Heteroatom Chemistry
Volume 2, Number 5, 1991

Stereochemistry of the Reaction of Ribonucleoside Cyclic 3′,5′-Phosphorothioates with Oxiranes

Andrzej Okruszek, Piotr Guga, and Wojciech J. Stec*

Polish Academy of Sciences, Centre of Molecular and Macromolecular Studies, Department of Bioorganic Chemistry, Sienkiewicza 112, 90-363 Lodz, Poland

Received 3 July 1991.

ABSTRACT

The stereochemical course of the epoxide-induced oxidative rearrangement of ribonucleoside cyclic 3′,5′-phosphorothioates into the corresponding 2′,3′-phosphates has been determined using styrene [^{18}O] oxide and (S_P)-uridine cyclic 3′,5′-phosphorothioate. The evidence of full stereoselectivity of this reaction is presented and mechanistic implications of the presence of the nucleoside 2′-hydroxyl group are discussed in terms of a classical Hamer Mechanism.

INTRODUCTION

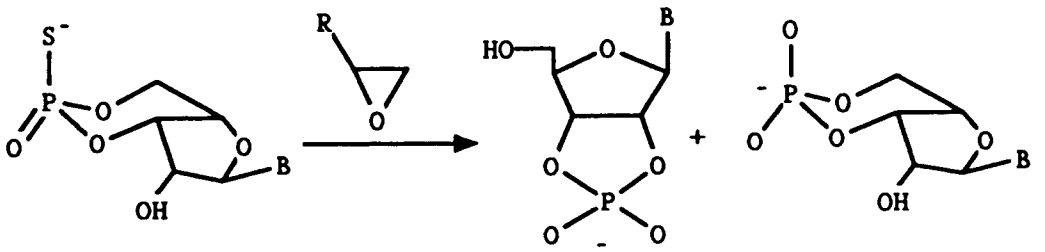
Numerous examples presented in the chemical and biochemical literature indicate that P-chiral phosphates and phosphorothioates have become indispensable tools in the elucidation of chemical reactions involving the phosphorus atom [1–3]. Especially interesting are examples of reactions of biophosphates catalyzed by enzymes where stereochemical analysis of substrates and products have provided fundamental information about the participation of covalently bound substrate-enzyme intermediates [4]. As a precondition to these studies the stereospecific syntheses of model compounds of known chirality at phosphorus had to be solved. After our successful design of methodology for the stereospecific conversion of dialkylphosphoranilidates into the corresponding phosphorothioates [5], attention has been focused on the application of these compounds in the stereospecific synthesis of P-chiral isotopomeric phosphates. Although it was demonstrated that this goal can be reached in several ways [6–10], reaction of dialkylphosphorothioates with alkylene oxides was particularly tempting due to its full stereospecificity [11, 12] and easy access to oxygen isotope-labeled oxiranes. Among the numerous examples of stereoretentive conversion of P-chiral phosphorothioates into isotopomeric phosphates, one particular reaction went in an unexpected direction. Treatment of ribonucleoside cyclic 3′,5′-phosphorothioates with the series of epoxides (ethylene, 1,2-butylene, and styrene oxide) produced ribonucleoside cyclic 2′,3′-phosphates (38–81%) as major products accompanied by variable amounts of the "expected" ribonucleoside cyclic 3′,5′-phosphates (7–38%) (Scheme 1). The tentative mechanism of the rearrangement accompanying the process of PS→PO conversion, based on the classical Hamer's scheme [13], has been proposed [14]. In this article, we present evidence that the conversion (PS → PO exchange and rearrangement) caused by the alkylene oxide is stereoselective, and provides the isotopomeric ribonucleoside cyclic 2′,3′-[^{18}O] phosphate of predetermined sense of chirality at phosphorus.

RESULTS

For detailed studies of the stereochemical course of the conversion process, we have chosen the reaction of uridine cyclic (S_P)-3′,5′-phosphorothioate (**1**) with racemic styrene [^{18}O]oxide. The stereospecific synthesis of S_P-**1** has been elaborated earlier in this

*To whom correspondence should be addressed.
Dedicated to Professor Leopold Horner on the occasion of his eightieth birthday.

1042-7163/92/$3.50 + .25

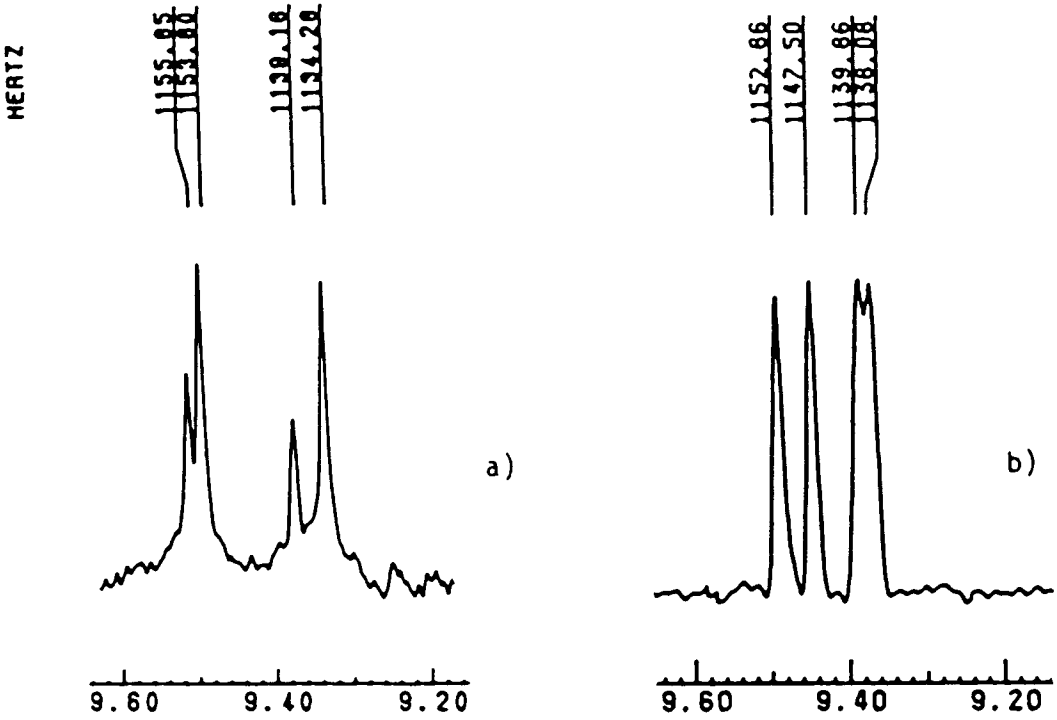


SCHEME 1

laboratory [15, 16] and its absolute configuration at phosphorus has been confirmed by independent X-ray diffraction studies [17]. We considered that the synthesis and absolute configuration of the diastereomeric uridine cyclic 2′,3′-phosphorothioates (**2**) was known from the work of Eckstein and Saenger [18]. Thus the stereoselective reaction of **2** with styrene [^{18}O]oxide should have given the isotopomeric uridine cyclic 2′,3′-[^{18}O]phosphates (**3**) of known configuration at the P-atom. Reaction of S_P-**1** with styrene [^{18}O]oxide was performed in ethanolic solution and its progress was followed by HPLC. After 2.5 h at 62 °C, the HPLC analysis showed the presence of starting **1** (11%), **3** (71%), and uridine cyclic 3′,5′-phosphate (**4**, 18%). The identity of these compounds has been confirmed by comparison with genuine samples. Since ^{31}P NMR resonances of both oxo-products were well separated ($\Delta\delta$ = 20), there was no need to isolate product **3**, and its stereochemical analysis was performed with the mixture of **3** and **2**. In order to discriminate substituents at the *exo*- and *endo*-positions in **3**, the products were transformed into a mixture of trimethylsilyl esters by treatment with *bis*(trimethylsilyl)trifluoroacetamide (BSTFA) and analyzed by means of ^{31}P NMR spectroscopy [19, 20]. The region of the spectrum corresponding to the trimethylsilyl esters of cyclic 2′,3′-phosphate is presented in Figure 1a.

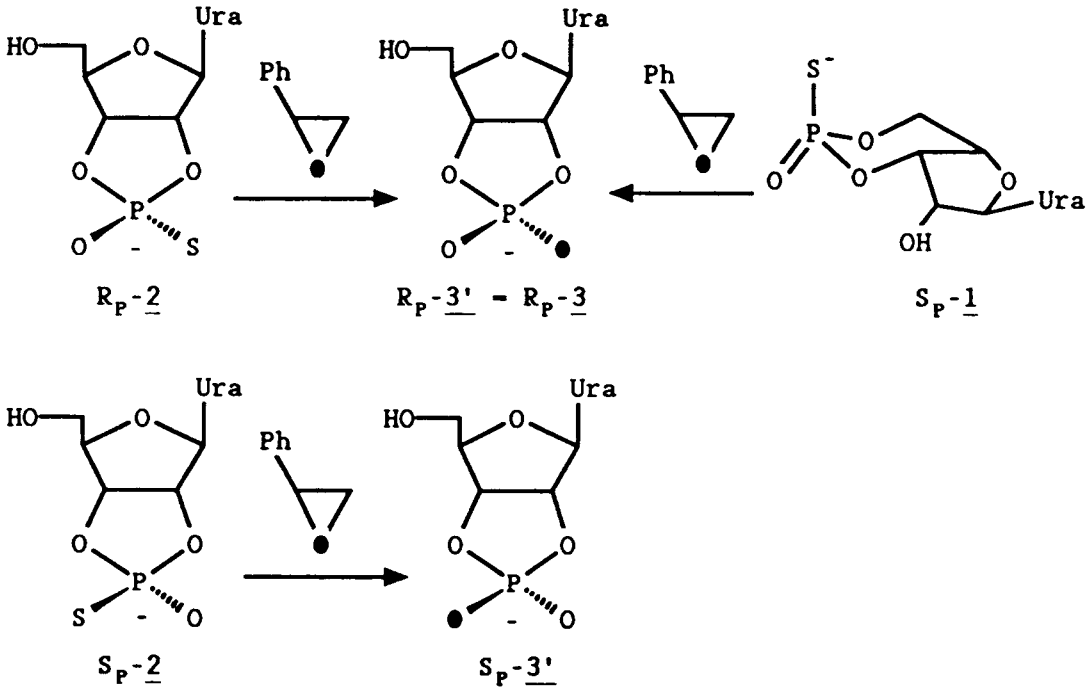
In an independent set of experiments, **2** was prepared by the phosphorodiamidite approach and separated into pure S_P-(*exo*) and R_P-(*endo*)-diastereomers by preparative HPLC. Their absolute con-

FIGURE 1 The ^{31}P NMR spectra of trimethylsilyl esters of uridine cyclic 2′,3′-[^{18}O] phosphates obtained from: **(a)** S_P-**1** and R_P-**2**, **(b)** S_P-**2**. Parameters: **(a)** sweep width 4350 Hz, time domain 8 K, Fourier transformation in 16 K; **(b)** sweep width 1200 Hz, time domain 4 K, Fourier transformation in 8 K.

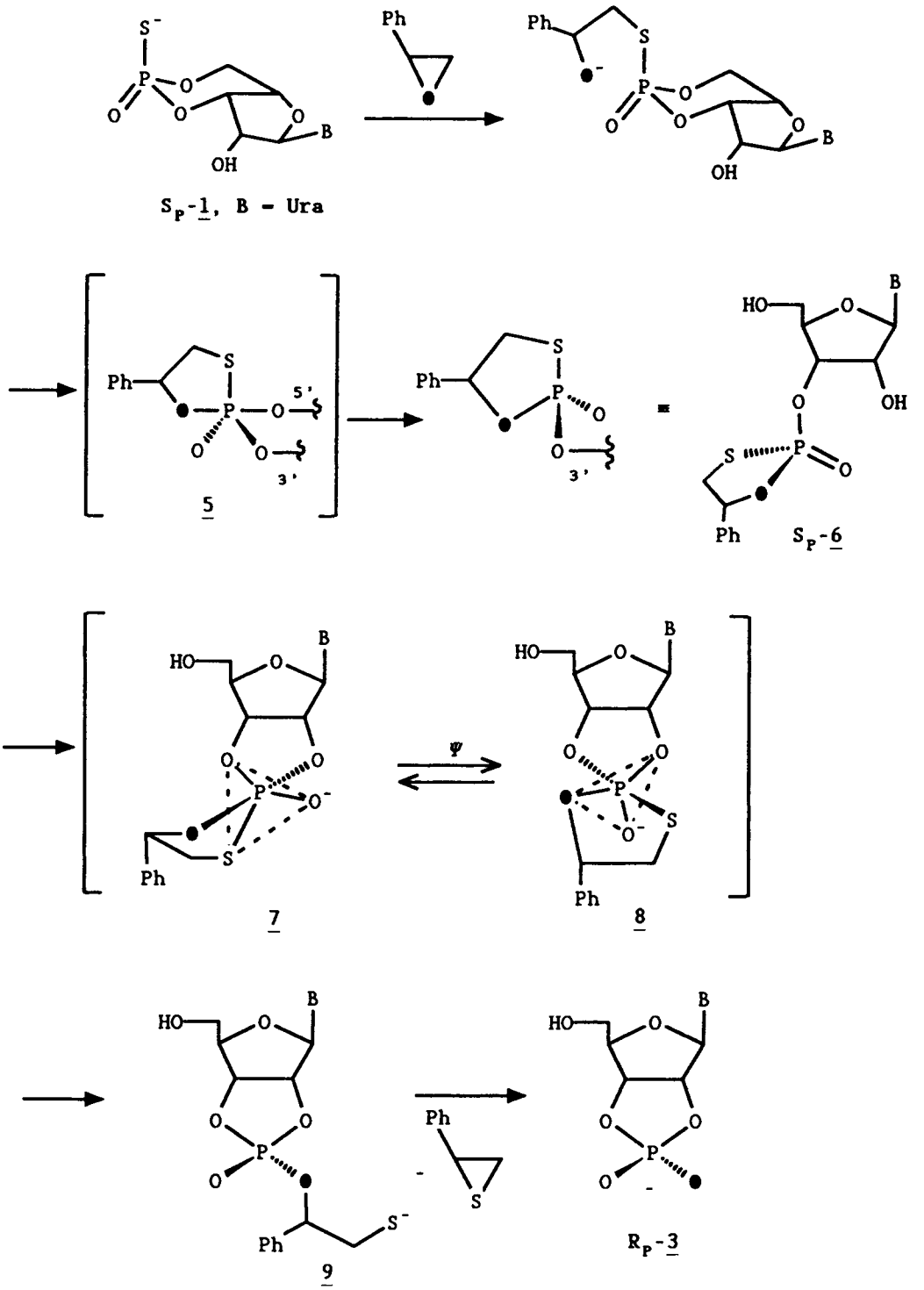


figurations were assigned by chromatographic and spectroscopic comparison with genuine samples of known configuration, obtained from the Laboratory of Prof. F. Eckstein. Each S_P-**2** and R_P-**2** isomer was independently treated with styrene [^{18}O]oxide in ethanolic solution and the products obtained (**3'**) had both chromatographic and spectroscopic properties characteristic for **3** (Scheme 2). Because the PS→PO exchange process under conditions of the Hamer reaction has been proven to occur with retention of configuration at phosphorus [11, 12], therefore, we could, with high probability, assign the R_P-configuration for **3'** resulting from R_P-**2** and, similarly, the S_P-configuration for **3'** obtained from S_P-**2**. Both compounds R_P-**3'** and S_P-**3'** were silylated by means of BSTFA and their high resolution ^{31}P NMR spectra were analyzed and compared with that recorded for silylated **3** (Figure 1a, b). The resonances of silylated **3**, as well as those of R_P-**3'** and S_P-**3'**, were composed of two signals of approximately equal intensity ($\Delta\delta$ = 0.18) corresponding to *exo*- and *endo*-isomers (with respect to the position of the trimethylsilyl group). Each signal was accompanied by an upfield peak corresponding to the ^{18}O-isotopomer with an isotope chemical shift difference depending upon the P-^{18}O bond order: $\Delta\delta$ = 0.017 for a single bond and 0.041 for a double bond [21]. This order of isotope chemical shift differences allowed us to assign the downfield pair of signals of the trimethylsilyl esters (δ = 9.5) to the *endo*-isomer and the upfield pair (δ = 9.3) to the *exo*-isomer. We could clearly observe that the spectra of silylated **3** and R_P-**3'** showed identical ^{18}O-isotope shift patterns with the lower isotope shift for the isomeric silyl ester absorbing at lower field. Therefore, we were able to conclude that the sense of chirality at phosphorus in both **3** and R_P-**3'** was the same. The lack of alternative ^{18}O-isotopomeric signals (e.g., with higher isotope shift for the low-field absorbing isomer) indicated that the two processes under study **1** → **3** and **2** → **3'** are fully stereospecific and lead to single diastereomers of **3** and **3'**, respectively.

The configurational identity of the cyclic isotopomeric nucleotides **3** and **3'** obtained via two different ways allows us to draw conclusions regarding the stereochemistry of the process **1** → **3** involving both sulfur–oxygen exchange and ring rearrangement. The reaction of uridine cyclic 3',5'-phosphorothioate with styrene [^{18}O]oxide proceeds with the stereochemistry S_P-**1** → R_P-**3** without detectable formation of S_P-**3**. This stereochemical result fully supports the mechanistic pathway suggested for the reaction of ribonucleoside cyclic 3',5'-phosphorothioates with epoxides [14] (Scheme 3). The key intermediate in this rearrangement is nucleoside 3'-O-(1,3,2-oxathiaphospholane) (**6**), which is formed from the initial adduct of phosphorothioate and oxirane after intramolecular attack of the β-hydroxyl group on the phosphorus atom leading to the cleavage of P—O5' bond [22]. We assume that this step proceeds with inversion via an $S_N2(P)$ "in-line" mechanism. Intermediate **6** undergoes an oxathiaphospholane ring opening reaction by an attack of the vicinal 2'-hydroxyl group of the ribose yielding triester **9** containing the β-mercaptoe-



SCHEME 2



SCHEME 3

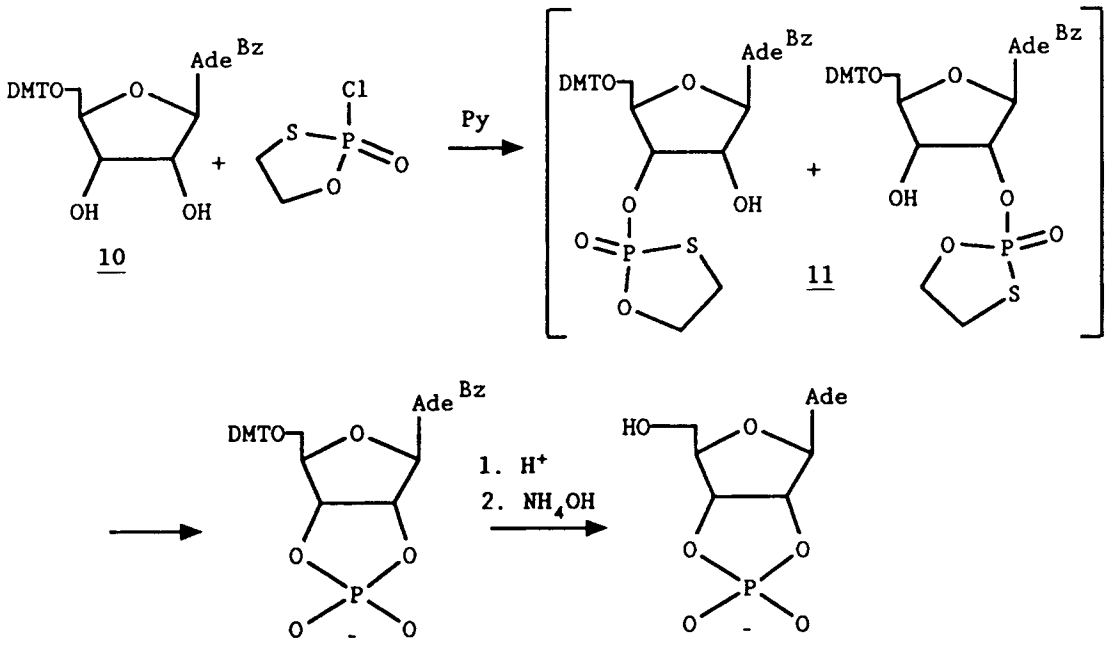
thoxyl ligand (which under reaction conditions undergoes elimination of episulfide giving the final product). The latter step must proceed with retention of configuration at phosphorus as a result of an "adjacent" process involving participation of a pentacovalent phosphorus intermediate **7**. The same stereochemical result was also found by Inch [25] for the solvolysis of acyclic S-alkyl esters. Since oxygen is more apicophilic than sulfur, the attack of the 2'-hydroxyl group occurs from the opposite side to the P—OR bond rather than to P—SR. Therefore, P—S bond cleavage can occur only after

pseudorotation of intermediate **7** resulting in translocation of the sulfur atom into the apical position (intermediate **8**). It should be noted, however, that when the less apicophilic nitrogen atom was present in the five-membered ring, the ring opening of 1,3,2-oxazaphospholanes by sodium alkoxides in alcohol was found to proceed with inversion of configuration at phosphorus [26, 27].

The evidence supporting the participation of oxathiaphospholane **6** in the reaction pathway was provided by an independent experiment, where *N*-6-benzoyl-5'-O-dimethoxytrityl-adenosine (**10**) was reacted with 2-chloro-2-oxo-1,3,2-oxathiaphospholane in pyridine solution (Scheme 4). The major product, ^{31}P NMR δ = 20.0, after full deprotection was identified by means of ^{31}P NMR and HPLC analysis to be adenosine cyclic 2',3'-phosphate. Since both 2'- and 3'-hydroxyl groups have similar reactivity toward phosphorylating reagents, it can be assumed that a significant part of the phosphorylation occurs in the 3'-position, thus giving rise to the ribonucleoside 3'-oxathiaphospholane product **11** analogous to **6**.

The observed preferential cleavage of the P—O5' bond (but not P—O3' bond) accompanying formation of **6** can be rationalized in terms of the stereoelectronic relations discussed by Tomasz [28] in the case of the alkaline hydrolysis of C5'-modified analogues of adenosine cyclic 3',5'-phosphate. This author suggested that, in the intermediate **5**, which exists in the form of a trigonal bipyramid with a twist-boat conformation of the dioxaphosphorinane ring (as shown by Bentrude [29]), the lone electron pair of the sp^2 hybridized oxygenatom at the 3' position occupies an energetically favored place in the plane of the equatorial substituents on the P-atom. This situation is possible if the O5' atom occupies the apical position; thus, the apical position of the O3' atom is discriminated, and hence the cleavage of the P—O3' bond does not occur. The regioselectivity of the ring opening reaction in the cyclic 3',5'-phosphate observed in the rearrangement under consideration is much higher than the regioselectivity reported by Tomasz [28] and Gerlt [30]. This fact, probably due to the entropy factor, may be connected with the difference in the nucleophile involved (intramolecular β-hydroxyethyl group vs. hydroxide ions present in an excess in the reaction medium).

The most intriguing question is posed by the fact that the conversion of the *2'-deoxy*ribonucleoside cyclic 3',5'-phosphorothioates into phosphates caused by styrene oxide in water/dimethylformamide as a solvent is accompanied by formation of observable amounts of neither 5'- nor 3'-nucleoside phosphates; this indicates that the cleavage of the P—O3' or P—O5' bond does not occur [12]. This observation emphasizes the puzzling role of the 2'-hydroxyl group in **1** after treatment with epoxides, since inspection of molecular models does not permit the assumption of the direct participation of the 2'-hydroxyl group in the formation and evolution of the transition state leading to cleavage of the P—O5' bond. From this point of view, the fundamental difference in reactivity between ribo- vs. deoxyribonucleoside cyclic 3',5'-phosphorothioates remains obscure. Therefore, this difference must be considered in terms of significant changes in con-



SCHEME 4

formations of both molecules or as a long distance interaction of the 2'-hydroxyl group with the phosphorus center with participation of solvent molecules. In the latter case, the presence of the 2'-hydroxyl group hampers the pseudorotation process of intermediate **5**. In this respect, the mechanistic aspects of reaction of ribonucleoside cyclic 3',5'-phosphorothioates with epoxides is reminiscent of those involved in nuclease-assisted hydrolysis of phosphodiesters undergoing "in-line" attack by water molecules where also no evidence of pseudorotation of the primary intermediate has been found [31].

EXPERIMENTAL

Materials

Nucleosides were purchased from Pharma-Waldhof (Germany). 2-Cyanoethyl *N,N,N',N'*-tetraisopropylphosphorodiamidite was synthesized according to the procedure described by Köster et al. [32]. [^{18}O]Benzaldehyde and uridine cyclic 3',5'-phosphorothioate (S_P isomer) were synthesized according to procedures described by J. Baraniak and W. J. Stec [16]. 5'-Methoxyacetyluridine was obtained from reaction of 2',3'-O-ethoxymethylidene uridine with methoxyacetic anhydride according to known methodology [33]. Gaseous nitrogen tetroxide obtained by thermal decomposition of lead (II) nitrate was absorbed in methylene chloride at −60 °C up to a concentration 8.5g N_2O_4/15 mL of solvent and stored at 4 °C. Trimethylsulfonium iodide was obtained from dimethyl sulfide and methyl iodide according to the procedure described by Corey and Chaykowski [34].

Instrumental Analysis

Gas chromatography analyses (GC) were performed on a Varian Aerograph 2700 instrument (capillary column OV-101; 2 m, temperature program 70–290 °C, 10 °C/min) equipped with a flame ionization detector with nitrogen as a carrier gas. HPLC was performed using the LDC Milton Roy system equipped with the UV detector working at 260 nm. ^{31}P NMR spectra were recorded on a Bruker MSL-300 spectrometer (121.5 MHz for ^{31}P) with deuterium lock and quadrature detection. Chemical shifts are reported referring to 85% H_3PO_4 used as an external standard. Mass spectra were recorded on a GCMS LKB 2091 instrument.

Synthesis of Styrene [^{18}O]oxide

A suspension of 0.3 g (13mmol) of sodium hydride in 9 mL of anhydrous dimethylsulfoxide (DMSO) was stirred at 70 °C for ca. 20 min (gaseous hydrogen evolved). The solution of the sodium salt was cooled to room temperature and diluted with 9 mL of anhydrous tetrahydrofuran and then cooled to −10 °C. At this temperature, a solution of trimethylsulfonium iodide (2.6 g, 13 mmol) in 9 mL of dry DMSO was added with stirring followed (after 1 min) by a solution of [^{18}O]benzaldehyde (1.2 g, 11.5 mmol, 92 atom % ^{18}O) in 2 mL of DMSO. The stirring was continued for 15 min at 0 °C and then 1 h at room temperature. The reaction mixture was poured into a separatory funnel containing 75 mL of water and the nonionic products were extracted with ethyl ether (100 + 50 mL). The organic layer was dried with anhydrous magnesium sulfate, concentrated, and the oily residue was distilled under reduced pressure to give 1.0 g of a mixture (bp 50–52 °C/0.01 mmHg) consisting of styrene [^{18}O]oxide (73%), DMSO (19%), and other contaminants (8%, GC analysis). This mixture was poured into 20 mL of water and extracted with pentane (3 × 5 mL). The organic layer was dried and evaporated giving 0.7 g of styrene oxide of 98.8% purity and enriched in 91 at.% ^{18}O as measured by means of mass spectrometry on molecular ions.

Synthesis of 2-Chloro-1,3,2-Oxathiaphospholane

Into the solution of phosphorus trichloride (27.5 g, 0.2 mol) in 400 mL of anhydrous diethyl ether a solution of anhydrous pyridine (15.8 g, 0.4 mol) and freshly distilled 2-mercaptoethanol (15.6 g, 0.2 mol) in 100 mL of anhydrous diethyl ether was added dropwise with stirring at room temperature. The reaction mixture was stirred for 2 h and the precipitate of pyridine hydrochloride was filtered off. The filtrate was evaporated and the residue was distilled under reduced pressure affording 14.3 g (50%) of product in the form of a colorless liquid (bp 76 °C/10 mmHg, ^{31}P NMR δ = 205.3 (C_6D_6)). MS (70 eV): m/z 142, M^+, 0,3%; 96, M^+ −HCHS, 20%; 83, M^+ −HCHS−CH, 16%; 60, 100%; Lit. [35] bp 57 °C/0.4 mmHg.

Synthesis of 2-Chloro-2-Oxo-1,3,2-Oxathiaphospholane

Into the stirred solution of 2-chloro-1,3,2-oxathiaphospholane (3.2 g, 22.5 mmol) in 20 mL of anhydrous methylene chloride was added dropwise at −20 °C, a solution of nitrogen tetroxide in methylene chloride until the mixture became permanently green. The solvent was evaporated and distillation of the residue under reduced pressure afforded 1.2 g of colorless liquid (bp 110 °C/0.01 mmHg; ^{31}P NMR δ = 50.5 ($CDCl_3$), 87% purity; MS(15 eV): m/z 158, M^+, 24%; 123, M^+ −Cl, 32%).

Synthesis of Uridine Cyclic 2',3'-Phosphorothioate

5'-Methoxyacetyluridine (130 mg, 0.3 mmol), 1*H*-tetrazole (50 mg, 0.66 mmol), and elemental sulfur (30 mg) were suspended in 3 mL of anhydrous acetonitrile, cooled to 0 °C, and a solution of 2-cyanoethyl *N,N,N',N'*-tetraisopropylphosphorodiamidite (100 mg, 0.33 mmol) in 0.4 mL of acetonitrile was added with vigorous stirring. The cooling bath was removed and stirring was continued for 3 h at room temperature. The solid material was filtered off; the filtrate was evaporated and dissolved in a mixture containing 30% aqueous ammonia (2.5 mL) and ethanol (2.5 mL). The solution was evaporated, and the residue was dissolved in 2 mL of water and applied onto a column filled with Sephadex A25 (bicarbonate form). The column was eluted with a linear gradient of water (700 mL) and 1.2 M triethylammonium bicarbonate (TEAB, 700 mL). The first 300 mL of eluate was discarded and then fractions of 15 mL were collected. The uridine cyclic 2',3'-phosphorothioate was found in fractions 13–26, which were pooled and concentrated. A ^{31}P NMR spectrum of this material showed the presence of two signals, δ = 77.2 and 75.6 (D_2O), of nearly equal intensity corresponding to both diastereomers of the desired product (Lit. [36]: ^{31}P NMR δ = 76.7 and 75.2). Pure isomers were isolated by means of preparative HPLC (ZORBAX-ODS, 21.2 mm × 25 cm, flow rate 8.5 mL/min,7% CH_3CN/0.1 M TEAB) to give 30 mg of *exo*- and 23 mg of *endo*-isomer (Rt = 5.2 and 6.7 min, respectively), total yield 43%.

Reaction of (S_P)-Uridine Cyclic 3',5'-Phosphorothioate with Styrene [^{18}O]oxide

A solution of the triethylammonium salt of (S_P)-uridine cyclic 3',5'-phosphorothioate (37 mg, 88 μmol) and 200 μL of styrene [^{18}O]oxide (65 atom % ^{18}O, 1500 μmol) in 2.5 mL of 96% ethanol was incubated at 62 °C for 3 h. The HPLC analysis (ODS Hypersil C18, 5–40% CH_3CN/0.1 M TEAB, 1%/min) showed the presence of **3** (71%, Rt = 6.8 min), uridine cyclic 3',5'-phosphate (18%, Rt = 8.7 min) and unreacted **1** (11%, Rt = 10.8 min). The solvent was evaporated and the sample was dried at high vacuum for 12 h. Anhydrous acetonitrile-d_3 (3 mL) was added followed by 200 μL of BSTFA. After a few minutes the solution was filtered through a membrane filter (0.45 μm) into an NMR tube. ^{31}P NMR analysis (T = 266 K, sweep width 4350 Hz, time domain 8 K, Fourier transformation in 16 K) showed in the region of the silyl ester of **3** (^{31}P NMR δ = 9.5–9.3) the following ^{18}O-isotope shift pattern: signals at 1155.8, 1153.8 (isotope effect 2 Hz), 1139.2, and 1134.3 Hz (isotope effect 4.9 Hz) downfield of standard frequency.

Reaction of Uridine Cyclic 2',3'-Phosphorothioate with Styrene [^{18}O]oxide

A solution of the triethylammonium salt of (S_P)-uridine cyclic 2',3'-phosphorothioate (30 mg, 71 μmol, *exo*-isomer) and styrene [^{18}O]oxide (55 atom % ^{18}O, 200 μL, 1500 μmol) in 2.5 mL of 96% ethanol was incubated at 62 °C for 3 h. The volatile components of the reaction mixture were evaporated and the residue was dissolved in CD_3OD. The ^{31}P NMR spectrum revealed the presence of uridine cyclic 2',3'-phosphate (81%, δ = 14.2). The mixture was silylated (*vide supra*). The spectrum recorded at 266 K (sweep width 1200 Hz, time domain 4 K, Fourier transformation in 8 K) showed the presence of signals shifted downfield of the standard frequency by 1152.8, 1147.5 (isotope effect 5.3 Hz), 1139.9, and 1138.0 Hz (isotope effect 1.9 Hz).

The same procedure was repeated with the sample of 23 mg of the *endo*-isomer of **2** using slightly more enriched styrene [^{18}O]oxide (65% atom % ^{18}O). The spectrum of the silylated product showed the presence of signals at 1154, 1152 (isotope effect 2 Hz), 1137, and 1132 Hz (isotope effect 5 Hz) downfield of the standard frequency.

Reaction of 5'-Dimethoxytrityl-N-6-Benzoyl-Adenosine with 2-Chloro-2-Oxo-1,3,2-Oxathiaphospholane

A solution of 5'-dimethoxytrityl-N-6-benzoyl-adenosine (250 mg, 0.37 mmol) and 2-chloro-2-oxo-1,3,2-oxathiaphospholane (57 mg, 0.37 mmol) in 1 mL of pyridine was incubated at 100 °C for 2 min, cooled, and evaporated to dryness. The residue was dissolved in deuterated chloroform and analyzed by means of ^{31}P NMR spectroscopy. The spectrum showed the presence of signals at δ = 20 (26%), 1.8 (13%), and −9.4 (11%) accompanied by a number of signals of lower intensity. The solvent was then evaporated and the residue was dissolved in a 2% solution of dichloroacetic acid in methylene chloride (3 mL). After 1 min, the resulting mixture was treated with 200 μL of methanol and 1 mL of pyridine. The volatile components were evaporated; the residue was dissolved in 25% aqueous ammonia (5 mL) and kept overnight at room temperature. The solvent was evaporated and the residue was dissolved in 2 mL of water and filtered. HPLC analysis (ODS Hypersil C18, 3–40% CH_3CN/0.1 M TEAB, 1%/min) showed the presence of adenosine (73%, Rt = 8.44 min), adenosine cyclic 2',3'-phosphate (10%, Rt = 9.62 min), and a number of peaks at longer retention times (17%, Rt = 10.1–12.3 min).

ACKNOWLEDGMENT

We are pleased to thank Prof. Fritz Eckstein for his kind gift of a sample of pure *endo*-uridine cyclic

2',3'-phosphorothioate that we used as the standard in HPLC analysis.

REFERENCES

[1] S. L. Buchwald, D. E. Hansen, A. Hasset, J. R. Knowles: Chiral [^{16}O, ^{17}O, ^{18}O] Phosphoric Monoesters as Stereochemical Probes of Phosphotransferases, in D. L. Purich (ed): *Methods in Enzymology*, Vol. 87, Academic Press, New York, pp. 279–301 (1982).

[2] J. A. Gerlt, J. A. Coderre, S. Mehdi: Oxygen Chiral Phosphate Esters, in A. Meister (ed): *Advances in Enzymology*, Vol. 55, Wiley & Sons, New York, pp. 291–380 (1983).

[3] F. Eckstein, *Ann. Rev. Biochem., 54,* 1985, 367.

[4] P. A. Frey, *Adv. Enzymol. Relat. Areas Mol. Biol., 62,* 1989, 119–201.

[5] W. J. Stec, *Acc. Chem. Res., 16,* 1983, 411.

[6] G. Lowe, G. Tansley, P. M. Cullis, *J. Chem. Soc., Chem. Commun.* 1982, 595.

[7] R. Iyengar, T. T. Ho, R. D. Sammons, P. A. Frey, *J. Am. Chem. Soc., 106,* 1984, 6038.

[8] B. A. Connolly, F. Eckstein, H. H. Füldner, *J. Biol. Chem., 257,* 1982, 3382.

[9] A. Okruszek, W. J. Stec, *Tetrahedron Lett., 23,*1982, 5203.

[10] A. Okruszek, W. J. Stec, *J. Chem. Soc., Chem. Commun.,* 1984, 117.

[11] P. Guga, W. J. Stec, *Tetrahedron Lett., 24,* 1983, 3899.

[12] P. Guga, A. Okruszek, *Tetrahedron Lett., 25,* 1984, 2897.

[13] N. K. Hamer, *Chem. Commun.,* 1968, 1399.

[14] A. Okruszek, P. Guga, W. J. Stec, *J. Chem. Soc., Chem. Commun.,* 1985, 1225.

[15] J. Baraniak, P. Mejbaum, W. J. Stec, *Pol. J. Chem., 56,* 1982, 441.

[16] J. Baraniak, W. J. Stec, *J. Chem. Soc., Perkin Trans. I,* 1987, 1645.

[17] W. Hinrichs, M. Steifa, W. Saenger, F. Eckstein, *Nucleic Acids Chemistry, 15,* 1987, 4945.

[18] The *endo-* configuration of the crystalline isomer of uridine cyclic 2',3'-phosphorothioate has been assigned by means of X-ray analysis: F. Eckstein, W. Saenger, *J. Am. Chem. Soc., 92,*1970, 4712.

[19] Conversion of **3** to the mixture of O-methyl esters was unsuccessful because of the high instability of the triesters containing the 5-membered 1,3,2-dioxaphospholane ring.

[20] The successful application of trimethylsilyl esters for stereochemical analysis of P-chiral isotopomeric phospholipids was originally reported by K. Bruzik and M.-D. Tsai, *J. Am. Chem. Soc., 104,* 1982, 863.

[21] G. Lowe, B. V. L. Potter, B. S. Sproat, W. E. Hull, *J. Chem. Soc., Chem. Commun.* 1979, 733.

[22] It should be mentioned that in a case of phosphorothioate diesters possessing at least one aryloxyl group attached to phosphorus, the same attack of the β-hydroxyl group leads to formation of the oxathiaphospholane ring after departure of the appropriate phenol molecule [23, 24].

[23] W. Kudelska, M. Michalska, *Tetrahedron, 42,* 1986, 629.

[24] A. Okruszek, P. Guga, W. J. Stec, *J. Chem. Soc., Chem. Commun.* 1987, 594.

[25] D. B. Cooper, C. R. Hall, J. M. Harrison, T. D. Inch, *J. Chem. Soc., Perkin I.,* 1977, 1969.

[26] D. B. Cooper, J. M. Harrison, T. D. Inch, *Tetrahedron Lett.,* 1974, 2697.

[27] D. B. Cooper, C. R. Hall, T. D. Inch, *Chem. Commun.,* 1975, 721.

[28] J. Tomasz, *Nucleosides and Nucleotides, 9,* 1990, 173.

[29] J. H. Yu, A. E. Sopchik, A. M. Arif, W. G. Bentrude, *J. Org. Chem., 55,* 1990, 3444.

[30] S. Mehdi, J. A. Coderre, J. A. Gerlt, *Tetrahedron, 39,* 1983, 3483.

[31] F. H. Westheimer: The Polytopal Rearrangement at Phosphorus, in P. deMayo (ed): *Rearrangements in Ground and Excited States,* Vol. 2, Academic Press, New York, p. 229 (1980).

[32] N. D. Sinha, J. Biernat, H. Köster, *Tetrahedron Lett., 24,* 1983, 5843.

[33] C. B. Reese, J. C. M. Stewart, J. H. Van Boom, H. P. M. de Leeuw, J. Nagel, J. F. M. Rooy, *J. Chem. Soc., Perkin Trans. I,* 1975, 934.

[34] E. Corey, M. Chaykowsky, *J. Am. Chem. Soc., 87,* 1965, 1353.

[35] R. S. Edmundson, *Dictionary of Organophosphorus Compounds,* Chapman and Hall, London, p. 637, 1988.

[36] F. Eckstein, H. H. Schulz, H. Ruterjans, W. Haar, W., Maurer, *Biochemistry, 11,* 1972, 3507.