*Sp*)-[^{18}O]d(TpA) (**9a**).

Following the procedure of Lowe *et al.*[17] the phosphodiesters of [^{18}O]d(TpA) (**12a** and **12b**) were methylated with MeI to give two diastereoisomers and two isotopomers according to Figure 2. The ^{31}P NMR spectrum of the reaction mixture showed six signals which were assigned according to Herdering and Seela.[16] Integration showed that the *Sp*-diastereoisomer‡ was formed in preponderance compared to the *Rp*-diastereoisomer‡ (3.5:1). The same reaction sequence as described for (**3a**) was carried out with (**3b**). It is noteworthy that the *Sp*-diastereoisomer is also the main product (2:1). As expected, a 1:1 mixture of (**3a**) and (**3b**), oxidized under the same conditions, shows an *Sp*/*Rp* ratio of 2.5:1. This indicates that the oxidation of phosphonate diesters with I_2–H_2O is not stereospecific. The diastereoisomeric excess (d.e.) was 55.6% in the case of (**3a**) and 33.3% starting with (**3b**). As d.e. = 43% was found for the 1:1 mixture of (**3a**)–(**3b**), oxygen isotopes can be introduced preferentially into the *Sp* position if mixtures of diastereoisomeric phosphonate diesters are oxidized. Similar behaviour was observed when oxidation of d(A*p*A) phosphonate was carried out.[18]

Cullis reported on the oxidation of a 2:1 (*Sp*, *Rp*) mixture of phosphite triesters of ApA with I_2–$H_2{}^{18}O$.[19] The conclusion that the reaction is stereospecific and proceeds with retention of configuration was drawn from a 2:1 ratio of [^{18}O]ApA triester obtained upon oxidation of a 2:1 mixture of phosphite triesters. We have carried out similar experiments with the pure *Sp*- or a *Rp*-diastereoisomer of the d(T*p*A) silylated at oxygen (**4a, 4b**). We obtained the preferred formation of *Sp*-configured phosphate diesters in both cases {(*Sp*)-[^{18}O]d(T*p*A) was always the main product}. Similar results were found without silylation (**3a, 3b**). This excludes a stereospecific reaction if I_2–$H_2{}^{18}O$ is used as oxidant.

‡ *Sp* and *Rp* refer to the position of [^{18}O] and not to the methyl esters.



	R^1	R^2	R^3
(9)	DMT	tBdMS	Bz
(10)	DMT	H	Bz
(11)	H	H	Bz
(12)	H	H	H

The different stereochemical outcome of I_2–water oxidation *vs.* reaction with sulphur must be attributed to the differences in the reaction mechanism and/or solvent effects. It has been found that sulphurization of optically active phosphines to phosphine sulphides with elemental sulphur proceeds with retention of configuration.[13] On the other hand, it has been reported that in the case of oxidation of phosphines retention, inversion, or racemization may take place depending on the oxidizing agent and the solvent employed.[20] In the case of I_2–H_2O oxidation pentavalent intermediates may be formed[21,22] which hydrolyse with racemization. However, if other chiral centres are present in the ligands the diastereotopic environment may control the stereochemical outcome of the hydrolysis products. As a consequence the *R*p configured phosphonate (**3a**) can be preferentially oxidized with retention of configuration and (**3b**) (*S*p) with inversion leading to the oxidation products with predominant *S*p configuration.

In conclusion oxidation of diastereoisomeric phosphonate diesters of d(T*p*A) with sulphur is stereospecific and proceeds with retention of configuration; oxidation with I_2–$H_2{}^{18}O$, however, leads to the *S*p isomer, preferentially. This may serve as a tool to build up an almost completely *S*p-configured isotopically labelled oligomer, even within an automated solid-phase synthesis, without separating phosphonate diesters. It remains to be proved whether the stereochemical outcome is the same in case of the oxidation of other dimeric phosphonate diesters or oligomeric phosphonates formed during oligonucleotide synthesis.

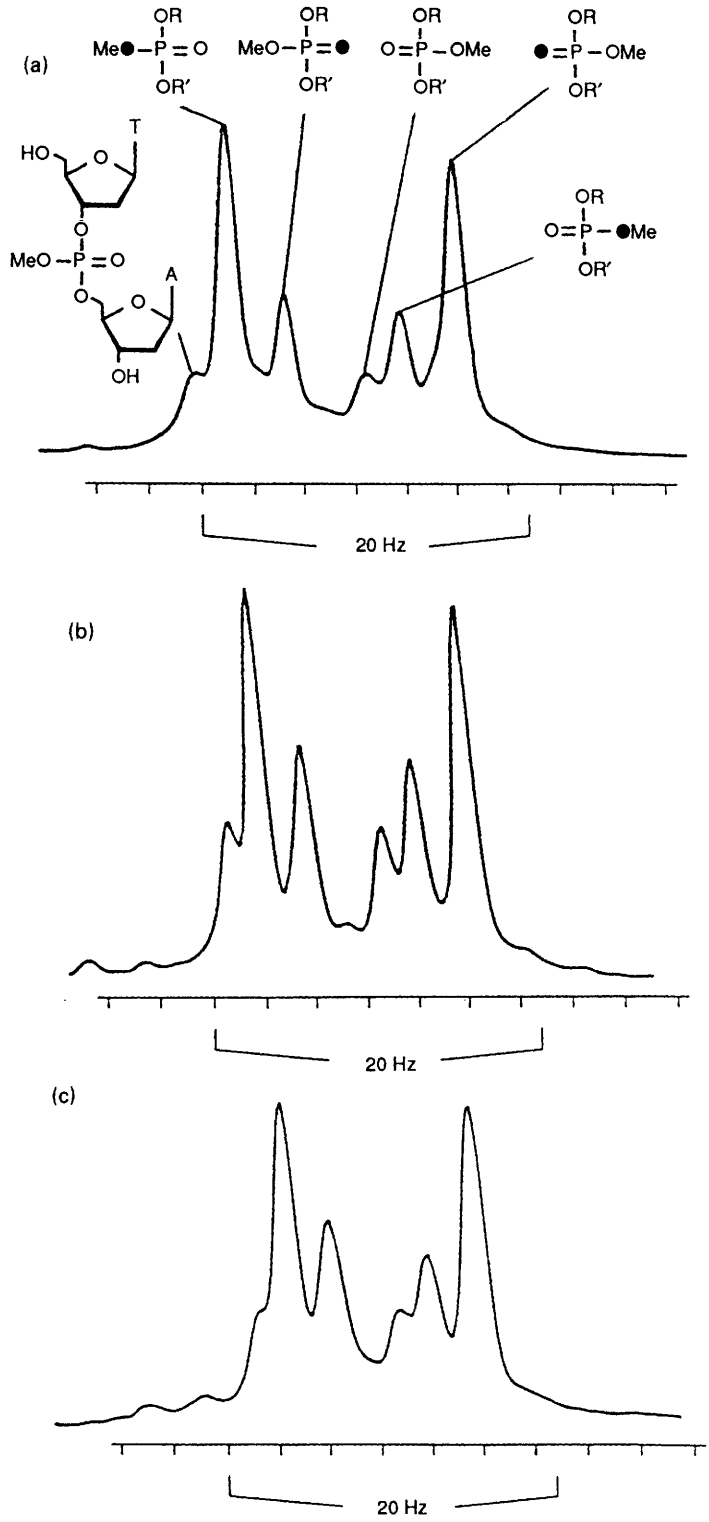


Figure 2. ^{31}P NMR spectra of diastereoisomeric [^{18}O]d[TpA'] methyl esters (15 mM) obtained from the phosphonates (**3a**) or (**3b**) by I_2–$H_2{}^{18}O$ oxidation followed by methylation. Solvent: CD_3SOCD_3 containing 8-hydroxyquinoline. (a) oxidation of (**3a**); (b) oxidation of (**3b**); (c) oxidation of a 1 : 1 mixture of (**3a**) and (**3b**).

We thank Dr H. Rosemeyer for the NMR spectra. Financial support by the Deutsche Forschungsgemeinschaft is gratefully acknowledged.

Received, 10th April 1990; Com. 0/01629B

References

1 P. J. Garegg, T. Regberg, J. Stawinski, and R. Strömberg, *Chem. Scri.*, 1986, **26**, 59.
2 P. J. Garegg, T. Regberg, J. Stawinski, and R. Strömberg, *Tetrahedron Lett.*, 1986, **27**, 4051.
3 P. J. Garegg, T. Regberg, J. Stawinski, and R. Strömberg, *Chem. Scri.*, 1985, **25**, 280.
4 C. L. Bodkin and P. Simpson, *J. Chem. Soc., Perkin Trans. 2*, 1973, 676.
5 B. C. Froehler, P. G. Ng, and M. D. Matteucci, *Nucleic. Acids Res.*, 1986, **14**, 5399.
6 K. K. Ogilvie, *Can. J. Chem.*, 1973, **51**, 3799.
7 P. J. Garegg, T. Regberg, J. Stawinski, and R. Strömberg, *J. Chem. Soc., Perkin Trans. 1*, 1987, 1269.
8 T. Hata, M. Sekine, *Tetrahedron Lett.*, 1974, **15**, 3943.
9 Applied Biosystems, User Bulletin No. 44, 'Oligonucleotide Synthesis with Hydrogen-Phosphonate Monomers.'
10 F. Eckstein, *Annu. Rev. Biochem.*, 1985, **54**, 367.
11 B. V. L. Potter, B. A. Connolly, and F. Eckstein, *Biochemistry*. 1983, **22**, 1369.
12 M. Koziolkiewicz, B. Uznanski, W. J. Stec, and G. Zon, *Chem. Scripta*, 1986, **26**, 251.
13 L. Horner, *Pure Appl. Chem.*, 1964, 225.
14 D. P. Young, W. E. McEwen, D. C. Velez, J. W. Johnson, and C. A. VanderWerf, *Tetrahedron Lett.*, 1964, **7**, 359.
15 F. Seela, J. Ott, and B. L. V. Potter, *J. Am. Chem. Soc.*, 1983, **105**, 5879.
16 W. Herdering and F. Seela, *J. Org. Chem.*, 1985, 50, 5314.
17 G. Lowe, B. V. L. Potter, B. S. Sproat, and W. E. Hull, *J. Chem. Soc., Chem. Commun.*, 1979, 733.
18 F. Seela and U. Kretschmer, unpublished results.
19 P. M. Cullis, *J. Chem. Soc., Chem. Commun.*, 1984, 1510.
20 D. E. C. Corbridge, 'Phosphorus,' in 'Studies in Inorganic Chemistry 2,' Elsevier, Amsterdam, 1980, p. 484.
21 A. Skowronska, M. Pakulski, J. Michalski, D. Cooper, and S. Trippett, *Tetrahedron Lett.*, 1980, **21**, 321.
22 C. L. Bodkin and P. Simpson, *J. Chem. Soc., Perkin Trans. 2*, 1972, 2049.