Heteroatom Chemistry
Volume 9, Number 2, 1998

Crystal and Molecular Structures of Isomeric 2-Morpholino-2-thiono-4-methyl-1,3,2-dioxaphosphinane and 2-Morpholino-2-oxo-4-methyl-1,3,2-dioxaphosphinane

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Received 8 July 1997; revised 22 September 1997

ABSTRACT

*Two isomeric pairs of compounds, cis- and trans-2-morpholino-2-thiono-4-methyl-1,3,2-dioxaphosphinane (***1A** + **1B***) and 2-morpholino-2-oxo-4-methyl-1,3,2-dioxaphosphinane (***2A** + **2B***) were obtained and separated into the pure compounds by silica gel chromatography. Attempts at crystallization afforded* **1A, 1B**, *and* **2B**. *Each crystalline isomer was studied by the X-ray technique, and each crystal and molecular structures assigned. These studies revealed that* **1B** *and* **2B** *have exocyclic 4-CH_3 and sulfur (for* **1B***) or 4-CH_3 and oxygen (for* **2B***) in the diequatorial position (cis-geometry), while compound* **1A** *possesses the 4-CH_3 group in equatorial position while sulfur is in an axial position (trans-geometry). For all the examined compounds, all the basic geometrical parameters, such as bond lengths, bond and torsion angles, and the deformation of a chair conformation of the six-membered heterocyclic rings, have been established. Such unambiguous assignment of cis-trans geometry in both pairs of* **1** *and* **2** *allowed us to confirm the stereoretentive PS → PO conversion by means of OXONE® and the stereoinvertive mechanism of formic-acid catalyzed hydrolysis of phosphorothiomorpholidates.* © 1998 John Wiley & Sons, Inc. Heteroatom Chem 9:271–279, 1998

Dedicated to Prof. William E. McEwen on the occasion of his seventy-fifth birthday.

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 CCC 1042-7163/98/020271-09

INTRODUCTION

Earlier works from this [1] and other laboratories [2,3] on the synthesis and stereochemistry of di(nucleoside phosphoromorpholidate)s and chimeric oligothymidylates possessing, in alternate internucleotide positions, phosphoromorpholidate linkages revealed that the stereochemistry at phosphorus strongly influences the avidity of such constructs to complementary stranded DNA.

However, the assignment of the absolute configuration at phosphorus in the oligonucleotide constructs bearing phosphoromorpholidate internucleotide linkages was only tentative [1,3]. In model studies, Wilk *et al.* [1] proposed the method of stereochemical correlation based upon the synthesis of

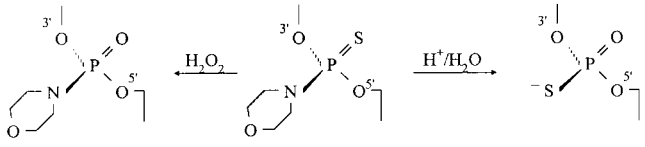
dithymidine 3′,5′-phosphorothiomorpholidates and their separation into fast- and slow-eluted isomers. Each isomer was oxidized with H_2O_2 to the appropriate dithymidine 3′,5′-phosphoromorpholidates (PS → PO conversion), and, independently, each was hydrolyzed by means of 98% formic acid to the dithymidine 3′,5′-phosphorothioates (PN → PO conversion, Scheme 1).

Since the absolute configuration of the resulting dithymidine 3′,5′-phosphorothioates was known from earlier studies [4], based upon an assumption that oxidation (PS → PO) occurs with retention of configuration at the P-atom [5] and acid-catalyzed hydrolysis (PN → PO) involves inversion of configuration [6], the absolute configuration for the fast-eluted dithymidine 3′,5′-phosphoromorpholidate was assigned as [S_P]- and that of the slow-eluted one was assigned to be [R_P]. Such tentative assignments allowed for the analysis and rationalization of melting properties of heteroduplexes formed between chimeric oligonucleotides and complementary DNA.

In these studies, we wish to report on the crystal and molecular structure assignment of the isomeric 2-morpholino-2-thiono-4-methyl-1,3,2-dioxaphosphinanes (**1**) and 2-morpholino-2-oxo-4-methyl-1,3,2-dioxaphosphinane (**2B**). Unambiguous assignment of *cis–trans* geometry in isomers of **1** and **2** allowed us to study the process of PS → PO conversion and PN → PO hydrolysis, and to prove the correctness of our earlier assumptions concerning stereoretentive (PS → PO) and stereoinvertive (PN → PO) conversions, and therefore, to confirm the correctness of our earlier assignment of absolute configuration in fast- and slow-eluted isomers of dithymidine 3′,5′-phosphoromorpholidates [1].

RESULTS

*Chemical Syntheses of the Isomeric Compounds, **1, 2**, and Their Transformations.* For an elucidation of the stereochemical course of the acid-catalyzed conversion of O,O-dialkyl phosphorothiomorpholidates into O,O-dialkyl phosphorothioates, we chose, as a model system, *cis* and *trans* isomers of 4-methyl-2-morpholino-2-thiono-1,3,2-dioxaphosphinane (**1**). Besides their simple accessibility (*vide supra*), an argument supporting our choice was the knowledge of the stereochemistry of the expected products of hydrolysis of **1,** namely, *cis*- and *trans*-2-hydroxy-2-thio-4-methyl-1,3,2-dioxaphosphinane (**3**), elucidated earlier as a result of X-ray studies by Bartczak [7,8]. Moreover, isomers of **1,** under treatment with OXONE® [9], provide isomeric 4-methyl-2-morpholino-2-oxo-1,3,2-dioxaphosphinanes (**2**). Two pairs of isomeric compounds *cis*- and *trans*-2-morpholino-2-thiono-4-methyl-1,3,2-dioxaphosphinanes (**1**) and 2-morpholino-2-oxo-4-methyl-1,3,2-dioxaphosphinanes (**2**) have been obtained according to Scheme 2.

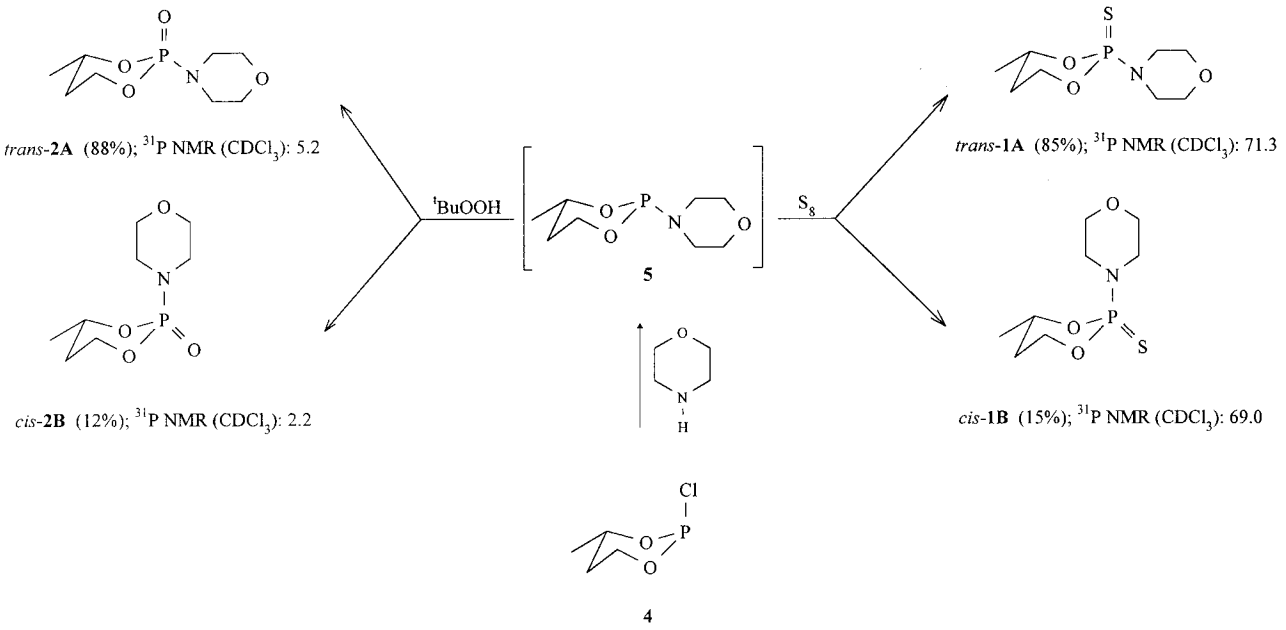


SCHEME 1

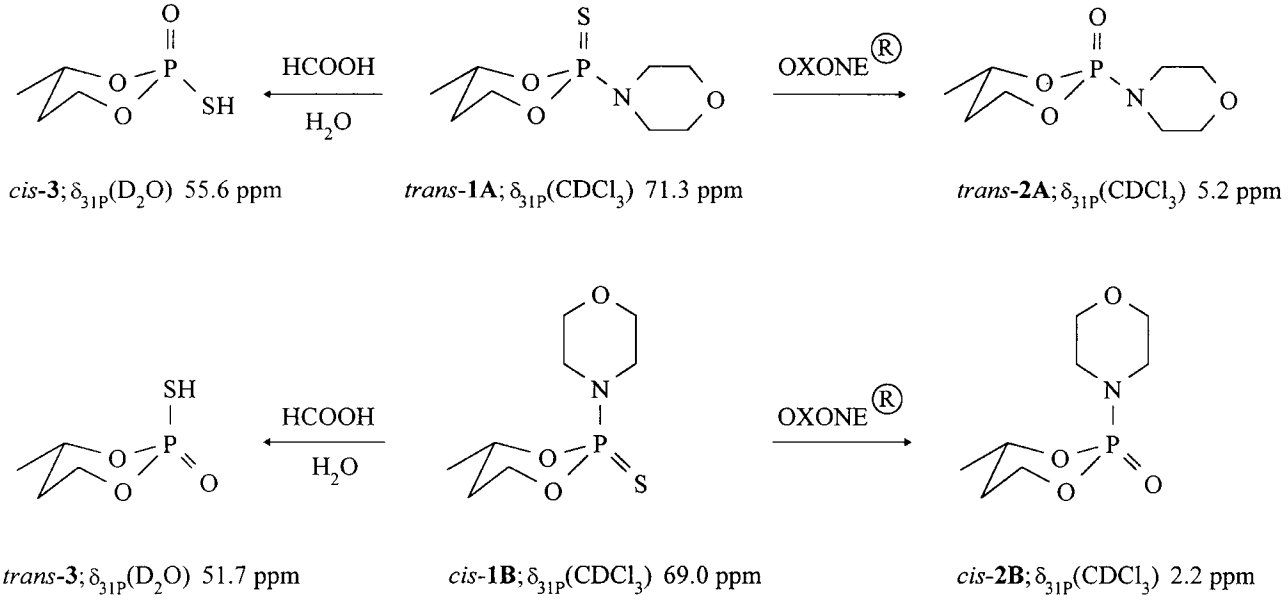
Condensation of 2-chloro-4-methyl-1,3,2-dioxaphosphinane (**4**) [10] with morpholine gave the phosphoromorpholidites **5,** key intermediates in the synthesis of **1** and **2.** Most probably, compound **5** equilibrates in the presence of morpholine hydrochloride to give the mixture of diastereomers with predominance of the isomer having the *cis*-orientation of C-methyl and morpholine groups. Preferential equatorial orientation of dialkylamino substituents at phosphorus, incorporated in the 4-methyl-1,3,2-dioxaphosphinanyl ring system, had been proved in earlier works [11]. Phosphoromorpholidite **5,** without isolation, has been oxidized with *tert*-butyl hydrogen peroxide into the mixture of **2A** + **2B** (ratio 88:12). Oxidation of **5** by means of elemental sulfur provided the mixture of **1A** + **1B** (ratio 85:15). Each diastereomeric mixture was separated into the individual isomers by means of silica gel chromatography, and each isomer was characterized by ^{31}P NMR spectroscopy. Isomer **1A** [^{31}P NMR ($CDCl_3$) δ 71.3] was crystallized from ethanol, and we obtained crystals (mp 59.6–60.1°C) that appeared to be suitable for X-ray analysis. Similarly, isomer **1B** [^{31}P NMR ($CDCl_3$) δ 69.0] was crystallized from benzene–hexane (1:1) providing crystals (mp 125.2 – 125.6°C); therefore, the crystal and structural analysis of **1A** and **1B** became feasible. In the case of **2,** only isomer **2B** [^{31}P NMR ($CDCl_3$) δ 2.2], when crystallized from ethyl acetate–methanol (100:1), gave crystals suitable for X-ray analysis (mp 99.9–100.4°C).

In the next set of experiments, each isomer of 2-morpholino-2-thiono-4-methyl-1,3,2-dioxaphosphinane (**1**) was treated at room temperature in acetonitrile solution with OXONE® [9] and, independently, with 98% formic acid for 4 hours at 95°C [12] (Scheme 3).

It was found that oxidation of **1A** [^{31}P NMR ($CDCl_3$) δ 71.3] by means of OXONE® ($2KHSO_5 \times KHSO_4 \times K_2SO_4$) gave 2-morpholino-2-oxo-4-methyl-1,3,2-dioxaphosphinane [**2A,** ^{31}P NMR ($CDCl_3$) δ 5.3], while the product of acid hydrolysis of **1A** was *cis*-2-hydroxy-2-thio-methyl-1,3,2-dioxaphosphinane (**3**) [^{31}P NMR ($CDCl_3$) δ 55.6] [13]. Reaction of **1B** [^{31}P NMR ($CDCl_3$) δ 69.0] with OXONE® led to **2B** [^{31}P NMR ($CDCl_3$) δ 2.2], and the product of formic acid solvolysis of **1B** was *trans*-**3** [^{31}P NMR ($CDCl_3$) δ 51.7] [13]. The relative configuration at the phosphorus atom in both diastereomers of 2-hy-



SCHEME 2



$$OXONE^{®} = 2KHSO_5 \times KHSO_4 \times K_2SO_4$$

SCHEME 3

droxy-2-thio-4-methyl-1,3,2-dioxaphosphinanes had been assigned earlier by X-ray analysis [7,8].

X-Ray Analysis of Isomers of **1** *and* **2.** Crystal and molecular structures of **1A, 1B,** and **2B** were determined using data collected at room temperature on a CAD-4 [16] diffractometer with graphite monochromatized CuKα radiation. All three compounds crystallized in the monoclinic system. Crystal data and experimental details are shown in Table 1.

The lattice constants were refined by least-squares fit of 25 reflections in the θ range 15.20°–29.70° for **1B,** 18.70°–30.48° for **1A,** and 20.38°–29.12° for **2B,** respectively. The decline in intensities of three control reflections (1,3,−1; 2,−2,6; −2,3,−2 for **1B,** −1,2,−7; 0,6,−1; −1,−1,−9 for **1A,** and −2,−2,6; 2,3,5; 2,−2,7 for **2B**) were 3.9% during 61.8 hours of exposure time, 13.3% during 64 hours, and 19.1% during 73.0 hours for **1B, 1A,** and **2B,** respectively; the intensity corrections (DECAY program) [14] were applied. An empirical absorption correction was applied by the use of the ψ-scan method (EAC program) [14,15]. A total of 2286 (**1B**), 2362 (**1A**), and 2128 (**2B**) observed reflections with $I \geq 0\sigma(I)$ were used to solve each structure by direct methods and to refine it by full matrix least-squares

TABLE 1 Crystal Data and Experimental Details

	1B	***1A***	***2B***
Molecular formula	$C_8H_{16}NO_3PS$	$C_8H_{16}NO_3PS$	$C_8H_{16}NO_4P$
Formula weight	237.25	237.25	221.19
Crystallographic system	monoclinic	monoclinic	monoclinic
Space group	$P2_1/c$	$P2_1/n$	$P2_1/c$
a (Å)	15.419(4)	6.589(3)	16.217(5)
b (Å)	6.344(3)	11.455(4)	6.311(2)
c (Å)	11.613(4)	15.619(6)	10.390(3)
β (°)	91.96(3)	99.72(4)	90.29(3)
V (Å^3)	1135.3(7)	1162.0(8)	1063.4(6)
Z	4	4	4
D_c (g/cm^3)	1.388	1.356	1.382
μ [cm^{-1}]	37.57	36.71	22.57
Crystal dimensions (mm)	0.08×0.32×0.80	0.10×0.30×0.40	0.15×0.30×0.40
Maximum 2θ (°)	150	150	150
Radiation, λ (Å)	CuKα, 1.54184	CuKα, 1.54178	CuKα, 1.54178
Scan mode	$\omega/2\theta$	$\omega/2\theta$	$\omega/2\theta$
hkl ranges h =	−19 19	−8 8	0 20
k =	−7 7	−14 14	−7 7
l =	0 14	0 19	−13 13
DECAY correction: min:	1.00001	1.00007	1.00016
max:	1.02002	1.07379	1.11153
ave:	1.00988	1.03725	1.05004
EAC correction: min:	0.7202	0.8426	0.9017
max:	0.9992	0.9988	0.9993
ave:	0.9183	0.9120	0.9497
No. of reflections: unique	2334	2401	2189
refine with $I > 0\sigma(I)$	2286	2362	2128
observed with $I > 2\sigma(I)$	2170	2244	1919
No. of parameters refined	192	192	192
Largest diff. peak (eÅ^{-3})	0.407	0.929	0.515
Largest diff. hole (eÅ^{-3})	−0.615	−0.431	−0.448
R_{obs}	0.0554	0.0627	0.0593
wR_{obs}	0.1477	0.1731	0.1505
Weighting coeff.[a] m	0.0952	0.01341	0.01107
n	0.3164	0.3180	0.0515
Extinction coef.[b] k	0.0033(7)	0.0075(13)	0.0049(11)
S_{obs}	1.089	1.056	1.104
Shift/esd max	0.000	0.001	0.000
R_{int}	0.0721	0.0255	0.0724
$T_{meas.}$	293(2)	293(2)	293(2)
F 000	504	504	472

[a]Weighting scheme $w = [\sigma^2(Fo^2) + (mP)^2 + nP]^{-1}$ where $P = (Fo^2 + 2Fc^2)/3$
[b]Extinction method SHELXL, extinction expression $Fc^* = kFc[1 + 0.001 \times Fc^2\lambda^3/\sin(2\theta)]^{-1/4}$

using F^2. Hydrogen atoms were found on a difference Fourier map and refined isotropically. Anisotropic thermal parameters were refined for all non-hydrogen atoms. The final refinement converged to R = 0.0554, 0.0627, and 0.0593 for **1B, 1A,** and **2B,** respectively.

Structure solution: SHELXS-86 [17]; structure refinement: SHELXL-93. Bond lengths for **1A, 1B,** and **2B** are given in Table 2, while bond angles for the same compounds are given in Table 3. Torsional angles for non-H atoms are given in Table 4. Other crystallographic data have been deposited in the Cambridge Crystallographic Data Centre [18].

TABLE 2 Bond Lengths (Å) for Non-H Atoms

		1B	*1A*	*2B*
O1	P2	1.578(2)	1.599(2)	1.578(2)
O1	C6	1.470(3)	1.454(4)	1.463(3)
P2	O3	1.5840(15)	1.578(2)	1.570(2)
P2	S8	1.9151(8)	1.9260(10)	1.4574(15)
P2	N9	1.654(2)	1.614(2)	1.638(2)
O3	C4	1.473(2)	1.469(3)	1.476(2)
C4	C5	1.517(3)	1.506(4)	1.508(4)
C4	C7	1.502(3)	1.505(4)	1.507(3)
C5	C6	1.501(4)	1.488(5)	1.497(4)
N9	C10	1.467(3)	1.469(3)	1.469(3)
N9	C14	1.467(3)	1.468(3)	1.462(2)
C10	C11	1.507(4)	1.515(4)	1.516(3)
C11	O12	1.416(4)	1.421(4)	1.416(3)
O12	C13	1.423(4)	1.414(4)	1.421(3)
C13	C14	1.504(4)	1.507(4)	1.516(3)

TABLE 3 Bond Angles (°) for Non-H Atoms

			1B	*1A*	*2B*
C6	O1	P2	117.71(14)	116.3(2)	117.23(14)
O1	P2	O3	105.93(8)	102.80(10)	105.59(9)
O1	P2	N9	104.24(9)	101.91(12)	104.09(8)
O3	P2	N9	103.51(8)	104.61(10)	104.51(8)
O1	P2	S8	112.42(6)	114.64(8)	111.40(10)
O3	P2	S8	111.46(6)	115.31(8)	111.17(10)
N9	P2	S8	118.19(7)	115.81(8)	118.99(10)
C4	O3	P2	117.33(12)	119.7(2)	117.68(13)
O3	C4	C7	106.6(2)	106.0(2)	106.7(2)
O3	C4	C5	108.4(2)	108.7(2)	108.3(2)
C7	C4	C5	114.0(2)	115.2(3)	114.6(2)
C6	C5	C4	112.6(2)	112.5(3)	113.1(2)
O1	C6	C5	110.9(2)	110.1(2)	111.1(2)
C14	N9	C10	111.0(2)	111.8(2)	111.6(2)
C14	N9	P2	116.57(15)	120.7(2)	119.50(14)
C10	N9	P2	120.62(15)	124.2(2)	121.83(13)
N9	C10	C11	107.6(2)	109.9(2)	107.9(2)
O12	C11	C10	111.6(3)	111.1(2)	111.0(2)
C11	O12	C13	109.7(2)	110.4(2)	110.9(2)
O12	C13	C14	111.1(2)	111.0(2)	111.7(2)
N9	C14	C13	108.7(2)	110.4(2)	108.7(2)

DISCUSSION

X-ray studies presented above allowed for unambiguous assignment of *cis/trans* geometry in both pairs of compounds **1** and **2** (Figures 1 and 2). **1A** was unambigously shown to possess *trans*-geometry of the 4-C-methyl group and sulfur atom in this compound, while **1B** has the *cis*-geometry (diequatorial 4-methyl and sulfur). Compound **2B** has both 4-methyl and oxygen in equatorial orientation (*cis*-geometry). Although **2A** was not obtained in crystalline form suitable for X-ray analysis, its geometry must be *trans* (equatorial 4-methyl and axial exocyclic oxygen). Each of the diastereomers, *trans*- and *cis*-2-morpholino-2-thiono-4-methyl-1,3,2-dioxaphosphinanes (**1A** and **1B**), exist in crystalline form in the chair conformation, and the 4-methyl group occupies an equatorial position, while the sulfur atom is in the axial or equatorial position, respectively. Reciprocal interactions between the sulfur atom and the 1,3,2-dioxaphosphinane ring, as well as the morpholine ring and the 1,3,2-dioxaphosphinane ring, can be different.

The magnitude of these interactions should be slightly modified if sulfur is replaced by oxygen. On comparison of isomeric *cis*- and *trans*-**1,** it was noticed that bond lengths in the examined structures

TABLE 4 Torsional Angles (°) for Non-H Atoms

				1B	*1A*	*2B*
C6	O1	P2	O3	44.0(2)	49.1(2)	45.4(2)
C6	O1	P2	S8	166.0(2)	−76.8(2)	166.2(2)
C6	O1	P2	N9	−64.8(2)	157.3(2)	−64.4(2)
O1	P2	O3	C4	−47.2(2)	−47.8(2)	−48.2(2)
S8	P2	O3	C4	−169.74(12)	77.7(2)	−169.1(2)
N9	P2	O3	C4	62.2(2)	−153.9(2)	61.3(2)
P2	O3	C4	C7	179.2(2)	177.8(2)	179.5(2)
P2	O3	C4	C5	56.0(2)	53.4(3)	55.7(2)
O3	C4	C5	C6	−59.1(2)	−55.8(3)	−57.7(2)
C7	C4	C5	C6	−177.6(2)	−174.7(3)	−176.6(2)
P2	O1	C6	C5	−51.2(3)	−58.4(3)	−51.8(3)
C4	C5	C6	O1	57.3(3)	59.8(4)	56.8(3)
O1	P2	N9	C14	172.1(2)	−54.9(2)	167.1(2)
O3	P2	N9	C14	61.5(2)	51.9(2)	56.6(2)
S8	P2	N9	C14	−62.3(2)	180.0(2)	−68.2(2)
O1	P2	N9	C10	−48.2(2)	147.3(2)	−44.7(2)
O3	P2	N9	C10	−158.8(2)	−105.9(2)	−155.3(2)
S8	P2	N9	C10	77.4(2)	22.2(2)	80.0(2)
C14	N9	C10	C11	−57.6(3)	−52.4(3)	−57.9(3)
P2	N9	C10	C11	160.7(2)	107.1(2)	151.7(2)
N9	C10	C11	O12	59.3(4)	56.1(3)	58.7(3)
C10	C11	O12	C13	−60.9(4)	−60.7(3)	−59.8(3)
C11	O12	C13	C14	59.7(4)	60.8(3)	58.4(3)
C10	N9	C14	C13	57.4(3)	52.7(3)	56.6(2)
P2	N9	C14	C13	−159.2(2)	−107.5(2)	−152.2(2)
O12	C13	C14	N9	−57.8(3)	−56.6(3)	−56.0(3)

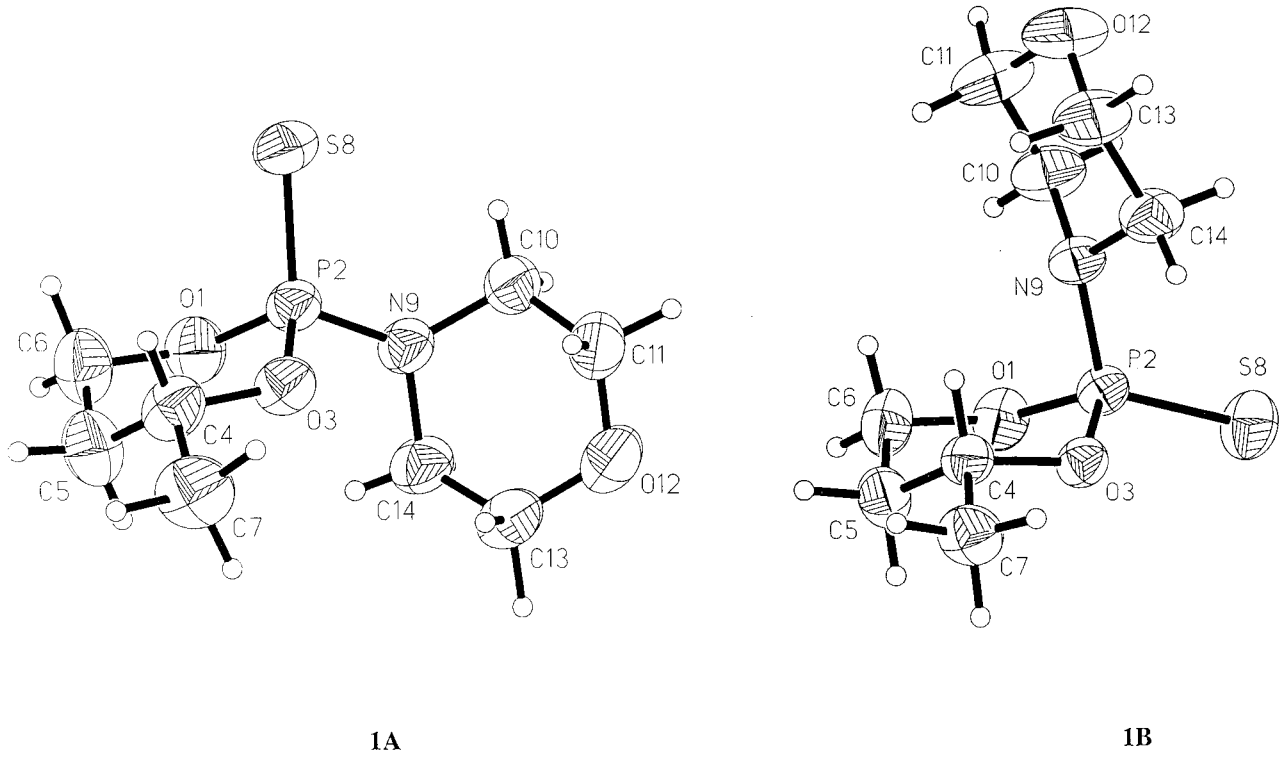


FIGURE 1 Molecular structure of **1A** and **1B** showing 50% probability displacement ellipsoids.

are significantly different in a heteroatom part of 1,3,2-dioxaphosphinane ring in both structures.

Bond lengths in compounds with an equatorially oriented sulfur (**1B**) or oxygen atom (**2B**) are longer than corresponding bonds in *trans* isomers:

$$\text{P2–O1} = \Delta\ 0.02\ \text{Å}\ (10\sigma)$$

$$\text{P2–O3} = \Delta\ 0.006\ \text{Å}\ (3\sigma)$$

$$\text{P2–N} = \Delta\ 0.024\text{–}0.04\ \text{Å}\ (12\sigma\text{–}20\sigma)$$

More profound differences are observed in valency angles around heteroatoms in the 1,3,2-dioxaphosphinanyl ring. In compounds with an equatorially oriented sulfur or oxygen atom (**1B** and **2B**), valency angles are greater:

$$\text{O1P2O3} = \Delta\ 3.0^\circ\ (30\sigma)$$

$$\text{C6O1P2} = \Delta\ 1.4^\circ\ (10\sigma)$$

$$\text{C4O3P2} = \Delta\ 2.4^\circ\ (20\sigma)$$

In all three compounds investigated, the 1,3,2-dioxaphosphinanyl ring as well as morpholine ring both possess the chair conformation. Distortions from the ideal chair conformation in the morpholine ring are small, but, in a case of the 1,3,2-dioxaphosphinanyl ring, they are more emphasized (see Tables 5 and 6).

The Newman projections around the N9–P2 bond (Figure 3) show that the electron pair on the nitrogen atom is *anti*-periplanar (*ap*) in compounds **1B** and **2B** and *syn*-clinal (*sc*) in compound **1A.** The dihedral angles α and β are nearly equal (Table 7).

Analysis of the intermolecular and intramolecular hydrogen bond contacts (not presented here; Table V deposited in the Cambridge Crystallographic Data Centre) reveals the importance of intramolecular interactions between nitrogen atom (N9) and "flag" hydrogen atoms H4 and $H6_{ax}$ in compounds **1B** and **2B,** as well as intramolecular interactions $O1\cdots H1_{10}$–C10 or $O1\cdots H1_{14}$–C14. In all the examined compounds, **1A, 1B,** and **2B,** these interactions are responsible for the definite geometry of both the 1,3,2-dioxaphosphinanyl and morpholine rings.

The unambiguous assignment of stereoisomerism in compounds **1, 2** (this work), and **3** [7,8] by means of X-ray crystallography and results of acid solvolysis of isomers of **1** and their OXONE®-promoted oxidation to **2** allowed for the following conclusions:

1. Reaction of 2-chloro-4-methyl-1,3,2-dioxaphosphinanes (*trans*-**4,** anancomeric) with morpholine occurs with predominant inversion of configuration [19] since *trans*-**1B** has appeared to be the prevailing product of sul-

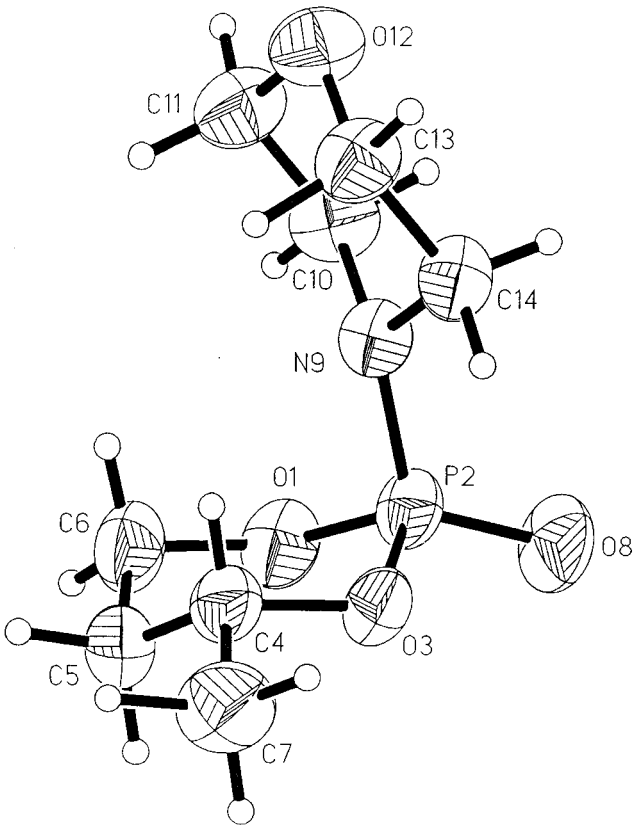


FIGURE 2 Molecular structure of **2B** showing 50% probability displacement ellipsoids.

TABLE 5 Asymmetry Parameters [1,2] of Six-Membered Ring: O1, P2, O3, C4, C5, C6

	1B	*1A*	*2B*
$\Delta C_s^{(O1)} = \Delta C_s^{(C4)}$	7.4(4)	8.9(5)	6.3(4)
$\Delta C_s^{(P2)} = \Delta C_s^{(C5)}$	3.5(4)	3.8(6)	2.8(4)
$\Delta C_s^{(O3)} = \Delta C_s^{(C6)}$	10.7(4)	5.1(5)	8.8(4)
$\Delta C_2^{(O1-P2)} = \Delta C_2^{(C4-C5)}$	3.0(4)	8.8(6)	2.7(4)
$\Delta C_2^{(P2-O3)} = \Delta C_2^{(C5-C6)}$	10.2(4)	3.6(6)	8.4(4)
$\Delta C_2^{(O3-C4)} = \Delta C_2^{(C6-O1)}$	12.6(4)	9.4(6)	10.5(4)

TABLE 6 Asymmetry Parameters [1,2] of Six-Membered Ring; N9, C10, C11, O12, C13, C14

	1B	*1A*	*2B*
$\Delta C_s^{(N9)} = \Delta C_s^{(O12)}$	1.1(6)	0.3(5)	1.9(5)
$\Delta C_s^{(C10)} = \Delta C_s^{(C13)}$	2.5(6)	5.6(6)	2.4(5)
$\Delta C_s^{(C11)} = \Delta C_s^{(C14)}$	1.5(6)	6.0(6)	0.8(5)
$\Delta C_2^{(N9-C10)} = \Delta C_2^{(O12-C13)}$	2.6(7)	3.8(6)	3.1(5)
$\Delta C_2^{(C10-C11)} = \Delta C_2^{(C13-C14)}$	2.8(6)	8.2(6)	1.9(5)
$\Delta C_2^{(C11-O12)} = \Delta C_2^{(C14-N9)}$	0.3(6)	4.5(6)	1.4(4)

furization of transient 2-morpholino-4-methyl-1,3,2-dioxaphosphinanes (**5**). Sulfurization is known to be stereospecific and occurs with retention of configuration [20].

2. Reaction of **5** with *t*BuOOH occurs with prevailing retention of configuration [21].
3. PS → PO conversion caused by OXONE® occurs with full retention of configuration.
4. Formic acid-catalyzed solvolysis of both isomers of **1** leading to 2-hydroxy-2-thio-4-methyl-1,3,2-dioxaphosphinanes **2** occurs with full inversion of configuration.

Besides these, the herein presented results validate earlier conclusions concerning the assignment of absolute configuration at phosphorus in both diastereomers of dithymidine (3′,5′)phosphoromorpholidates [1,2,3] and dithymidine (3′,5′)phosphorothiomorpholidates [1].

EXPERIMENTAL

*Synthesis of 2-Morpholino-2-thiono-4-methyl-1,3,2-dioxaphosphinane (**1A** and **1B**) [22]*

Morpholine (5 mmol, 4.35 g) was added dropwise at room temperature over a period of 1 hour into a solution at 2-chloro-4-methyl-1,3,2-dioxaphosphinane (25 mmol, 3.88 g) in dry benzene (150 mL). Then elemental sulfur (1 g) was added in one portion, and the reaction mixture was stirred at room temperature for 12 hours. The precipitated morpholine hydrochloride was filtered off, washed with benzene, and the filtrate was concentrated to dryness. The crude product was purified and separated into individual diastereoisomers by column chromatography on silica gel (Kieselgel 60H) with hexane–ethyl acetate (8:2) and methanol (0 → 8%) as eluants. The more abundant isomer, isolated as fast-eluted **1A,** obtained in 65% yield (3.8 g), was crystallized from ethanol, mp 59.6–60.1°C, ^{31}P NMR ($CDCl_3$) δ 71.3. Elemental analysis for $C_8H_{16}O_3NPS$ calculates: C 40.49, H 6.80, N 5.90, P 13.10, S 13.51; found: C 40.36, H 6.82, N 5.97, P 13.17, S 13.22. The second isomer, slow-eluted **1B,** was obtained in 11% yield (0.65 g). Its crystallization from benzene–hexane (1:1) gave the crystals, mp 125.2–125.6°C, ^{31}P NMR ($CDCl_3$) δ 69.0. Elemental analysis for $C_8H_{16}O_3NPS$ calculated: C 40.49, H 6.80, N 5.90, P 13.10, S 13.51; found: C 40.74, H 6.68, N 5.98, P 13.13, S 13.73.

Synthesis of 2-Morpholino-2-oxo-4-methyl-1,3,2-dioxaphosphinane (**2A** *and* **2B**)

Morpholine (50 mmol, 4.35 g) was added dropwise at room temperature over a period of 1 hour into a solution of 2-chloro-4-methyl-1,3,2-dioxaphosphin-

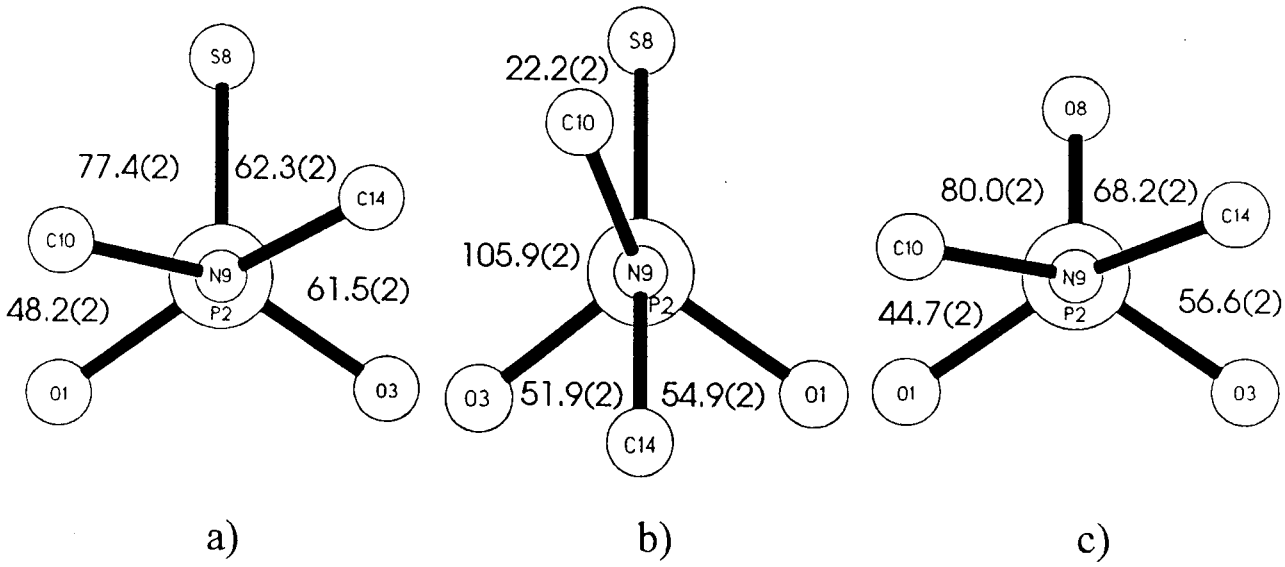
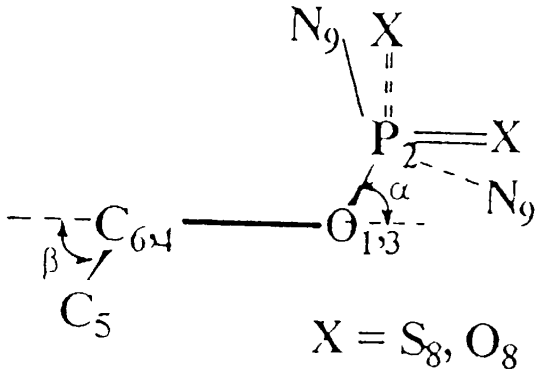


FIGURE 3 The Newman projections perpendicular to the N9-P2 bond of (a) **1B,** (b) **1A,** and (c) **2B.**

TABLE 7 Dihedral angles in 1,3,2-dioxaphosphinanyl ring



Compound	***1A***	***1B***	***2B***
α	41.02° (10)	39.73° (7)	40.50° (8)
β	52.55° (21)	53.61° (21)	52.60° (18)

ane (25 mmol, 3.88 g) in dry benzene (150 mL). Then *tert*-butyl hydroperoxide (25 mmol, 5 mL) was added, and the reaction mixture was stirred at room temperature for 12 hours. The mixture was concentrated *in vacuo* to give a solid, which was redissolved in ethyl acetate and separated into individual isomers by column chromatography on silica gel (Kieselgel 60H) with hexane–ethyl acetate (8:2) and methanol ($0 \rightarrow 8\%$) as eluents. The preponderant isomer, fast-eluted **2A,** was obtained in 74% yield (4.06 g) as a thick oil, which solidified upon storage at room temperature. ^{31}P NMR ($CDCl_3$) δ 5.2. All attempts of its crystallization have failed. Elemental analysis for $C_8H_{16}O_4NP$ calculated: C 43.44; H 7.29, N 6.33, P 14.00; found: C 43.25; H 7,26; N 6.30; P 13.60. Slow-eluted **2B** was obtained in 10% yield (0.57 g) as crystals (from ethyl acetate–methanol, 100:1), mp 99.9–100.4°C. ^{31}P NMR ($CDCl_3$) δ 2.2. Elemental analysis for $C_8H_{16}O_4NP$ calculated: C 43.44, H 7.29, N 6.33, P 14.00; found: C 43.14, H 7.36, N 6.17, P 13.50.

Formic-Acid Hydrolysis of **1A** *and* **1B** *[22]*

Each isomer, **1A** or **1B** (0.42 mmol, 100 mg), was added to 98% HCOOH (300 μL), and the resulting solution was stirred at 95°C. TLC control [silica gel plates Merck $60F_{254}$, developing system: hexane–ethyl acetate (8:2)] has shown the complete vanishing of substrates after 4 hours. Formic acid was evaporated, and the resulting solid was dissolved in D_2O. ^{31}P NMR spectra were recorded for each solution.

The spectrum of the product resulting from hydrolysis of *trans*-**1A** showed the lack of the resonance signal of the substrate, and the presence of a resonance signal at δ 55.6 (96%) was characteristic for *cis*-**3** [13]. In the spectra recorded for the product of hydrolysis of *cis*-**1B** [^{31}P NMR ($CDCl_3$) δ 69.0], the resonance signal observed at δ 51.7 (87%), characteristic of *trans*-**3** [13], was accompanied by two resonance signals at δ 26.0 and 26.6 (ca. 9.0%) and a broad signal at ca. δ 1.0 (4.0%), which were not identified.

Oxone-oxidation of **1**

In separate experiments, each isomer of **1** (240 mg, 1 mmol) was dissolved in acetonitrile (20 mL), and to this solution was added dropwise, with stirring, the solution of OXONE® (2 mmol, 1.23 g) in water (20 mL). The reaction was maintained at room temperature for 30 minutes. TLC control [silica gel plates Merck $60F_{254}$, developing system: hexane–ethyl acetate–methanol (8:2:1)] showed the complete vanishing of **1.** Aqueous Na_2SO_3 (3.1 mL) was added for neutralization of an excess of OXONE®. A solid precipitate was filtered off, and the filtrate was extracted with chloroform (3 × 20 mL). After drying over anh. $MgSO_4$, solvent was removed from the filtrate and the residual solid was crystallized from an appropriate solvent.

From *trans*-**1A** [^{31}P NMR ($CDCl_3$) δ 71.3], compound *trans*-**2A** [^{31}P NMR ($CDCl_3$) δ 5.2] was obtained with the yield 93% as an oil that solidified upon storage. From *cis*-**1B** [^{31}P NMR ($CDCl_3$) δ 69.5], compound *cis*-**2B** [^{31}P NMR ($CDCl_3$) δ 2.2] was obtained with the yield 97%. There was no evidence for cross-contamination of **2A** or **2B** resulting from oxidation of **1A** or **1B,** respectively.

ACKNOWLEDGMENTS

Results presented in this article were obtained within the frame of projects financially assisted by the State Committee for Scientific Research, Grant No. 4PO5F 023 10, and Human Science Promotion Foundation of Japan (both to WJS).

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