

ratios. We feel this is the most critical evaluation of cell toxicity available.

**Viruses.** Rhinovirus types were obtained from the Research Resources Branch of the NIAID. The viruses were passed in WI-38 cells in our laboratory and titered in HeLa cells using plaque formation.

**X-ray Structure Determination.** The monohydrate of 5 crystallizes from methanol-water as colorless, highly refractive polyhedra in the centrosymmetric monoclinic space group,  $P2_1/n$ , with four molecules in a unit cell having the dimensions:  $a = 16.400 \pm 0.003 \text{ \AA}$ ;  $b = 10.045 \pm 0.003 \text{ \AA}$ ;  $c = 11.187 \pm 0.003 \text{ \AA}$ ;  $\beta = 106.32 \pm 0.02$ . The density calculated for  $C_{17}H_{18}N_4O_3S \cdot H_2O$  ( $M_r$  376.4) is  $1.41 \text{ g cm}^{-3}$ , and the density observed by flotation is  $1.42 \text{ g cm}^{-3}$ . The intensities of 2576 reflections, of which 170 were considered unobserved, were measured on a four-angle au-

tomated diffractometer, using monochromatic copper radiation. The structure was solved by direct methods, using the program MULTAN. All 26 of the nonhydrogen atoms (including the oxygen of the previously unsuspected water of hydration) showed up on the first  $E$  map. The structure was refined to an  $R$  factor of 0.080 using anisotropic temperature factors for the heavy atoms and isotropic temperature factors for the hydrogen atoms, which were placed at assumed positions.

**Acknowledgment.** The authors thank David W. Smith for computer assistance.

**Supplementary Material Available:** Table II, atomic coordinates and  $U_{ij}$  values; Table III, bond distances and bond angles (4 pages). Ordering information is given on any current masthead page.

## Synthesis and Antitumor Activity of Cyclophosphamide Analogues. 3.<sup>1</sup> Preparation, Molecular Structure Determination, and Anticancer Screening of Racemic *cis*- and *trans*-4-Phenylcyclophosphamide

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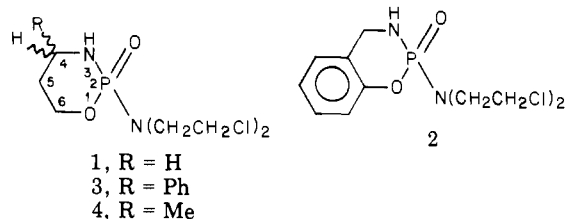
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Cyclization of racemic 3-amino-3-phenyl-1-propanol with bis(2-chloroethyl)phosphoramidic dichloride gave a diastereomeric mixture of 4-phenylcyclophosphamide (**3**), which was chromatographically separated into the faster and slower eluting components. A combination of  $^1\text{H}/^{31}\text{P}$  NMR and IR spectral data indicated that the faster and slower racemates correspond to *cis*-**3** (mp 129–130 °C) and *trans*-**3** (mp 112–114.5 °C), respectively. The molecular structure of the former compound was determined by X-ray crystallography and thereby unambiguously established the *cis* relationship between equatorially disposed phenyl and P=O substituents in a chair conformation. These results confirm the stereochemical assignments for *cis*- and *trans*-**3** which have been independently deduced by Y. E. Shih, J. S. Wang, and C. T. Chen [*Heterocycles*, 9, 1277 (1978)]. Anticancer screening tests against L1210 lymphoid leukemia in mice have revealed that, while both diastereomers of **3** afford toxic metabolites, *trans*-**3** led to therapeutic activity and *cis*-**3** did not. The relevance of these findings to results reported for 4-methylcyclophosphamide and cyclophosphamide is briefly discussed.

The clinical utility of racemic cyclophosphamide (**1**)



against a relatively broad spectrum of human cancers has prompted numerous investigations regarding the metabolism, mechanism of action, and influence of structural modification upon the therapeutic efficacy of this drug.<sup>2-4</sup> Metabolic details for **1** are not fully understood at present;

however, knowledge that enzymatic C-4 oxidation ("activation") is followed by competing toxification, detoxification, and delayed toxicity processes has allowed the conception of diverse strategies for predictably altering and/or improving chemotherapeutic activity. The consequences of lowering the oxidation potential of the C-4 position by modifying the structure of **1** has been of interest to us and led to the synthesis of 5,6-benzocyclophosphamide (**2**) as a candidate system;<sup>5</sup> however, lack of activity for **2** against L1210 lymphoid leukemia in mice diverted our attention to its exocyclic cognate, 4-phenylcyclophosphamide (**3**), 2-[bis(2-chloroethyl)amino]-4-phenyl-2H-1,3,2-oxazaphosphorinane 2-oxide.

Monosubstitution at C-4 in racemic **1** generates a second chiral center, and the resultant diastereomeric racemates, which may simply be referred to as *cis*- and *trans*-**3** (*cis* = *RS/SR*; *trans* = *RR/SS*),<sup>6</sup> were of further interest relative to stereochemical studies with enantiomers of **1**<sup>7,8</sup> and

(1) For paper 2, see S. M. Ludeman, G. Zon, and W. Egan, *J. Med. Chem.*, **22**, 151 (1979).

(2) D. L. Hill, "A Review of Cyclophosphamide", Charles C. Thomas, Springfield, Ill., 1975.

(3) O. M. Friedman, A. Myles, and M. Colvin in "Advances in Cancer Chemotherapy", A. Rosowsky, Ed., Marcel Dekker, New York, 1979, pp 143–204.

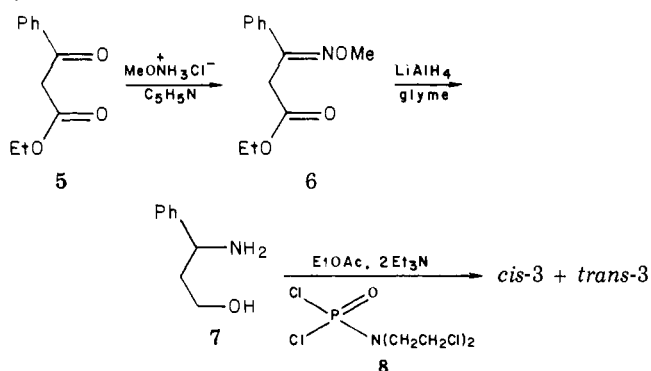
(4) M. Colvin in "Clinical Pharmacology of Anti-Neoplastic Drugs", H. M. Pinedo, Ed., Elsevier/North-Holland Biomedical Press, 1978, pp 245–261.

(5) S. M. Ludeman and G. Zon, *J. Med. Chem.*, **18**, 1251 (1975).

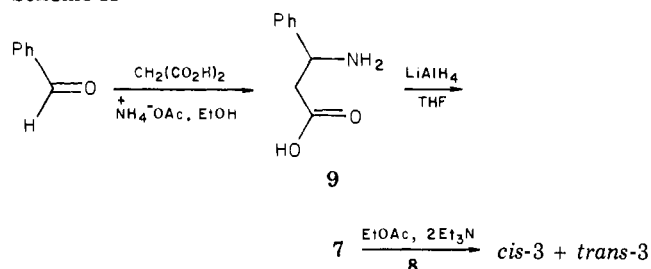
(6) The *cis* and *trans* relationships refer to the P=O and phenyl substituents, in accordance with IUPAC rules for nomenclature [*J. Org. Chem.*, **35**, 2849 (1970)].

(7) M. Jarman, R. A. V. Milsted, J. F. Smyth, R. W. Kinan, K. Pankiewicz, and W. J. Stec, *Cancer Res.*, **39**, 2762 (1979), and references to earlier work cited therein.

## Scheme I



## Scheme II



diastereomeric 4-methylcyclophosphamides (4).<sup>9-12</sup> During the latter stages of our work with *cis*- and *trans*-3, Shih et al.<sup>13</sup> published the synthesis of a series of 4-aryl-cyclophosphamides, which included 3, and suggested assignments of *cis* and *trans* relationships based upon a generalized spectroscopic argument. We now wish to report the results of our investigations with 3 which include the direct determination of molecular structure by X-ray crystallography and the first comparative assessment of *in vivo* anticancer activity for the *cis* and *trans* diastereomers.<sup>14</sup> The structural findings are in accord with a number of reported spectroscopic correlations, while the screening results contrast with those found for *cis*- and *trans*-4.

## Results and Discussion

**Synthesis, Spectroscopic Analysis, and X-Ray Data.** Preparation of racemic *cis*- and *trans*-3 according to Scheme I takes advantage of a new and improved method<sup>16</sup> for  $\gamma$ -amino alcohol synthesis utilizing 1,3-dicarbonyl compounds as starting materials. Treatment of ethyl benzoylacetate (5) with *O*-methylhydroxylamine hydrochloride and pyridine gave a 75% yield of intermediate oxime 6, which was subsequently reacted with  $\text{LiAlH}_4$  to give 3-amino-3-phenyl-1-propanol (7) in 79% yield.<sup>16</sup>

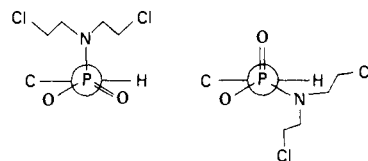
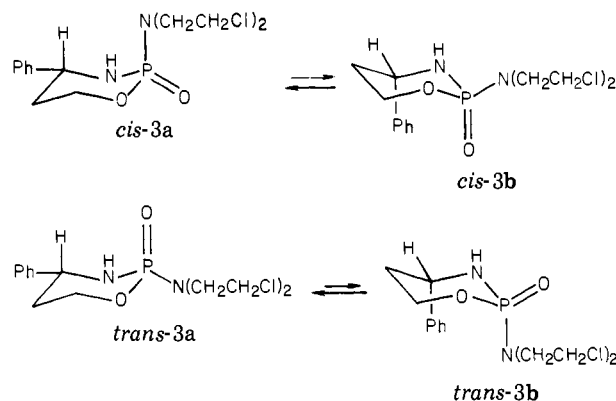


Figure 1. Newman-type projections along the endocyclic P-N bond in *cis*-3a (on the left) and *trans*-3a (on the right).

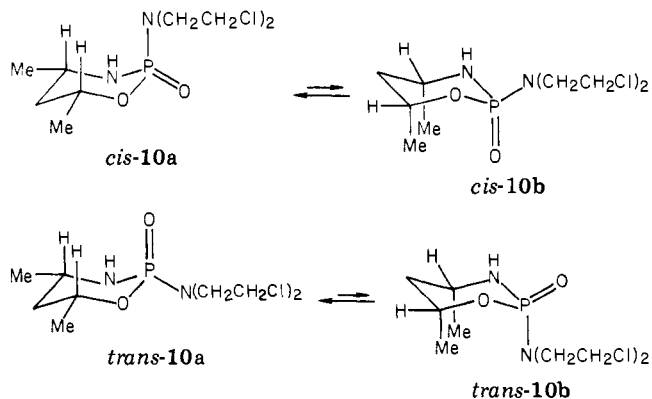
Cyclization of 7 with bis(2-chloroethyl)phosphoramidic dichloride (8) in the presence of 2 equiv of  $\text{Et}_3\text{N}$  afforded crude 3, which was chromatographed on silica gel with  $\text{EtOAc}$  to give analytically pure crystals of the faster ( $R_f$  0.47; mp 129–131 °C) and slower ( $R_f$  0.23; mp 112–114.5 °C) eluting diastereomers of 3 in nearly quantitative yield. Scheme II outlines the route to 3 published by Shih et al.,<sup>13</sup> wherein condensation of benzaldehyde with malonic acid to give  $\beta$ -aminocarboxylic acid 9 (52%) was followed by  $\text{LiAlH}_4$  reduction to 7 (86%) for an overall yield of 45% that is somewhat less than the 59% efficiency for 5  $\rightarrow$  7.

Assignment of *cis*- and *trans*-3 structures to the faster



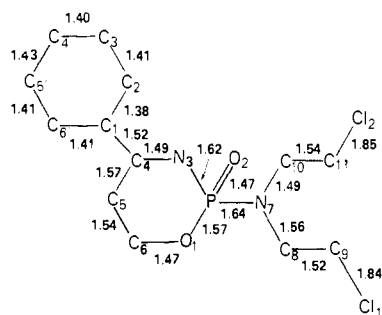
and slower eluting diastereomeric cyclization products has been suggested<sup>13</sup> on the basis of a correlation between their relative P=O stretching frequencies (1230 and 1212  $\text{cm}^{-1}$ , respectively) and substituent stereochemistry.<sup>12</sup> Our measurements with 3 indicated a significantly smaller difference between these IR absorption frequencies (1232 vs. 1228  $\text{cm}^{-1}$ ); nevertheless, the following observations were judged by us to be consistent with the suggested configurational assignments.

The *cis* and *trans* diastereomers of 3 should each exist in primarily one solution conformer (*cis*- and *trans*-3a) due to the greater equatorial preference of the phenyl group and, consequently, should closely resemble the *cis* and *trans* diastereomers of 10, which have been shown<sup>15</sup> to



adopt conformations with both methyl groups equatorial (*cis*- and *trans*-10a). These conformationally biased model compounds give rise to <sup>31</sup>P NMR chemical shifts<sup>15</sup> at 10.2 and 12.9 ppm, whereas the proposed *cis* and *trans* dia-

- (8) F. P. Tsui, J. A. Brandt, and G. Zon, *Biochem. Pharmacol.*, **28**, 367 (1979), and references to earlier work cited therein.
- (9) R. F. Struck, M. C. Thorpe, W. C. Coburn, Jr., and M. C. Kirk, *Cancer Res.*, **35**, 3160 (1975).
- (10) P. J. Cox, P. B. Farmer, and M. Jarman, *Biochem. Pharmacol.*, **24**, 599 (1975).
- (11) P. B. Farmer, M. Jarman, T. Facchinetti, K. Pankiewicz, and W. J. Stec, *Chem.-Biol. Interact.*, **18**, 47 (1977).
- (12) R. Kinas, K. Pankiewicz, W. J. Stec, P. B. Farmer, A. B. Foster, and M. Jarman, *J. Org. Chem.*, **42**, 1650 (1977).
- (13) Y. E. Shih, J. S. Wang, and C. T. Chen, *Heterocycles*, **9**, 1277 (1978).
- (14) Professor N. D. Heindel (Lehigh University) has kindly informed us of his independent synthesis of 3 and intentions of publishing these studies at a later date.
- (15) D. W. White, D. E. Gibbs, and J. G. Verkade, *J. Am. Chem. Soc.*, **101**, 1937 (1979).
- (16) H. V. Secor and E. B. Sanders, *J. Org. Chem.*, **43**, 2539 (1978).



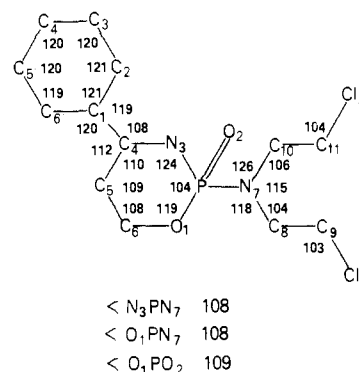
**Figure 2.** Bond lengths for *cis*-3. For simplicity, the bond distances have been given to two figures after the decimal point; however, the actual estimated standard deviations range between 0.005 and 0.015 Å.

stereomers of **3** resonate at 8.8 and 13.1 ppm, respectively. The detailed origin of this type of empirical relationship between  $^{31}\text{P}$  NMR chemical shifts and axial vs. equatorial orientations of the phosphorus substituents in 1,3,2-oxazaphosphorinane 2-oxides is not fully understood; however, it appears to hold for *cis*- and *trans*-4<sup>12</sup> (11.0 and 13.5 ppm, respectively) as well as various 1,3,2-dioxaphosphorinane 2-oxides.<sup>17,18</sup>

Further spectroscopic evidence derives from the  $^1\text{H}$  NMR chemical-shift difference between the NH resonances at 2.98 and 2.68 ppm for the faster and slower eluting diastereomers of **3**, respectively. The substantial deshielding (0.3 ppm) thus exhibited by the faster moving compound suggests more efficient intramolecular H bonding to the adjacent P=O functionality, which would be the case for *cis*-**3** as is illustrated by comparative Newman-type projections along the endocyclic P-N bond shown in Figure 1. This difference in H bonding was also suggested by the very broad IR absorption centered at 3450  $\text{cm}^{-1}$  for the NH group in the proposed *cis*-**3** compound, as opposed to the relatively sharp NH band for *trans*-**3** centered at 3120  $\text{cm}^{-1}$ . Operation of such four-membered ring intramolecular H bonding in **1** has been thoroughly discussed<sup>15</sup> and is also present in analogous phosphoramidates.<sup>19</sup>

While the foregoing NMR and IR data are consistent with *cis* and *trans* geometries for the faster and slower eluting diastereomers of **3**, alternative interpretations and counterarguments can be offered. It was necessary, therefore, to establish these structures directly by X-ray crystallographic methods and, consequently, provide an unambiguous test of the assignments based on spectroscopic data.

A crystal of the faster eluting diastereomer of **3** suitable for X-ray analysis was obtained by recrystallization from  $\text{CHCl}_3\text{-Et}_2\text{O}$ . The compound crystallizes in the monoclinic space group  $P2_1/c$  with  $Z = 4$  and cell dimensions  $a = 7.998(1)$ ,  $b = 10.280(2)$ ,  $c = 19.998(4)$  Å,  $\beta = 102.12(1)^\circ$ . The observed density (floatation) of 1.39  $\text{g cm}^{-3}$  agrees with the calculated value of 1.393  $\text{g cm}^{-3}$ . The structure was solved by direct methods. A preliminary least-squares refinement on all atoms except hydrogen was carried out and gave an  $R$  value of 0.090 for all reflections. Bond distances, angles, and a stereoscopic view of the molecule are presented in Figures 2-4. Figure 4 clearly reveals the expected chair-like structure with an equatorially disposed phenyl substituent.<sup>20</sup> More importantly, the X-ray



**Figure 3.** Bond angles for *cis*-3. For simplicity, the bond angles have been rounded off to three significant figures; however, the estimated standard deviations were found to range between 0.3 and 0.8°.

structure is seen to possess a *cis* relationship between the equatorial phenyl and P=O moieties, which confirms the assignment reported by Shih et al.<sup>13</sup> and independently deduced herein. The difference Fourier map reveals the N-H hydrogen atom which participates in an intermolecular H bond to the P=O moiety of a centrosymmetrically related molecule. A final refinement on the complete model which will include this hydrogen atom and all C-H hydrogen atoms will be carried out, and a complete structural paper including all positional parameters will be published elsewhere.

**Anticancer Screening Data.** The *in vivo* anticancer activity of diastereomerically pure (>99.5%,  $^{31}\text{P}$  NMR) samples of *cis*- and *trans*-**3** was evaluated against L1210 lymphoid leukemia in mice according to National Cancer Institute standard protocol for analogues of **1**.<sup>21</sup> Test samples were administered intraperitoneally as aqueous solutions on day 1 only, at various doses, and results were evaluated on day 30. Mean survival time was used as the evaluation parameter, and compounds exhibiting a test/control (T/C) percentage >125 are considered to be active in this preliminary testing system.<sup>22</sup>

The toxicity day survivors data for *cis*- and *trans*-**3** in Table I indicates that both of these diastereomers give rise to toxic metabolites, presumably via C-4 oxidation and ultimate release of phosphoramidate mustard. A more significant finding is that *trans*-**3** is therapeutically active while *cis*-**3** is not. These results contrast with L1210 screening data for racemic *cis*- and *trans*-**4**, which showed no appreciable difference in activity of the isomers, and led to the suggestion of essentially equal facility for "activation" by mouse liver microsomes.<sup>9</sup> More recent screening studies with the diastereomeric racemates of **4** against ADJ/PC6 mouse plasma cell tumor have also found very similar therapeutic indices, and the extent of their metabolism by isolated rat liver microsomes was reported to be comparable.<sup>11</sup> The complexity of the metabolism of **1** and its derivatives precludes, at this stage,

(17) J. G. Verkade, *Phosphorus Sulfur*, **2**, 251 (1976).

(18) J. A. Mosko and J. G. Verkade, *J. Org. Chem.*, **42**, 1549 (1977).

(19) L. C. Thomas, "Interpretation of the Infrared Spectra of Organophosphorus Compounds", Heyden & Sons, London, 1974.

(20) For recent NMR and X-ray studies of a novel 1,3,2-oxazaphosphorinane 2-oxide twist conformation in both solution and solid phase, see G. S. Bajwa, W. G. Bentrude, N. S. Pantaleo, M. G. Newton, and J. H. Hargis, *J. Am. Chem. Soc.*, **101**, 1602 (1979).

(21) R. I. Geran, N. H. Greenberg, M. M. Macdonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep., Part 3*, **3** (2), 1 ff. (1972).

(22) Instruction 14, "Screening Data Summary Interpretation and Outline of Current Screen", Drug Evaluation Branch, Drug Research and Development, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD, 1973. Consult all subsequent insert pages for most recent updating.

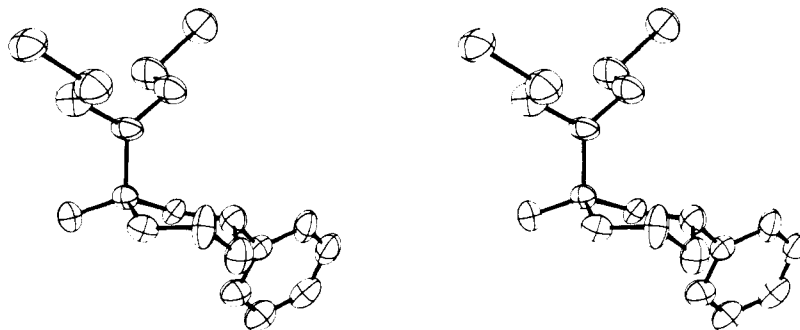


Figure 4. A stereoscopic view (ORTEP) for *cis*-3. Thermal ellipsoids are drawn at the 50% level of probability.

Table I. Anticancer Screening Data for Racemic *cis*- and *trans*-4-Phenylcyclophosphamide (3) against Mouse L1210 Lymphoid Leukemia

compd <sup>a</sup>	NSC no.	dose, <sup>b</sup> mg/kg	toxicity day survivors <sup>c</sup>	T/C <sup>d</sup>
<i>cis</i> -3	313676	500.00	5/6	108
		250.00	6/6	105
		125.00	6/6	94
		62.50	6/6	109
		31.30	6/6	106
		15.63	6/6	103
<i>trans</i> -3	313675	500.00	3/6 (5/6) <sup>e</sup>	f (220) <sup>e</sup>
		250.00	5/6 (6/6)	149 (120)
		125.00	6/6 (6/6)	124 (106)
		62.50	6/6 (6/6)	106 (104)
		31.30	6/6	100
		15.63	6/6	100
<i>cis</i> - and <i>trans</i> -3 (ca. 1:1)	306110	500.00	4/6	204
		250.00	6/6	122
		125.00	6/6	120
		62.50	6/6	105

<sup>a</sup> All compounds are racemic. <sup>b</sup> Day 1 intraperitoneal injection of  $10^5$  cells in an aqueous media. <sup>c</sup> Number of survivors on day 5 for each group of female mice. <sup>d</sup> Test/control evaluation incorporating mean survival time over 30 days. <sup>e</sup> Values in parentheses refer to male mice. <sup>f</sup> Not determined.

a detailed interpretation of these results for 3 and 4; however, it is reasonable to speculate that the steric inequity between phenyl and methyl is an important factor. Thus, for example, the diastereomers of 3 may be conformationally restricted, relative to those of 4, and may therefore be subject to subtle selectivity factors during enzymatic "activation". In this regard it is interesting to note that the dominant solution conformer of 1, which has been shown<sup>15</sup> by NMR to have axial P=O and equatorial N(CH<sub>2</sub>CH<sub>2</sub>Cl)<sub>2</sub> substituents, has been tentatively suggested<sup>15</sup> to be enzymatically activated more efficiently than its equatorial P=O/axial N(CH<sub>2</sub>CH<sub>2</sub>Cl)<sub>2</sub> conformational counterpart. Assuming that *cis*- and *trans*-3a are dominant conformations in solution, then the aforementioned toxicity findings indicate that enzymatic selectivity between the relative configurations at phosphorus is not very large, with the somewhat higher toxicity of *trans*-3 being consistent with the suggested<sup>15</sup> enzymatic activity difference.

With the limited amount of testing data currently available for 3, it is difficult to comment on the significance of the higher T/C value in female mice obtained with an ca. 1:1 mixture of *cis*-3/*trans*-3 vs. that for pure *trans*-3 (see Table I), as slight biological variations could account for the difference.

In order to further probe the influence of C-4 substitution on anticancer activity of cyclophosphamide analogues, we have begun the synthesis of the 4-hydroxy metabolites of *cis*- and *trans*-3 for enzyme "activation" kinetics and

multinuclear NMR studies of their solution chemistry.

### Experimental Section

Melting points were obtained with a Thomas-Hoover capillary melting point apparatus and are uncorrected. Elemental analyses were performed by Chemalytics, Inc. IR measurements were obtained with KBr disks using a Perkin-Elmer Model 337 spectrometer. <sup>1</sup>H NMR spectra at 220 MHz were obtained in the continuous-wave mode on a Varian HR 220 spectrometer. <sup>31</sup>P NMR spectra were obtained with a JEOL FX-100 instrument; the accumulated free-induction decay signal (8132 data points) from 90° pulses with <sup>1</sup>H decoupling was sampled over a 5-kHz spectra window and was zero-filled and exponentially multiplied (1-Hz broadening) prior to Fourier transform. A coaxial insert containing 85% H<sub>3</sub>PO<sub>4</sub> in D<sub>2</sub>O was used as an external chemical-shift reference. TLC analysis utilized 2.5 × 10 cm plates with a 250-μm layer of silica gel GF, while column chromatography employed Baker 60–200 mesh silica gel. The reported R<sub>f</sub> values are approximate.

**2-[Bis(2-chloroethyl)amino]-4-phenyl-2H-1,3,2-oxazaphosphorinane 2-Oxide (4-Phenylcyclophosphamide, 3).** A freshly prepared<sup>16</sup> sample of 3-amino-3-phenyl-1-propanol (7; 9.5 g, 0.063 mol) was dissolved in EtOAc (300 mL) containing Et<sub>3</sub>N (17.5 mL, 0.126 mol), and a solution of bis(2-chloroethyl)phosphoramidic dichloride (8; 16.3 g, 0.063 mol) in EtOAc (100 mL) was then added dropwise (30 min) with vigorous stirring. After 72 h of continued stirring, the reaction mixture was suction filtered and the filtrate was concentrated at reduced pressure to give the crude product (ca. 100%) as a pale yellow oil. Column chromatography using EtOAc led to isolation of fractions containing faster eluting *cis*-3 (R<sub>f</sub> 0.47) and slower eluting *trans*-3 (R<sub>f</sub> 0.23). Combined fractions of *cis*- and *trans*-3 were concentrated, and the residue in each case was dissolved in a minimal amount of CHCl<sub>3</sub>, diluted with 3–5 volumes of Et<sub>2</sub>O, and was then kept at 5 °C to give crystals with mp 129–131 °C (lit.<sup>13</sup> mp 130.5–132 °C) and 112–114.5 °C (lit.<sup>13</sup> mp 114–116 °C), respectively. For *cis*-3: <sup>1</sup>H NMR (CDCl<sub>3</sub>/Me<sub>4</sub>Si) δ 2.00–2.25 (m, 2, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 2.98 (br s, 1, NH), 3.30–3.59 (m, 4 H, NCH<sub>2</sub>), 3.68 (t, J = 7 Hz, 4, CH<sub>2</sub>Cl), 4.14–4.32 (m, OCH<sub>A</sub>H<sub>B</sub>), 4.32–4.50 (m, 1, OCH<sub>A</sub>H<sub>B</sub>), 4.55–4.73 (m, 1, NCHPh), 7.23–7.45 (m, 4, *o*- and *m*-Ph), 7.45–7.59 (d, J = 7 Hz, *p*-Ph); <sup>31</sup>P NMR (CDCl<sub>3</sub>/ext H<sub>3</sub>PO<sub>4</sub>) δ 8.8; IR (KBr disk) 3700–3200 and 3160 (N–H), 1232 (P=O) cm<sup>-1</sup>. Anal. (C<sub>13</sub>H<sub>19</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>2</sub>P) C, H, N. For *trans*-3: <sup>1</sup>H NMR (CDCl<sub>3</sub>/Me<sub>4</sub>Si) δ 1.82–2.11 (m, 2, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 2.68 (s, 1, NH), ~3.4–3.6 (m, 4, NCH<sub>2</sub>), 3.66 (t, J = 7 Hz, 4, CH<sub>2</sub>Cl), 4.18–4.43 (apparent d of d with ~10-Hz spacing and additional small coupling, 1 H), 4.50–5.75 (apparent d of d with ~10–20-Hz spacing and additional small coupling, 2 H), 7.36 (br s, 5 H, Ph); <sup>31</sup>P NMR (CDCl<sub>3</sub>/ext H<sub>3</sub>PO<sub>4</sub>) δ 13.1; IR (KBr disk) 3120 (N–H) and 1228 (P=O) cm<sup>-1</sup>. Anal. (C<sub>13</sub>H<sub>19</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>2</sub>P) C, H, N.

**Acknowledgment.** This investigation was supported in part by Research Grant CA-21345 to G.Z. from the National Institutes of Health, PHS/DHEW. <sup>1</sup>H NMR spectra were obtained at the National Institutes of Health Laboratory of Chemical Physics and <sup>31</sup>P NMR spectra were obtained at the Food and Drug Administration Bureau of Biologics. The X-ray structure determination described in this paper was supported by the Food and Drug Administration, FDA–NBS Interagency Agreement 224-75-8253.