Synthesis and Absolute Configuration Assignments of Enantiomeric Forms of Ifosphamide, Sulfosphamide, and Trofosphamide

K. Pankiewicz,[1a] R. Kinas,[1a] W. J. Stec,*[1a] A. B. Foster,[1b] M. Jarman,[1b] and Jan M. S. Van Maanen[1b]

Contribution from the Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, 90-326 Łódź, Boczna 5, Poland, and the Institute of Cancer Research, Royal Cancer Hospital, Chester Beatty Research Institute, London SW3 6JB, England. *Received April 23, 1979*

Abstract: Using *N*-(*S*)-α- or *N*-(*R*)-α-methylbenzyl-3-aminopropan-1-ol as the starting material, the diastereomers of 2-chloro-3-[(*S*)-α- or (*R*)-α-methylbenzyl]tetrahydro-2*H*-1,3,2-oxazaphosphorine 2-oxide were obtained in the ratio 80:20. Their conversion via *P*-aziridines (**14**) into 2-chloroethylamino derivatives (**15**) and separation of the latter into diastereometrically pure forms allowed the further synthesis of enantiomers of ifosphamide [2-(2-chloroethylamino)-3-(2-chloroethyl)-tetrahydro-2*H*-1,3,2-oxazaphosphorine 2-oxide] and sulfosphamide [2-(2-mesyloxyethylamino)-3-(2-chloroethyl)tetrahydro-2*H*-1,3,2-oxazaphosphorine 2-oxide]. Chloroacetylation of previously reported enantiomers of cyclophosphamide {2-[bis(2-chloroethyl)amino]tetrahydro-2*H*-1,3,2-oxazaphosphorine 2-oxide} and conversion of the *N*-chloroacetyl group into an *N*-(2-chloroethyl) group gave both enantiomers of trofosphamide {2-[bis(2-chloroethyl)amino]-3-(2-chloroethyl)tetrahydro-2*H*-1,3,2-oxazaphosphorine 2-oxide}. Absolute configurations were assigned as (+)-**2**(*R*), (+)-**3**(*S*), (+)-**4**(*R*) by the following chemical transformations: **2** was related to **1** by the stereospecific synthesis from the common intermediate **15** and, independently, by conversion into a common bicyclic product **21**, **3** by direct synthesis from **1**, and **4** by synthesis of its enantiomers and those of **2** from common intermediates.

Introduction

Recognition of a chiral biologically active compound by the active center of enzyme(s) responsible for its metabolism depends on the absolute configuration of one or more asymmetric centers in the molecule. With that in mind a program was undertaken to synthesize the enantiomeric forms of the family of known anticancer drugs based on the 1,3,2-oxazaphosphorinane skeleton (**1–4**), which are chiral by virtue of the asymmetric phosphorus atom (Figure 1). The first of these, known by the trivial name cyclophosphamide {**1**, 2-[bis(2-chloroethyl)amino]tetrahydro-2*H*-1,3,2-oxazaphosphorine 2-oxide}, was introduced as a racemic mixture into clinical use in 1966 and is now accepted worldwide as an effective drug against lymphoreticular tumors and hemoblastoses.[2a] Recently three other compounds, ifosphamide [**2**, 2-(2-chloroethylamino)-3-(2-chloroethyl)tetrahydro-2*H*-1,3,2-oxazaphosphorine 2-oxide],[2b] trofosphamide {**3**, 2-[bis(2-chloroethyl)amino]-3-(2-chloroethyl)tetrahydro-2*H*-1,3,2-oxazaphosphorine 2-oxide},[2b,3] and sulfosphamide [**4**, 2-(2-mesyloxyethylamino)-3-(2-chloroethyl)tetrahydro-2*H*-1,3,2-oxazaphosphorine 2-oxide],[4] were demonstrated to possess even better antitumor activity and, as has been demonstrated very recently, they indicate the relationship between chemical constitution and organotropism.[5] According to Brock et al., sulfosphamide also possesses promising effects in induction of both humoral and cellular immunotolerance.[6] It is necessary to point out that all reported results prior to our studies[7] were obtained with the use of racemic mixtures of **1**, **2**, **3**, and **4**. These compounds are inactive in vitro and are activated in the liver of warm-blooded organisms to cytostatically active metabolites. It is well established that the first step in the activation pathway of **1** and **2** is hydroxylation at the carbon atom (C-4) adjacent to the endocyclic nitrogen atom.[8]

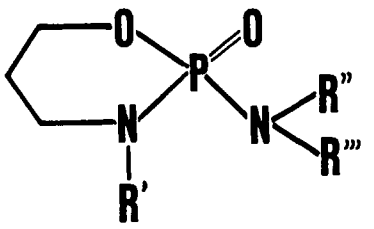
A new chiral center is thereby formed. Assuming that chiral prefragmentation metabolites[8] are configurationally stable at phosphorus atom in vivo, and that their interactions with biological species, like liver cytosol[9] and/or sulfhydryl group containing proteins,[10] are enantio- and/or diastereoselective, one could expect differences in metabolism and therapeutic effects of the drugs considered herein.

For the above reasons and in the light of recently reported results concerning stereodifferentiated metabolism of cyclophosphamide[7,11,12] and 4-methylcyclophosphamides,[13] a systematic program of research was undertaken of which the first step was the search for efficient methods of synthesis of the enantiomeric forms of drugs **2–4**, so far evaluated in the racemic forms only.

Results

Synthesis. Enantiomers of cyclophosphamide (**1**) were first reported from this laboratory in 1975.[14] The synthetic approach was based on introduction of an asymmetric group attached to the endocyclic nitrogen atom of molecule **1** and, after separation of diastereomers, the removal of the N-attached chiral moiety (Scheme I). The enantiomers of α-methylbenzylamine were chosen as the resolving agents. Reaction of (*S*)-α-methylbenzylamine with 3-chloropropan-1-ol led to *N*-(*S*)-α-methylbenzyl-3-aminopropan-1-ol [**5**(*S*)], which after treatment with *N*,*N*-bis(2-chloroethyl)phosphoramidodichloridate (**6**) gave diastereomeric 2-[bis(2-chloroethyl)amino]-3-[(*S*)-α-methylbenzyl]tetrahydro-2*H*-1,3,2-oxazaphosphorine 2-oxides[15] [**7**(*S*,R_p) and **7**(*S*,S_p)]. Separation of diastereomers by means of column chromatography and hydrogenolytic removal of the α-methylbenzyl group from each of the diastereomers **7** gave the desired enantiomers of **1**. The use of optically active α-1-naphthylethylamine in this approach is described here (see Experimental Section), and demonstrates the superiority of this resolving amine since one of the diastereomers can be purified throughout crystallization. The use of virtually optically pure amines and full resolution of diastereomers (TLC and ^{31}P NMR assay) should, as we pointed out in our original work,[14] lead to optically pure enantiomers of **1** since removal of the α-methylbenzyl group does not involve any bond reorganization around the phosphorus atom. Zon and co-workers have verified by means of ^{1}H and ^{31}P NMR that compounds obtained according to our procedure were enantiomerically homogenous.[16] Recently Verkade et al.[17] have also obtained both enantiomers of **1** using optically active (−)-(*S*)- or (+)-(*R*)-1-naphthylphenylmethylsilyl chloride (**8**) as the resolving

0002-7863/79/1501-7712$01.00/0



1 R'=H, R''=R'''=CH_2-CH_2Cl CYCLOPHOSPHAMIDE

2 R'=R''=CH_2CH_2Cl, R'''=H IFOSPHAMIDE

3 R'=R''=R'''=CH_2CH_2Cl TROFOSPHAMIDE

4 R'=$ClCH_2CH_2Cl$, R''=$CH_2CH_2OSO_2CH_3$, R'''=H, SULFOSPHAMIDE

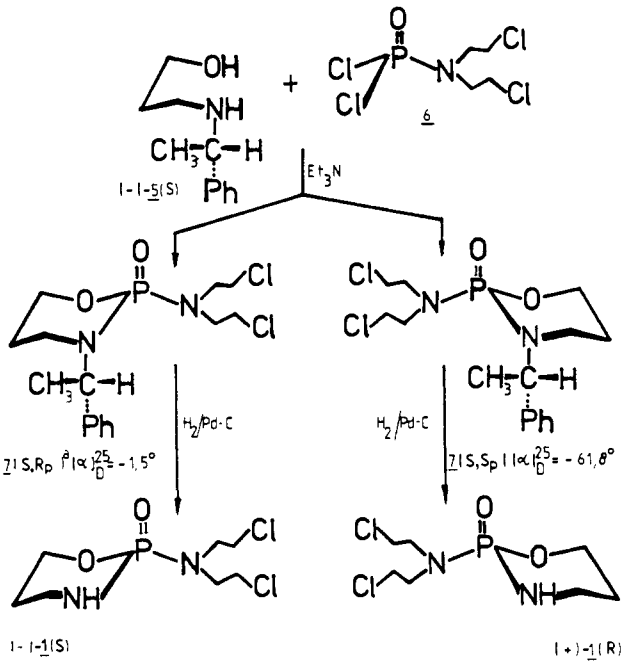
Figure 1.

agents. Treatment of racemic **1** with **8** gave a diastereomeric mixture of 2-[bis(2-chloroethyl)amino]-3-(1-naphthylphenylmethylsilyl)tetrahydro-2*H*-1,3,2-oxazaphosphorine 2-oxides (**9**), which after separation was desilylated to give enantiomers of **1**. This procedure, although shorter and applicable to commercially available racemic **1**, has several disadvantages, e.g., laborious preparation of **8** and the need to avoid contact with any nucleophile or acid which may cause the removal of the chiral silicon moiety during separation of the diastereomers of **9**.

The absolute configuration of the enantiomers of **1** was established by X-ray crystallography and the *S* configuration was assigned for levorotatory (−)-cyclophosphamide[18] and, independently, *R* for (+)-cyclophosphamide.[19] Preliminary results on the differential metabolism of (+)- and (−)-**1**[7] prompted the synthesis of the enantiomers of **2**.[20] Although a similar synthetic route was used (Scheme II), separation of the diastereomers of **12** and hydrogenolytic removal of the asymmetric group attached to the exocyclic nitrogen atom were found to be very inefficient, and enantiomers of **2** were obtained in a very low yield. In this paper we report a new procedure which allows the preparation of large quantities of the enantiomers of **2** as well as those of **3** and **4**. Reaction of the amino alcohol **5**(*S*) with phosphoryl chloride gives a mixture of diastereomers of 2-chloro-3-[(*S*)-α-methylbenzyl]tetrahydro-2*H*-1,3,2-oxazaphosphorine 2-oxide [**13**(S,R_p) (20%) and **13**(S,S_p) (80%) (^{31}P NMR assay)]. This mixture, on reaction with ethylenimine, gave the mixture of phosphorethylenimides **14**(S,S_p) and **14**(S,R_p) in the ratio 8:2. The predominant diastereomer **14**(S,S_p) was separated by crystallization from carbon tetrachloride. The mother liquor contained residual **14**(S,S_p) together with the (S,R_p) diastereomer. On treatment with anhydrous hydrogen chloride it was converted into a diastereomeric mixture of 2-(2-chloroethylamino)-3-[(*S*)-α-methylbenzyl]tetrahydro-2*H*-1,3,2-oxazaphosphorine 2-oxides [**15**(S,R_p) and **15**(S,S_p)], and these diastereomers were then separated by column chromatography. Treatment of pure **14**(S,S_p) with hydrogen chloride gave pure **15**(S,S_p). The yield of ethylenimide ring opening products was quantitative. Hydrogenolytic removal of the α-methylbenzyl group from **15**(S,R_p) and **15**(S,S_p) gave enantiomers of 2-(2-chloroethylamino)tetrahydro-2*H*-1,3,2-oxazaphosphorine 2-oxide [**16**(*S*) and **16**(*R*)].[21]

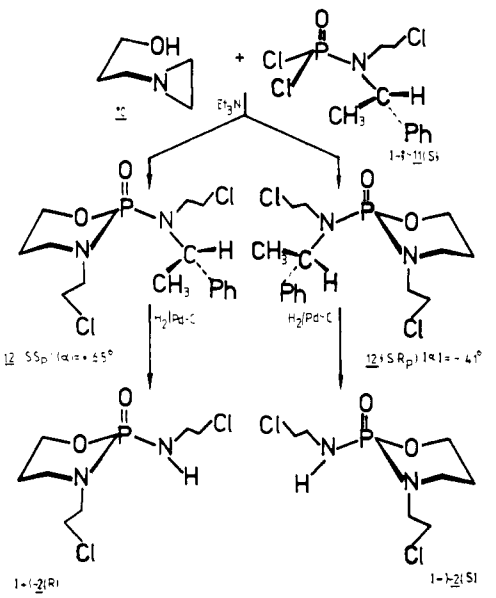
The enantiomers of **16** were the key intermediates for the synthesis of **2** and **4**. Reaction of **16**(*S*) and **16**(*R*) with a molar amount of chloroacetyl chloride in THF solution at room temperature gave the corresponding 2(*R*)- and 2(*S*)-(2-chloroethylamino)-3-chloroacetyltetrahydro-2*H*-1,3,2-oxazaphosphorine 2-oxides [**17**(*R*) and **17**(*S*)] (Scheme IV). This process is fully regioselective. Besides the high yield of **17**, there was no ^{31}P NMR evidence for the formation of either the

Scheme I



[a]The assignment of absolute configuration in both **7** is based on the knowledge of that in **1** and assumption that conversion of **7** → **1** does not involve any direct bond to P atom.

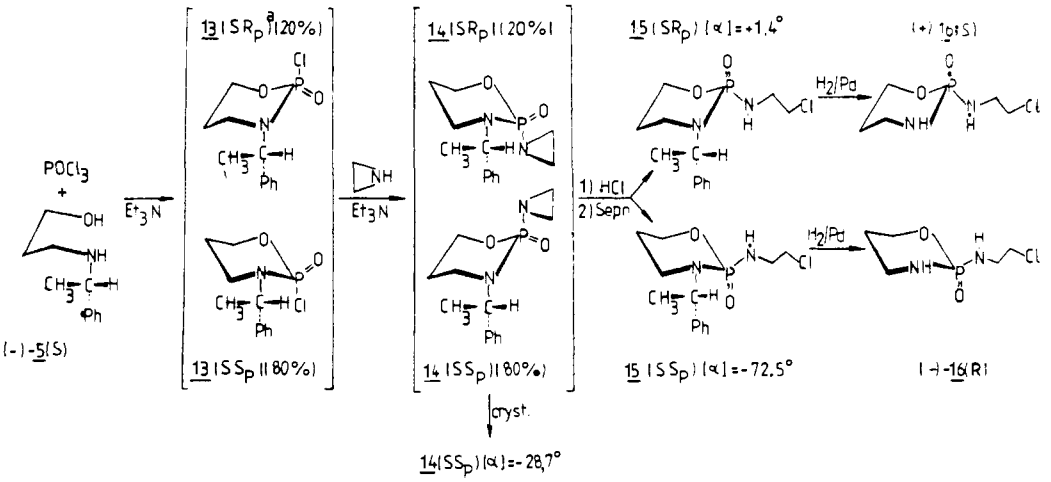
Scheme II



product containing chloroacetyl group attached to the exocyclic nitrogen atom or the compound containing two chloroacetyl groups linked to N-3 and exocyclic nitrogen atoms. The reduction of the carbonyl group in each enantiomer of **17** with diborane[23] gave the corresponding enantiomers of ifosphamide, **2**(*R*) and **2**(*S*), which were identical with those obtained by our earlier procedure.[20]

Availability of enantiomers of **2** allowed us to perform the synthesis of enantiomeric sulfosphamides **4**(*S*) and **4**(*R*). Reaction of **2**(*S*) and **2**(*R*) with sodium hydride led solely to 2-ethylenimino-3-(2-chloroethyl)tetrahydro-2*H*-1,3,2-oxazaphosphorine 2-oxides **18**(*S*) and **18**(*R*), respectively. No other isomers of **21** (vide infra) were formed.[26] Each enantiomer of **18** when reacted with methanesulfonic acid[4] gave enantiomeric **4**(*S*) and **4**(*R*) (Scheme V).

Scheme III



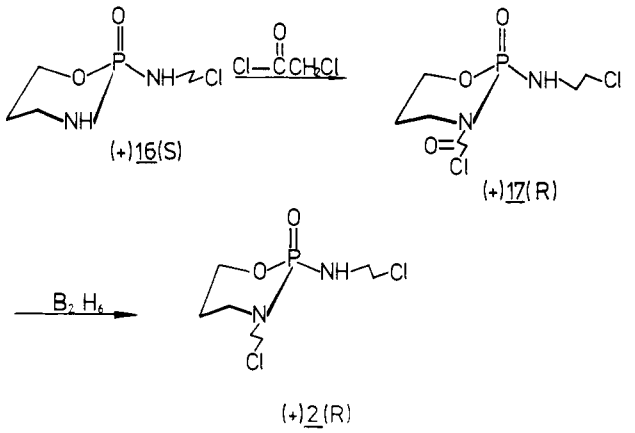
[a]The assignment of absolute configuration in both **13** is based on assumption that their reactions with ethylenimine, like these of diastereomeric 2-chloro-2-oxo-4-methyl-1,3,2-dioxaphosphorinans, proceed with inversion of configuration at P atom (Kinas R. W., Ph.D. Thesis, 1979).

The successful conversion of **16** to **2** inspired us to use the same approach to the synthesis of the enantiomers of trofosphamide (**3**). Reaction of **1**(*S*) and **1**(*R*) with chloroacetyl chloride gave 2-[bis(2-chloroethyl)amino]-3-chloroacetyltetrahydro-2*H*-1,3,2-oxazaphosphorine 2-oxides **19**(*R*) and **19**(*S*), respectively (Scheme VI). The reduction of the carbonyl group with diborane gave the corresponding enantiomers of trofosphamide, **3**(*S*) and **3**(*R*). The optical rotation values and melting points of **2**, **3**, **4**, and **16** are given in Table I. Other physical parameters (δ_{31P}, R_f, and mass spectral fragmentation) were identical with those of racemic compounds.

Optical Purity and Absolute Configuration Assignments. Optical purity of enantiomeric **2** and **16** was checked by means of ^{31}P NMR spectroscopy. Spectra were determined in the presence of Eu(tfc)$_3$.[24] The proportions of 1,3,2-oxazaphosphorine 2-oxide/shift reagent, solvent, molar concentrations, and induced chemical shift values are given in Table II. The accuracy of measurement was established as ±3% on the basis of results obtained with samples of known (by weight) enantiomeric composition. Assignment of the absolute configuration *R* to the levorotatory isomer of **3** was straightforward, because each step in the conversion **1** → **3** occurs without cleavage of bonds to the phosphorus atom. The assignment of absolute configuration to enantiomers of **2** and **4** is based on the conversion of common intermediate **15**(R,R_p), $[\alpha]^{25}_D$ +72.5°, to (−)-**1**(*S*) and (+)-**2**(*R*) using reactions wherein no bond attached to the dissymmetric phosphorus in **15** is cleaved (Scheme VII and also III and IV). Reaction of **15**(R,R_p) with chloroacetyl chloride gave 2(*S*)-[*N*-(2-chloroethyl)-*N*-chloroacetyl]amino-3-[(*R*)-α-methylbenzyl]tetrahydro-2*H*-1,3,2-oxazaphosphorine 2-oxide [**20**(R,S_p)], which under treatment with diborane gave the previously described[14,15] precursor of (−)-**1**(*S*), namely, 2(*R*)-[bis(2-chloroethyl)amino]-3-[(*R*)-α-methylbenzyl]tretrahydro-2*H*-1,3,2-oxazaphosphorine 2-oxide [**7**(R,R_p)]. Because the same diastereomer of **15**, $|\alpha|^{25}_D$ +72.5°, was converted to (+)-**2** in the sequence of reactions (+)-**15**(R,R_p) → (+)-**16** → (+)-**17** → (+)-**2**, the absolute configuration of (+)-**2** must be *R*. The correctness of this assignment was supported by the conversion of (−)-**1**(*S*) and (+)-**2** into a common product **21** and comparison of the induced δ_+ and δ_- parameters in ^{31}P NMR Eu(tfc)$_3$ spectra.

Treatment of (−)-**1**(*S*) with sodium hydride gave 1-(2-chloroethyl)tetrahydro-1*H*,5*H*-[1,3,2]diazaphospholo[2,1-*b*][1,3,2]oxazaphosphorine 9-oxide as an oil [**21**(*S*), $[\alpha]^{25}_D$ +10.8°, δ_{31P} 24.0, MS *m/z* 224 (8%) molecular ion, 175 (100%)] which was optically pure as shown by ^{31}P NMR Eu(tfc)$_3$ assay. On the other hand, **18**(*R*) when heated under reflux in anisole solution for 48 h also isomerized to **21**. However, the isomerization **18** → **21** was not complete and the product, besides desired **21** (30%), contained starting material **18** (60%) and some other organophosphorus compounds. Attempts to increase the extent of the conversion **18** → **21** by prolonged heating were unsuccessful. Preparative isolation of

Scheme IV



Scheme V

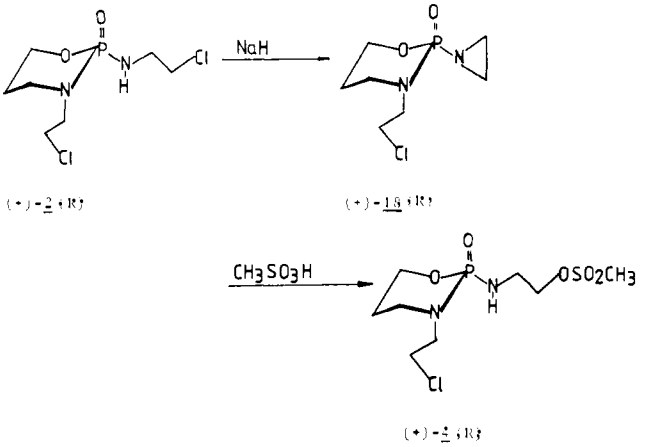
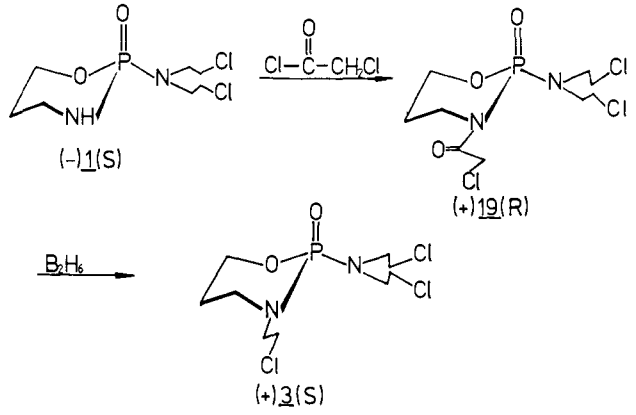


Table I. Melting Points and Specific Rotation Values of **1**, **2**, **3**, **4**, and **16**

compd		mp, °C	$[\alpha]^{25}_D$ (methanol), deg
cyclophosphamide	(**1**)	65–66	±2.3[a]
ifosphamide	(**2**)	62–63	±39.0[a]
trofosphamide	(**3**)	oil	±28.6
sulfosphamide	(**4**)	oil	±37.5
metabolite	(**16**)	108–111	±15.1[a]

[a] According to ^{31}P NMR these values are characteristic for optically pure species.

Scheme VI



21 from this mixture was not possible owing to the high reactivity of this compound (vide infra). The change of chemical shift in the ^{31}P NMR spectra of **21**, which had been prepared from (−)-**1**(*S*), in the presence of Eu(tfc)$_3$ was the same as that of the sample of **21** which had been obtained as the product of isomerization of **18** {$[\alpha]^{25}_D$ +24.4° (*c* 2.4, methanol)} (see Figure 2). Consequently the stereochemical environment of phosphorus in (−)-**1**(*S*) and in (+)-**18** [and consequently in (+)-**2**] must be the same. On the basis of the above arguments we have assigned the absolute configurations to enantiomers of **14**, **15**, **16**, **17**, and **4**. However, the conclusive assignment of absolute configuration must be obtained from X-ray crystallography (work in progress).

A striking feature of racemic **21** is that its physical parameters differ from those reported by Zon et al.[25] The isolation of **21** from the reaction mixture obtained from anhydrous, racemic **1** was achieved by means of its extraction with ethyl ether and crystallization from this solvent (white crystals, mp 75–79 °C, satisfactory elemental analysis, MS *m/z* 224 (10%) molecular ion, δ_{31P} 24 ($CHCl_3$)); compound **21** according to

Scheme VII

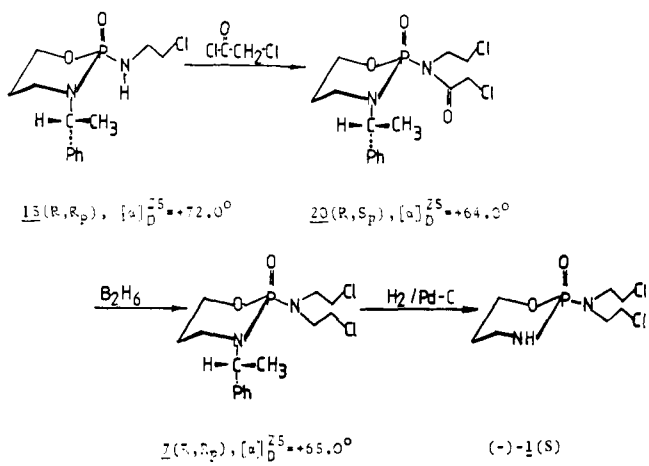


Table II. Conditions of Assignment of Optical Purity of **2** and **16**

compd		ratio compd/Eu(tfc)$_3$	solvent	δ_+[a]	δ_-[a]
ifosphamide	(**2**)	1:0.7	CCl_4	−68.0	−67.2
metabolite	(**16**)	1:0.5	C_6D_6	−36.8	−39.3

[a] ^{31}P NMR chemical shift values (ppm) of enantiomeric species run in the presence of Eu(tfc)$_3$. Molar concentrations of **2** and **16** 0.020 and 0.025 mol/L, respectively.

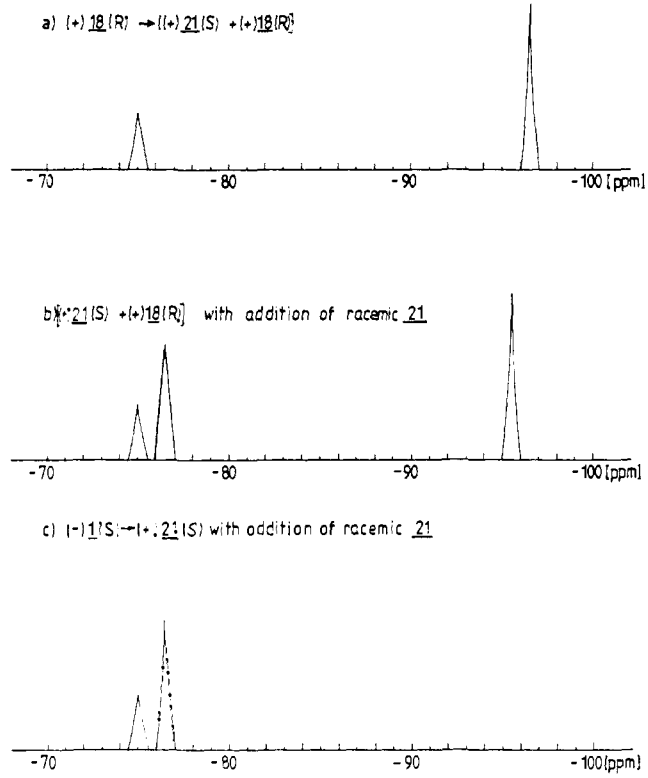
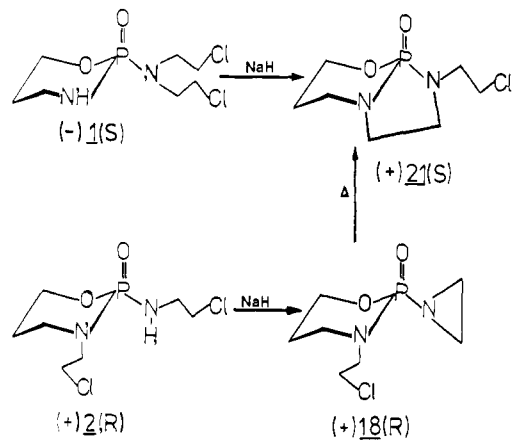


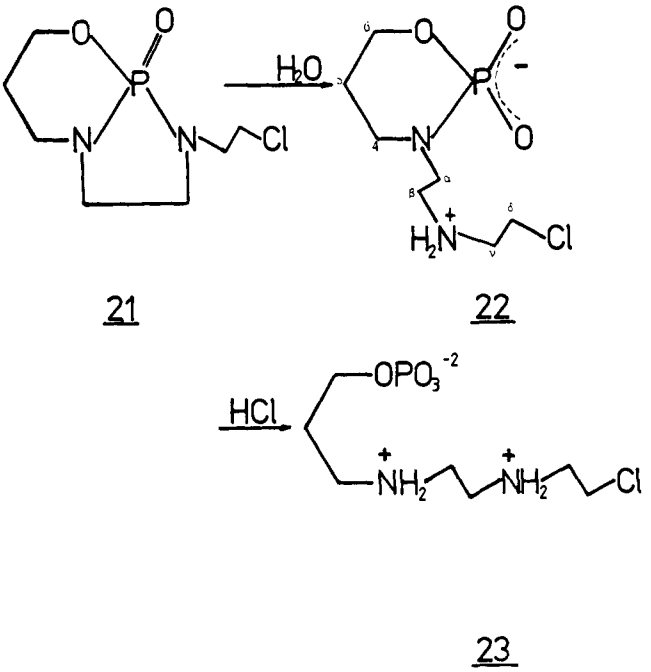
Figure 2. Schematic ^{31}P NMR Eu(tfc)$_3$ spectra of enantiomeric **21** obtained from **18** and **1**.

Zon et al. was a colorless oil that turned into a waxy solid, mp 142–143 °C, unsatisfactory elemental analysis.[25] Because the assignment of structure for the product of reaction of **1** with sodium hydride was essential for elucidation of the absolute configuration of **2** by means of stereospecific conversions **2** → **18** → **21** and **1** → **21**, we undertook further studies on hydrolysis of **21** which speak for the correctness of our structural assignments. Compound **21**, when dissolved in water, gave **22**, mp 180°C, δ_{31P} 7.7 (H_2O), which in the presence of hydro-

Scheme VIII



Scheme IX



21 22

23

chloric acid further hydrolyzed to **23** (Scheme IX). Compound **23** possessed the same ^{13}C NMR characteristics as the product of the acid hydrolysis of the bicyclic compound reported by Zon et al.[25] Compound **21** when dissolved in D_2O gave dideuterated **22.** Its mass spectrum contained M − DCl ion (*m/z* 207). Also, the dissolution of **21** in $H_2{}^{18}O$ (50% of $H_2{}^{18}O$) gave the product, which in the mass spectrum contained ions *m/z* 206, 208, 175 (100%), and 177 (36%) and corresponded to **22** (*m/z* 206 and 175) and ^{18}O-labeled **22** (*m/z* 208, 177). Dissolution of enantiomeric **21** ($[\alpha]^{25}_D$ +10.8°) in water led to optically inactive product. These results strongly support the correctness of our assignment of the structure of the product of cyclization of **1** under the treatment with NaH. Further studies on biological evaluation of enantiomeric **2–4** are in progress.

Experimental Section

All melting points and boiling points are uncorrected. Solvents and commercial reagents were distilled and dried by conventional methods before use.

$^{31}P\{^1H\}$ NMR and ^{13}C NMR spectra were recorded at 24.3 and 15.03 MHz, respectively, with a JEOL C-60H spectrometer equipped with hetero-spin decoupler SNH-SD-HC. Positive chemical shift values (ppm) are reported for compounds absorbing at lower fields than 85% H_3PO_4 and Me_4Si, respectively. Mass spectra were obtained on a LKB 2091 spectrometer at 70 eV ionizing energy. Optical activity measurements were made with a Perkin-Elmer 241 MC photopolarimeter. Product purities were determined from integrated ^{31}P NMR spectra and TLC (silica gel 60, F_{254}). Silica gel for column chromatography was 100–200 mesh. Solvent systems were $CHCl_3$–acetone, 3:1 (A); $CHCl_3$–EtOH, 9:1 (B); benzene–$CHCl_3$–acetone, 8:2:1 (C); benzene–acetone–$CHCl_3$, 12:3:1 (D).

2(*S*)-Ethylenimine-3-[(*S*)-α-methylbenzyl]tetrahydro-2*H*-1,3,2-oxazaphosphorine 2-Oxide [14(S,S_p)] and Its Enantiomer 14(R,R_p). Into a cooled (−30 °C) solution of *N*-[(*S*)-α-methylbenzyl]-3-aminopropan-1-ol [**5**(*S*), 28.0 g, 0.15 mol, $[\alpha]^{25}_D$ −41.2° (*c* 5.9, benzene)] and triethylamine (32.0 g, 0.31 mol) in methylene chloride (400 mL) was added dropwise a solution of phosphoryl chloride (24.0 g, 0.15 mol) in the same solvent (100 mL). A temperature of −30 to −20 °C was maintained during addition, and stirring of the reaction mixture was continued for 1 h at ambient temperature. A ^{31}P NMR spectrum of a sample of the reaction mixture showed the presence of **13**(S,R_p), δ 8.9, and **13**(S,S_p), δ 8.2, in the ratio 1:4. A mixture of ethylenimine (6.8 g, 0.15 mol), triethylamine (17.0 g, 0.16 mol), and CH_2Cl_2 (100 mL) was then added at a temperature of 0–5 °C, and stirring was continued at ambient temperature for 1 h. Precipitated triethylamine hydrochloride was filtered off, and the filtrate was concentrated. ^{31}P NMR assay showed that the residual oily material consisted mainly of **14**(S,R_p), δ 16.6, and **14**(S,S_p), δ 17.9, in 1:4 ratio (measurement in CH_2Cl_2). The residue was dissolved in carbon tetrachloride–*n*-hexane (3:1, 300 mL). The colorless crystals of **14**(S,S_p) (24 g, 57.5%) were obtained: mp 121–123 °C; $[\alpha]^{25}_D$ −28.7° (*c* 3.7, MeOH); $\delta_{^{31}P}$ 16.8 (benzene); R_f(A) 0.47; MS *m/z* 266 ($M^{+\cdot}$, 21%), 251 (100%), 224 (20%), 105 (62%). Anal. C, H, N, P.

Mother liquor was concentrated and the oily residue (12 g) was diluted with $CHCl_3$ and then treated with dry ethyl ether saturated with HCl giving a mixture of diastereomers of **15.** In analogous manner, starting from **5**(*R*) [28.0 g, 0.15 mol, $[\alpha]^{25}_D$ +41.5° (*c* 4.3, benzene)], **14**(R,R_p) was obtained in 60% yield (25.0 g), mp 119–122 °C, $[\alpha]^{25}_D$ +30.0° (*c* 3.5 MeOH), $\delta_{^{31}P}$ 16.8 (benzene), R_f(A) 0.47.

2-(*S*)-(2-Chloroethyl)amino-3-[(*S*)-α-methylbenzyl]tetrahydro-2*H*-1,3,2-oxazaphosphorine 2-Oxide [15(S,S_p)] and Isomeric 15(S,R_p), 15(R,R_p), and 15(R,S_p). To a solution of **14**(S,S_p) (26.6 g, 0.1 mol, $[\alpha]^{25}_D$ −28.7°) in CH_2Cl_2 (200 mL), an ethereal solution of HCl (0.1 mol) was added in one portion and after 10 min solvents were evaporated. The solid residue was crystallized from acetone and afforded 25.0 g (82.7%) of **15**(S,S_p) as colorless crystals: mp 114–116 °C; $[\alpha]^{25}_D$ −72.5° (*c* 3.2, MeOH); $\delta_{^{31}P}$ 9.9 (benzene); R_f(A) 0.41; MS *m/z* 302 ($M^{+\cdot}$, 20%), 287 (100%), 253 (4%), 105 (84%). The mother liquor from this experiment was combined with that obtained from the experiment described above and the mixture of **15**(S,S_p) and **15**(S,R_p) was separated by column chromatography with silica gel (200 g). Benzene–chloroform–acetone (4:3:1) was used as eluent. The "faster" migrating **15**(S,S_p) {4.0 g, mp 114–115 °C, $[\alpha]^{25}_D$ −72.6° (*c* 4.0, MeOH), other parameters as above} was obtained as well as 4.5 g of "slower" migrating **15**(S,R_p), which was a thick oil: $[\alpha]^{25}_D$ +1.4° (*c* 5.7, MeOH); $\delta_{^{31}P}$ 11.6 (benzene); R_f(A) 0.32; MS *m/z* 302 ($M^{+\cdot}$, 22%), 287 (100%), 253 (5%), 105 (62%). The overall yield of **15**(S,S_p) from **5**(*S*) was 55%. Starting from **14**(R,R_p), $[\alpha]^{25}_D$ +30.0° (*c* 3.5, MeOH), and using an analogous procedure **15**(R,R_p), $[\alpha]^{25}_D$ +72.5° (*c* 3.2, MeOH), and **15**(R,S_p), $[\alpha]^{25}_D$ −1.6° (*c* 4.1, MeOH), were obtained. Anal. C, H, N, P.

2(*R*)-(2-Chloroethylamino)tetrahydro-2*H*-1,3,2-oxazaphosphorine 2-Oxide [16(*R*)] and 16(*S*). **15**(S,S_p) (11.0 g, 0.036 mol, $[\alpha]^{25}_D$ −72.5°) was dissolved in EtOH (500 mL) and after addition of 10% Pd/C (0.5 g) was then hydrogenated at room temperature under normal pressure. The progress of C–N bond splitting was followed by means of TLC. When **15**(S,S_p) disappeared (1–4 days), the catalyst was filtered off, solvent was evaporated, and the residue was purified on a SiO_2 column with $CHCl_3$–EtOH (9:1) as eluent. Compound **16**(*R*) was obtained in 83% yield (6.0 g): mp 109–112 °C (from CCl_4–acetone); $[\alpha]^{25}_D$ −15.1° (*c* 3.0, MeOH); $\delta_{^{31}P}$ 12.2 (MeOH); R_f(B) 0.25; MS *m/z* 198 ($M^{+\cdot}$, 4%), 149 (100%), 56 (51%). Hydrogenolysis of **15**(R,R_p) ($[\alpha]^{25}_D$ +72.5°) has been performed in an analogous manner and **16**(*S*), mp 107–110 °C, $[\alpha]^{25}_D$ +15.2° (*c* 3.1, MeOH), was obtained. In this manner **15**(R,S_p), $[\alpha]^{25}_D$ −1.6°, was converted to **16**(*R*), $[\alpha]^{25}_D$ −13.6° (*c* 3.0, MeOH), other colligative data as described above. Anal. C, H, N, P.

2(*R*)-(2-Chloroethylamino)-3-chloroacetyltetrahydro-2*H*-1,3,2-oxazaphosphorine 2-Oxide [17(*R*)] and Its Enantiomer 17(*S*). To a solution of **16**(*S*) (1.98 g, 0.01 mol, $[\alpha]^{25}_D$ +15.2°) in THF (30 mL), chloroacetyl chloride (1.12 g, 0.01 mol) in THF (20 mL) was added. Progress of acetylation was followed by means of TLC. When starting **16** disappeared, solvent was evaporated, the residue was introduced into a short column of SiO_2 (10 g), and the product was eluted with acetone–chloroform (3:1). The crude **17**(*R*) was purified by crystallization from acetone–ethyl ether. Colorless crystals of **17**(*R*) (2.0 g, 73%) were collected: mp 87–88 °C; $[\alpha]^{25}_D$ +45.2° (*c* 4.0, MeOH); $\delta_{^{31}P}$ 3.4 (MeOH); MS *m/z* 274 ($M^{+\cdot}$, 0.3%), 150 (100%), 238 (9%), 225 (50%). In the same way **16**(*R*), $[\alpha]^{25}_D$ −15.2°, was converted to **17**(*S*), $[\alpha]^{25}_D$ −45.4° (*c* 4.1, MeOH), other parameters as for **17**(*R*). Anal. C, H, N, P.

2(*R*)-(2-Chloroethylamino)-3-(2-chloroethyl)tetrahydro-2*H*-1,3,2-oxazaphosphorine 2-Oxide [2(*R*)] and Its Enantiomer 2(*S*) (Ifosphamide). Into a stirred solution of **17**(*R*) (2.74 g, 0.01 mol, $[\alpha]^{25}_D$ +45.2°) in THF (70 mL) was added a saturated solution of diborane in THF (5 mL). After exhaustive reduction of the carbonyl group (TLC assay) water (2 mL) was added, solvent was evaporated, and the residue was dissolved in $CHCl_3$ (50 mL). This solution was washed with 5% aqueous Na_2CO_3 and water, dried over anhydrous $MgSO_4$, and introduced onto a column of silica gel (70 g). Product was eluted with $CHCl_3$–acetone (3:1) and was crystallized from ethyl ether. **2**(*R*)

(1.3 g) was obtained in 50% yield: mp 62–63 °C; $[\alpha]^{25}_D$ +39.0° (*c* 4.0, MeOH); δ_{31P} 12.2 ($CHCl_3$); R_f(B) 0.45; MS *m/z* 260 ($M^{+\cdot}$, 1%), 211 (100%), 134 (38%). Similar treatment of **17**(*S*), $[\alpha]^{25}_D$ −45.4°, gave **2**(*S*), $[\alpha]^{25}_D$ −38.8° (*c* 4.2, MeOH), other parameters as for **2**(*R*).

2(*R*)-Ethylenimine-3-(2-chloroethyl)tetrahydro-2*H*-1,3,2-oxazaphosphorine 2-Oxide [18(*R*)] and Its Enantiomer 18(*S*). Into a stirred solution of **2**(*R*) (0.52 g, 0.002 mol, $[\alpha]^{25}_D$ +39.0°) in THF (20 mL), sodium hydride (1 equiv) was added at ambient temperature in one portion and stirring was continued until **2**(*R*) disappeared (TLC monitoring). The reaction mixture was filtered through Celite, solvent was evaporated, and the residue was purified on a silica gel column. $CHCl_3$–acetone (3:1) was used as eluent. Evaporation of solvents left crystalline **18**(*R*) (0.23 g) [mp 64–66 °C; $[\alpha]^{25}_D$ +24.5° (*c* 2.4, MeOH); δ_{31P} 18.3 (MeOH); R_f(A) 0.34] in 51% yield, MS *m/z* 224 ($M^{+\cdot}$, 1%), 175 (100%). Analogous reaction of **2**(*S*) gave a 50% yield of **18**(*S*), $[\alpha]^{25}_D$ −24.5° (*c* 2.5, MeOH), other parameters the same as those of **18**(*R*). Anal. C, H, N, P.

2(*R*)-(2-Mesyloxyethylamino)-3-(2-chloroethyl)tetrahydro-2*H*-1,3,2-oxazaphosphorine 2-Oxide [4(*R*)] and Its Enantiomer 4(*S*). (Sulfosfamide). A solution of methanesulfonic acid (0.096 g, 0.001 mol) in THF (1 mL) was added to a solution of **18**(*R*) (0.224 g, 0.001 mol, $[\alpha]^{25}_D$ +24.5°) in THF (10 mL). After 10 min of stirring at room temperature, THF was evaporated and the residue was purified on a silica gel column with $CHCl_3$–ethyl acetate–ethanol (9:4:1) as eluent. **4**(*R*) (0.190 g) was obtained in 60% yield as a colorless oil: $[\alpha]^{25}_D$ +37.8° (*c* 6.1, MeOH); δ_{31P} 12.5 (MeOH); R_f(B) 0.33; MS *m/z* 320 ($M^{+\cdot}$, 0.2%), 271 (86%), 211 (32%), 213 (10%), 175 (100%). Starting from the **18**(*S*) enantiomer, **4**(*S*) was obtained, $[\alpha]^{25}_D$ −38.0° (*c* 5.8, MeOH), other parameters as for **4**(*R*).

2(*R*)-Bis[(2-chloroethyl)amino]-3-chloroacetyltetrahydro-2*H*-1,3,2-oxazaphosphorine 2-Oxide [19(*S*)] and Its Enantiomer 19(*R*). Conversion of **1** to **19** was performed in a way analogous to that of **16** → **17**. Thus, starting from **1**(*R*) (2.6 g, $[\alpha]^{25}_D$ +2.3°), the crude product was purified by column chromatography with silica gel using benzene–acetone–$CHCl_3$ (8:2:1). Compound **19**(*S*) (1.92 g) was obtained as a pale-yellow oil in 60% yield: $[\alpha]^{25}_D$ −20.0° (*c* 2.5, MeOH); δ_{31P} 3.6 (MeOH); R_f(B) 0.50; MS *m/z* 287 (100%), 212 (82%). From **1**(*S*) the desired **19**(*R*) was obtained, $[\alpha]^{25}_D$ +19.4° (*c* 3.0, MeOH). Anal. C, H, N, P.

2(*R*)-[Bis(2-chloroethyl)amino]-3-(2-chloroethyl)tetrahydro-2*H*-1,3,2-oxazaphosphorine 2-Oxide [3(*R*)] (Trofosphamide). Reduction of **19**(*S*) with diborane was performed in a manner analogous to the reaction **17** → **2**. Starting from **19**(*S*) (3.4 g, 0.01 mol, $[\alpha]^{25}_D$ −20.0°), the crude product was purified on a silica gel column (100 g) with chloroform–ethanol (18:1) as the eluent system. **3**(*R*) was obtained (1.6 g) as a colorless oil in 50% yield: $[\alpha]^{25}_D$ −28.6° (*c* 2.0 MeOH); δ_{31P} 13.3 (MeOH); R_f(B) 0.60; MS *m/z* 322 ($M^{+\cdot}$, 0.8%), 273 (100%), 182 (44%), 154 (58%), 134 (52%), 118 (28%). The results of ^{31}P NMR, TLC, and MS examinations were identical with those obtained for racemic **3**.

2(*S*)-{[*N*-(2-Chloroethyl)-*N*-chloroacetyl]amino}-3-[(*R*)-α-methylbenzyl]tetrahydro-2*H*-1,3,2-oxazaphosphorine 2-Oxide [20(*R*,S_p)]. Into chloroacetyl chloride (5 mL), **15**(*R*,R_p) (3.0 g, 0.01 mol, $[\alpha]^{25}_D$ +72.5°) was added in small portions and the mixture was stirred for 15 min at room temperature. Excess chloroacetyl chloride was removed under reduced pressure. The residue was dissolved in $CHCl_3$ (100 mL) and the solution was washed with water (3 × 20 mL) and then dried over anhydrous $MgSO_4$. Solvent was evaporated and the residue was introduced onto a silica gel column (100 g). Product was eluted with benzene–$CHCl_3$–acetone (8:2:1). **20**(*R*,S_p) was obtained as a pale-yellow oil in 30% yield: $[\alpha]^{25}_D$ +64.0° (*c* 2.2, MeOH); δ_{31P} 0.1 (MeOH); R_f(C) 0.53; MS *m/z* 378 ($M^{+\cdot}$, 0.5%), 273 (18%), 240 (32%), 138 (74%), 119 (94%), 117 (99%), 105 (100%). Anal. C, H, N, P.

2(*R*)-[Bis(2-chloroethyl)amino]-3-[(*R*)-α-methylbenzyl]tetrahydro-2*H*-1,3,2-oxazaphosphorine 2-Oxide [7(*R*,R_p)]. Reduction of **20**(*R*,S_p) (1.0 g, $[\alpha]^{25}_D$ +64.0°) with diborane was performed by analogy to that described for conversion of **17**(*R*) → **2**(*R*) (vide supra). Crude product was purified on a silica gel column using benzene–$CHCl_3$–acetone (4:3:1) as eluent. **7**(*R*,R_p) was obtained as a colorless oil in 52% yield (0.5 g): $[\alpha]^{25}_D$ +65.0° (*c* 2.2, benzene); δ_{31P} 10.2 (benzene); R_f(C) 0.28;[14] MS *m/z* 364 ($M^{+\cdot}$, 4.8%), 315 (50%), 224 (44%), 211 (35%), 120 (37%) 105 (100%). Hydrogenation of **7**(*R*,R_p) leading to (−)-**1**(*S*) was described earlier.[14]

1-(2-Chloroethyl)tetrahydro-1*H*,5*H*-[1,3,2]diazaphospholo[2,1-*b*][1,3,2]oxazaphosphorine 9-Oxide [21(*S*)] and 21(*R*). A. To a solution of anhydrous **1** [prepared by reaction of *N*,*N*-bis(2-chloroethyl)-phosphoramidodichloridate with 3-aminopropan-1-ol, 2.6 g, 0.01 mol] in THF (100 mL) was added sodium hydride (0.24 g) in one portion and stirring was continued until **1** disappeared (TLC assay). The precipitated sodium chloride and an excess of sodium hydride were filtered off (or centrifuged) and solvent was evaporated. The solid residue was extracted with ethyl ether and the extract was evaporated to half volume. Crystalline product was filtered off to give **21** in 50% yield (1.1 g): mp 75–79 °C; δ_{31P} 24.0 ($CHCl_3$); R_f($CHCl_3$–EtOH, 18:1) 0.56; MS *m/z* 224 ($M^{+\cdot}$, 8%), 175 (100%). Anal. C, H, N, P, Cl.

The ^{31}P NMR spectrum of **21** (22.4 mg) run in the presence of Eu(tfc)$_3$ [molar ratio **21**:Eu(tfc)$_3$ 1:2] in CCl_4 solution (2 mL) revealed the presence of two signals at −74.7 (−) and −75.6 ppm (+) in a 1:1 ratio. Starting from (−)-**1**(*S*) {$[\alpha]^{25}_D$ −2.3° (*c* 2.5, MeOH)}, **21**(*S*) was obtained as a pale-yellow oil, $[\alpha]^{25}_D$ +10.8° (*c* 2.0, MeOH). From (+)-**1**(*R*), enantiomeric **21**(*R*) was obtained, $[\alpha]^{25}_D$ −10.2° (*c* 2.5, MeOH). ^{31}P NMR analysis performed in the presence of Eu(tfc)$_3$ (conditions as above) showed that **21**(*S*) was an optically pure compound, δ_{31P} −75.6. Addition of racemic **21** caused the appearance of a second signal at δ −74.7 (see Figure 2c).

B. A solution of **18**(*R*) [0.224 g, 0.001 mol, $[\alpha]^{25}_D$ +24.5° (*c* 2.4, MeOH)] in anisole (10 mL) was heated under reflux during 72 h. The ^{31}P NMR spectrum of this mixture revealed the presence of **18**(*R*), δ 16.9 (80%), and **21**(*S*), δ 22.3 (20%). Into 1 mL of reaction mixture, racemic **21** (0.022 g) was added and ^{31}P NMR showed the increase of intensity of the signal at δ 22.3 (40%). The remaining (9 mL) reaction mixture was concentrated under high vacuum and the residue was dissolved in CCl_4 (9 mL). Into 1 mL of this solution, Eu(tfc)$_3$ (0.05 g) dissolved in CCl_4 (1 mL) was added and the resulting mixture was examined by means of ^{31}P NMR. The spectrum contained signals at δ −96.6 [**18**(*R*), 80%] and −75.1 [**21**(*S*), 20%] (see Figure 2a). Into the same sample a solution of racemic **21** (0.005 g) and Eu(tfc)$_3$ (0.009 g) in CCl_4 (0.5 mL) was added and the ^{31}P NMR spectrum then revealed the presence of signals at δ −95.2 [**18**(*R*), 65%], −76.4 [**21**(*S*), 25%], and −73.4 [**21**(*R*), 10%] (see Figure 2b).

2-Hydroxy-3-[2-(2-chloroethylamino)ethyl]tetrahydro-2*H*-1,3,2-oxazaphosphorine 2-Oxide (22) and the Product of Its Acidic Hydrolysis, 23. Compound **21** (1 g) was dissolved in water (1 mL) and water was then removed under reduced pressure. **22** (1 g) was obtained: mp 179–180 °C; δ_{31P} 7.7 (H_2O); MS *m/z* 224 (8%, M − H_2O), 226 (2.8%), 206 (12%, M − HCl), 175 (100%); ^{13}C NMR δ 64.1 (d, J_{CP} = 6.2 Hz, C-6), 49.8 (d, J_{CP} = 3.9 Hz), 45.3 (d, J_{CP} = 2.3 Hz), 44.2, 43.6 (singlets, C-γ, C-δ), 42.0 (d, J_{CP} = 4.7 Hz), 26.4 (d, J_{CP} = 3.9 Hz, C-5).

The sample of **21** (224 mg) was dissolved in D_2O (4 mL) and hydrochloric acid (1 mL of 1 N HCl) and the ^{13}C NMR spectrum was recorded: δ 64.5 (d, $^2J_{CP}$ = 6 Hz), 50.7, 47.2, 44.4, 44.4, 40.4, 28.0 (d, $^3J_{CP}$ = 7.3 Hz). This spectrum corresponds to that reported by Zon et al.[25] for **23** oxalate.

2-[Bis(2-chloroethyl)amino]-3-[1(*S*)-(α-naphthyl)ethyl]tetrahydro-2*H*-1,3,2-oxazaphosphorine 2-Oxide [24(*S*,R_p)] and 24(*S*,S_p). A mixture of γ-chloropropanol (14.1 g, 0.15 mol) and 1(*S*)-α-naphthylethylamine [34.2 g, 0.2 mol, $[\alpha]^{25}_D$ −61.0° (*c* 3.3, EtOH)] was heated on the oil bath at 120 °C during 16 h. After cooling, the reaction mixture was dissolved in water and 30% NaOH was added for neutralization of hydrogen chloride. Extraction with $CHCl_3$ followed by typical workup afforded the pale-yellow solid residue, which after crystallization from *n*-hexane gave 14 g (41%) of *N*-[1(*S*)-(α-naphthyl)ethyl]-3-aminopropan-1-ol: mp 91–92 °C; $[\alpha]^{25}_D$ −39.7° (*c* 3.7, MeOH); MS *m/z* 229 ($M^{+\cdot}$, 1.9%), 214 (100%), 155 (68%). A dioxane solution (20 mL) of this compound (1.12 g, 0.005 mol) and triethylamine (1.1 g, 0.011 mol) was added dropwise into a dioxane (20 mL) solution of *N*,*N*-bis(2-chloroethyl)phosphoramidodichloridate (1.3 g, 0.005 mol). The mixture was left for 48 h at room temperature with stirring, triethylamine hydrochloride was filtered off, and the filtrate was then concentrated. The residue was diluted with benzene and was chromatographed on a silica gel (100 g) column using solvent system D. "Faster" migrating **24**(*S*,R_p), mp 89 °C, R_f(D) 0.47, $[\alpha]^{25}_D$ −2.0° (*c* 3.3, benzene), δ_{31P} 9.5 (benzene), was obtained in 14.5% yield (0.3 g): MS *m/z* 414 ($M^{+\cdot}$, 58%), 399 (86%), 365 (100%), 274 (84%), 155 (53%).

After fractions containing unseparated diastereomers, the "slower" **24**(*S*,S_p) was isolated as a thick oil: $[\alpha]^{25}_D$ +23.0° (*c* 1.5, benzene); δ_{31P} 11.6 (benzene); R_f(D) 0.35; 9.6% (0.2 g); MS *m/z* 414 ($M^{+\cdot}$,

100%), 399 (78%), 365 (43%), 274 (54%), 155 (42%). Separation conditions were not optimized.

Cyclophosphamide 1(*R*). The hydrogenolysis of compound **24**(S,R_P), $[\alpha]^{25}_D$ −2.0°, under the conditions described previously[14] afforded (+)-**1**(*R*) in 85% yield, $[\alpha]^{25}_D$ +2.3° (*c* 4.4, MeOH).

Acknowledgments. This investigation was supported by grants to the Polish Academy of Sciences, Centre of Molecular and Macromolecular Studies from the National Cancer Program, and to Institute of Cancer Research from the Medical Research Council. J.M.S. van M. thanks the Koningin Wilhelmina Fonds for a Research Fellowship.

References and Notes

(1) (a) Polish Academy of Sciences. (b) Chester Beatty Research Institute.
(2) (a) Arnold, H.; Bourseaux, F.; Brock, N. *Naturwissenschaften* **1958**, *45*, 64. Hill, D. L. "A Review of Cyclophosphamide", Charles Thomas: Springfield, Ill., 1975. (b) Burket, H.; Voigt, H. C. "Proceedings of the International Holoxan-Symposium", Düsseldorf, March 21–23, 1977.
(3) Brock, N. *Med Monatsschr.* **1973**, *27*, 390–394.
(4) Brock, N.; Kuhlmann, I. *Arzneim.-Forsch.* **1974**, *24*, 1139–1149.
(5) Druckey H. In ref 2b, pp 11–13.
(6) Brock, N.; Potel, J. *Arzneim.-Forsch.* **1974**, *24*, 1149–1160. Botzenhard, V.; Lemeel, E.-M. *Ibid.* **1974**, *24*, 1167–1172.
(7) Cox, P. J.; Farmer, P. B.; Jarman, M.; Jones, M.; Stec, W. J.; Kinas, R. *Biochem. Pharmacol.* **1976**, *25*, 993–996.
(8) Connors, T. A.; Cox, P. J.; Farmer, P. B. *Biochem. Pharmacol.* **1974**, *23*, 115–129.
(9) Cox, P. J.; Phillips, B. J.; Thomas, P. *Cancer Res.* **1975**, *35*, 3755–3761.
(10) Draeger, U.; Peter, G.; Hohorst, H.-J. *Cancer Treat. Rep.* **1976**, *60*, 355–359.
(11) Cox, P. J.; Farmer, P. B.; Foster, A. B.; Griggs, L. J.; Jarman, M.; Kinas, R.; Pankiewicz, K.; Stec, W. J. *Biomed. Mass Spectrom.* **1977**, *4*, 371–375. Cox, P. J.; Farmer, P. B.; Jarman, M.; Kinas, R. W.; Stec, W. J. *Drug Metab. Dispos.* **1978**, *6*, 617–622. Milsted, R. A.; Jarman, M.; Smyth, J. F.; Kinas, R. W.; Pankiewicz, K.; Stec, W. J. *Proc. Am. Assoc. Cancer Res.* **1978**, *19*, 50.
(12) Tsui, Fai-Po; Brandt, J. A.; Zon, G. *Biochem. Pharmacol.* **1979**, *28*, 367–374.
(13) Farmer, P. B.; Jarman, M.; Facchinetti, T.; Pankiewicz, K.; Stec, W. J. *Chem.-Biol. Interact.* **1977**, *18*, 47–57. Abel, G.; Cox, P. J.; Farmer, P. B.; Haskins, N. J.; Jarman, M.; Merai, K.; Stec, W. J. *Cancer Res.* **1978**, *38*, 2592–2599.
(14) Kinas, R.; Pankiewicz, K.; Stec, W. J. *Bull. Acad. Pol. Sci., Ser. Sci. Chim.* **1975**, *23*, 981–984.
(15) Zon, G. *Tetrahedron Lett.* **1975**, 3139–3142.
(16) Zon, G.; Brandt, J. A.; Egan, W. *J. Natl. Cancer Inst.* **1977**, *58*, 1117–1119.
(17) Kawashima, T.; Kroshefsky, R. D.; Kok, R. A.; Verkade, J. G. *J. Org. Chem.* **1978**, *43*, 1111–1114.
(18) Adamiak, D. A.; Saenger, W.; Kinas, R.; Stec, W. J. *Angew. Chem.* **1977**, *89*, 336. Adamiak, D. A.; Saenger, W.; Kinas, R.; Stec, W. J. *Z. Naturforsch. C* **1977**, *32*, 672–677.
(19) Karle, I. L.; Karle, J. M.; Egan, W.; Zon, G.; Brandt, J. A. *J. Am. Chem. Soc.* **1977**, *99*, 4803–4807.
(20) Kinas, R.; Pankiewicz, K.; Stec, W. J.; Farmer, P. B.; Foster, A. B.; Jarman, M. *Bull. Acad. Pol. Sci., Ser. Sci. Chim.* **1978**, *26*, 39–42.
(21) **16** (of unspecified enantiomeric composition) has been previously isolated from human urine.[22] We have isolated crystalline **16** (in addition to **2**) from the urine of the patients and have determined enantiomer ratios by ^{31}P NMR (manuscript in preparation).
(22) Norpoth, K.; Müller, G.; Raidt, H. *Arzneim.-Forsch.* **1976**, *26*, 1376–1377.
(23) Brown, H. C.; Heim, P. *J. Am. Chem. Soc.* **1964**, *86*, 3566–3567.
(24) Kashman, Y.; Awerbough, O. *Tetrahedron* **1971**, 5593–5599.
(25) Zon, G.; Ludeman, S. M.; Egan, W. *J. Am. Chem. Soc.* **1977**, *99*, 5785–5795.
(26) Ludeman, S. M.; Zon, G.; Egan, W. *J. Med. Chem.* **1979**, *22*, 151.