Synthesis and Antitumor Activity of Cyclophosphamide Analogues. 2.[1] Preparation, Hydrolytic Studies, and Anticancer Screening of 5-Bromocyclophosphamide, 3,5-Dehydrocyclophosphamide, and Related Systems

Susan Marie Ludeman, Gerald Zon,* and William Egan[2]

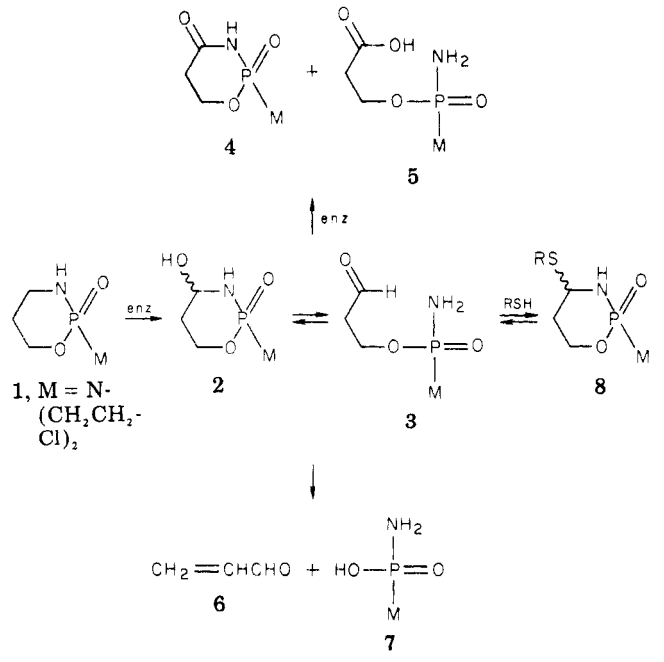
Maloney Chemical Laboratory, The Catholic University of America, Washington, D.C. 20064. *Received July 21, 1978*

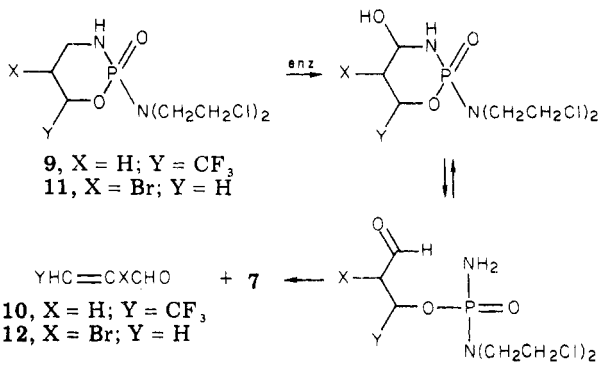
Reaction of 3-amino-2-bromopropanol hydrobromide (**22**) with 3 molar equiv of triethylamine in the presence of phenylphosphonic dichloride [$C_6H_5P(O)Cl_2$], phenyl dichlorophosphate [$C_6H_5OP(O)Cl_2$], or bis(2-chloroethyl)-phosphoramidic dichloride [$(ClCH_2CH_2)_2NP(O)Cl_2$] afforded low to moderate yields (12–34%) of 5-bromo-1,3,2-oxazaphosphorinane 2-oxide derivatives having phenyl (**23**), phenoxy (**24**), or bis(2-chloroethyl)amino (**11**) substituents bonded to phosphorus at the 2 position of this heterocyclic ring system which is common to the anticancer drug cyclophosphamide (**1**). In each of these cases, two diastereomers (cis/trans isomers) were separable by chromatography; however, the relative stereochemical relationships between the C-5 bromine and phosphorus substituents could not be assigned on the basis of ^1H NMR spectra. Treatment of the experimental anticancer drug isophosphamide (**27**) with sodium hydride (NaH) led to a high yield (84%) of 2-aziridinyl product **29** rather than the fused bicyclic material **26**. This preference for "3-exo-tet" rather than "5-exo-tet" intramolecular cyclization was utilized to convert **11** (5-bromocyclophosphamide) into 3,5-dehydrocyclophosphamide (**13**), and the generality of such a closure process was briefly studied by carrying out similar NaH reactions with 4,5-benzocyclophosphamide (**32**) and a 1,3-diazacyclophosphamide analogue (**33**). The hydrolytic behavior of **11**, **13**, **23**, and **24** was examined by ^1H NMR techniques, and it was deduced that unbuffered hydrolysis of **11** does not proceed by initial formation of **13**. Ring systems **13** and, surprisingly, **23** were found to be quite sensitive to catalytic hydrolysis. In vivo screening tests against L1210 lymphoid leukemia in mice with samples of **13**, **23**, **24**, **26**, and **29** were uniformly negative [test/control (T/C) percentage < 125], except for a diastereomer of **11** (**11a**) which gave a value for T/C = 146. This lower level of anticancer activity relative to **1** was also reflected in the lower therapeutic index ($TI = LD_{50}/ED_{90}$) obtained for **11** (25) as compared to **1** (95) against the ADJ/PC6 mouse plasma cell tumor. Of the aforementioned screening samples, only diastereomer **13a** showed activity in vitro (KB cell culture, 2.2 μg/mL), and the marked toxicity of **13a** without microsomal activation was evident from growth-inhibition tests with Walker 256 cells, which gave ID_{50} = 1.5 μg/mL. This toxicity level for **13a** is comparable to that observed with microsomally activated **1** (ID_{50} = 0.5–1.0 μg/mL) and is thus ascribed to the facile hydrolytic conversion of **13a** into an active phosphoramide mustard alkylating agent.

Cyclophosphamide (**1**) is a well-known antineoplastic agent which provides therapeutic activity against a relatively broad spectrum of human cancers.[3] The primary metabolic transformations for **1** are shown in Scheme I, and it has been proposed[4] that the cytotoxic selectivity of this drug toward cancerous, as opposed to normal, cells may result from rate inequities between the competing enzymatic detoxification and nonenzymatic toxification pathways involving 4-hydroxycyclophosphamide (**2**) and aldophosphamide (**3**), viz., **2**/**3** → **4**/**5** and **6**/**7**, respectively. Two other factors which may be of significance with regard to the marked oncostatic selectivity of **1** are the reversible conversion of labile metabolites **2** and **3** into thioether conjugates **8**[5,6] and stereospecific phenomena, e.g., enzyme reactions, involving (+)-(*R*)-**1**,[7] (−)-(*S*)-**1**,[8] and the chiral metabolites **2**–**5** and **8** derived from each of these enantiomers of **1**.[9-11]

Structural cognates of **1** have been studied as an indirect means of both elaborating the molecular details associated with the anticancer activity of **1** and, hopefully, providing new and improved drugs for cancer chemotherapy. For example, investigations with C-4 through C-6 methylated[12] and deuterated[13] analogues of **1** have led to, inter alia, substantial metabolic insights, while the synthetic accessibility of 4-hydroperoxycyclophosphamide affords a convenient entry into "preactivated" systems that are of clinical interest.[14,15] Our previously reported[1] investigation of 5,6-benzocyclophosphamide somewhat paralleled the latter approach in that 5,6-benzo annelation of **1** provides oxidatively reactive C-4 benzylic hydrogens for facilitated liver-enzyme oxidation; however, this structural activation approach gave negative results in preliminary screening tests. In 1975, Farmer and Cox[16] reported the use of 6-(trifluoromethyl)cyclophosphamide (**9**) to generate β-(trifluoromethyl)acrolein (**10**) which was regarded[16] as a metabolite of greater toxicity, relative to acrolein (**6**, cf. Scheme I), due to the enhanced electrophilic character of

Scheme I

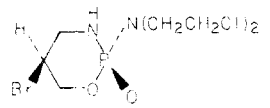




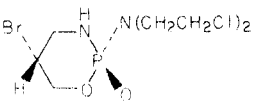
0022-2623/79/1822-0151$01.00/0

the conjugated system. A similar rationale was operative during our concurrent work with 5-bromocyclophosphamide (**11**), since **11** could reasonably be expected to undergo C-4 oxidation and ultimately yield α-bromoacrolein (**12**) by analogy to the formation of **6** from **1**. Alternative novel metabolites might include an electrophilic epoxide produced via cyclization of 4-hydroxy-**11** and/or a tris-alkylating agent generated by endocyclic P–N bond hydrolysis.

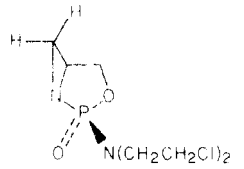
The present paper reports the synthesis and separation of cis and trans diastereomers of **11** and the chemical



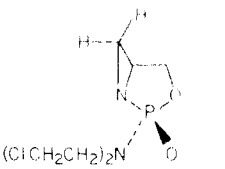
cis-**11**



trans-**11**



exo-**13**

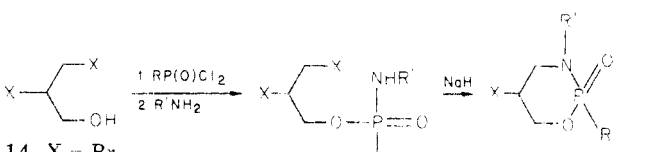


endo-**13**

conversion of **11** into diastereomeric *exo*- and *endo*-3,5-dehydrocyclophosphamides (**13**), which are of interest because of their inherent ring strain.[17] In vivo and in vitro anticancer screening results for these compounds and a number of related systems are briefly discussed with regard to structure, hydrolytic stability, and pertinent literature data.

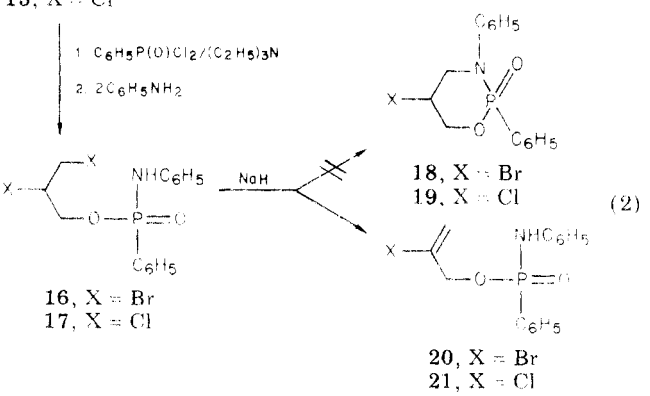
Results and Discussion

Synthesis of 5-Bromocyclophosphamide (11), 3,5-Dehydrocyclophosphamide (13), and Related Systems. Our initial synthetic approach to **11** and related 5-halo-1,3,2-oxazaphosphorinane 2-oxide derivatives is represented by eq 1 and was predicated on reports by Savignac



14, X = Br
15, X = Cl

(1)



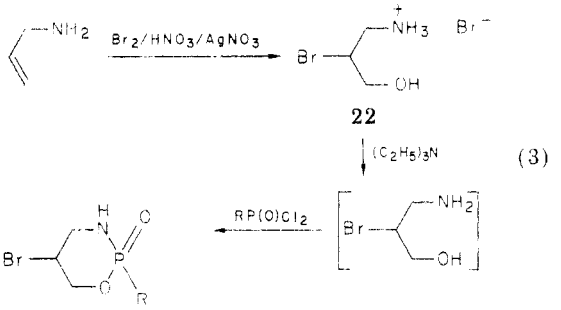
16, X = Br
17, X = Cl

18, X = Br
19, X = Cl

20, X = Br
21, X = Cl

(2)

and co-workers[18] in which sodium hydride (NaH) was employed for analogous cyclizations. Preliminary investigations regarding this synthetic plan were carried out according to eq 2, and it was found that treatment of intermediates **16** and **17** with NaH did not cause the expected intramolecular displacement to give cyclic products **18** and **19** but instead led to 1,2-dehydrohalogenation, which afforded olefins **20** and **21**. It is assumed that initial generation of a sodium anilide is followed by inter- and/or intramolecular proton abstraction with concommitant loss of the terminal halide ion; however, the steric and electronic factors which favor CHX removal over CH_2O are not obvious to us. A more reliable cyclization scheme was pursued using 3-amino-2-bromopropanol hydrobromide (**22**) and triethylamine to generate 3-amino-2-bromopropanol in situ (eq 3). In the presence



22

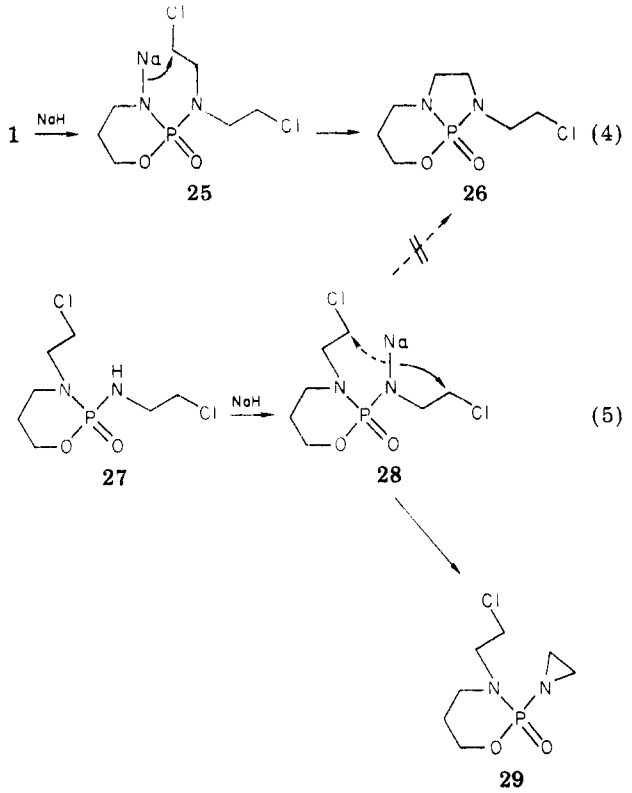
(3)

23, R = C_6H_5
24, R = OC_6H_5
11, R = $N(CH_2CH_2Cl)_2$

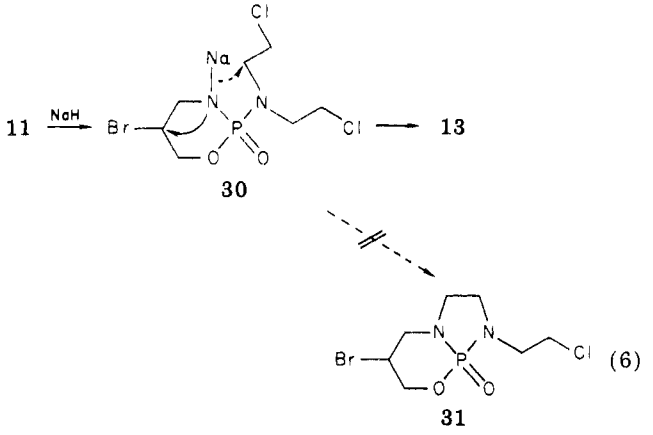
of phenylphosphonic dichloride, $C_6H_5P(O)Cl_2$, the unstable 2-haloethylamine could be successfully trapped via formation of product **23**; however, the combined yield of only 15% for the chromatographically separated cis and trans diastereomers of **23** is most likely due to competing intramolecular cyclization of free 3-amino-2-bromopropanol to the aziridine system. The observation of only six[19] carbon NMR peaks for the minor (slower eluting) isomer of **23** at both 40 and -60 °C is consistent with relatively rapid ring inversion on the NMR time scale and is, therefore, analogous to experimental[20] and theoretical[21] findings for **1**. While persuasive spectroscopic arguments have been recently offered for assigning the relative (cis vs. trans) stereochemical relationships in diastereomeric 4-methylcyclophosphamides,[12c] we were unable to decipher those features in the high-resolution (220 MHz) ^1H NMR spectra for **23**, which might be indicative of the relative stereochemistry between the C-5 and phosphorus substituents. Consequently, the faster and slower eluting diastereomers of **23** are referred to as **23a** and **23b**, respectively.

Reactions of phenyl dichlorophosphate, $C_6H_5OP(O)Cl_2$, and bis(2-chloroethyl)phosphoramidic dichloride, $(ClCH_2CH_2)_2NP(O)Cl_2$, with **22** (eq 3) gave, respectively, diastereomeric mixtures of products **24** (12%) and **11** (34%). In each case, careful silica gel chromatography afforded faster (**24a** and **11a**) and slower (**24b** and **11b**) eluting components which were shown by TLC and/or NMR to be diastereomerically pure (≥95%). As with **23**, the observation of single sets of ^1H NMR absorption signals for diastereomers of **24** and **11** at 20 °C is consistent with conformationally mobile 1,3,2-oxazaphosphorinane 2-oxide frameworks. The proton spectra for diastereomeric samples of **24** and **11** exhibited a high degree of complexity at 220 MHz, and telling features concerning cis vs. trans relationships were not apparent.

During the course of our previous investigations concerning the hydrolytic chemistry of **1**,[22] it was established that reaction of anhydrous **1** with NaH led to the isolation of fused-bicyclic product **26**, which results from intramolecular alkylation of the indicated intermediate sodium salt **25** shown in eq 4. By way of contrast, the presently reported work has shown that isophosphamide (**27**) undergoes NaH-induced cyclization to afford an ca. 85% isolated yield of aziridinyl system **29** rather than **26** (eq 5). The highly regioselective mode of ring closure thus exhibited by sodium derivative **28** is consistent with

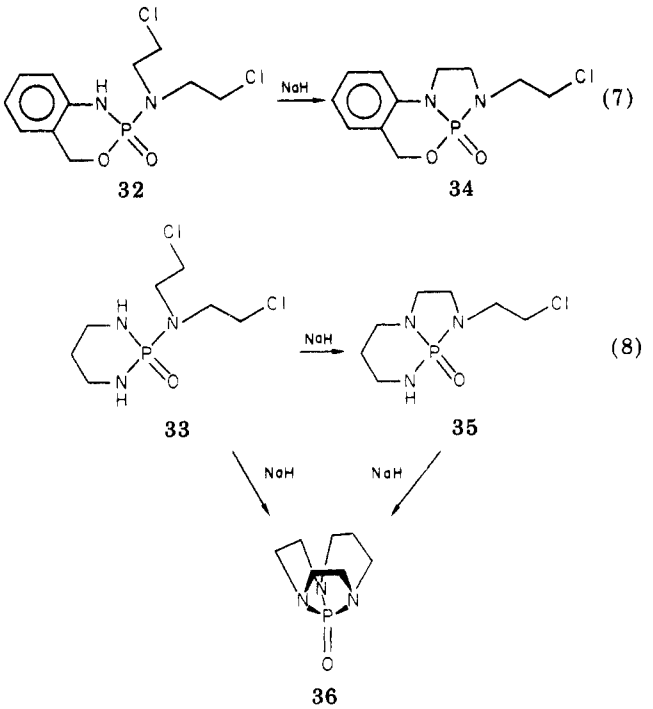


reported[23] rules for cyclization reactions, viz., that "3-exo-tet" processes (28 → 29) are favored over "5-exo-tet" transformations (28 → 26). These findings led to the prediction that treatment of 11 with NaH would be followed by closure of intermediate 30 in a 3-exo-tet fashion to give 13 rather than 5-exo-tet cyclization to yield ring system 31 (eq 6). Consideration of the time-average planar

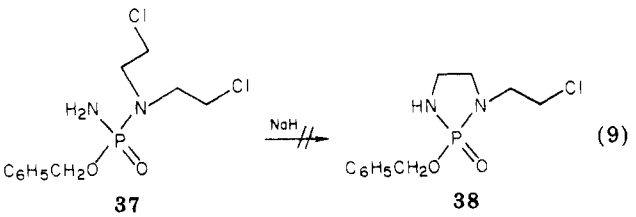


geometry about the anionic nitrogen position in 30 and the conformational mobility of this heterocyclic intermediate suggested further that 3-exo-tet closure rates for diastereomers 11a and 11b should be roughly comparable. Hence, an ca. 2:1 mixture of 11a/11b was reacted with NaH, and subsequent thick-layer or column chromatography on silica gel provided for the separation of faster (13a) and slower (13b) eluting components which were isolated in the expected 2:1 ratio (10:6%). Both of these components exhibited high-resolution ^1H and ^{13}C NMR spectra that were consistent with the expected bicyclic aziridinyl ring structure (13). The proton spectrum in $CDCl_3$ for 13b exhibited an eight-line multiplet (AM portion of an AMX spin system) for the aziridinyl CH_2N nuclei, with a $\Delta\nu_{AM}$ value which was significantly greater than the corresponding chemical-shift difference for these nuclei in 13a (80 vs. 15 Hz). Based upon the reported[24,25] anisotropy effects for a P=O group, we therefore assume that 13b has the assigned endo structure in which the P=O can exert a greater influence upon the chemical-shift difference between *H*a and *H*m.[26]

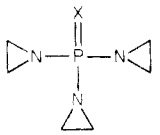
The 5-exo-tet closure reaction initially observed with 1 was briefly explored as a synthetic method for obtaining additional phosphoramides that possessed relatively uncommon heterocyclic skeletons. Given the structural similarities between 1, 32, and 33 (cf. eq 7 and 8), it was



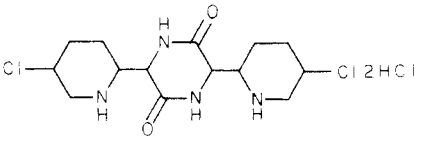
not surprising that NaH treatment of the latter two compounds led to the respective isolation of products 34 (9%) and 35 (67%), which were identified solely on the basis of high-resolution ^1H NMR spectra due to their instability. A more notable set of transformations involves the NaH-induced reactions of either 33 or 35 to yield the same product that was tentatively assigned (^1H NMR) tricyclic structure 36; however, the spectral complexity, small quantities, and instability of this material prevented its unambiguous identification as the interesting "bowl-shaped" molecule 36. As a final point with regard to these cyclization processes, it appears that the endocyclic, and therefore rotationally-restricted, nature of the reacting nitrogen center in 1, 32, 33, and 35 favors the 5-exo-tet closures shown in eq 7 and 8. This conclusion follows from our failure to convert acyclic starting material 37 into isolable amounts of product 38 via NaH reaction, as indicated by eq 9.



Hydrolytic Studies of 5-Bromocyclophosphamide (11), 3,5-Dehydrocyclophosphamide (13), and Related Systems. The instability of anticancer agents such as 1, 27, TEPA (39), and thioTEPA (40) in aqueous media and the mechanistic details concerning their hydrolysis reactions have been areas of considerable interest and some misconception.[3,22,27] Given this background and the fact

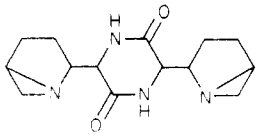


39, X = O
40, X = S

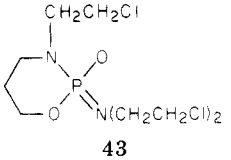


41

that the chemotherapeutic (alkylating) effects of 2,5-piperazinedione derivative **41** have been discussed in terms of in vivo intramolecular cyclizations to bisaziridinyl analogue **42**,[28] we investigated the feasibility of either **11**

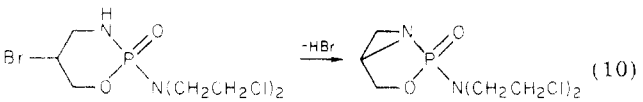


42



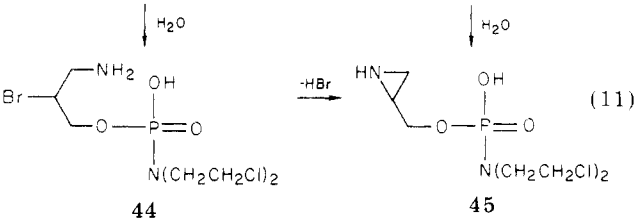
43

→ **13**, **11** → **44**/**45**, or **13** → **45** under aqueous conditions (eq 10 and 11). Such reactions imply latent or "masked"



(10)

11 13

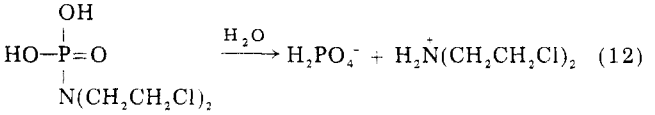


(11)

44 45

tris-alkylating capabilities for **11**/**13** and therefore provide an operational basis for some analogy between these systems and anticancer drugs such as **39**, **40**, and "trophosphamide" (**43**).[15]

NMR has proved to be very useful in studies[22,27,29] related to the solution chemistry of various alkylating agents; therefore, we investigated the hypothetical reactions in eq 10 and 11 with ^{1}H NMR at 220 MHz. The reaction of **11** (0.07 M) in 1:2 (v/v) D_2O/Me_2SO-d_6 was surprisingly slow and was only ca. 50% complete after 40 days at 37 °C; consequently, the sample was heated at 100 °C for an additional 5 days in order to achieve essentially complete disappearance of the absorption signals due to starting material. The complexity of the final spectrum was such that it precluded detailed interpretation; however, the more obvious presence of $(ClCH_2CH_2)_2N^+D_2$ was confirmed by paper chromatography. Our primary interest was to assess whether the hydrolysate mixture was derived from the initial conversion of **11** to **13**, which then underwent further reaction, or if the overall hydrolysis process was triggered by cleavage of the labile endocyclic P–N bond (vide infra), **11** → **44**. Control experiments which involved heating solutions of diastereomerically pure **13a** and **13b** in 1:1 (v/v) D_2O/Me_2SO-d_6 at 80 °C revealed that, while both of these stereoisomers were much more susceptible to hydrolysis ($\tau_{1/2}$ < 30 min) than **11**, neither compound afforded a hydrolysate mixture which was the same as that obtained from **11**. We therefore believe that the hydrolytic pathway for **11** in D_2O/Me_2SO-d_6 involves **11** → **44**/**45** and that intermediates **44**/**45** undergo further hydrolytic reactions that include P–O bond cleavage to give bis(2-chloroethyl)phosphoramidic acid (**46**), which by analogy to phosphoramidic mustard $[(HO)P(O)NH_2$-$N(CH_2CH_2Cl)_2]$[30] would be unstable with regard to the formation of nor-nitrogen mustard (eq 12) and accounts

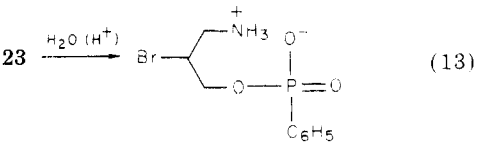


(12)

46

for the NMR detection of $D_2N^+(CH_2CH_2Cl)_2$.

As a final note in this section, it is worthwhile to elaborate upon the endocyclic P–N bond hydrolysis deduced for **11**, which is normally rather sluggish in the absence of strong acidic catalysis (pH <2).[22] Evidence indicating that such hydrolytic ring opening of 1,3,2-oxazaphosphorinane 2-oxides can, however, be subject to much milder and sometimes unexpected catalysis was initially obtained when compound **23** was dissolved in $CDCl_3$ for NMR analysis. A highly insoluble precipitate formed within 2 h and was subsequently identified as **47** (eq 13) by ele-



(13)

47

mental analysis and Fourier transform ^{1}H NMR. Deuteriochloroform obtained from a different source had no effect on **23**, which suggested trace H_2O/HCl contamination in the original $CDCl_3$, and this possibility was supported by finding that stirring a chloroform solution of **23** with a few drops of dilute HCl led to immediate precipitation of **47**. The unusual susceptibility of **23** toward hydrolysis under mild conditions was also seen in its conversion to **47** after 24 h in 1:2 (v/v) D_2O/Me_2SO-d_6 at 37 °C, whereas analogue **24** was inert under these conditions. Aziridinyl system **13** was likewise prone toward adventitious hydrolysis; the disappearance of **13a** was found to obey a first-order rate law with $\tau_{1/2} \simeq 5$ min at 50 °C; however, repeat analyses gave substantially longer half-lives for **13** even at 80 °C (vide supra).

Anticancer Screening Data. The in vivo anticancer activity of diastereomerically pure samples of **11**, **13**, **23**, and **24**, as well as the activity for samples of **26** and **29**, was evaluated against L1210 lymphoid leukemia in mice according to the National Cancer Institute standard protocol for cyclophosphamide analogues.[31] Test samples were administered intraperitoneally in either a pure water or aqueous Tween-80 (polysorbate) vehicle on day 1 only, at various doses, and results were evaluated on day 30. Mean survival time was utilized as the evaluation parameter, and compounds exhibiting a test/control (T/C) percentage greater than or equal to 125 are considered to be active in this preliminary testing system.

From Table I, which lists the highest T/C values obtained for each compound tested, it can be seen that the therapeutic effectiveness of **11a** (T/C = 146) is considerably less than that of **1** (T/C = 252[32]) and that analogues of **11a** without the nitrogen mustard group, viz., **23** and **24**, failed to show activity. The latter results parallel those reported[16] for the 2-diethylamino analogue of **9** and suggest that if **11a**, **23**, or **24** yields α-bromoacrolein (**12**) in vivo the anticancer effect of this metabolite is negligible. Additional information concerning the therapeutic effectiveness of **11** relative to **1** was obtained[33] with ADJ/PC6 plasma cell tumor in mice, and from the therapeutic indices (TI) given in Table II it can be seen that **11** is approximately four times less effective than **1**, as was also the case for the 6-(trifluoromethyl) analogue **9**. Fur-

Table I. Selected Anticancer Screening Data for Mouse L1210 Lymphoid Leukemia

compd[a]	dose,[b] mg/kg	T/C
1	250	252[32]
11a	500	146
13a	62.5	120
13b	500	106
23a	250	100
24a	62.5	107
24b	150	113
26	500	105
29	62.5[c]	110

[a] All compounds are racemic. [b] Dose at which highest T/C was achieved. Except as noted, this dosage was accompanied by 100% toxicity-day survivor (3/3-6/6). [c] 83% toxicity-day survivors (5/6).

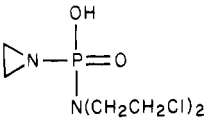
Table II. Screening Data for Mouse ADJ/PC6 Plasma Cell Tumor

compd[a]	ref	LD_{50}, mg/kg	ED_{90}, mg/kg	therapeutic index (TI = LD_{50}/ED_{90})
1	this work	450	4.75	95
11	this work	450	18.0	25
9	16	400	14.0	29
cyclophosphamide				
cis-6-Me-	12b	540	9.7	56
trans-6-Me-	12b	385	9.2	42
cis-4-Me-	12b	225	4.4	51
trans-4-Me-	12b	270	4.7	57

[a] All compounds are racemic.

thermore, it is evident that these lower TI values for **11** and **9** vs. **1** are the result of comparable toxicities (LD_{50}) being offset by the larger doses of **11** and **9**, which are required for tumor cell kill (ED_{90}).[34] The fact that **11a**, like **1**, is inactive in vitro (KB cell culture[35]) but active in vivo provides prima facie evidence for our assumption that **11a** undergoes in vivo "activation" and further metabolism in basically the same manner as **1**(Scheme I). While it is not possible at this time to reliably specify the cause(s) for ED_{90} differences between **11** and **1**, the added bromine substituent in **11** most likely exerts a decelerating effect upon the substrate turnover rate for liver enzyme-mediated C-4 oxidation ("activation").[36] In any event, the screening data for **1**, **11**, and other cyclophosphamide analogues listed in Table II clearly indicate that introduction of substituents at the ring-carbon positions in **1** decreases therapeutic effectiveness. It is also noted that, while small quantities of diastereomerically pure **11b** precluded anticancer testing as a means of evaluating stereochemical factors, the relatively small TI differences between cis and trans diastereomers of both 4- and 6-methylcyclophosphamide (Table II) suggest that **11a** and **11b** would exhibit roughly comparable levels of therapeutic activity. However, caution in this regard follows from the greater toxicity of **13a** (0% survivors at ≥125 mg/kg) relative to **13b** (100% survivors at 500 mg/kg).

A perhaps more important aspect of the toxicity of **13a** is that this compound does *not* require microsomal "activation" to exhibit its toxic effect, which contrasts significantly with **1**. More specifically, tests with Walker 256 cells indicated that **13a** causes 100% growth inhibition at dose levels of 80–320 μg/mL and that the ID_{50} was equal to 1.5 μg/mL.[33] By way of comparison, this ID_{50} value is almost as low as that found for microsomally activated **1** (ID_{50} = 0.5–1.0 μg/mL), and untreated **1** has virtually no effect on Walker 256 cells (ID_{50} > 500 μg/mL).[33] The potency of **13a** is tentatively ascribed to its hydrolytic instability (vide supra), as cleavage of the "strained" endocyclic P–N bond according to eq 10/11 can lead to **45**, which is structurally related to the highly toxic phosphoramide mustard **48**.[37]



48

In ending this section, we note that the feasibility for intramolecular cyclizations of **1** → **26** (eq 4) and **27** → **29** (eq 5) has been previously evaluated in vitro, and it was concluded that such processes would be extremely slow, relative to the time scale for metabolism of **1** and **27**; however, the role of in vivo enzymatic assistance could not be excluded.[22] Consequently, the inactivity now reported for **26** and **29** in the L1210 test system reveals that even if these cyclized compounds are formed as metabolites during treatments with **1** and **27**, respectively, they will most likely make no significant contribution to the observed anticancer activity.

Conclusions

The present work has demonstrated that incorporation of a C-5 bromine substituent into **1** does not lead to enhanced anticancer activity in either the L1210 or ADJ/PC6 animal screening systems. The negative impact of this structural modification upon the anticancer activity of **1** parallels what has been reported[1,12,13,16] for a number of ring carbon-substituted cyclophosphamide analogues and thereby indicates, once again, that the metabolic pathway for **1** is highly responsive to structural perturbations. On the other hand, successful conversion of **11** into **13**, which has been found to be highly toxic without microsomal "activation", represents a new type of synthetic transformation and an explorable approach toward "preactivation" of cyclophosphamide analogues. Our current work with latentiated mustard carriers capable of hydrolytic "activation" in vivo will be reported in the future.

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 a Perkin-Elmer Model 337 spectrometer. ^1H NMR spectra at 60 MHz were recorded on a Varian A 60 instrument at ambient probe temperature, using 10% (v/v) solutions in either $CDCl_3$ or D_2O with tetramethylsilane or TSP[38] as an internal reference, except as noted. ^1H NMR spectra at 220 MHz were obtained in either the continuous-wave or Fourier transform mode on a Varian HR 220 spectrometer equipped with a Fourier transform accessory and 620 L computer. The accumulated free-induction decay signal (8K data points) was transformed to give spectra with either 2500- or 1000-Hz sweep widths; ambient probe temperature was 20 ± 1 °C.

^{13}C NMR spectra were obtained in the pulse Fourier transform mode utilizing a Bruker superconducting magnet with a "homebuilt" spectrometer and probe. A Nicolet 1080 computer system, modified for quadrature phase detection, was used for data collection and transformation. The accumulated free-induction decay signal (32K data points) was tranformed to give a spectrum with 15 151.5-Hz sweep width. A 90° ^{13}C pulse was approximately 27 μs. Spectra were recorded under broad-band pseudo random-noise proton-decoupling conditions at a probe temperature of 28 ± 2 °C, unless otherwise noted. Precooled

nitrogen gas was used to obtain the low-temperature ^{13}C spectra. A chloroform solution containing a copper–constantan thermocouple connected to a precalibrated Doric Trendicator 400 Type T/C digital readout temperature meter was used to establish the probe temperature before and after sample runs.

Except as noted, analytical thin-layer chromatography utilized either 2.5 × 10 or 5 × 20 cm Analtech plates with a 250-μm layer of silica gel GF, while analogous preparative separations were performed with 20 × 20 cm plates having a 1000-μm coating; component visualization as brown-colored spots was achieved by exposure to iodine vapor. Column chromatography employed Baker 60–200-mesh silica gel. All of the reported R_f values are approximate.

All reactions performed in nonaqueous media were conducted with protection from atmospheric moisture.

***O*-(2,3-Dibromopropyl) *N*-Phenylphenylphosphonamidate (16).** A solution of 2,3-dibromo-1-propanol (10.3 mL, 0.1 mol) in ether (140 mL) was added (1 h) to a mechanically stirred solution of phenylphosphonic dichloride (14.2 mL, 0.1 mol) and triethylamine (14.6 mL, 0.105 mol) in ether (200 mL) at ice bath temperatures. After 24 h at ambient temperature, the reaction mixture was suction filtered, and the filtrate was then concentrated on a rotary evaporator. The residual crude 2,3-dibromopropyl phenylphosphonochloridate (32 g, 0.085 mol) was dissolved in ether (120 mL) and the chilled solution was then treated with an equal volume of ether which contained aniline (15.5 mL, 0.17 mol). After mechanical stirring at room temperature for 60 h, the mixture was suction filtered, and the filtrate was concentrated at reduced pressure. Column chromatography of the residual product (82%) using Florisil (60–100 mesh) and methylene chloride/ether (1:1) eluent afforded **16**, which crystallized from methylene chloride/petroleum ether as small white needles: mp 111–113 °C; ^1H NMR (220 MHz) δ 7.89 (m, $^3J_{HH}$ = 8 Hz, $^4J_{HH}$ = 2 Hz, $^3J_{HP}$ = 15 Hz, 2 H, *o*-H of *P*-phenyl), 7.59–7.39 (m, 3 H, *m*- and *p*-H of *P*-phenyl), 7.16 (apparent t, $^3J_{HH}$ = 8 Hz, 2 H, *m*-H of *N*-phenyl), 6.94 (d, $^3J_{HH}$ = 8 Hz, 2 H, *o*-H of *N*-phenyl), 6.90 (t, $^3J_{HH}$ = 8 Hz, 1 H, *p*-H of *N*-phenyl), 6.59 (apparent t, *J* = 5 Hz, NH, exchange with D_2O), 4.70–4.55 (m, CHBr), 4.52–4.30 (m, CH_2O), 3.86–3.66 (m, CH_2Br); ^{13}C NMR[39] δ 140–117 (aromatic), 64.87 (d, $^2J_{CP}$ = 5.4 Hz, CH_2O), 48.13 (d, J_{CP} = 7.7 Hz, CHBr), 32.10 (s, CH_2Br). Anal. ($C_{15}H_{16}NO_2PBr_2$) C, H, N; Br: calcd, 36.90; found, 39.59.

***O*-(2,3-Dichloropropyl) *N*-Phenylphenylphosphonamidate (17).** Use of 2,3-dichloro-1-propanol in the above procedure for **16** yielded (93%) crude product as a yellow oil, which was similarly purified to afford **17** as a microcrystalline powder: mp 88–94 °C; ^1H NMR (220 MHz) δ 7.86 (m, $^3J_{HH}$ = 8 Hz, $^4J_{HH} \simeq$ 2 Hz, $^3J_{HP} \simeq$ 14 Hz, 2 H, *o*-H of *P*-phenyl), 7.61–7.36 (m, 3 H, *m*- and *p*-H of *P*-phenyl), 7.14 (apparent t, $J \simeq$ 7 Hz, 2 H, *m*-H of *N*-phenyl), 6.93 (m, 3 H, *o*- and *p*-H of *N*-phenyl), 6.82 (ca. d with fine splitting, $^2J_{HP} \simeq$ 7 Hz, NH), 4.59–4.45 (m, CHCl), 4.41–4.20 (m, CH_2O), 3.84 (d, *J* = 6 Hz, 1 H of CH_2Cl), 3.79 (d, *J* = 6 Hz, 1 H of CH_2Cl). Anal. ($C_{15}H_{16}NO_2PCl_2$) C, H, N.

***O*-(2-Bromoallyl) *N*-Phenylphenylphosphonamidate (20).** A solution of **16** (1.51 g, 4 mmol) in tetrahydrofuran (15 mL) was added (1 h) to a magnetically stirred suspension of sodium hydride (0.11 g, 4.4 mmol) in an equal volume of solvent. After 5 h at 50 °C, the cooled reaction mixture was suction filtered, and the filtrate was then concentrated on a rotary evaporator. Column chromatography of the residual oil using methylene chloride/ether (1:1) as eluent, followed by crystallization from ether/petroleum ether, yielded (39%) a sample of **20** (mp 90–110 °C) which was found by NMR analyses to contain 20% of unreacted **16**: ^1H NMR (220 MHz) δ 7.88 (m, $^3J_{HH}$ = 8 Hz, $^4J_{HH}$ = 2 Hz, $^3J_{HP}$ = 14 Hz, 2 H, *o*-H of *P*-phenyl), 7.77 (d, $^2J_{HP}$ = 6 Hz, NH, exchange with D_2O), 7.59–7.32 (m, 3 H, *m*- and *p*-H of *P*-phenyl), 7.10 (apparent t, $^3J_{HH}$ = 8 Hz, 2 H, *m*-H of *N*-phenyl), 6.95 (d, $^3J_{HH}$ = 8 Hz, 2 H, *o*-H of *N*-phenyl), 6.84 (t, $^3J_{HH}$ = 8 Hz, 1 H, *p*-H of *N*-phenyl), 5.98 (s with ~1 Hz fine splitting, 1 H, vinylic), 5.61 (s with ~1 Hz fine splitting, 1 H, vinylic), 4.91–4.54 (AB part of ABX with ~1 Hz fine splitting, CH_2O); ^{13}C NMR[39] δ 126.19 (d, $^3J_{CP}$ = 8.4 Hz, =CBr), 118.59 (s, $=CH_2$), 67.00 (d, $^2J_{CP}$ = 5.4 Hz, CH_2O), and aromatic signals (12) virtually identical to those in **16**.

***O*-(2-Chloroallyl) *N*-Phenylphenylphosphonamidate (21).** Use of **17** in the procedure described above for **20**, together with a 25-h reaction period at 50 °C, led to preparative TLC isolation (24%) of a sample of **21** that contained 12% unreacted **17**, as determined from ^1H NMR signal integrations. Repeated attempts to obtain a homogeneous sample of **21** by fractional crystallization were unsuccessful. The identity of partially purified **21** was based on the close similarity of its ^1H NMR (60 MHz) spectrum with that of **20**.

5-Bromo-2-phenyl-2*H*-1,3,2-oxazaphosphorinane 2-Oxide (23). Isomerically pure 3-amino-2-bromo-1-propanol hydrobromide was prepared from allylamine according to literature procedures[40] and was used as a viscous oil, after unsuccessful attempts to obtain crystalline material. A solution of phenylphosphonic dichloride (0.67 mL, 4.7 mmol) in ethyl acetate (10 mL) was added (15 min) to a magnetically stirred solution (10 °C) of the hydrobromide (1.1 g, 4.7 mmol) and triethylamine (1.96 mL, 14.1 mmol) in an equal volume of the same solvent; after 48 h at ambient temperature, the reaction mixture was suction filtered, and the filtrate was then concentrated at reduced pressure. The resultant oil was carefully column chromatographed using methylene chloride/ether (1:1) as eluent, and faster eluting **23a** (mp 145–146.5 °C) and then **23b** (mp 162–163.5 °C) were obtained in 12 and 3% yields, respectively. Anal. for **23a** ($C_9H_{11}NO_2PBr$) C, H, N, Br. Anal. for **23b** ($C_9H_{11}NO_2PBr$) C, H, N. For **23a**: ^1H NMR (220 MHz) δ 7.89–7.75 (m, 2 H, *o*-H), 7.59–7.36 (m, 3 H, *m*- and *p*-H), 4.59–4.11 (m, CHBr and CH_2O), 4.2 (br s, NH), 3.70–3.48 (m, CH_2N); ^{13}C NMR δ 132.77 (d, J_{CP} = 3.7 Hz, aromatic), 131.79 (s, aromatic), 128.96 (d, J_{CP} = 12.9 Hz, aromatic), 70.83 (d, $^2J_{CP}$ = 5.6 Hz, CH_2O), 48.12 (d, $^2J_{CP}$ = 3.7 Hz, CH_2N), 43.27 (s, CHBr). For **23b**, ^1H NMR (220 MHz) δ 7.93–7.75 (m, 2 H, *o*-H), 7.64–7.43 (m, 3 H, *m*- and *p*-H), 4.70–4.39 (m, CHBr), 4.48 (br s, NH), 4.39–4.20 (m, 1 H of CH_2O), 4.11–3.95 (m, 1 H of CH_2O), 3.82–3.55 (m, 1 H of CH_2N), 3.32–3.16 (m, 1 H of CH_2N).

5-Bromo-2-phenoxy-2*H*-1,3,2-oxazaphosphorinane 2-Oxide (24). Use of phenyl dichlorophosphate in the above procedure for **23** gave, after column chromatography, **24a** (9%, R_f 0.65), mp 123.5–125.5 °C, and **24b** (3%, R_f 0.43), mp 115–117.5 °C. For **24a**: ^1H NMR (220 MHz) δ 7.41–7.11 (m, 5 H, aromatic), 4.77 [d (*J* = 12.5 Hz) of t (*J* = 2.5 Hz), 1 H of CH_2O], 4.50 [doubled (*J* = 20.5 Hz) d (*J* = 12.5 Hz) of t (*J* ≃ 2.5 Hz), 1 H of CH_2O], 4.20 (hextet, *J* = 2.5 Hz, CHBr), 4.00 (br s with fine splitting, NH), 3.75 [doubled (*J* = 14.5 Hz) d (*J* = 6.5 Hz) of t (*J* ≃ 2 Hz), 1 H of CH_2N], 3.59–3.32 (m, 1 H of CH_2N). A sample enriched in **24b** led to a ^1H NMR (220 MHz) spectrum which indicated signals for this isomer at δ 4.7–4.3 (CH_2O), 4.3–4.1 (CHBr), 3.5–3.3 (CH_2N). Anal. for mixture of **24a**/**24b** ($C_9H_{11}NO_2PBr$) C, H.

2-[Bis(2-chloroethyl)amino]-5-bromo-2*H*-1,3,2-oxazaphosphorinane 2-Oxide (5-Bromocyclophosphamide, 11). Reaction of 3-amino-2-bromo-1-propanol hydrobromide with bis(2-chloroethyl)phosphoramidic dichloride according to the above procedure used in the preparation of **23** led to the column chromatographic isolation of **11a** (29%, R_f 0.41), mp 88–91 °C, and **11b** (5%, R_f 0.19) as a pale-yellow colored viscous oil. Repeat preparations of **11** sometimes afforded only minute quantities of diastereomer **11b**. For **11a**: ^1H NMR (220 MHz) δ 4.51–4.35 (m, CH_2O), 4.28–4.15 (m, CHBr), 3.91–3.83 (m, NH), 3.73–3.61 (t, $^3J_{HH}$ = 7 Hz, 4 H, CH_2Cl), 3.61–3.36 (m, 6 H, CH_2N). Anal. for mixture of **11a**/**11b** ($C_7H_{14}N_2O_2PBrCl_2$) C, H, N.

2-(1-Aziridinyl)-3-(2-chloroethyl)-2*H*-1,3,2-oxazaphosphorinane 2-Oxide (29). A solution of isophosphamide (**27**, 0.26 g, 1 mmol) in anhydrous benzene was added (1 h) to a magnetically stirred suspension of sodium hydride (0.03 g, 1.2 mmol) in ether (2 mL), and the extent of reaction was monitored by TLC using chloroform/methanol (99:1) as eluent. After 24 h, no **27** was detectable; the reaction mixture was suction filtered, and the filtrate was then chromatographed using chloroform/methanol (9:1) as eluent, and **29**, which elutes slightly ahead of **27**, was isolated (84%) as a colorless oil that crystallized upon standing in the freezer: mp 30–32 °C; ^1H NMR (220 MHz) δ 4.59–4.25 (m, CH_2O), 3.59 (t, $^3J_{HH}$ = 6 Hz, CH_2Cl), 3.68–3.51, 3.32–3.09 (two m, 1 H each, endocyclic CH_2N), 3.48–3.32 (m, exocyclic CH_2N), 2.27–2.02 and 1.76 [m and br d (*J* = 13 Hz), respectively, 1 H each, $CH_2CH_2CH_2$], 2.12 (apparent d, $^3J_{HP}$ = 15 Hz, 4 H, aziridinyl).

3,5-Dehydrocyclophosphamide (13). Reaction of an isomeric mixture of 5-bromocyclophosphamide (**11a**/**11b**, ca. 2:1) with sodium hydride for 48 h was carried out as described above for

27. Preparative TLC using methylene chloride/methanol (98:2) as eluent and iodine vapor spraying of the plate edges led to the isolation of **13a** (10%, R_f 0.26) and **13b** (6%, R_f 0.14). For **13a**: ^{1}H NMR (220 MHz, $CDCl_3$) δ 4.52–4.23 (AB part of ABX, CH_2O), 3.67 and 3.65 (two t, $^3J_{HH}$ = 6 Hz, 2 H each, CH_2Cl), 3.46 (apparent quintet, $^3J_{HH}$ = 6 Hz, $^3J_{HP}$ = 12 Hz, 4 H, exocyclic CH_2N), 3.21–3.05 (m, 1 H, CHN), 2.48–2.30 (AM part of AMX, $\Delta\nu_{AM}$ = 15 Hz, 2 H, endocyclic CH_2N); ^{13}C NMR δ 66.61 (d, $^2J_{CP}$ = 4.4 Hz), 49.43 (d, $^3J_{CP}$ = 3.8 Hz, CH_2Cl), 41.94 (s, CH_2N), 39.71 (s, aziridinyl CH_2N), 29.45 (d, $^3J_{CP}$ = 5.7 Hz, aziridinyl CH). For **13b**: ^{1}H NMR (220 MHz, $CDCl_3$) δ 4.54–4.23 (2 H, CH_2O), 3.75–3.22 (8 H, CH_2CH_2Cl), 3.20–3.07 (m, 1 H, CHN), 2.43–1.95 (AM part of AMX, $\Delta\nu_{AM}$ = 80 Hz, 2 H, endocyclic CH_2N); ^{13}C NMR δ 64.37 (d, $^2J_{CP}$ = 5.5 Hz), 49.96 (d, $^3J_{CP}$ = 3.6 Hz, CH_2Cl), 41.94 (s, CH_2N), 36.90 (s, aziridinyl CH_2N), 29.13 (d, $^3J_{CP}$ = 7.4 Hz, aziridinyl CH).

2-[Bis(2-chloroethyl)amino]-2*H*-1,3,2-diazaphosphorinane 2-Oxide (33). A solution of bis(2-chloroethyl)phosphoramidic dichloride (5.05 g, 19.5 mmol) in ethyl acetate (30 mL) was added (2 h) to a magnetically stirred solution of 1,3-diaminopropane (1.6 mL, 19.5 mmol) and triethylamine (5.4 mL, 39 mmol) in the same solvent (50 mL). After 48 h, the filtrate from the reaction mixture was concentrated at reduced pressure, and the residue was then column chromatographed using acetone eluent. The combined fractions containing **33** (20%, mp 98–103 °C; lit.[41] mp 106–107 °C) were used without further purification: ^{1}H NMR (60 MHz) δ 3.90–2.90 (m, 14 H), 1.90–1.50 (m, 2 H, $CH_2CH_2CH_2$); for 0.4 M **23** containing 0.25 equiv of Eu(tfc)$_3$: δ 8.70–8.25 (m, 2 H, NH), 6.15–5.55 (m, 4 H), 5.55–5.10 (m, 4 H), 5.10–4.40 (m, 4 H), 3.05–2.55 (m, 2 H).

Repeat preparations of **33** gave material which could not be crystallized. Storage of this viscous oil at room temperature led to polymerization that was clearly evident from the complexity of the ^{13}C NMR spectrum; consequently, low-temperature storage is advised.

Reaction of 33 and 35 with Sodium Hydride. A solution of **33** (0.52 g, 2 mmol) in benzene (15 mL) was added (0.5 h) to a magnetically stirred suspension of sodium hydride (0.053 g, 2.2 mmol) in ether (4 mL). After 42 h, TLC analysis using chloroform/methanol (9:1) eluent revealed the presence of unreacted **33**, as well as a new component which eluted slightly ahead of **33** and gave a more persistent color upon exposure to iodine vapors; consequently, more sodium hydride (0.007 g, 0.3 mmol) was added, and hydrogen evolution was noted. After an additional 20 h of stirring and subsequent workup as in the case of **27**, product **35** was isolated (67%) as an unstable colorless oil: ^{1}H NMR (220 MHz) δ 4.05 (br s, NH), 3.66 (t with additional fine splitting, $^3J_{HH} \simeq$ 6 Hz, 2 H, CH_2Cl), 3.59–2.86 (two complex m, 5 H each, CH_2N), 2.00–1.55 (m, 2 H, $CH_2CH_2CH_2$).

The above reaction of **33** was repeated using a large excess of sodium hydride (0.57 g, 24 mmol), and the elutable column chromatographic material (70 mg) was further purified by preparative TLC, wherein the central portion of the band (R_f 0.3) visualized by iodine vapor spraying of the plate edges was collected. The small amount of resultant unstable product (7 mg) was tentatively assigned structure **36** (hexahydro-5*H*-2a,4a,7a-triaza-7b-phosphacyclopent[*cd*]indene 7b-oxide) on the basis of ^{1}H NMR spectral comparisons with **33** and **35**. For **36**: ^{1}H NMR (220 MHz) δ 3.84–3.59 (m, 4 H, CH_2N protons shifted to lower field presumably due to anisotropy of P=O), 3.27–3.05, and 2.95–2.69 (two m, 4 H each, typical chemical-shift range for CH_2N protons), 2.34–2.14, 2.14–1.86 (two m, 1 H each, $CH_2CH_2CH_2$).

Reaction of **35** with 1.2 equiv of sodium hydride was monitored by TLC, and after 48 h the relatively small amount of material having approximately the same retention time (R_f 0.32) as **36** (R_f 0.28) was collected by preparative TLC; however, this sample underwent decomposition before spectral analysis could be performed.

3-(2-Chloroethyl)-2,3-dihydro-1*H*,6*H*-[1,3,2]diazaphospholo[1,2-*a*][3,1,2]benzoxazaphosphorine 4-Oxide (34). A solution of **32**[1] (0.31 g, 1 mmol) in benzene was added to a magnetically stirred suspension of sodium hydride (0.28 g, 12 mmol) in ether, and after 18 h at room temperature the reaction mixture was worked up as in the case of **27**. Chromatography on silica gel using chloroform/methanol (9:1) as eluent afforded product **34**, which had the same R_f value (0.57) as **32**, in 9% yield as an unstable pale-yellow oil that was identified on the basis of its characteristic ^{1}H NMR (220 MHz) spectrum: δ 7.41–7.25 (m, 1 H, aromatic), 7.20–6.98 (m, 3 H, aromatic), 5.33–5.00 (m, CH_2O), 4.40–4.27 and 4.18–4.05 (two m, 1 H each, CH_2N proximate to aromatic ring), 3.94–3.25 (m, 4 H, remaining CH_2N), 3.72 (d of t, $^3J_{HH}$ = 6 Hz, $^4J_{HP} \simeq$ 2 Hz, 2 H, CH_2Cl).

Hydrolytic Conversion of 23a into 47 under Acidic Conditions. A magnetically stirred solution of **23a** (0.25 mmol) in chloroform was treated with hydrochloric acid (0.1 mL of 10^{-3} M), which caused immediate formation of a white precipitate. After 24 h, the microcrystalline powder (mp 180–195 °C) was collected and spectroscopically identified as **47**: ^{1}H NMR (220 MHz, D_2O) δ 7.89–7.68 (m, 2 H, *o*-H), 7.66–7.45 (m, 3 H, *m*- and *p*-H), 4.45–4.30 (m, CHBr), 4.20–3.98 (m, CH_2O), 3.56–3.37 (AB part of ABX: J_{AX} = 3.6 Hz and J_{BX} = 8.9 Hz, or J_{AX} = 4.0 Hz and J_{BX} = 9.1 Hz; CH_2N^+). Anal. ($C_9H_{13}NO_3PBr$) H, N; C: calcd, 36.76; found, 36.09. ^{13}C NMR δ 133.28 (s, *p*-H), 132.81 (d, $^3J_{CP}$ = 9.3 Hz, *m*-H), 130.34 (d, $^2J_{CP}$ = 14.8 Hz, *o*-H), 67.58 (d, $^2J_{CP}$ = 3.7 Hz, CH_2O), 47.93 (d, $^3J_{CP}$ = 9.3 Hz, CHBr), 44.84 (s, CH_2N^+); IR (Nujol) 3450 (br), 1190 (s), 1050, 925, 870, and 695 cm^{-1}.

Anticancer Screening Tests. The essential aspects of the L1210 screening tests are noted in the text and in Table I; for further details, consult ref 31 and 42. Similar comments hold for the KB cell culture tube assay,[35] while the methods of procedure for the ADJ/PC6 test data in Table II may be found in ref 16.

Acknowledgment. This investigation was supported in part by Research Grants CA-16158 and CA-17421 to G.Z. from the National Institutes of Health, PHS/DHEW. High-resolution NMR spectra were obtained at the National Institues of Health Laboratory of Chemical Physics.

References and Notes

(1) For paper 1, see S. M. Ludeman and G. Zon, *J. Med. Chem.*, **18**, 1251 (1975).
(2) Division of Bacterial Products, Bureau of Biologics, Food and Drug Administration, Bethesda, Md. 20014.
(3) D. L. Hill, "A Review of Cyclophosphamide", Charles C. Thomas, Springfield, Ill., 1975; S. E. Salmon and M. Apple, "Review of Medicinal Pharmacology", F. H. Meyers, E. Jawetz, and A. Goldfein, Eds., Lange Medical Publishers, Los Altos, N.M., 1976.
(4) P. J. Cox, B. J. Phillips, and P. Thomas, *Cancer Treat. Rep.*, **60**, 321 (1976), and pertinent references cited therein.
(5) H. J. Hohorst, V. Draeger, G. Peter, and G. Voelcker, *Cancer Treat. Rep.*, **60**, 309 (1976).
(6) V. Draeger, G. Peter, and H. J. Hohorst, *Cancer Treat. Rep.*, **60**, 355 (1976).
(7) I. L. Karle, J. M. Karle, W. Egan, G. Zon, and J. A. Brandt, *J. Am. Chem. Soc.*, **99**, 4803 (1977).
(8) D. A. Adamiak, W. Saenger, R. Kinas, and W. J. Stec, *Z. Naturforsch., C: Biosci.*, **32**, 672 (1977).
(9) P. J. Cox, P. B. Farmer, M. Jarman, M. Jones, W. J. Stec, and R. Kinas, *Biochem. Pharmacol.*, **25**, 993 (1976).
(10) P. J. Cox, P. B. Farmer, A. B. Foster, L. J. Griggs, M. Jarman, R. Kinas, K. Pankiewicz, and W. J. Stec, *Biomed. Mass Spectrom.*, **4**, 371 (1977).
(11) F.-P. Tsui, J. A. Brandt, and G. Zon, *Biochem. Pharmacol.*, in press.
(12) (a) N. Brock, *Cancer Treat. Rep.*, **60**, 301 (1976); (b) P. B. Farmer, M. Jarman, T. Facchinetti, K. Pankiewicz, and W. J. Stec, *Chem.-Biol. Interact.*, **18**, 47 (1977); (c) R. Kinas, K. Pankiewicz, and W. J. Stec, *J. Org. Chem.*, **42** 1650 (1977).
(13) P. J. Cox, P. B. Farmer, A. B. Foster, E. D. Gilby, and M. Jarman, *Cancer Treat. Rep.*, **60**, 483 (1976).
(14) (a) A. Takamizawa, S. Matsumoto, T. Iwata, K. Katagiri, Y. Tochino, and K. Yamaguchi, *J. Am. Chem. Soc.*, **95**, 985 (1973); (b) G. Peter, T. Wagner, and H. J. Hohorst, *Cancer Treat. Rep.*, **60**, 429 (1976).
(15) J. A. Montgomery and R. F. Struck, *Cancer Treat. Rep.*, **60**, 381 (1976).
(16) P. B. Farmer and P. J. Cox, *J. Med. Chem.*, **18**, 1106 (1975), and pertinent references cited therein.
(17) This "cis" and "trans" nomenclature adopted for **11** follows the convention which has been previously used for 4-methylcyclophosphamide,[12c] while the "exo" and "endo"

designations for 13 result from arbitrarily focusing upon the stereochemical disposition of the phosphoryl oxygen in this bicyclic structure. For the sake of simplicity in this paper, only one enantiomer of 11 and 13 is shown, and in other chiral molecules no attempt is made to specify relative and/or absolute stereochemical relationships.
(18) P. Savignac, J. Chenault, and M. Dreux, *J. Organomet. Chem.*, **66**, 63 (1974); J. F. Brault and P. Savignac, *J. Organomet. Chem.*, **66**, 71 (1974). See also, B. Corbel, J. P. Paugam, M. Dreux, and P. Savignac, *Tetrahedron Lett.*, 835 (1976).
(19) Either diasteriomer of 23 has seven anisochronous carbon positions; however, the ^{13}C nucleus directly bonded to phosphorus was not observed under the employed NMR conditions due to its relatively long spin–lattice relaxation time.
(20) W. Egan and G. Zon, *Tetrahedron Lett.*, 813 (1975).
(21) W. Egan, G. Govil, and G. Zon, unpublished conformational energy calculations for 1 using a classical potential function.
(22) G. Zon, S. M. Ludeman, and W. Egan, *J. Am. Chem. Soc.*, **99**, 5785 (1977).
(23) J. E. Baldwin, *J. Chem. Soc., Chem. Commun.*, 734 (1976); J. E. Baldwin, R. C. Thomas, L. I. Kruse, and L. Silberman, *J. Org. Chem.*, **42**, 3846 (1977).
(24) P. Laszlo and P. Stang, "*Organic Spectroscopy: Principles and Applications*", Harper and Row, New York, N.Y., 1971.
(25) W. G. Bentrude and H.-W. Tan, *J. Am. Chem. Soc.*, **95**, 4666 (1973), and pertinent references cited therein.
(26) If 30 → 13 proceeds via an S_N2 mechanism, it follows that *cis*- and *trans*-11 will give *endo*- and *exo*-13, respectively. Cis/trans assignments for faster and slower eluting 11 can, therefore, be made by identifying the NaH product of either pure 11a or 11b as pure *endo*- or *exo*-13. Diminishing supplies of 11, however, precluded pursuit of this experiment.
(27) G. Zon, W. Egan, and J. B. Stokes, *Biochem. Pharmacol.*, **25**, 989 (1976).
(28) R. W. Brockman, S. C. Shaddix, M. Williams, and R. F. Struck, *Cancer Treat. Rep.*, **60**, 1317 (1976). For the analogous cyclization of 3-chloro-1-ethylpiperidine, see C. F. Hammer, M. McCarthy Ali, and J. D. Weber, *Tetrahedron*, **29**, 1767 (1973).
(29) For example, see P. L. Levins and Z. B. Papanastassiou, *J. Am. Chem. Soc.*, **87**, 826 (1965).
(30) Unpublished ^{31}P NMR studies of phosphoramidic mustard by G. Zon and W. Egan have indicated a 33-min half-life at pH 7.9 and ca. 37 °C. See also, P. L. Levins and I. W. Rogers, *Cancer Chemother. Rep.*, **44**, 15 (1965).
(31) 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).
(32) The value of T/C = 252 for 1 is the average of three randomly selected T/C values which were obtained at doses of 250 mg/kg.
(33) We thank Dr. Peter B. Farmer of the Chester Beatty Research Institute for providing us with these results. See Farmer and Cox[16] for a lead reference to details regarding this test.
(34) In contrast to the identical LD_{50} values for 1 and 11 in Table II, the L1210 tests with a dosage of 500 mg/kg for 1 gave ≤50% survivors vs. the 100% survivor rate listed in Table I for 11a at this dose.
(35) Human epidermoid carcinoma of the nasopharynx is the tumor utilized in the KB cell culture tube assay.[31] Synthetic compounds with an ED_{50} value ≤4.0 μg/mL are considered active in this test system.
(36) Aside from the obvious steric "bulk" of an added bromine substituent, solubility perturbations of a more subtle nature may also be a factor in controlling the relative rate of substrate turnover by the microsomal enzyme system. For the role of substrate lipophilicity in determining type I microsomal P-450 binding characteristics, see K. A. S. Al-Gailany, J. B. Houston, and J. W. Bridges, *Biochem. Pharmacol.*, **27**, 783 (1978).
(37) O. M. Friedman and co-workers, *Adv. Cancer Chemother.*, in press.
(38) All chemical shifts in aqueous solutions are internally referenced to the sodium salt of 3-(trimethylsilyl)propionic acid (TSP), unless specified otherwise.
(39) We thank Dr. S. Sojka (Naval Research Laboratory) for obtaining these spectral data and for performing ^1H spin-decoupling experiments to allow for signal assignments.
(40) D. H. Ball, J. M. Williams, and L. Long, Jr., *J. Org. Chem.*, **28**, 1589 (1963).
(41) O. M. Friedman, E. Bogner, V. Grubliauskas, and H. Sommer, *J. Med. Chem.*, **6**, 50 (1963).
(42) 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.

Aryloxyalkyloxy- and Aralkyloxy-4-hydroxy-3-nitrocoumarins Which Inhibit Histamine Release in the Rat and Also Antagonize the Effects of a Slow Reacting Substance of Anaphylaxis

Derek R. Buckle,* D. James Outred, Janet W. Ross, Harry Smith, Raymond J. Smith, Barbara A. Spicer, and Brian C. Gasson

Beecham Pharmaceuticals-Research Division, Brockham Park, Betchworth Surrey RH3 7AJ, England. *Received July 26, 1978*

The syntheses and structure–activity relationships of a number of 4-hydroxy-3-nitrocoumarins, which are both antagonists of a slow reacting substance of anaphylaxis and potent inhibitors of antigen-induced histamine release in the rat, are described. Most active among these are 7-[3-(4-acetyl-3-hydroxy-2-*n*-propylphenoxy)-2-hydroxypropoxy] derivatives having hydrogen or lower alkyl substituents at the C-8 position of the coumarin ring, **168**, **171**, **173**, and **174**.

Disodium cromoglycate (DSCG) has been shown to inhibit the antigen-induced release of the mediators of allergic reaction[1] but it is not equally effective at inhibiting the release of all mediators. In particular, it is poor at inhibiting the release of a slow reacting substance of anaphylaxis (SRS-A) when antibodies other than IgE are involved.[2] It has been suggested that the failure of DSCG to benefit some patients with bronchial asthma might be due to the involvement of IgG antibodies, which might release SRS-A from sources other than the mast cell by a mechanism resistant to treatment with DSCG.[3] If this is so, then a compound with the ability to antagonize the effects of SRS-A, in addition to the stabilization of mast cells, might be expected to be of greater therapeutic value, than DSCG, in the treatment of bronchial asthma.

We have reported previously that some compounds containing the 2-nitro-1,3-dicarbonyl moiety showed potent antiallergic activity, as determined by the inhibition of rat

0022-2623/79/1822-0158$01.00/0